

Vitamin C and the Common Cold in the Asthmatic Population

by

Kathryn Michelle Earhart

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Graduate Supervisory Committee:

Carol Johnston, Chair  
Karen Sweazea  
Christy Lespron

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## ABSTRACT

Asthma is a high-stress, chronic medical condition; 1 in 12 adults in the United States combat the bronchoconstriction from asthma. However, there are very few strong studies indicating any alternative therapy for asthmatics, particularly following a cold incidence. Vitamin C has been proven to be effective for other high-stress populations, but the asthmatic population has not yet been trialed. This study examined the effectiveness of vitamin C supplementation during the cold season on cold incidence and asthmatic symptoms. Asthmatics, otherwise-healthy, who were non-smokers and non-athletes between the ages of 18 and 55 with low plasma vitamin C concentrations were separated by anthropometrics and vitamin C status into two groups: either vitamin C (500 mg vitamin C capsule consumed twice per day) or control (placebo capsule consumed twice per day). Subjects were instructed to complete the *Wisconsin Upper Respiratory Symptom Survey-21* and a short asthma symptoms questionnaire daily along with a shortened vitamin C Food Frequency Questionnaire and physical activity questionnaire weekly for eight weeks. Blood samples were drawn at Week 0 (baseline), Week 4, and Week 8. Compliance was monitored through a calendar check sheet. The vitamin C levels of both groups increased from Week 0 to Week 4, but decreased in the vitamin C group at Week 8. The vitamin C group had a 19% decrease in plasma histamine while the control group had a 53% increase in plasma histamine at the end of the trial, but this was not statistically significant ( $p>0.05$ ). Total symptoms recorded from WURSS-21 were  $129.3\pm 120.7$  for the vitamin C and  $271.0\pm 293.9$ , but the difference was not statistically significant ( $p=0.724$ ). Total asthma symptoms also slightly varied between

the groups, but again was not statistically significant ( $p=0.154$ ). These results were hindered by the low number of subjects recruited. Continued research in this study approach is necessary to definitively reject or accept the potential role of vitamin C in asthma and cold care.

I dedicate this work to my husband, Jared Earhart. My love of nutrition thrived with his encouragement to pursue this career path. His support and expertise in respiratory pathophysiology propelled my success in this research. My husband has given me the confidence to excel in nutrition research in respiratory patients. Without him, I would not be where I am today.

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## CHAPTER 1: INTRODUCTION

### Overview

Asthma impacts 1 in 12 individuals in the United States (Akinbami, 2012). Although this disease affects a huge population, little research has been done to examine a natural remedy for its debilitating symptoms. Complementary and alternative care methods are needed since the traditional medications are expensive and possess potential side effects. Asthmatics are also more prone to experience a common cold every year than individuals without asthma. Vitamin C supplementation has been proven effective in normal, healthy populations to prevent colds (Pauling, 1971). This effectiveness of vitamin C for preventing colds is increased in populations experiencing high stress conditions, such as athletes and military personnel (Jacob, 2002). Although vastly different physiologically, asthmatics are subject to high stress during asthma attacks, when their body is unable to control the reactive oxygen species (ROS) and inflammation caused by leukotrienes and histamine (Liu, 2013). Due to vitamin C's role as an antioxidant, its role as a possible Complementary and Alternative Medicine (CAM) therapy is still untapped in the asthmatic population.

The relationship between vitamin C and colds has been tested for many decades (Akinbami, 2012), and the most effective outcomes of vitamin C supplementation are for high stress subjects (Jacob, 2002). However, in the general population, the benefits of high dose vitamin C on combating the common cold are mixed. We recently completed a study in Spring 2011 in male non-smokers at Arizona State University. Comparing 15

subjects in treatment and 13 subjects in control, there was a significant difference in plasma vitamin C and cold symptoms between the two groups (Johnston, 2014).

On the other hand, current studies examining the effect of vitamin C in the asthmatic populations have limited results. Studies have not shown significant effect of vitamin C on decreasing cold symptoms in asthmatic populations. However, no adverse effects have been documented (Milan, 2013). One randomized controlled trial examining leukocyte vitamin C level in asthmatics after one month supplementation showed a significant difference in leukocyte vitamin C levels in the treatment group compared to the controlled group (Nadi, 2012). As indicated by the latest Cochrane review, more research is necessary to confirm the usefulness of vitamin C as a complementary therapy in asthmatics (Milan, 2013).

The study at hand attempted to bridge the two areas of research. This study examined the effect of vitamin C supplementation for preventing colds in asthmatics and for decreasing asthma symptoms over an 8-week session. The study took place at the height of cold and flu season in January through April 2014. To thoroughly examine the effects, a placebo group was compared to the study group (1000 mg vitamin C daily). Through randomization, this double-blind study attempted to provide a missing link in asthma and vitamin C research. This study was the first of its kind to examine whether vitamin C supplementation reduces the incidence and severity of colds in an adult asthmatic population.

## **The Study Purpose**

The objective of this study is to examine the effect of vitamin C supplementation (1000mg/day) for decreasing cold symptoms in asthmatic adults through a validated cold and respiratory symptom questionnaire administered daily over an 8-week period. To determine participation consistency, self-reported ingestion of capsules is indicated through a calendar submitted at the last blood draw (Week 8). Subjects were advised to consume the pill with meals and record consumption on a provided, simple weekly calendar.

## **Hypotheses**

H1: 8 weeks of 1000 mg daily vitamin C supplementation will decrease the incidence of colds and/or cold symptoms in adults with asthma.

H2: 8 weeks of 1000 mg daily vitamin C supplementation will decrease asthma symptoms and blood histamine concentrations in adults with asthma.

## **Definition of Terms**

Asthmatic adults: Participant over the age of 18 who has been diagnosed by a Medical Doctor sometime in their lifespan with asthma.

BMI: A ratio of weight to height. Calculated as  $\text{weight (in pounds)} / \text{height}^2 (\text{in}^2) \times 703$ .

Common cold: An infectious disease of the upper respiratory system that includes symptoms of stuffy/runny nose, persistent cough, weakness, fatigue, and sore throat.

Excessive exercise: An exercise regimen to train a person for a high-intensity activity, such as a marathon. This schedule typically entails multiple hours of daily exercise.

Gluten intolerance: A condition where a person experiences discomfort when ingesting gluten.

Histamine: A common blood marker used to indicate inflammatory status in the body

Low vitamin C status: A plasma vitamin C level  $\leq 0.75$  mg/dL.

Smoker: Someone who consistently uses  $\geq 10$  cigarettes per week.

Vegan: A person who avoids any foods related to or coming from any animal.

### **Delimitations**

Applicable subjects include individuals ages 18 to 55. Subjects must have been previously diagnosed with asthma by a Medical Doctor. Smokers will be excluded from the study, due to the increasing inflammatory conditions associated with smoking and increasing cold or asthma symptoms (Omenaas, 2003). Participants with normal to low plasma vitamin C status will be included. Finally, interested candidates who already take supplements containing vitamin C will be excluded. Generalizability will also be limited by the small sample size. The study will last from January 2014 to April 2014.

### **Limitations**

Limitations include self-reporting ingestion of capsules, symptoms relating to cold and asthma for the Wisconsin Questionnaire, and diagnosis by an MD. Also, observation of cold symptoms only lasts for the specific 8 weeks in the study. Thus, the possibility for missing any cold or symptom events outside the time block exists.

## CHAPTER 2: LITERATURE REVIEW

### Review of Vitamin C

**History.** As with most micronutrients, the history of vitamin C is tightly intertwined with its deficiency disease: scurvy (Gropper, 2013). Once humans began to explore the world by sea around the sixteenth century, sailors, particularly British sailors, fell ill to this disease. It was not until the 20<sup>th</sup> century that vitamin C was isolated and defined by its structure (Gropper, 2013). Even though present-day societies understand the role of vitamin C in scurvy, the disease still exists today. However, the impact of scurvy is significantly less than it was in the sea-faring days. Rather, scientists currently focus on the potential effect of vitamin C on various illnesses and conditions; one in particular is health prevention by its antioxidant roles.

Scurvy has been around much longer than the sailors. One of the earliest cases of scurvy was found in the skeletal remains of a child in England. Archeologists found lesions in the endocranial and ectocranial sections of the skull. The skeleton is considered to be as old as 2200-1970 BC (Mays, 2008). A more abundant record of scurvy starts with the Spanish and Portuguese explorers. One of the lead explorers, Vasco da Gama, observed swollen extremities and gums with a lack of appetite among his crew members. These symptoms went away after trading for oranges from Moorish traders. A few months later on their return journey, the symptoms returned and 30 men died from the illness. Yet, da Gama associated the illness with displeasure of the gods, rather than from the citrus (Carpenter, 1986; Baron, 2009).

In the sixteenth century, British sailors recorded their experience with scurvy. Sir Richard Hawkins, an English sea captain, claims he saw at least 10,000 men develop the deadly symptoms of scurvy in his 20 years of sailing (Carpenter, 1986). Sir Hawkins eventually began trading for citrus on his journeys to prevent the scorbutic issues (Baron, 2009). In 1590, Sir Hawkins bought hundreds of lemons and oranges from Brazil just to prevent scurvy in his men. From speculation, Sir Hawkins had experience with scurvy from being a prisoner of war in Spain prior to his sailing expeditions. He wrote, “that which I have seene more fruitful is sower oranges and lemons...I wish that some learned man would write of it, for it is the plague of the sea,” (Baron, 2009). A primary reason for this high incidence of scurvy among his men was due to the fact that only a surgeon was on board. Physicians who would write and investigate diseases did not contact many people with scurvy, since it mostly happened while at sea (Carpenter, 1986). One of the first research trials related to scurvy involved James Lancaster in 1601. He gave each of his sailors on one of the British three spoonfuls of lemon juice each morning while three other ships did not have lemon juice. Interestingly, there were little differences in the mortality rates of all four ships; however Lancaster still considered his methodology a success (Baron, 2009).

Major writings of vitamin C preventing and treating scurvy happened in the 17<sup>th</sup> century. A surgeon on the HMS Salisbury, James Lind, was one of the first few people to complete a case-controlled study regarding scurvy (Baron 2009; Bartholomew, 1753). On May 20, 1747, he selected 12 individuals with a similar degree of severity with scurvy, placed them in the same quarters, and fed them similar diets. He split them into pairs,

with each pair receiving one of the following treatments: cider, elixir of vitriol, vinegar, sea water, purgative electuary, or two oranges with one lemon. The only pair of scurbutic sailors to heal was the last pair fed the citrus (Baron 2009; Bartholomew, 1753). Even with this proof, most people were hesitant to accept citrus as a treatment due to the stubbornness of scholars and their traditional mannerisms (Carpenter, 1986).

Although Lind associated the cure for scurvy with citrus fruits in early 17<sup>th</sup> century, it was not until the early 20<sup>th</sup> century when vitamin C was officially discovered. In 1907, Axel Horst and Alfred Frohlich utilized their research in Guinea pigs to indicate a single, specific anti-scurvy component. However, these two researchers did not isolate the single component. Rather, a Hungarian biochemist named Albert Szent-Gyorgyi identified the structure of vitamin C as well as vitamin P. He worked with Charles G. King, a researcher at the University of Pittsburgh, and published their findings of the isolated vitamin C in April 1932 (Grzybowski, 2013). Perhaps the largest obstacle in the isolation of vitamin C was the amount of samples; Szent-Gyorgyi used Guinea pigs and bovine renal tissue during his studies. He soon discovered he could find vitamin C from the Hungarian pepper, which was far more abundant in the city he researched in, Szeged (Grzybowski, 2013). One of the scientists working with Szent-Gyorgyi, Norman Haworth, gave vitamin C the name “ascorbic acid”, which means “without scurvy” in Latin (Grzybowski, 2013). Szent-Gyorgyi’s work with vitamin C allowed him to win a Nobel Prize in physiology and medicine (Gropper, 2013; Grzybowski, 2013).

Today, most research revolves around the antioxidant function of vitamin C in the body. This antioxidant function theoretically supports actions to prevent and perhaps

cure common chronic illnesses. By essentially satiating the electron needs of reactive oxygen species (ROS), vitamin C prevents damage to DNA in the cells, thus preventing chronic diseases and hindering their progression in the body (Yu, 2013). One particular focus is its use in preventing the common cold. Although limited groups have shown statistically significant benefits of vitamin C in this regard, in the general population Vitamin C supplementation has little success. Yet, vitamin C is still one of the first sought remedies during cold and flu season (Hemila, 2013). More detail about the role of vitamin C in diseases will be discussed in later sections of this review.

**Biochemistry.** Vitamin C, also called L-ascorbic acid or ascorbate, is one of the essential water-soluble vitamins in the human diet. In fact, some researchers go as far as to say that vitamin C is “the most important water-soluble antioxidant in human plasma,” (Liang, 2001). Ascorbate is a 6-carbon  $\alpha$ -ketolactone with two freely donateable hydrogens from the two hydroxyl carbons (Gropper, 2013; Rose, 1993). Vitamin C is typically found in one of two forms: ascorbic acid (reduced form) or dehydroascorbic acid (the oxidized form after donation of hydrogens). Semidehydroascorbic acid is considered to be the intermediate, as the mono-oxidized form. However, this form is mostly found in the body in the interconversion of ascorbic acid to dehydroascorbic acid, DHA (Gropper, 2013; Rose, 1993). Although existing in both L- and D-isomers, the L-isomer of ascorbic acid is the biologically active form in humans (Rose, 1993).

In humans, ascorbic acid does not need any kind of alteration in order to be absorbed from the lumen of the small intestine (Gropper, 2013). In the small intestine, ascorbic acid transports into the brush border cells with the assistance of sodium-

dependent vitamin C transports 1 and 2 (SVCT 1 and 2), also called sodium-ascorbate co-transporters (Savini, 2008). SVCT 1 and 2 show specific affinity for the L-ascorbic acid. A  $\text{Na}^+/\text{K}^+$ -ATPase provides optimal energy for the ascorbic acid to cross the electrochemical barrier (Savini, 2008). However, ascorbic acid is not the only form of vitamin C that can be absorbed. Dehydroascorbic acid (DHA) is also absorbed from the small bowel. Through the use of glucose transporters (GLUTs) 1, 3, and 4, DHA is able to cross through the intestinal cells. This is accomplished because of the similarities of DHA with glucose. However, this also poses a problem. Often, these transporters can be saturated with glucose, providing a competition for DHA (Liang, 2001). This is particularly apparent as glucose in the human diet is over one thousand times more evident than vitamin C, considering humans consume glucose in grams versus the milligrams of vitamin C contained in human foods. In the cell, DHA is quickly reduced to ascorbic acid by the oxidation of glutathione (Gropper, 2013). In fact, ascorbic acid accounts for 95% of vitamin C in human plasma (Liang, 2001). If DHA is too high in the body, it would act as a pseudo-ROS, causing the very problem ascorbic acid works to prevent.

Ascorbic acid and DHA generally flow in the blood unbound (Wilson, 2005). The body tightly regulates vitamin C concentration (Padayatty, 2004). However, most adults are considered to be deficient, yet not scorbutic. Some researchers use the following scale to interpret serum vitamin C levels:  $<11 \mu\text{mol/L}$  is considered to be deficient,  $11\text{-}28 \mu\text{mol/L}$  is inadequate, and  $>28 \mu\text{mol/L}$  is adequate (Lam, 2013). Other studies have found that a plasma vitamin C concentration  $\geq 50 \mu\text{mol/L}$  is reflective of

decreased risk of damage from free radicals (Krajcovicova-Kudlackova, 2007). The adrenal and pituitary glands have the highest amounts of ascorbic acid, yet vitamin C can also be found in liver, heart, brain, white blood cells and muscle (Gropper, 2013; Wilson, 2005; Hornig, 1975). Similarly to cells in the small intestine, absorption of ascorbic acid into cells in the rest of the body occurs by SVCT 1 and, primarily, SVCT 2 while DHA competes with glucose for its absorption through GLUT 1, 3, and 4 (Wilson, 2005).

Vitamin C is considered to be generally non-toxic although it has a tolerable Upper Limit. Excess ascorbic acid is usually excreted in the urine. Part of this “nontoxic” association is due to the bioavailability of ascorbic acid. Doses of vitamin C up to 200mg resulted in 100% absorption in the general population; however intakes above 200mg resulted in decreased absorption from the intestinal lumen (Deruelle, 2008). Patients with abnormal iron metabolism or kidney stones may experience dangerous side effects as intake increases, particularly through the use of supplements (Gropper, 2013; Deruelle, 2008).

**Sources, recommended intake: natural and supplemental.** Arguably, the best natural sources of vitamin C are fresh colorful fruits, vegetables, and juices. Common sources are included in Table 1.

Table 1.

*Common Sources of Vitamin C (Gropper 2013; Levine, 1999).*

Food	Amount of vitamin C
Strawberries, sliced, 1 cup	90-95 mg
Orange, 1 medium	80 mg
Red pepper, raw, ½ cup	65 mg
Kale, cooked, 1 cup	55 mg
Kiwi, 1 medium	75 mg
Orange Juice	50-90 mg

Vitamin C is considered to be a very labile chemical (Levine, 1999). Cooking, freezing, and exposure to air cause a decline in ascorbic acid in foods. Even the purchaser source of vegetables and fruits can cause differentiation in amounts of ascorbic acid. For instance, broccoli purchased from a larger company-owned market such as Fry's or Safeway will have 33% less vitamin C than from the independent grower (Levine, 1999). Additionally, cooking can have a variety of effects. One study found a 20% decrease in plasma vitamin C from subjects who ate cooked broccoli compared to those who consumed fresh broccoli (Deruelle, 2008). Another study found that boiling can decrease 50-80% of available ascorbic acid in vegetables (Levine, 1999). Finally,

orange juice, arguably the most popular source of vitamin C in society, can have vastly differing amounts of vitamin C levels. In terms of long term storage, it has been found that freezing orange juice and thawing prior to consumption is the optimal way to utilize orange juice as a source of vitamin C (Johnston, 2002).

The Recommended Dietary Allowance for vitamin C is 75 mg for females and 90 mg for men (Monsen, 2000). The RDA for vitamin C was originally established to prevent scurvy (Omenaas, 2003). However, those with increased states of inflammation are recommended to consume more daily. Some individuals who may have increased needs of vitamin C are smokers, pregnant women, and athletes (Gropper, 2013; Monsen, 2000; Omenaas, 2003). In fact, research has established that smokers should consume an additional 35 mg of vitamin C compared to their nonsmoking counterparts (Gropper, 2013). Some individuals should moderate their intake of vitamin C just below the RDA. These groups include those who metabolize oxalate into kidney stones and those who have impaired iron metabolism; vitamin C can increase iron absorption, which could be deleterious for those with hemochromatosis, or excessive iron in the body (Deruelle, 2008; Massey, 2005).

The requirement of exogenous vitamin C is due to the fact that humans are one of the few mammals that cannot produce vitamin C from glucose. Homo sapiens lack the L-gulonolactone oxidase enzyme, which would normally allow for glucose to be converted into ascorbic acid in the liver (Delanghe, 2011). Humans who do not consume vitamin C for approximately 5-6 weeks have undetectable levels of plasma ascorbic acid (Delanghe,

2011). For this reason, vitamin C intake is so critical that some doctors may recommend supplementation beyond just the five fruits or vegetables a day (Omenaas, 2003).

A tolerable Upper Limit has been established for vitamin C intake, 2000 mg intake daily, because vitamin C may cause gastrointestinal problems such as cramping, osmotic diarrhea, and bloating (Monsen, 2000; Omenaas, 2003). Supplemental vitamin C may be provided in pills or intravenous supplementation (Padayatty, 2004). Most physicians recommend an intake of 1000 mg daily (Omenaas, 2003). In the current human diet, getting that much vitamin C from food alone is difficult. In rare cases, some individuals may not respond as well to supplementation. One study found that several patients with scurvy had no significant change in plasma vitamin C levels when taken by supplement. However, ingestion of natural lemon juice provided an increase in plasma concentrations to rectify the classification of the scorbutic state (Deruelle, 2008). Generally, most studies have shown a significant change in plasma vitamin C levels when given supplemental vitamin C.

**Functions: antioxidant and enzyme necessity.** One of the crucial actions of vitamin C revolves around its antioxidant roles in the blood. Vitamin C acts as a strong reducing agent in the aqueous solutions of the body, primarily including blood and cytosol (Gropper, 2013). The structure of vitamin C permits its actions as a strong antioxidant. Vitamin C donates the two H<sup>+</sup> from the hydroxyl groups, forming the semidehydroascorbic acid and DHA. However, the aspect of ascorbic acid that makes it such an effective antioxidant is its stability. When ascorbic acid donates its hydrogens, the resulting structures are actually very stable, especially in comparison to the dangerous

free radicals in the body (Heo, 2013). Then, some of the DHA is converted back to ascorbic acid, while the majority is metabolized through the actions of hydrolysis (Heo, 2013). Some of the free radicals that vitamin C neutralizes includes hydroxyl, hydroperoxyl, superoxide, alkoxyl, and peroxy radicals (Gropper, 2013; Shanmugasundaram, 2001).

In addition to neutralizing ROS, vitamin C's mechanisms as a reducing agent are strongly applicable to other antioxidants. With the help of niacin, vitamin C takes a supporting role in preventing lipid peroxidation. Niacin helps in the Thiol Cycle, with glutathione, and reduces the inactive DHA to the active ascorbic acid. After vitamin C donates the hydrogens to the oxidized vitamin E, vitamin E is able to act as the primary lipid antioxidant (Packer, 1979). Dehydroascorbic acid is reduced by glutathione, another antioxidant, via dehydroascorbate reductase. Glutathione donates its hydrogens from GSH to form GSSG. In fact, the GSH: GSSG ratio is used to measure oxidative stress in patients (Urso, 2003). Vitamin C interacts in many different ways with other antioxidants in the body.

It should be noted that vitamin C is also considered to act as a pro-oxidant in addition to its popular antioxidant properties. The biological decision for vitamin C to act as a pro-oxidant specifically depends on the concentrations of free metal ions such as iron and copper. Ascorbic acid can reduce ferric iron ( $\text{Fe}^{+3}$ ) to ferrous iron ( $\text{Fe}^{+2}$ ). Unfortunately, in this reaction, the ROS hydroxyl radical  $\text{O}_2^-$  is produced, as well as  $\text{H}_2\text{O}_2$ . This could potentially pose some problems in lipid peroxidation. However, this would occur if there was a higher concentration of free iron or other metal ions floating in

the blood stream. Free iron in the form of  $\text{Fe}^{+3}$  can be a threat to the body; the free iron can cause critical damage to cellular components. Patients with iron-binding issues in the blood should be cautious about taking vitamin C without an iron-chelator due to possible complications regarding the pro-oxidant effects and duties of ascorbic acid in the body (Du, 2012).

The role of vitamin C in collagen formation is tightly associated with the symptoms of scurvy, and perhaps the reason for the discovery of vitamin C. Bleeding gums and difficult-to-heal wounds associated with a scorbutic state are reflective of insufficient collagen formation. Ascorbic acid is key for the early steps of collagen formation. It acts as a cofactor in the hydroxylation reactions of proline and lysine which will eventually form the stability of the triple-stranded helix formation of procollagen. Prolyl hydroxylase and lysyl hydroxylase oxidize iron from  $\text{Fe}^{+2}$  to  $\text{Fe}^{+3}$ . Vitamin C acts to reduce the iron back to the ferrous state. The reduction of ferric iron allows for the continuation of enzyme activity, resulting in collagen synthesis (Sørensen, 2006).

Vitamin C is also involved in the formation of carnitine. A lack of carnitine caused by decreased vitamin C status is thought to be the genesis of fatigue or weakness in patients with scurvy. Ascorbic acid is involved in two specific hydroxylation reactions in the formation of carnitine in the body. The two enzyme reactions involve  $\alpha$ -ketoglutarate-dependent dioxygenases. These enzymes utilize ferrous iron ( $\text{Fe}^{+2}$ ) and a reducing agent in the form of ascorbic acid. In scorbutic Guinea pigs, a decrease of muscle carnitine concentrations are found specifically in the skeletal muscle, hepatic and renal tissues, and heart muscle (Rebouche, 1991). Decreases in carnitine concentrations

were rectified with supplementation of ascorbic acid (Rebouche, 1991). Although scurvy is more associated with the decreased collagen formation, decreased carnitine is still prevalent in scorbutic people.

Another way vitamin C acts in the immune system is by managing histamine levels. In the body, histamine is a protein made from the amino acid histidine (Gropper, 2013). Histamine is crucial for hydrochloric acid release in the stomach from stimulation via the vagus nerve. Then, histamine binds to the H<sub>2</sub> receptors allowing acid secretion into the stomach (Gropper, 2013; Hagel, 2013). Additionally, histamine simultaneously plays a positive and negative role in immune function. Histamine causes the bronchial smooth muscle to constrict, leading to a cough (Gropper, 2013). Also, histamine dilates the capillaries throughout the body to increase white blood cell delivery to areas of infection. This dilation may then result in other symptoms related to a cold, such as a runny nose (Gropper, 2013). Due to the outcomes of higher histamine levels, antihistamine medications are common treatment for cold-like symptoms (De Sutter, 2012). However, vitamin C helps to stop the action of histamine once the body has found the foreign invader. As studied in vitro, vitamin C breaks the imidazole ring of histamine; this break alters the protein, ceasing its function. Vitamin C also decreases the activity of histidine decarboxylase, the enzyme that creates histamine from histidine (Hagel, 2013; Uchida, 1989; Gropper, 2013). In Guinea pig studies, supplementing vitamin C led to a significant decrease in plasma histamine concentration (Hagel, 2013; Johnston, 1996). These Guinea pig studies are relatable to humans because Guinea pigs cannot make vitamin C from glucose, like humans. Human studies have shown a similar

result: an inverse relationship between serum vitamin C levels and serum histamine levels (Hagel, 2013). Mega-dosing vitamin C (7.5g) intravenously has shown to decrease serum histamine levels by 31.3% in both healthy patients and patients experiencing allergy symptoms within 60 minutes of infusion (Hagel, 2013). Vitamin C's role in decreasing histamine concentrations is relative to its use to fight symptoms from the common cold virus.

Histamine-receptors and histamine levels can be increased by leukotrienes (LT) (Ogawa, 2006). Although vitamin C can influence histamine levels, vitamin C does not affect LTs as well. Ascorbic acid has been shown to decrease Arachidonic Acid, a LT precursor, in Guinea pigs. However, the same activity cannot be said for humans. However, synthetic forms of vitamin C have been shown to decrease lipoxygenase activity; these enzymes result in damage to the lipid bilayer of cell membranes (Mohamed, 2014). Currently, vitamin C does not have a strong impact on LTs, but can decrease the other contributors to inflammation, specifically ROS and histamine.

**Actions in diseases.** Vitamin C's role in diseases is primarily recognized in its deficiency disease called scurvy. Although rare today, humans may still get scurvy if their plasma vitamin C drops below 0.2 mg/dL (Jacob, 2002).

Health professionals have developed a mnemonic of the four Hs to summarize scurvy symptoms: hypochondriasis, hematology, hemorrhages, and hyperkeratosis of hair (Gropper, 2013). Hypochondria, a psychological disorder, relates to vitamin C's role as a reducing agent for mineral cofactors or cosubstrates in enzymatic reactions that produce

neurotransmitters such as norepinephrine and serotonin (Gropper, 2013). Hematology refers to abnormalities in collagen production and iron absorption that occur when vitamin C levels are too low. This category includes the fatigue that many patients experience from decreased carnitine synthesis as well as the increased time in wound healing (Gropper, 2013). Hemorrhages refer to the petechiae, or small red spots on the skin from ruptured capillaries, bleeding gums, and bruising. Collagen formation is required to heal or maintain these tissues; when vitamin C is low, the triple helix cannot form due to impaired synthesis of hydroxylysine and hydroxyproline and the strength and integrity of these vascular tissues are compromised. Finally, hyperkeratosis relates to the weak, corkscrew-shaped hair that grows out of follicles with red-purple bruises at the root. Similar to the hemorrhaging symptom, the lack of collagen formation results in structurally weak vessels and hair, which normally rely on collagen formation to maintain their shape (Gropper, 2013).

Apart from the primary function of collagen formation, the high antioxidant function of vitamin C is applicable to many studies of other disease states. Vitamin C intake, status, and mega-dose treatment plans have been studied in many problematic diseases and conditions humans face today. Arguably the most prevalent, deadly disease humans face today is heart disease; this disease, primarily atherosclerotic in genesis, has caused millions of deaths across the world and is the number one cause of death in the United States for adults over the age of 65 (Gebhard, 2014; Sharma, 2013). At first glance, vitamin C's role in preventing and protecting against heart disease and its complications seems fairly straight-forward. Cholesterol builds up in the walls of blood

vessels from increased inflammation. This causes the blood vessels to constrict, increasing blood pressure and stressing the myocardial tissue. Eventually, a cardiac event occurs. Unfortunately, most adults do not know they have increased inflammation until the cardiac event happens and they are hospitalized. Because of the high incidence of death, a focus in today's society is to prevent heart disease and decrease the current inflammatory state ideally through diet, primarily with antioxidants such as vitamin C, and exercise. However, researchers today are still unsure of ascorbic acid's action; whether vitamin C acts as a primary defense or simply as a cofactor to additional medications is still being examined today (Gebhard, 2014).

Adequate vitamin C intake has been shown to prevent heart disease (Sharma, 2013). 500 mg supplementation of vitamin C has been shown to significantly lower blood cholesterol levels within 30 days (Sharma, 2013). One study looked at increasing fat or cholesterol in the diet and its effect on blood cholesterol levels. Those who added fat or cholesterol to their diets increased their serum blood cholesterol levels. However, when supplemented with vitamin C, participants with an increased fat consumption did not have an increase in blood cholesterol levels when supplemented with vitamin C (Sharma, 2013). Vitamin C works *in vivo* to decrease cholesterol in two primary ways. First, vitamin C lowers LDL cholesterol by increasing its excretion to bile. Second, vitamin C's antioxidant capacities prevent oxidation of cholesterol; this oxidation of cholesterol can lead to increased build up in the arterial walls (Gropper, 2013; Sharma, 2013). Additionally, many studies have indicated an inverse relationship between vitamin C and cardiovascular disease risk (CVD); higher intake of vitamin C has been

shown in multiple studies to decrease risk of CVD (Ashor, 2014). Supplementing at least 700 mg vitamin C daily can attribute to a 25% decrease in risk for coronary heart disease (Ashor, 2014). Overall, studies related to intake of vitamin C have shown that it decreases risk for cardiovascular issues.

An individual's vitamin C status can also impact a person's risk for disease or the progression of a disease state. Similar to most nutrients, vitamin C has the most effect on an individual when the subject initially has a lower vitamin C status at the beginning of the trial. For example, studies examining effects of vitamin C on endothelial function (EF) related to CVD indicated that subjects with adequate vitamin C status had no effect on EF markers when supplemented with vitamin C (Ashor, 2014). However, those who had a lower vitamin C status had shown improvement in EF markers. The dose of exogenous vitamin C also impacted EF markers; the higher the dose of vitamin C, the greater the response in EF markers (Ashor, 2014). Evidence of vitamin C status affecting the effectiveness of vitamin C treatment has been researched in individuals with genetic single nucleotide polymorphisms (SNPs) affecting vitamin C absorption. One study looked at both men and women who had SNP for the SVCT 2 transporter. These individuals would generally have a lower vitamin C status due to a disruption in absorption of ascorbic acid into the metabolically active tissues, including the aorta and other cardiac tissues (Dalgård, 2013; Gropper, 2013; Hediger, 2002). The study found that women, but not men, with the SVCT 2 SNP had a higher risk for acute coronary syndrome (ACS). This was true for the women who consumed both high ( $\geq 127$  mg) and low amounts of vitamin C ( $\leq 100$  mg) in the diet, but was not consistent in men (Dalgård,

2013). Sex was an important discriminating factor in the assessment for cardiac risk; the researchers concluded that hormonal response may play a role in this SNP, but the direct causation is still being researched. The results of this study suggested a “ceiling” for plasma vitamin C levels in this participatory group. This was further indicated by a lack of response to vitamin C supplementation (Dalgård, 2013). This study found that those with a consistently low vitamin C status, due to genetic variation, had an increased risk for developing cardiac issues. Overall, a relationship between vitamin C status and cardiovascular health risk is evident.

Another deadly, chronic disease that has been the focus of vitamin C research is cancer, specifically lung cancer. Every year, approximately 1.3 million new cases of lung cancer are recorded (Luo, 2014). In 2006, lung cancer was responsible for 162,460 deaths (Slatore, 2008). Vitamin C has been examined as a preventive measure and as a form of CAM. In general, research in Vitamin C and lung cancer has shown mixed results. One primary focus with vitamin C and its potential role in lung cancer relates to prevention. Vitamin C’s role has two parts: decreasing damage from cigarette smoking and supplemental protection. Cigarette smoke is thought to be related to 90 percent of lung cancers (Slatore, 2008). Cancer is still eminent after stopping the habit (Slatore, 2008). In research, vitamin C is shown to decrease the impact of cigarette smoke. In vivo, cigarette smoke can increase cell proliferation, which increases the activity of cancerous cells. This happens primarily from a compound called p-benzoquinone (p-BSQ) (Dey, 2011). The damage to cells occurs when p-BSQ converts to p-benzoquinone (p-BQ) (Dey, 2011). A cascade of reactions happen, including excessive

epidermal growth factor receptor (EGFR) and Akt, a protein kinase that allows the cancerous cells to thrive, which ultimately result in tumors on the lungs (Dey, 2011). In Guinea pig studies, those deficient in vitamin C had extensive tissue damage and tumor growth from cigarette smoke. Vitamin C reduces p-BQ to a hydroquinone, which renders it inactive (Dey, 2011). Because of its clear role in decreasing damaging semiquinones, vitamin C supplementation's effect on lung cancer risk has been examined through studies. In those with lung cancer, serum vitamin C levels are significantly lower than healthy control patients (Mahdavi, 2009). Those with lung cancer had  $0.13 \pm 0.03$  mg/dL vitamin C while the healthy counterparts had  $0.89 \pm 0.07$  mg/dL (Mahdavi, 2009). Additionally, malondialdehyde is significantly higher at  $4.6 \pm 2.4$  nmol/L in those with cancer compared to  $0.27 \pm 0.11$  nmol/L (Mahdavi, 2009). Malondialdehyde is associated with lipid peroxidation and DNA malformation, which is caused by uncontrolled ROS activity (Mahdavi, 2009). The lower serum vitamin C level may be attributed to the higher malondialdehyde. Since those with cancer had such low levels of vitamin C, supplementation is thought to reduce the risk of getting lung cancer due to vitamin C's antioxidant capacity. However, studies have shown no difference in risk. In the VitAL (Vitamins and Lifestyle) study, 77126 subjects were followed for 4 years and supplementation regimen was documented. 521 of all subjects developed lung cancer (Slatore, 2008). The researchers found there was no significant difference in risk in those who took 1000mg supplemental vitamin C and those who did not. The study did note that there may be promise in male subjects with vitamin C supplementation and decreased cancer risk (Slatore, 2008). Another study confirmed this observation in males

over females, noting that for every 100mg vitamin C taken, the risk for getting cancer decreases by 7% (Luo, 2014). The study also discussed that those who consumed vitamin C in the form of fresh fruits and vegetables do have a protective factor against getting lung cancer (Slatore, 2008). Overall, it seems that the form of consumed vitamin C has an effect on cancer risk, but vitamin C in general has been shown to decrease the dangerous effect of cigarette smoking.

Vitamin C has also been studied as a form of CAM in those who do have lung cancer already. In cancer patients, high-dose vitamin C is administered intravenously (IV), ranging from 5-50g (Mikirova, 2012). Inflammation in the body is typically associated with increased cancer cell growth. One study examined the effect of IV vitamin C on inflammation levels; C-reactive protein and pro-inflammatory cytokines, such as IL-2, IL-8, and TNF- $\alpha$ , were examined. An inverse correlation has been found in inflammatory levels and tumor markers. The study found that vitamin C did have a significant effect on CRP levels and inflammatory status (Mikirova, 2012). This study shows that vitamin C may be a suitable complementary therapy to other cancer treatment plans. There is not enough evidence to use vitamin C as a sole treatment for cancer, although studies are still being completed.

Vitamin C has been recently studied in relation to protection against gout. However, the results are varied. Gout, in general, is a buildup of uric acid in the body (Stamp, 2013, Choi, 2009). Gout is controlled by lowering the serum urate to less than 0.36 millimoles per liter (Stamp, 2013). There are two main ways to decrease serum urate in the body. The first is to decrease the production of uric acid by medications that

inhibit the xanthine oxidase enzyme (Stamp, 2013). The second is to increase the excretion of urate from the body; this is achieved by slightly manipulating the kidneys through medical treatment (Stamp, 2013). However, vitamin C has been proposed as an alternative way to manage gout. Vitamin C competes with uric acid in the proximal tubules of the kidneys as both utilize anion-exchange transport mechanism for reabsorption; this increases reabsorption of vitamin C and excretion of uric acid in the urine (Choi, 2009). One small study examined the effects of mega-dosing vitamin C and its effects on serum urate concentrations. There was a notable increase in urate excreted via urine. Interestingly, the serum urate concentrations did not show any significant change (Stamp, 2013). On the other hand, another randomized controlled trial showed a statistically significant 0.02mmol/L decrease of serum urate in healthy individuals when supplemented 500mg vitamin C daily for 60 days (Stamp, 2013). In the same study, the subgroup that had baseline serum urate levels  $> 0.42$  mmol/L had a reduction of 0.09 mmol/L; this change was four times greater than the general population, indicating that those with gout may be likely to see a change in serum urate concentrations (Stamp, 2013). However, another study that compared 20 subjects given daily 500 mg vitamin C to 20 subjects given standard xanthine oxidase inhibitor allopurinol (50-100mg, depending on the subject's physician and renal status), which inhibits uric acid production, did not find a clinically significant decrease in serum urate. It should be noted that this study still saw the same change from baseline (0.02 mmol/L). The researchers noted that 500 mg/day of vitamin C, which is the common dosage in these trials, may be too low to see a clinically significant change in serum urate (Stamp, 2013).

In terms of preventing gout, one 20-year longitudinal study of 51529 males in health profession found that those who consumed more total vitamin C (via food or supplement) had a statistically significant lower risk for developing gout than those who had less than 250 mg/day (Choi, 2009). Interestingly, the males who consumed over 1000 mg/day vitamin C had no significant change than those who consumed between 500 and 999 mg/day. This study concluded that an intake of 500 mg/day vitamin C is strongly associated with a decreased risk for gout (Choi, 2009). Altogether, current usage of vitamin C as alternative medicine is still mixed in research.

## **Review of Asthma**

**Definition and diagnosis.** The official diagnosis of asthma occurs with the clinical assessment of a licensed medical doctor in tandem with laboratory results. Patients with asthma will show the common symptoms, which include cough, chronic wheezing, shortness of breath, and tightness in the chest. Any extent of these symptoms may be present, including a small cough. After observation of the symptoms, pulmonary function tests (PFT) will show what is called reversible airflow obstruction. Decreased FEV<sub>1</sub> and FEV<sub>1</sub>:FVC ratio is indicative of airway obstruction. Because asthmatics are at a state of chronic inflammation, elevated IgE and eosinophilia will be present, but not solely indicative of asthma. Additionally, a chest x-ray may indicate asthma-induced inflammation. One popular test that can be run is the methacholine provocation test, also called the histamine challenge test. All tests together can indicate if a patient has asthma (Omenaas, 2003).

The diagnosis of asthma is not as easy as it appears. One common saying among respiratory experts is that “all that wheezes is not asthma” (Omenaas, 2003). Some other disorders may copy the signs and symptoms of asthma; many of these are considered to be much more serious than treatable asthma. These mimicking conditions include allergic inflammation of the sinuses, congestive heart failure, pulmonary embolism, and, perhaps the most commonly confused condition, chronic obstructive pulmonary disorder (COPD) (Corren, 2013). The difference between asthma and COPD is simple: COPD is irreversible (Omenaas, 2003). Asthma symptoms are completely, or at least partially, reversible. Analyzing whether or not something is irreversible takes some time. Therefore, many studies go ahead and couple COPD patients and asthma patients together.

The definition of asthma has changed over time. Previously, most definitions focused on the association of airway hyperresponsiveness, a condition where the human airway abnormally constricts from a stress response or inhaled allergens. Today, the focus of what classifies a patient as having asthma has shifted. Asthma is now considered a “primary inflammatory disease of the airways, with clinical manifestations of increased bronchial hyperreactivity and airflow obstruction due to the inflammation,” (Wilkins, 2009). Basically, asthmatics live in a constant state of inflammation, particularly in the trachea. This inflammation can lead to blocked airways, preventing normal respiration. Asthma, along with COPD, is one of the most common conditions under the larger spectrum of obstructive lung diseases, which also includes cystic fibrosis (Wilkins, 2009).

Asthma has been around for centuries. Interestingly, the term asthma derives from the Greek word for “panting”. Hippocrates gave the name asthma to the condition around 400 B.C. One early 20<sup>th</sup> century book called *Principles and practice of Medicine* described asthma as “swelling of the nasal or respiratory mucous membrane, increased secretion, and...spasms of the bronchial muscles with dyspnea, chiefly expiratory,” (Maslan, 2014). The author of the book, Sir William Osler, also indicated that treatment could happen with tobacco-based cigarettes. Obviously, this treatment has been dismissed, as smoking has shown to increase inflammatory conditions leading to asthmatic episodes (Omenaas, 2003).

Asthma is not universal among all patients. A group of asthmatics can have a myriad of different experiences. However, attempt has been made to identify common experiences among asthmatics. Through analysis of common themes among patients, different subgroups of asthmatic patients can be created (Wilkins, 2009). Generally, these themes revolve around the different phenotypes of asthmatic patients, or outward, observable characteristics. One theme that has been examined is whether the patient has atopic asthma or not. Atopic conditions are genetic-based allergic reactions that happen immediately after short exposure to a certain antibody (Omenaas, 2003). Another theme that is looked at is the time at which asthma began. More than often, asthma begins in childhood; this onset is considered early. In less frequent cases, asthma may occur later in life (Wilkins, 2009). The need for daily or emergency medication can separate asthmatics. Additionally, analysis of sputum to see the differences at the cellular level can differentiate asthmatics from one another. The sputum analysis can show

differences in antibodies present as well as inflammatory markers such as interleukin (IL) (Wilkins, 2009). Other themes among asthma patients include any decrease to lung function and Nitric Oxide (NO) expiration. The level of NO exhaled can show a correlation with IL-13 activity (Wilkins, 2009).

Very recently, more cases of exercise-induced asthma in adults have appeared. However, the term “exercise-induced asthma” is no longer used. Rather, professionals name this condition as exercise-induced bronchoconstriction (EIB). This is a reversible tightness of the airways brought on by at least 5-10 minutes of strenuous exercise. Common exercises that induce this condition include running and cycling. It can occur in swimmers, however much less often. Ninety percent of people with the diagnosis of asthma later in life show exercise-induced bronchoconstriction as one of the first signs of their asthma. EIB can impact all athletes on any skill level, from novices to elites. Diagnosis of this condition is done with an exercise challenge on a treadmill or bike. EIB affects 7-20% of the general population, and thus is crucial in the discussion of asthma (Randolph, 2009).

**Assessment tests.** Some people with asthma have decreased pulmonary function as a result of the constant inflammatory condition (Wilkins, 2009). Often, pulmonary function tests (PFTs) are used in asthma research to determine the success of interventions (Bender, 2004). However, other forms of tests, including Histamine Challenge and the Rint technique can also assess asthma status. Specific pulmonary function tests look at expiratory air and its volume or contents.

Spirometry looks at a multitude of pulmonary function tests useful for analysis of the severity of many different obstructive lung diseases; asthma falls under this category of respiratory diseases (Wilkins, 2009). Spirometry can also diagnose obstructive diseases (Mayo Clinic Staff, 2014). One pulmonary function test included in spirometry used is FEV<sub>1</sub>. FEV<sub>1</sub> is forced expiratory volume at 1 second. This measures the maximum volume of air a patient expires during the first second of a forceful exhale (Wilkins, 2009). In many research studies, FEV<sub>1</sub> is used to validate alternative tests to spirometry (Kooi, 2006). Asthmatics will find a decrease in FEV<sub>1</sub> as the severity of the disease increases (Oakes, 2008). Another PFT included in spirometry is FEF, or Forced Expiratory Flow. FEF measures the speed of the expired air in a certain amount of time. FEF tests can range from 25 to 1200 milliseconds (Wilkins, 2009). FEF will decrease in severe asthma (Oakes, 2008). These tests included in spirometry generally decrease in asthmatics due to the increase in airway obstruction and resistance (Wilkins, 2009). However, some PFTs can increase in asthmatics; most of these measure lung volume (Oakes, 2008). While the rates of speed and air expired generally increase, the volume of the lungs, interestingly, increases in the obstructive diseases. This happens because the body is trying to compensate for the difficulty breathing (Oakes, 2008; Wilkins, 2009). One of the PFTs that reflect this is RV, or Residual Volume. An increased RV can indicate the presence of air trapping, which happens in obstructive diseases. Because the body cannot expire the normal amount of volume, air is “trapped” in the lungs (Wilkins, 2009). Table 2 shows the trends in asthma with common PFTs.

Table 2.

*Pulmonary Function Tests (Oakes, 2008)*

Pulmonary Function Tests	Results in Asthma
RV	Increase
TLC (Total Lung Capacity)	Slight increase but within normal range
FEV <sub>1</sub>	Decrease
FEF 25-75	Decrease
FEF 200 – 1200	Slight decrease but within normal range

Another test that can measure the activity of obstructive diseases is the Histamine Challenge. The Histamine Challenge test involves administered exogenous 0.06 to 3.9  $\mu\text{mol}$  histamine in the form of histamine acid phosphate (Backer, 1991). Prior to administration of histamine, a baseline test of lung function is completed. Then, PFTs are completed after each dose. A Histamine Challenge can show bronchial hyperresponsiveness when PFTs, such as FEV<sub>1</sub>, decrease. The bronchial hyperresponsiveness correlates with the asthmatic condition. This test has been shown to be fairly accurate, with a 95% confidence interval (Backer, 1991; Schoeffel, 1980). Most asthmatics will show a significant decrease between 2 and 20% of baseline FEV<sub>1</sub> (Backer, 1991). The Histamine Challenge test is a tool that can show how responsive an

asthmatics airway is to foreign particles in the body. An exercise challenge test may be completed as well, but is not as reliable as the Histamine Challenge (Schoeffel, 1980). Most Histamine Challenge and exercise challenge tests have been researched for disease diagnosis in children with asthma rather than adults (Haby, 1994).

PFTs may be difficult to obtain in children, due to the discomfort of the mask and its effect on normal breathing (Kooi, 2006). When a mask is used, PFTs can vary as much as 10% (Child, 2005). To rectify this complication, another test can be performed. The Interrupter Technique (Rint) is used to measure the pressure of the mouth. This method was discovered in 1927 by Von Neergaard (Child, 2005). Recently, a portable, inexpensive device has been created. The device is essentially a handheld box, about the size of a phone, with a short tube. The patient puts his or her mouth on the tube and the patient breathes as normal as possible. Five to ten measurements are taken and the results are compared to the equation: Expiratory Rint ( $\text{kPa} \cdot \text{L}^{-1} \cdot \text{s}$ ) =  $1.972 - 0.00992 \times \text{standing height (cm)}$  (Kooi, 2006; Child, 2005). Although much less invasive than spirometry, the Rint method has been shown to be as valid as FEV<sub>1</sub> (Kooi, 2006; Child, 2005). The Rint technique has shown significant differences in healthy versus asthmatic patients (Kooi, 2006). Patients as young as 2-years-old can utilize the Rint technique. Although not completely established, current research is determining whether or not the Rint technique can be used to diagnose asthma in children (Child, 2005).

**Etiology of symptoms.** Although symptoms may differ between individuals, the most common clinical symptoms of asthma are shortness of breath, tightness of chest, wheezing, and coughing (Ogawa, 2006). These symptoms are related to a

hyperresponsive airway, chronic inflammation, and bronchoconstriction. The primary components of these processes are leukotrienes and their stimulation of histamine.

Leukotrienes (LT) are made from phospholipids and enzymatic reactions. Phospholipase A<sub>2</sub> releases arachidonic acid, which is then oxidized by 5-lipoxygenase at C-5 to make leukotriene A<sub>4</sub>, which is unstable. Leukotriene A<sub>4</sub> is then converted to CystLT (Liu, 2015). This specific type of leukotriene is most active in asthma (Liu, 2015). Therefore, some asthma research has examined the effect of an anti-LT medication, such as zafirlukast, montelukast and zileuton, to reduce asthmatic episodes (Wenzel 2003). Research has shown these drugs can improve FEV<sub>1</sub> (7% - 15% increase) while also decreasing episodes of nocturnal asthma symptoms by 30-40% (Wenzel, 2003). However, some research has also shown no improvement when coupled with a standard corticosteroid, which do not decrease CysLT levels (Ogawa, 2006). CysLTs specifically work in asthma by stimulating smooth muscle in the airway, which then increases inflammation by secreting cytokines, such as T<sub>H</sub>2, and chemokines that increase generation of IL-5 and TNF- $\alpha$  from the mast cells (Ogawa, 2006). They also promote inflammation by increasing histamine receptor expression (Ogawa, 2006). Research of anti-LT medications have shown no significant decrease in histamine receptors compared to corticosteroid therapy. Additionally, LTs increase eosinophil expression, promote chemotaxis (by stimulating endothelial cells to make platelet activating factor), and support the survival of these cells (by promoting progenitor cell activity and thus, increasing eosinophilopoiesis), thus promoting more asthmatic reactions in the near future (Ogawa, 2006). There is a strong correlation between eosinophilic inflammation of the

airway and the severity of asthma (Liu, 2015). CysLTs are also active in cell remodeling of the airway by promoting mitogen-stimulated lung fibroblasts. This condition is very common in asthmatics (Ogawa, 2006).

While LT activity is very strong and prominent in the asthma syndrome, not all asthmatics are responsive to anti-LT drug therapy. Approximately 6% of asthmatics have a genetic polymorphism that results in decreased productions of LTs (Ogawa 2006). Asthmatic smokers may have an increased benefit from anti-LT medications. Additionally, a correlation between urinary LT and effectiveness of treatment has been established; those patients with an increase in urinary excretion of LTs would have a strong reaction to anti-LT medications (Ogawa, 2006). Some patients may see improvements by pharmaceutically-mediated decreased bronchoconstriction: either by corticosteroids or by inhibiting the effects of LTs by anti-LT medications.

**Prevalence and economic impact.** Asthma is a dangerous medical condition that affects multiple populations worldwide (Milan, 2013).

In the United States, 1 in 12 adults and 1 in 11 children have been diagnosed with asthma by a medical doctor (Urbano, 2008). That means in the typical elementary classroom size (approximately 30), 3 children have difficulty breathing on a daily basis. Asthma affects women ages 18-24 more commonly than any other age group. Also, African-Americans are more likely than Caucasians to have asthma (Urbano, 2008). Asthma has a surprisingly high mortality rate; every day, 9 people in the United States die from asthma-related complications (Urbano, 2008). In 2010, approximately 3500

deaths can be attributed to complications associated with asthma (Urbano, 2008). The frequency of this disease is highly indicative of its economic effect on the United States.

Asthma's total cost on the United States is \$56 billion annually (Urbano, 2008). The main two components of this expense are hospital visits and emergency-room visits. In 2009, 1.9 million emergency-room visits and 8.9 million doctor visits were related by asthma symptoms. Asthma also attributed to 479,300 hospitalizations in 2009 (Urbano, 2008). The high rate of visits may be attributed to a few factors. One factor may be that asthmatics tend to have an overly high level of confidence that they are managing their asthma (Randolph, 2009). Because of this, a clear decline in medication use is evident. Among children, it has been found that prescribed daily medications are used only 40-48% of days per year (Center for Disease Control and Prevention, 2013). Asthmatics may not feel like they need their medication, so they do not follow directions for optimal use. This may lead to sudden attacks, thus requiring a trip to the emergency-room, followed by a trip to the doctor (Center for Disease Control and Prevention, 2013). Another factor may be unclear causes of asthma attacks. Many asthmatics only know a few of their triggers. New environments or experiences may trigger an unforeseen attack (Omenaas, 2003). Much work is being done to try to decrease medical visits caused by asthma (Karaca-Mandic, 2012).

Another cost of asthma is the medication itself. In general, people with asthma have an annual household income less than \$75,000. Although this is not poverty level, it still indicates that money may be more of a concern for these individuals. Asthma medications are another bill that needs to be budgeted. As asthma medications increase

in effectiveness, the prices increase (Karaca-Mandic, 2012). The cost is also an ethnicity issue. 1 in 5 Hispanics and 1 in 4 African-Americans cannot afford asthma medications prescribed by their doctor, even off-brand medications (Urbano, 2008). For children with asthma, average out of pocket expenses are \$154 yearly. With the low adherence to medication, this may reflect a possible waste (Center for Disease Control and Prevention, 2013; Karaca-Mandic, 2013). However, the low adherence may be due to the expense of the medication. Parents may try to stretch out medications to last longer than the typical 30 days (Center for Disease Control and Prevention, 2013). Ironically, children can identify asthmatic symptoms better than adults (Urbano, 2008). Therefore, ignorance may play a huge factor in the medication adherence and possible cause of an increase in medical visits. 1 in 5 asthmatic children went to the emergency room for asthma-related issues in 2009 (Urbano, 2008).

Finally, the cost of asthma is also reflected in missed work or school days. In children, 50% of those with asthma miss at least one day of school because of their severe asthma symptoms (Urbano, 2008). Although one day of missed school does not seem like much, it may hinder their learning progression. Additionally, working adults may have to stay home to care for their children on sick days, causing a measurable financial loss. In older asthmatics, 1 in 3 working adults have to miss at least one day of work due to asthma issues (Urbano, 2008). Since most asthmatic adults have a lower household income, finances is already a concern. One missed day of work can stress finances even further. The cost of asthma is multi-factorial and extremely high.

**Triggers.** Asthma symptoms can be triggered by a multitude of forces. The most commonly assumed triggers are physical allergens, such as animal hair or foreign air particles (Ritz, 2006; Wood, 2007). However, emotional and psychological factors are currently emerging as recognized huge players in asthma control. Research has shown that perceived control of asthma symptoms can have a huge impact on quality of life (Ritz, 2006).

Most asthma triggers are determined by questionnaires. However, a large range of results occur from questionnaires. A review of different questionnaires has found that 4 to 74% of triggers are captured by questionnaires (Ritz, 2006). Due to the large range, a higher quality survey is necessary. The Asthma Trigger Inventory (ATI) is a detailed questionnaire that covers 32 items on a 5-point rating scale: 0=never, 1=rarely, 2=sometimes, 3=most of the time, 4=always (Ritz, 2006). The ATI evaluates six overarching topics of common triggers; these triggers include emotional/psychological (including stress), animal, pollen, exercise, air irritants, and infection (Ritz, 2006).

A study of the ATI had many findings. One primary discovery related to psychological triggers. Psychological triggers have been found to have a significant decrease in overall health and quality of life (Ritz, 2006). This finding is key as psychological triggers were often overlooked in discussions with primary physicians (Ritz, 2006; Wood, 2007). Another finding was that animal allergens significantly increased required hospital care compared to the other triggers (Ritz, 2006). In general, there seems to be a synergistic effect of the triggers; multiple trigger types are interconnected in causing asthma symptoms.

Different populations were studied with the ATI for the internal and external validity of the questionnaire. Gender may play a role in triggering symptoms. Females are more likely to have triggers from physical activity, pollution, and infection over their male counterparts (Wood, 2007). This difference seems to stem from gender psychology; males are “taught” to ignore symptoms or “get over” the symptoms. The culture of males versus females has shown results in what triggers an asthma attack (Wood, 2007). In addition to gender having an effect, different ethnicities have different reactions to triggers. Non-Caucasian asthma patients have an increase in asthma symptoms from physical activity triggers and pollution (Wood, 2007). Finally, when asthma is diagnosed can impact the susceptibility of triggers. Those who are diagnosed earlier in life, early onset of asthma, are more reactive to all six types of triggers than those who were diagnosed later in life (Wood, 2007). Since asthma is a highly-individualized disease, the ATI can help in the discussion of asthma control with the primary care physician.

**Nutrient status.** Much research has been completed analyzing the overall nutrient status in asthmatics. Some groups of nutrients are similar between equivalent normal subjects and asthmatics. However, some nutrients, antioxidants in particular, are generally lower in asthmatics than their equivalent control counterparts.

Perhaps the most critical nutrient deficit in asthmatic individuals relates to micronutrients. One study examined the intake of common antioxidants over a 7-day food diary and incidences of asthma attacks during the previous 12 months. This study found that, on an individual basis, decreased intake of vitamin C, folate, calcium, and manganese were associated with increased asthma. Additionally, this study examined the

relationship between plasma vitamin C concentrations and increased risk of asthmatic attacks. Although the control and the study group had fairly equal plasma vitamin C levels, the plasma levels were significantly lower in those who had symptomatic asthma rather than those who had asymptomatic asthma (Bender, 2004).

In addition to micronutrient status, intake of polyunsaturated fatty acids has an effect on asthmatic conditions. The omega-6 PUFA's, such as arachidonic acid, are associated with inflammation while the omega-3 PUFA's, such as eicosapentaenoic acid and docosahexaenoic acid, are associated with anti-inflammatory status. Since the United States consumes a diet with a higher omega-6:omega-3 ratio, this should have a place in asthma research. Some studies have shown that consumption of omega-3 PUFA in the form of fish has been shown to decrease the risk for developing asthma in adults. However, it may actually increase the risk of developing asthma in children. The association is not quite clear. Additionally, increased consumption of omega-6 in the form of margarine has been shown to increased risk of wheezing symptoms of asthma. Due to the conflicting nature of the results involving studies with PUFA's, no recommendations for a possible intervention can be made (Patel, 2006).

Perhaps the most critical component of nutrient status in asthmatics revolves around antioxidants. In fact, this dietary association with asthma is one of the most well-studied (Bender, 2004). Many studies look at vitamin C status and consumption in relation to asthmatic symptoms. Studies have shown that lower vitamin C status is associated with lower lung function levels in children (Bender, 2004). Higher intakes of vitamin C were associated with better FEV<sub>1</sub> scores. A double-blind crossover study

examined exercise-induced bronchoconstriction (EIB) protection through vitamin C supplementation. A group of 8 patients who have had EIB in previous exercise experiences were given 1500mg vitamin C per day or placebo. PFTs were measured before and after exercise. One week later, the groups were switched, making it a crossover study. The study found that the treatment diet decreased the fall of PFTs post-exercise ( $-6.4 \pm 2.4\%$ ) whereas the placebo group fell much worse ( $-12.9 \pm 2.4\%$ ) (Tecklenburn, 2007). Obviously these results are not fully conclusive. In fact, the recent Cochrane Review states that there is insufficient evidence to clearly link vitamin C with asthma (Milan, 2013).

**Increased risk for colds.** Although little research has been done on asthmatics and colds, the two conditions are highly related (Milan, 2013). However, some research has indicated that asthmatics have increased worsening of symptoms following a cold. One study followed 413 adults with asthma. Using the Wisconsin Upper Respiratory Symptom Survey-21 (WURSS-21), they calculated the severity of the colds. They also used the mini-Asthma Control Questionnaire (mini-ACQ) to evaluate possible changes in stability of their asthma. The researchers found that the more severe the symptoms of the first two days of the cold will predict a decrease in control of the patient's asthma. From these results, it is very important for asthmatics to avoid getting the common cold. However, research preventing a cold is still mixed, giving asthmatics an unclear understanding of how to prevent worsening of their asthmatic symptoms (Allan, 2011).

## **Asthma and Vitamin C.**

Understanding the common functions of vitamin C in the body propels research for understanding the role of vitamin C in specific diseases. Generally, most vitamin C research in disease is fairly controversial. Vitamin C has mixed results in cancer treatment studies (Du, 2012). Most progress is made regarding certain types of individuals with diseases. For example, vitamin C has proven to significantly reduce the risk developing the common cold specifically in populations of athletes and military personnel (Milan, 2013).

The current research shows success of vitamin C in reducing the incidence of colds in highly stressed populations, specifically military cadets and athletes. There are major physiological differences between stress from asthma and exercise-induced stress. Stress is simply defined as any internal or external triggers or conditions that affect homeostasis (Mastorakos, 2005). Asthmatic stress is primarily related to uncontrolled ROS and eosinophilic action in the lungs leading to obstructive episodes (Liu, 2015). On the other hand, exercise-induced stress is very different physiologically, and this stress includes multiple organ systems (Mastorakos, 2005). ROS are produced during exercise; 2-5% of oxygen used in the mitochondria during exercise produces ROS (Urso, 2003). The central nervous system plays a major role in stress as well. Norepinephrine, typically associated with the fight-or-flight response, is increased to increase heart rate and attention or focus (Mastorakos, 2005). The hypothalamic-pituitary-adrenal (HPA)

reactions lead to increased cortisol; this steroid hormone is primarily increases blood sugar and metabolism while also suppressing the immune system (Cevada, 2014).

The success of vitamin C supplementation in these populations can relate to the physiological stress process. Vitamin C is required for the production of norepinephrine (Gropper, 2013). As more vitamin C is used for exercise-induced stress, more is absorbed to replenish the needs. Also, the release of cortisol increases metabolism, thus requiring more vitamin C to keep the mineral cofactors reduced (Gropper, 2013). Additionally, the immune system is suppressed during times of stress, leading to increased needs for antioxidant vitamins (Gropper, 2013). Athletes and military cadets utilize vitamin C much more frequently due to their daily stresses and initiation of the physiological stress response.

Asthma is another condition with highly mixed results from treatment with vitamin C. Utilizing vitamin C for treatment of asthma symptoms stems from the ideal conditions where vitamin C has a large room for effect. The role of vitamin C in the body, mostly as an antioxidant and as a nutrient recycler, relates strongly to the conditions of a typical adult asthmatic.

Asthmatics typically have low intakes of vitamin C. One study showed that decreased consumption of vitamin C in the diet has a positive relationship with forced vital capacity (FVC), or how much air a patient can expire as quick as possible (Ochs-Balcom, 2006; Allen, 2009). The FVC test is done in asthmatics to determine significance of symptoms. Therefore, increased vitamin C consumption should ideally

result in increased respiratory function. However, reported intake of vitamin C alone has high room for error, due to the reactive abilities of vitamin C and errors in reporting systems (Ochs-Balcom, 2006). Vitamin C is highly reactive in the air, making estimates in food difficult. Additionally, patients frequently overestimate intake of fruits and vegetables when asked by medical professionals, making the accuracy of food diaries questionable (Gropper, 2013).

In addition to general low intake of vitamin C, asthmatic adults generally have low plasma vitamin C levels. Decreased serum vitamin C levels are strongly associated with oxidative imbalances (Ruprai, 2011). Asthma is an inflammatory disease. Therefore, more vitamin C is required to try to subdue the inflammation by its actions as a secondary lipid antioxidant by regeneration of vitamin E (Ruprai, 2011).

Another condition of asthmatics that show a large possibility of a relationship involves the higher incidence of reactive oxidative species (ROS). Asthmatic adults have been shown to have significantly increased levels of ROS in the blood. Additionally, enzymes that are utilized to neutralize ROS, such as superoxide dismutase, and other free radical scavengers in the blood were noticeably lower in asthmatics than normal population. Clearly, in tandem with the common actions of vitamin C, ascorbic acid has the potential to show a change in the status of asthmatics. Perhaps with vitamin C, ROS and inflammation causing symptoms in asthma would significantly decrease, thus lowering risk for mortality related to asthma (Shanmugasundaram, 2001).

The potential usefulness of vitamin C is further supported by the assessment of medication use in asthmatics with ascorbic acid supplementation. One study examined the relationship between vitamin C supplementation and corticosteroid use in asthmatic adults. Compared with placebo and magnesium supplementation, participants in the vitamin C supplementation groups showed a significant decrease in corticosteroid use by 49 doses unused (Fogarty, 2006). The decrease in medication use in asthmatics in the vitamin C group show potential causal relationship. This association is critical, especially with the considerable economic impact of asthma.

Vitamin C supplementation to treat asthmatic symptoms has been explored in research. Nutrient supplementation is considered to be Complementary and Alternative Medicine. As shown with the results of the study decreasing corticosteroid use, vitamin C can potentially take the place of pharmaceuticals prescribed by medical doctors. It is important to note that 41% of asthma patients in the United States and 59% of asthma patients in the United Kingdom use some form of Complementary and Alternative Medicine (CAM) (Brustsche, 2002). However, most success stories with utilizing CAM in asthma were cases where CAM was used in addition to classical medications. Additionally, popularity cannot be correlated with safety; studies looking at CAM versus traditional medication are difficult to achieve due to placebo effects and inability to perform randomized controlled trials ethically (Brustsche, 2002).

To test the theory behind vitamin C's usefulness in decreasing asthmatic conditions, animal studies must be completed. One study examined a mega-dose of vitamin C to combat the inflammatory conditions of asthma. Experimental mice were

injected with pharmaceutical drugs to replicate the conditions of asthma. After the mice became “asthmatic”, 3-5 mg of vitamin C were administered 6 consecutive days. The results had shown a significant decrease in inflammatory cells in the lungs of the mice (Jeong, 2010). Only one human study, done in Nigeria, has shown significant changes from vitamin C supplementation on lung function tests in asthmatics (Jeong, 2010; Choi, 2009). It has been assessed that there is not enough information in vitamin C studies to determine significant effects on asthmatic populations (Milan, 2013).

## CHAPTER 3: METHODS

### Participants

The study was advertised through available list serves (APPENDIX A). Interested candidates followed a provided link on the email that will direct them to [surveymonkey.com](https://www.surveymonkey.com) (APPENDIX B). Those whose survey answers match the candidate requirements were then emailed and asked to come to the Downtown Phoenix campus for an initial blood draw. The subjects of the study are adults, ages 18 to 55 years, who have been diagnosed with asthma by a Medical Doctor. The subjects must also have a plasma vitamin C level  $\leq 0.75$  mg/dL. This indication of normal to low vitamin C status is critical for showing the effects of vitamin C supplementation on lessening symptoms of asthma or the common cold. Smokers were excluded, due to the increased antioxidant requirement (Omenaas, 2003). Additionally, those partaking in excessive exercise, such as training for a marathon, were also excluded because of the high correlation of exercise and asthmatic symptoms (Omenaas, 2003). Vegans were also excluded from the study because the placebo pills and vitamin C supplement contain gelatin-based capsules. To prevent any possible discomfort, gluten intolerant individuals were excluded since the placebo pill contains wheat flour. Flu shots could potentially also interfere with the susceptibility of contracting a cold. However, we did not exclude for this. One clear exclusion criteria was any vitamin C supplement use within the past 30 days. This time period is adequate to allow the additional vitamin C to be excreted from the body (Gropper, 2013). IRB approval of this study is complete, and written consent was obtained from all participants (APPENDIX C).

## **Study Design**

The study design was a double-blind randomized controlled trial. There was a treatment group (1000 mg daily vitamin C) and a control group (placebo). 1000 mg of vitamin C were used to ensure all treatment participants had absorption of vitamin C. Subjects were stratified by age, gender, BMI, and vitamin C status and randomized by a third party into the two groups. As the study was double-blinded, neither the subjects nor the researchers knew which specific intervention any subject underwent. The treatment of the study included 500 mg vitamin C supplement consumed twice daily with meals. The placebo capsule was indistinguishable from the vitamin C capsule and contained white flour. The study lasted for 8 weeks. Interested participants took a survey through [surveymonkey.com](https://www.surveymonkey.com) (APPENDIX B) in the month of December. Qualified individuals were contacted and invited to come to the Downtown Phoenix campus to the ABC 1 building to sign consent (APPENDIX D), to draw blood after a 5-hour fast, and complete a health history questionnaire (APPENDIX E). After measuring plasma vitamin C levels, subjects were asked to come to pick up questionnaires for Weeks 1-4 and capsules to consume twice daily (APPENDIX F, APPENDIX G, APPENDIX H). An official baseline overnight fasting blood draw (Week 0) was taken and subjects received the first incentive, a \$10 Target gift card. During Week 4, participants returned for a midterm blood draw. They received partial compensation, \$15 Target gift card, and questionnaires for Weeks 5-8. One final travel to the Downtown Campus occurred at Week 8 to determine final blood values and provide the final portion of compensation of \$20 Target gift card, which totaled to \$45 in Target gift card.

## **Measurements**

Blood samples from all participants were taken at Weeks 0, 4, and 8. Each subject fasted overnight for approximately 8 hours. A Registered Nurse collected the blood samples in lavender top vacutainers. Vitamin C was analyzed calorimetrically (APPENDIX F) (Omaye, 1987). Histamine was analyzed by ELISA assay (APPENDIX G) (ALPCO, 2013).

Additionally, questionnaires related to cold symptoms were completed by the subjects. The Wisconsin Upper Respiratory Symptom Survey-21 was specifically used (Walter, 2008). These questionnaires helped to examine symptoms experienced during the day as well as any medication use or exercise done throughout the day (APPENDIX D). An asthma symptom survey was also recorded daily. Weekly, a physical activity questionnaire and a vitamin C-focused FFQ were answered by the subjects. Questionnaires were scored according to the subjects' answers.

## **Statistical Analysis**

Data was represented as mean  $\pm$  SD. All data was analyzed using SPSS 20 system (Chicago, IL). Originally, normality testing was planned to be done, with potential transformation of data to reflect a normally distributed sample. Once normality was established, an independent t-test was planned to be calculated. However, the low number of subjects indicated for nonparametric testing. Therefore, nonparametric independent samples test, Mann-Whitney U, was run to analyze data. A p-value less than 0.05 was considered statistically significant.

## CHAPTER 4: DATA & RESULTS

Subjects were recruited for the study beginning of January 2014 until the end of March 2014. A total of 76 interested participants completed the SurveyMonkey questionnaire. Of those interested, approximately 50 qualified and were contacted via email for a screening date. 12 individuals set an appointment to meet with a researcher in ABC1 of the Downtown Phoenix campus. Of those candidates, only nine subjects attended their appointments and were enrolled. The nine subjects were stratified by gender, age, BMI, height, weight, body fat percentage, and screening plasma vitamin C into either the vitamin C treatment group or the control/placebo group. Two subjects voluntarily dropped from the study between the Week 0 and Week 4; one participant discovered she was pregnant and the other participant ceased contact with the researchers for the follow-up appointments. Thus, the results encompass full assessments of the data from seven subjects, four in the vitamin C group (two males and two females) and 3 in the control group (one male and two females). Compliance was adequate:  $93.43\pm 7.2\%$  for vitamin C and  $87.5\pm 7.8\%$  for control (FIGURE 1). These should not impact the results. All data was analyzed through SPSS-22 (Chicago, IL), and normality was not tested due to the low subject number (n). After discussion with lead researchers, the group concluded that utilizing nonparametric testing would be the most appropriate method for analyzing the small data sample. The non-parametric t-test, Mann Whitney U, was calculated for all comparisons.

Baseline data was similar for both groups. Both groups were very similar in age ( $28.3\pm 9.9$  years in vitamin C and  $30.0\pm 9.6$  years for the control group,  $p\text{-value}=1.000$ ).

The groups were nearly identical in height, with  $69.8 \pm 1.0$  inches for vitamin C and  $68.8 \pm 7.6$  inches for the control group. There was a slight difference in weight ( $148.8 \pm 25.1$  pounds in vitamin C and  $164.6 \pm 45.0$  pounds in control), BMI ( $21.5 \pm 3.4$   $\text{kg/m}^2$  in vitamin C and  $24.3 \pm 4.7$   $\text{kg/m}^2$  in control), and body fat percentage ( $20.8 \pm 12.1\%$  in vitamin C and  $25.8 \pm 12.2\%$  in control). However, none of these differences were large enough to be statistically significant (p-value was greater than 0.05). Initial vitamin C intake and physical activity patterns were similar for both groups. Yet, the large standard deviations indicate possible outliers. With such a small sample, the outliers could not be removed. Finally, the plasma vitamin C levels taken at the initial screen were below the recommended limits for the adult population ( $0.430 \pm .257$  mg/dL in vitamin C and  $0.585 \pm .085$  mg/dL in control, p-value = 0.480). Overall, the stratification of subjects was appropriate, with no statistically significant difference in the major anthropometric and biochemical data.

Table 3

*Subject Data*

Table 3: Subject Data <sup>a</sup>			
	Vitamin C (n=4)	Control Group (n=3)	p-value <sup>b</sup>
Gender	2 Female, 2 Male	2 Female, 1 Male	---
Age (y)	28.3± 9.9	30.0± 9.6	1.000
Height (in)	69.8±1.0	68.8±7.6	0.480
Weight (lb)	148.8±25.1	164.6±45.0	0.480
BMI (kg/m <sup>2</sup> )	21.5±3.4	24.3±4.7	0.480
Body Fat (%)	20.8±12.1	25.8±12.2	0.480
Initial Vitamin C (mg/dL)	0.430±.257	0.585±.085	0.480
Initial FFQ Scores	138.2±64.7	99.0±17.4	0.480
Initial Physical Activity Scores	30.3±19.4	68.3±64.9	0.480
Pill Compliance	93.43±7.2	87.5±7.8	0.275 <sup>c</sup>
<sup>a</sup> Values are mean ± SD			
<sup>b</sup> Analysis by nonparametric independent samples test (Mann-Whitney U)			
<sup>c</sup> Subject 9 (Vitamin C group) did not fill out the calendar to indicate compliance			

Both groups started out with low vitamin C blood levels (0.512±0.217 mg/dL for vitamin C and 0.596±0.109 mg/dL for control). The highest values in vitamin C occurred during Week 4 for vitamin C (0.922±0.190 mg/dL) and during Week 8 for control (0.745±0.123 mg/dL). The most noticeable change occurred between Week 0 and Week 4 (0.410±0.041 mg/dL vitamin C and 0.122±0.082 mg/dL control). This difference was not statistically significant (p=0.057). There was a larger change in vitamin C levels from Week 0 to Week 8 (0.283±0.110 mg/dL for vitamin C and 0.149±0.021 mg/dL for control). However, this difference is not statistically significant (p=0.077). The control group had a lower initial histamine level (0.858±1.103 ng/mL versus 1.818±0.909 ng/mL for vitamin C). However, the difference is not statistically significant (p=0.229).

Overall, the vitamin C group decreased in histamine level throughout the eight weeks ( $-0.344 \pm 1.270$  ng/mL) while the control group had an increase in histamine level ( $0.451 \pm 1.162$  ng/mL) but the difference was not statistically significant ( $p=0.724$ ). Again, large standard deviations indicate a potential outlier, but the small n prevents the data from being thrown out.

Table 4

*Blood Data*

Table 4: Blood Data <sup>a</sup>			
	Vitamin C (n=4)	Control (n=3)	p-value <sup>b</sup>
Vitamin C Week 0 (mg/dL)	0.512±0.217	0.596±0.109	0.857
Vitamin C Week 4 (mg/dL)	0.922±0.190	0.718±0.089	0.114
Vitamin C Week 8 (mg/dL)	0.795±0.276	0.745±0.123	0.629
Vitamin C Mid-Change (Week 4 – Week 0) (mg/dL)	0.410±0.041	0.122±0.082	0.057
Vitamin C Change (Week 8 – Week 0) (mg/dL)	0.283±0.110	0.149±0.021	0.077
Histamine Week 0 (ng/mL)	1.818±0.909	0.858±1.103	0.229
Histamine Week 4 (ng/mL)	1.280±1.010	0.680±0.142	0.629
Histamine Week 8 (ng/mL)	1.474±1.693	1.309±0.216	0.400
Histamine Mid-Change (Week 4 – Week 0) (ng/mL)	-0.538±1.740	-0.178±0.970	1.000
Histamine Change (Week 8 – Week 0) (ng/mL)	-0.344±1.270	0.451±1.162	0.724
<sup>a</sup> Values reported as mean ± SD			
<sup>b</sup> Analysis by nonparametric independent samples test (Mann-Whitney U)			

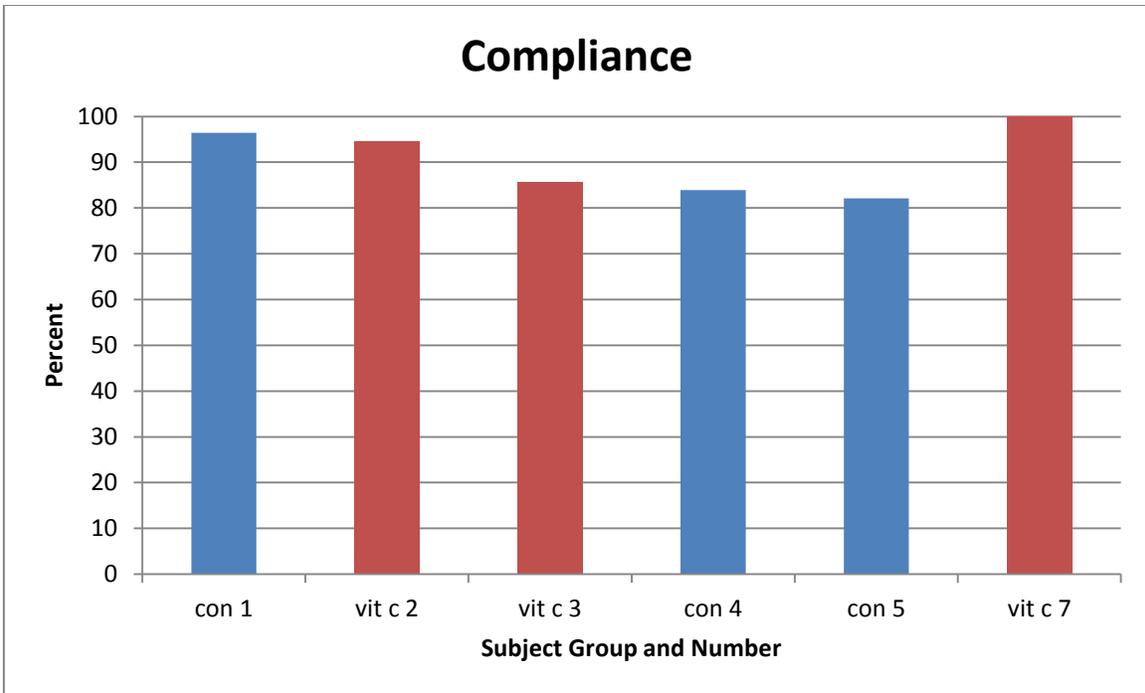


Figure 1. Comparison of pill compliance among subjects.

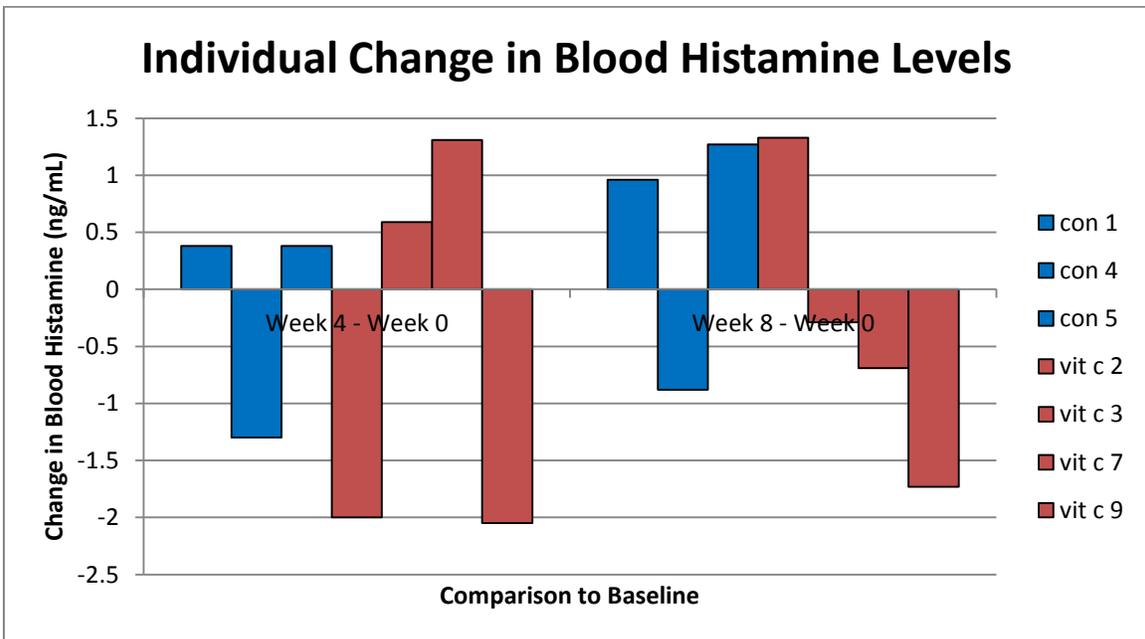


Figure 2. Graph of changes in blood histamine levels.

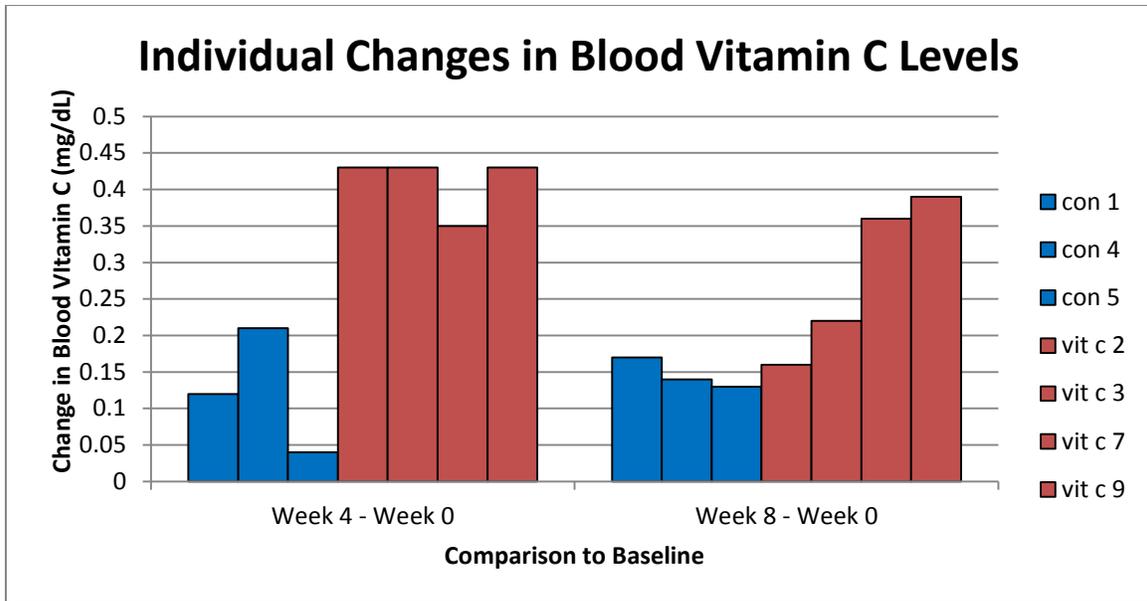


Figure 3. Graph of changes in blood vitamin C levels.

There were noticeable differences between the groups regarding the cold symptoms recorded by the WURSS-21 questionnaires. Overall, the vitamin C group seemed to have a lower recorded number of symptoms than the control group ( $129.25 \pm 120.7$  versus  $271.00 \pm 293.9$ ) but the difference is not statistically significant ( $p=0.724$ ). The cold symptoms appeared to have affected the daily living of the control group slightly more than the vitamin C group but the difference is not significant ( $p=0.157$ ). The vitamin C group experienced cold symptoms from days 4-12 and then did not seem to have significant cold symptoms. On the other hand, the control group experienced high cold symptoms day 16-36, according to FIGURE 4. The colds had a slightly lesser effect on the living scores, but the length of colds are still reflected in FIGURE 4. The differences are not statistically significant. Due to the small population, each subject is graphed independently.

Table 5

*WURSS-21 Data*

Table 5: WURSS-21 Data <sup>a</sup> over 8 weeks			
	Vitamin C (n=4)	Control (n=3)	p-value <sup>b</sup>
Total Symptoms	129.3±120.7	271.0±293.9	0.724
Total Living	114.0±219.4	184.7±245.9	0.157

<sup>a</sup>Values reported as mean ± SD  
<sup>b</sup>Analysis by nonparametric independent samples test (Mann-Whitney U)

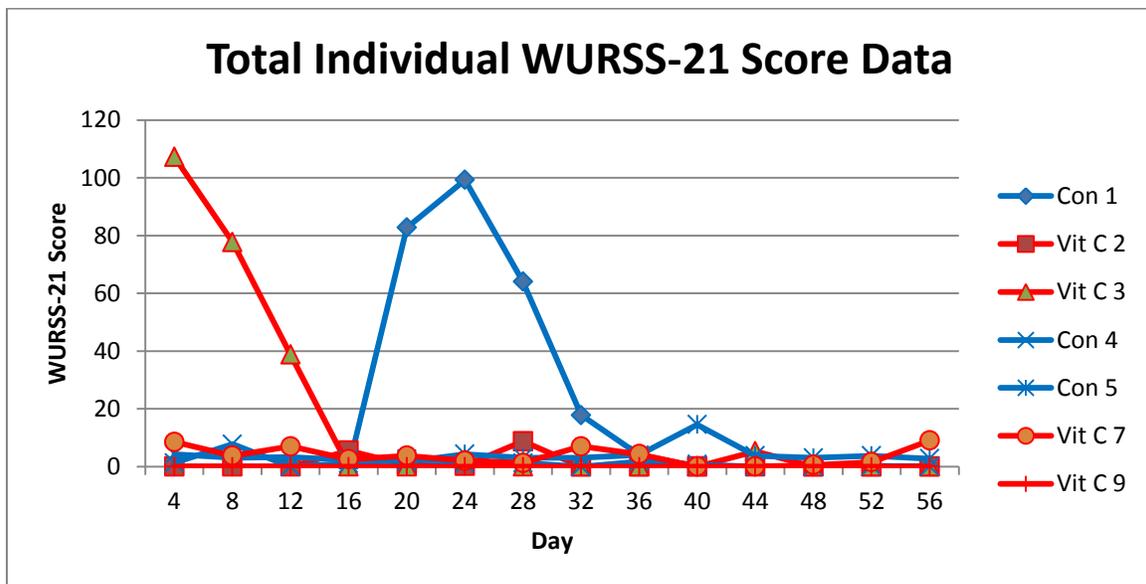


Figure 4. Total WURSS-21 scores throughout eight weeks.

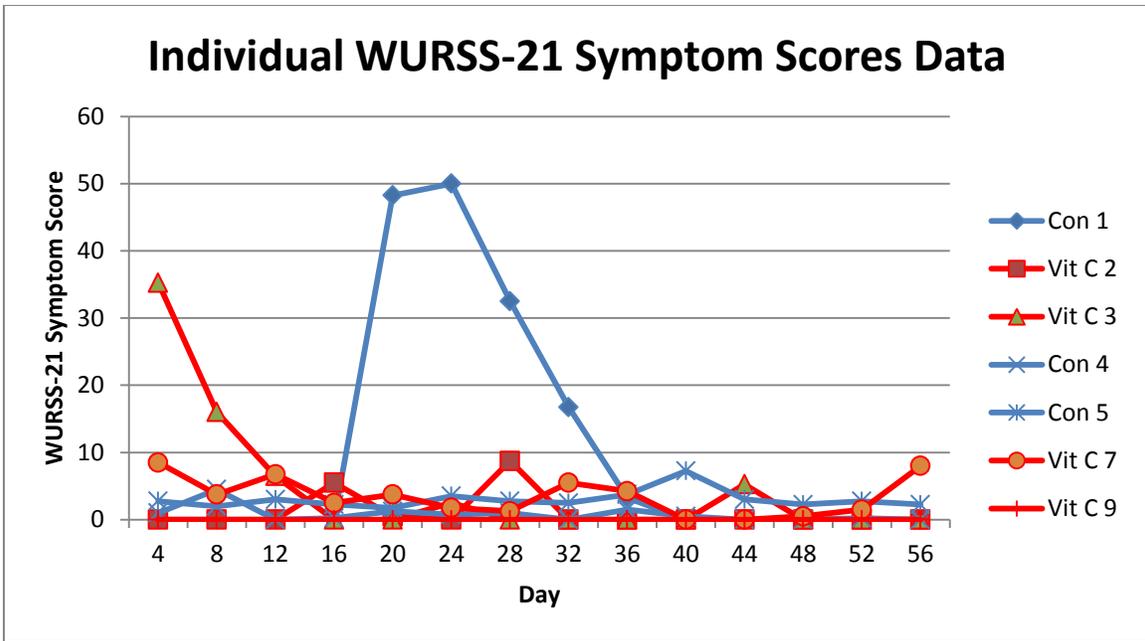


Figure 5. WURSS-21 cold symptoms section scores.

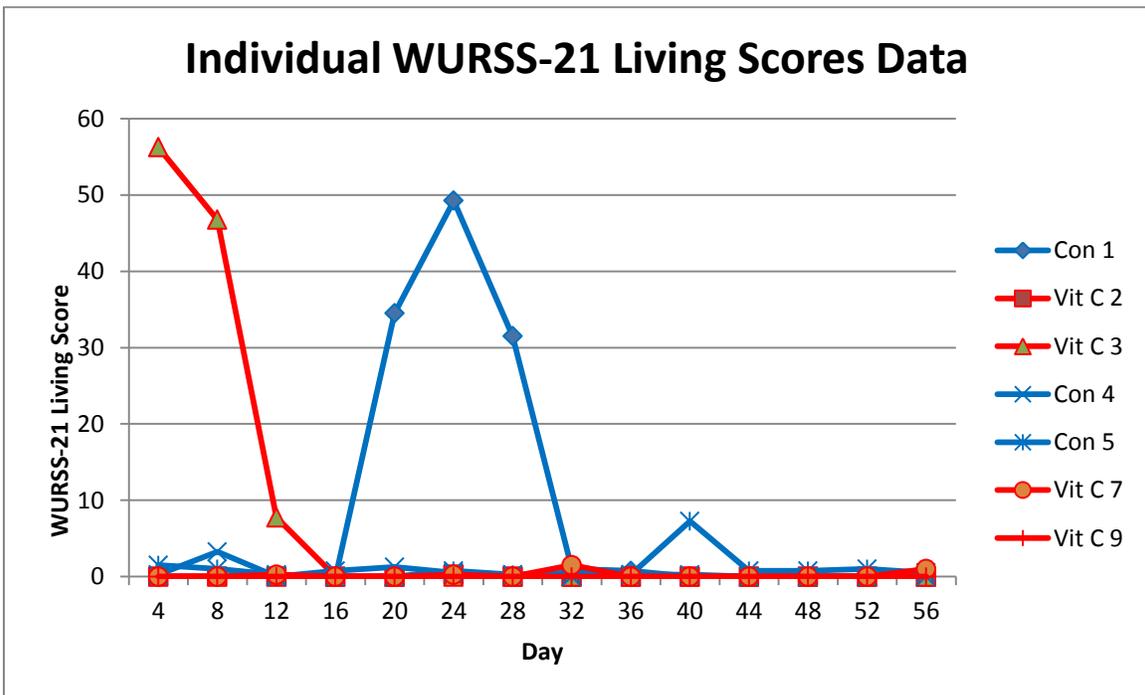


Figure 6. WURSS-21 daily living section scores.

Asthma symptoms were tracked by a daily asthma questionnaire. Again, differences are only noticeable and not statistically significant ( $p>0.05$ ). The vitamin C group had a slightly lower average asthma score ( $2.2\pm 2.8$ ) than the control group ( $5.0\pm 2.4$ ), but not a significant difference ( $p=0.154$ ). At the end of the eight weeks, the vitamin C group recorded a total of  $17.8\pm 22.5$  while the control group recorded  $40.3\pm 18.8$  ( $p=0.154$ ). The vitamin C group appeared to have stable asthma scores throughout the study while the control group had more variation as seen in FIGURE 7. Subjects 2 and 9 have no recorded asthma symptoms during the eight weeks of vitamin C supplementation.

Table 6

*Asthma Symptom Data*

Table 6: Asthma Symptoms Data <sup>a</sup>			
	Vitamin C (n=4)	Control (n=3)	p-value <sup>b</sup>
Average Asthma Score	2.2±2.8	5.0±2.4	0.154
Total Asthma Score	17.8±22.5	40.3±18.8	0.154
<sup>a</sup> Values reported as mean ± SD			
<sup>b</sup> Analysis by nonparametric independent samples test (Mann-Whitney U)			

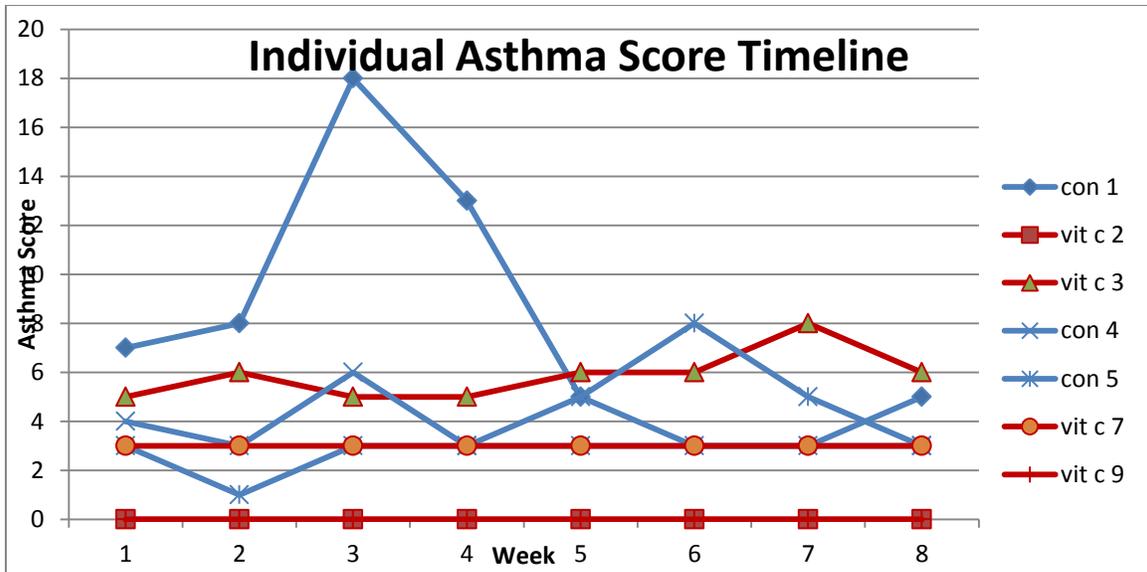


Figure 7. Asthma scores throughout eight weeks.

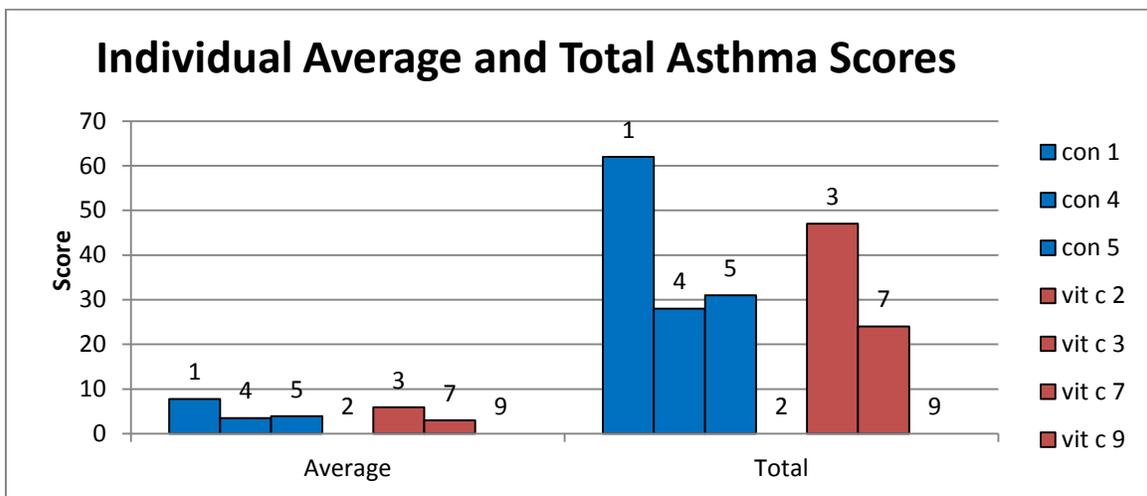


Figure 8. Average and total asthma scores of the eight weeks.

Physical activity was monitored throughout the study, as shown in FIGURE 9. The control group appeared to be more physically active throughout the study than the vitamin C group. The vitamin C group appeared to have more consistency in physical activity throughout the eight weeks than the control group. The physical activity did not seem to impact the blood data.

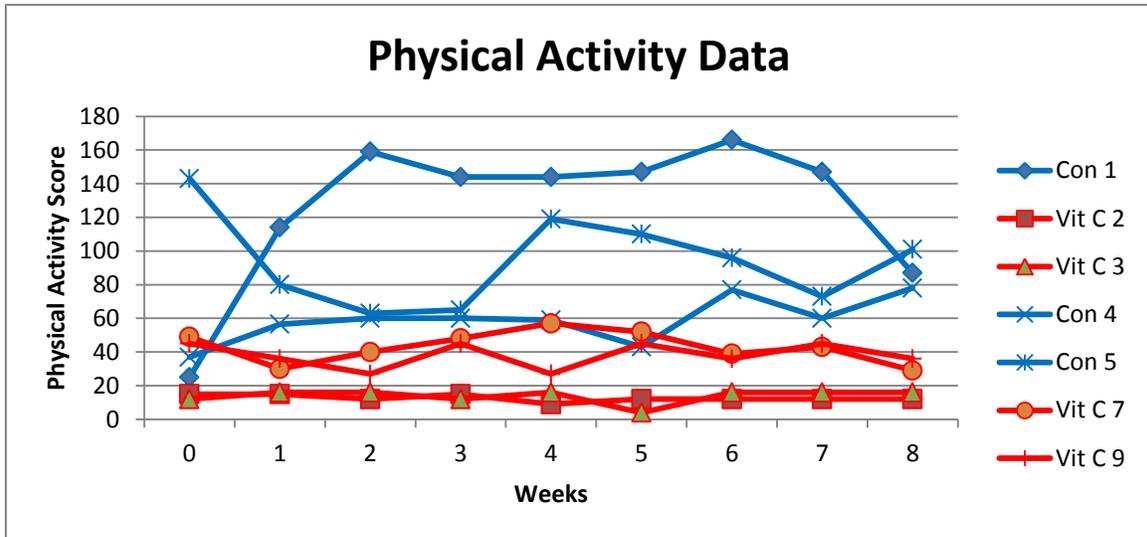


Figure 9. Trend of physical activity patterns of subjects.

Vitamin C consumed was monitored through a weekly FFQ. Overall, most subjects appeared to be fairly stable with vitamin C intake. One subject had very high intake compared to the other subjects. However, the subject's blood vitamin C level was not reflective of this increased intake.

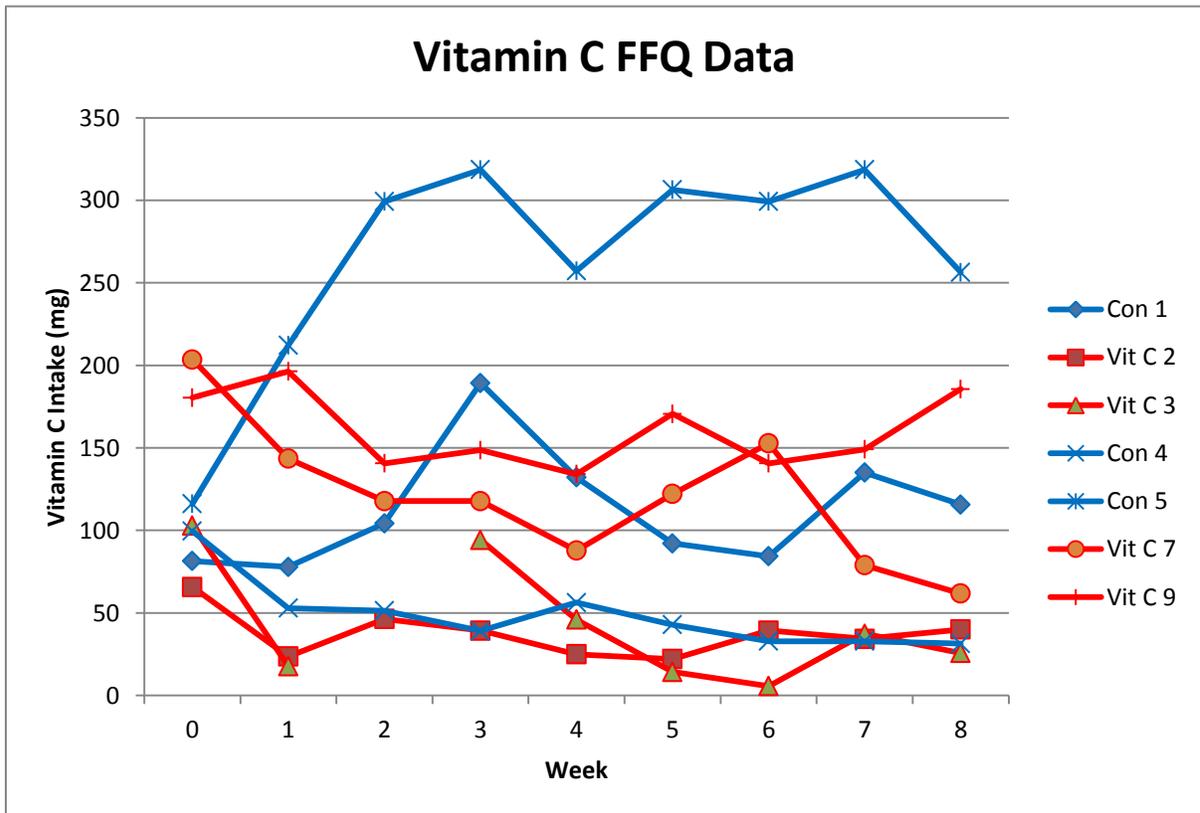


Figure 10. Individual subjects' vitamin C intake.

## CHAPTER 5: DISCUSSION

With the current results of this double-blind, randomized controlled trial, there is not enough evidence to support the hypotheses. In concurrence with the Cochrane review, the study conducted did not have enough subjects to strongly confirm or reject that vitamin C supplementation decreases cold symptoms or asthma symptoms in asthmatic adults (Milan, 2013). The majority of studies from the Cochrane review have medium to low strength level; this study, at the current point, would be classified as low strength. The study design called for at least 30 asthmatic individuals to reach a power of 0.9. However, major issues with recruiting and maintaining contact with interested respondents contributed to the low subject outcome. These low numbers prevented the researchers from seeing a clear trend, as evidenced by the p-values as well as the individual graphic representation. This study's current power does not reflect current evidence. Vitamin C supplementation has been shown to significantly decrease bronchoconstriction in asthmatics post-exercise (Tecklenburg, 2007). Another study found little to no effect from vitamin C in asthmatics and severity of bronchoconstriction; however, this study was also limited in small sample size (Nadi, 2012). In colds, vitamin C research is vastly dependent on the subject population (Hemila, 2013). As reflected with this study, the role of vitamin C supplementation in asthmatics and the common cold are still unconfirmed. A higher power of study is necessary to accept or reject the hypotheses.

Previous research has shown mixed results for vitamin C as a complementary treatment for asthma. Studies have consistently shown that asthmatics have lower levels

of plasma vitamin C than their non-asthmatic counterparts (Allen, 2009). However, supplementation may not always increase plasma vitamin C. Children may see as much as a 50% decrease in plasma vitamin C during asthma episodes; this is thought to be attributed to an increased utilization of vitamin C to negate free radical behavior (Shanmugasundaram, 2001). Researchers have shown a connection between the lower plasma vitamin C level and wheezing (Forastiere, 2000). Lower vitamin C status is associated with decreased lung function, leading to episodes of wheezing (Forastiere, 2000). Yet, even if the plasma vitamin C increases, some studies have found no change in spirometer measurements of respiratory activity (Nadi, 2012).

Overall, the studies of vitamin C's effectiveness against asthma have resulted in inconsistent answers (Riccioni, 2006). Many studies have shown a negative relationship between vitamin C status and asthmatic episodes. Those with a low vitamin C level have a 12% increased chance of having an asthma attack (Allen, 2009). One study found that the intensity of asthma symptoms decreased after following a 1500mg vitamin C daily diet for two weeks (Tecklenburg, 2007). Another study has found that 1000mg/day vitamin C supplementation for 16 weeks have no clinical benefits over standard medical therapy (Riccioni, 2006 and Milan, 2013).

One major aspect of vitamin C's action as a potential complementary therapy in asthmatics depends on the state of asthma. Studies that have shown no clinical benefit are typically in adults with well-established asthma (Fogarty, 2003 and Allan, 2011). In this study, all participants had asthma for at least 7 years. Thus, this population may not see a strong benefit to vitamin C supplementation. Although the study showed that the

vitamin C group had more control over their asthma symptoms over the eight weeks, a baseline asthma score was not taken.

Another aspect of vitamin C's effectiveness in protecting against asthma symptoms is the source of the asthma attack. Vitamin C has proven to be effective against attacks generating from the environment and air pollutants (Riccioni, 2006 and Nadi, 2012). These attacks are related to increase ROS production in the body from the multitude of particles in the air, and vitamin C has been shown to be effective in neutralizing ROS (Riccioni, 2006). As this study occurred in central Phoenix alone, there was no difference in asthma attacks among the subjects. Each subject has been an Arizona resident for at least two years and is likely adjusted to the pollution levels of this city. Additionally, vitamin C would have no effect on LT-induced inflammatory response in the airway. Vitamin C can decrease histamine levels, but cannot decrease LT levels (Liu, 2015). Overall, this study was not adequately powered and cannot reliably contribute to the discussion on vitamin C and asthma link.

One important observation of this study relates to the blood data trends. In both groups, serum vitamin C levels increased from baseline to week 4, but had a slight decrease in the treatment group. This pattern is also reflected with the histamine blood data: a decrease from baseline to Week 4, but an increase from Week 4 to Week 8. This is consistent with the inverse relationship established in other research (Gropper, 2013). Self-reported subject intake of vitamin C was fairly consistent, except for one participant. Additionally, physical activity does not seem to have played a part in the odd trend in blood data. The vitamin C group had consistent physical activity patterns while the

control group had a greater variety. The overall compliance rate was very high, and the dates of missed pills varied between subjects. The difference between Week 4 and Week 8 may indicate the study may be successful at only 4 weeks in length. The decrease in length would assist in increasing subject compliance and participation, as length of study can defer some participants. However, the 8-week long study has shown promise in previous studies in both asthma (Nadi, 2012) and colds (Johnston, 2014). No specific timeline for vitamin C research in the asthmatic population is evident; current studies range from two weeks to six months in length (Milan, 2013).

The WURSS-21 was utilized to measure any incidence of colds throughout the study. During the eight weeks, only two participants, one in the control group and one in the vitamin C group, had a strong cold. However, the low numbers prevent throwing out these “outliers”. The symptoms section of the WURSS-21 was higher than the living section. This could be representative of the asthmatic population as a whole. The asthmatic individual is used to dealing with slightly obstructive airways. Therefore, decreased breathing from stuffy or runny nose may not impact the asthmatic as much as a non-asthmatic individual. Additionally, there may be overlap in symptoms from asthma and an upper respiratory airway infection, or cold. These considerations may indicate that the WURSS-21 may not be appropriate for the asthmatic population. However, it is still used in other asthma and common cold research (Busse, 1999 and Nadi, 2012). One study has utilized the WURSS-21 in asthmatics to track the worsening of asthma symptoms following a cold (Walter, 2008).

The asthma questionnaire results indicate the need for continuation of the study for vitamin C supplementation in asthmatics. The subjects in the vitamin C supplementation group had consistent asthma symptoms compared to the control group, as evidenced in FIGURE 7, including the subject in the vitamin C group who had a severe cold. Although not statistically significant, the vitamin C group had lower asthma scores, comparatively. This incidence requires a higher study population to support its importance. Previous studies have indicated that control over asthma symptoms decreases following an upper respiratory airway infection (Busse, 1999). Other studies have shown that vitamin C supplementation can assist in stabilizing asthma symptoms (Milan, 2013). Studies have shown a potential protective factor of vitamin C in asthma, particularly in adults. Asthma increases with oxidative stress, and vitamin C's action as an antioxidant works to decrease the symptoms. Although the population of this particular study was small, there is promise in showing a protective factor of 1000 mg vitamin C supplementation in adults. Future studies are necessary.

**Limitations and strengths.** The major limitation with this study is the low subject turn-out. The low subject population is consistent with the struggles in asthma studies (Cochrane). No firm conclusion can be made from the data currently available. Only 25% of the goal participation was met in the year 2014. For this study to contribute to the research in nutrition and asthma, the study should be repeated. using larger sample sizes and be conducted during the cold season. . Since the cold virus may change from year-to-year, the additional years of study would strengthen the validity of the study. Another limitation of the study is the subjective measure. Although objective data is

taken at three different times in the study, the majority of the data is subjectively reported by the subjects themselves. Especially among asthma, symptoms that may be severe to one participant may be mild to another. More subjects participating in the study would help to balance out the subjectivity. Utilizing other biomarkers of the asthmatic condition would have strengthened data interpretation, such as leukotriene B4.

While the success of this study is primarily hindered by the limitation, this study still has many strengths. One strength was the high compliance rate. Subjects consumed almost all the pills, and the lower compliance occurred in the placebo group. Additionally, the study is population-specific in an area in dire need of research data. All subjects were diagnosed with asthma by a physician and provided a detailed medical history. The methodology has been well-established for other populations with vitamin C and the common cold research studies. This methodology is fairly appropriate for the asthmatic population. Daily and weekly questionnaires and blood data allowed the researchers to analyze multiple aspects of vitamin C status and possible outside causes of increased inflammation. Using questionnaires on a weekly basis allowed the participants to give a representation of their asthma, dietary, and physical activity patterns (Allen 2009). Additionally, using both biological data and questionnaires together to assess the interaction between the treatment and asthma status has been shown to be strong in previous studies (Allen 2009). The classification of the study adds to its strength. The third-party involvement of dividing the subjects into groups increased the validity of the study.

**Conclusion.** This study does not show a relationship between vitamin C and the asthmatic populations; however, the study was underpowered and the data cannot be adequately interpreted. Future trials are needed given the importance of this topic and the fact that the currently literature is equivocal. CAM treatments for the asthma are becoming more popular, particularly antioxidant supplementation coupling prescribed medications. Vitamin C supplementation has been shown to help populations with abnormal ROS activity prevent colds. More research is needed to establish its specific role in the asthmatic population.

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APPENDIX A  
STUDY ADVERTISEMENT

## **Adults with Asthma Needed for ASU Asthma Trial**

The ASU Nutrition Program is recruiting adults with doctor-diagnosed asthma (18-65 years of age). This 8-week trial will examine whether a dietary supplement may improve respiratory tract symptoms in asthmatics. If you are willing to provide 4 blood samples and record respiratory tract symptoms daily for 8 weeks, you may be interested in this trial. \$45 in Target gift card will be awarded to participants at the end of the study.

For more information or to apply for the study, please visit our recruitment site:

<https://www.surveymonkey.com/s/ASUAsthmaStudy>

APPENDIX B  
SURVEYMONKEY.COM QUESTIONS

1. Select gender.

1. Male
2. Female

2. Has your doctor diagnosed you with asthma?

1. Yes
2. No

3. Do you currently smoke cigarettes?

1. Yes
2. No

4. Do you take prescription medications every day to control your asthma?

1. Yes
2. No

5. Do you use prescription medications (such as an inhaler) to treat your asthma symptoms when you have an asthma attack?

1. Yes
2. No

6. Are you between 18 and 65 years old?

1. Yes
2. No

7. Do you exercise vigorously over 4 times weekly and/or consider yourself a competitive athlete?

1. Yes
2. No
3. Unsure

8. Do you weigh at least 110 pounds?

1. Yes
2. No
3. Unsure

9. Are you willing to have a small amount of blood taken from an arm vein (<1 tablespoon) on 4 occasions during the trial?

1. Yes
2. No

10. Are you currently being treated by a physician for a chronic disease or condition other than asthma (e.g., cancer, diabetes, arthritis, inflammatory bowel disease, heart disease, hepatitis, etc.)?

1. Yes
2. No

11. Do you regularly take vitamin/mineral supplements?

If yes, please list.

1. Yes
2. No

12. Are you willing to maintain your current diet pattern and activity level for 8 weeks?

1. Yes
2. No

13. Are you willing to record respiratory tract symptoms daily for 8 weeks?

1. Yes
2. No

14. Are you able to come to the ASU Downtown Phoenix Campus on 4 occasions in the next several month? (The ASU nutrition laboratories are located at 5th Street and Van Buren.)

1. Yes
2. No
3. Unsure

APPENDIX C  
IRB APPROVAL FORM

**To:** Carol Johnston  
ABC 132

*for* **From:** Carol Johnston, Chair *SM*  
Biosci IRB

**Date:** 04/05/2013

**Committee Action:** **Expedited Approval**

**Approval Date:** 04/05/2013

**Review Type:** Expedited F2 F4 F7

**IRB Protocol #:** 1303008996

**Study Title:** Vitamin C supplementation and sICAM-1 Concentrations in Asthmatic Adults

**Expiration Date:** 04/04/2014

The above-referenced protocol was approved following expedited review by the Institutional Review Board.

It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date. You may not continue any research activity beyond the expiration date without approval by the Institutional Review Board.

**Adverse Reactions:** If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Biosci IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

**Amendments:** If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Biosci IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.

APPENDIX D  
CONSENT FORM

**CONSENT FORM**  
**Impact of a Nutritional Supplement on Cold Symptoms in Asthmatics**

**INTRODUCTION**

The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

**RESEARCHERS**

Dr. Carol Johnston, Associate Director and Professor, School of Nutrition and Health Promotion, and Kate Bratrud, a graduate student, have invited your participation in a research study that will last 8 weeks.

**STUDY PURPOSE**

The purpose of this research is to evaluate the effect of a dietary supplement on cold and asthma symptoms.

**DESCRIPTION OF RESEARCH STUDY**

You have informed us that you have been diagnosed by a doctor with asthma and that you currently suffer from asthma. If you decide to participate in this 8-week trial you will join a study examining the effect of a dietary supplement on respiratory tract symptoms. You will be randomly assigned (by a coin toss) to receive either a nutritional supplement or a placebo. Neither you nor the researcher you are working with will know if you were assigned to the supplement group or the placebo group. You will be asked to take a capsule twice a day during the study and to keep a record of respiratory symptoms each day during the study. You will be asked to not change your typical diet or physical activity patterns during the study and to not drink fruit/vegetable juice during the study. The study involves four visits to the test site at the ASU Nutrition Labs on the Phoenix Downtown Campus (~15-20 minutes each). To measure nutrition markers, you will be asked to provide a fasting blood sample on three occasions. A trained phlebotomist or registered nurse will draw all blood samples; each sample of blood is about 2 tablespoons. You will be asked to complete a symptom questionnaire daily and physical activity and diet questionnaires weekly.

If you say YES, then your participation will last about 8 weeks at the Arizona State University Downtown Phoenix Campus. Approximately 40 subjects will be participating in this study.

**RISKS**

Potential risks of participating in this study involve general risks associated with giving a blood sample such as bruising or irritation at the site of venipuncture and faintness. Ingestion of the dietary supplement may be associated with GI tract disturbances, which are usually alleviated when the supplement is ingested with food. As with any research, there is some possibility that you may be subject to risks that have not yet been identified.

**BENEFITS**

Although there are no direct benefits to you, the possible benefits of your participation in the research are that you are contributing to scientific knowledge regarding the efficacy of a dietary supplement on symptoms of asthma.

**NEW INFORMATION**

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

**CONFIDENTIALITY**

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. In order to maintain confidentiality of your records, Dr. Carol Johnston will assign you a participant number. Your name will not appear on any records aside from this consent form. This form will be kept in Dr. Carol Johnston’s locked office to maintain your confidentiality.

**WITHDRAWAL PRIVILEGE**

It is OK for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

**COSTS AND PAYMENTS**

The researchers want your decision about participating in the study to be absolutely voluntary. Yet they recognize that your participation may pose some costs related to time and travel. Participants will receive a gift card to Target at visits 2, 3, and 4 (\$45 total).

**COMPENSATION FOR ILLNESS AND INJURY**

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

**VOLUNTARY CONSENT**

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Carol Johnston, carol.johnston@asu.edu or (602)827-2265 and Kate Bratrud, kbratrud@asu.edu.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788. This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be offered to you.

Your signature below indicates that you consent to participate in the above study.

---

_____	_____	_____	_____
Subject's Signature	Printed Name	Email	Date

**INVESTIGATOR’S STATEMENT**

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent

conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided (offered) the subject/participant a copy of this signed consent document."

Signature of Investigator\_\_\_\_\_ Date\_\_\_\_\_

APPENDIX E  
HEALTH HISTORY QUESTIONNAIRE

HEALTH HISTORY QUESTIONNAIRE

ID# \_\_\_\_\_

Height \_\_\_\_\_ Weight \_\_\_\_\_  
*to be completed by investigator*

1. Gender: M F

2. Age: \_\_\_\_\_

3. What year were you diagnosed by a physician for asthma? \_\_\_\_\_

Have you experienced asthma-related symptoms in the recent months?

\_\_\_\_\_ Yes \_\_\_\_\_ No

Describe your asthma status:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

4. Ethnicity: (please circle one) Native American African-American Caucasian  
Hispanic Asian Other

5. Education (please circle): HS diploma Current college student BS degree MS  
degree PhD degree

6. Do you smoke? \_\_\_\_\_ No, never  
\_\_\_\_\_ Yes # Cigarettes per day = \_\_\_\_\_  
\_\_\_\_\_ I used to, but I quit \_\_\_\_\_ months ago [or \_\_\_ years ago]

7. Do you take steroidal drugs for asthma (such as Symbicort, Advair, Flonase or Medrol)  
\_\_\_\_\_ Yes \_\_\_\_\_ No

8. Do you take any medications regularly? \_\_\_\_\_ Yes \_\_\_\_\_ No *If yes, list type  
and frequency:*

<u>Medication</u>	<u>month/year initiated</u>	<u>Dosage</u>	<u>Frequency</u>
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

9. Do you currently take supplements (vitamins, minerals, herbs, etc.)? \_\_\_Yes \_\_\_No  
*If yes, list type and frequency:*

Supplement  
Frequency

month/year initiated

Dosage

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10. Are you currently being treated by a physician for any condition other than asthma?  
\_\_\_ Yes \_\_\_ No If yes, please elaborate

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11. How would you rate your lifestyle?

Not active \_\_\_\_\_ Active \_\_\_\_\_ Somewhat active \_\_\_\_\_ Very Active \_\_\_\_\_

12. During the previous **7-day period** (one week), how many times on the average did you do the following kinds of exercise for **more than 15 minutes** during your free time?

**Light activities:**

(e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)

Times per week: 0 1 2 3 4 5 6 7 8 9 10+

**Moderate activities:**

(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

Times per week: 0 1 2 3 4 5 6 7 8 9 10+

**Vigorous activities:**

(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country

skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

Times per week: 0 1 2 3 4 5 6 7 8 9 10+

13. Do you consider yourself a competitive athlete? \_\_\_\_\_ Yes \_\_\_\_\_ No

14. How would you rate your lifestyle?

Not active \_\_\_\_\_

Active \_\_\_\_\_

Somewhat active \_\_\_\_\_

Very Active \_\_\_\_\_

15. Do you have any food allergies or restrictions? Yes No If yes, explain:  
\_\_\_\_\_

16. How many times per week do you drink fruit or vegetable juice? \_\_\_\_\_

17. Circle how often you eat these foods (circle appropriate frequency):

- Cruciferous vegetables (broccoli, kale, Brussels sprouts, cauliflower, cabbage, collard greens, turnip greens, asparagus)  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Melons (cantaloupe, honeydew, watermelon, etc)  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Citrus fruits (oranges, grapefruit, lemons, etc)  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Citrus juices  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Strawberries  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Papaya, mangos, kiwi (including juices)  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Peppers (sweet green, red, yellow, hot green chili, hot red chili, jalapeno)  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Highly fortified breakfast cereals (total, all bran, 100% bran, honey buckwheat crisp, bran buds, product 19, ovaltine, maypo, instant breakfast, etc)  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less
- Fortified energy/fitness bars/drinks (power bars, Powerade, etc)  
daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

APPENDIX F  
WURSS-21 QUESTIONNAIRE

Day:	Date:	Time [To be completed at or near bedtime]:	ID:
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Please fill in one circle for each of the following items:

	Not sick 0	Very mild 1	2	Mild 3	4	Moderate 5	6	Severe 7
In terms of respiratory tract illness only, how sick do you feel <b>today</b> ?	<input type="radio"/>							

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

	Do not have this symptom 0	Very mild 1	2	Mild 3	4	Moderate 5	6	Severe 7
Runny nose	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Plugged nose	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sneezing	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sore throat	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Scratchy throat	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cough	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hoarseness	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Head congestion	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chest congestion	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Feeling tired	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

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

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

**Compared to yesterday**, I feel that my cold is: [If you did not have cold symptoms yesterday, please leave blank.]

Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse
<input type="radio"/>						

**Please list** any products (including prescription or over-the-counter medicines, herbal preparations or supplements, and/or lozenges) taken to relieve respiratory symptoms.

Product name:	Dosage:	Time(s) taken:

APPENDIX G  
DAILY ASTHMA QUESTIONNAIRE



APPENDIX H  
FOOD FREQUENCY QUESTIONNAIRE

Circle how often you eat these foods (circle appropriate frequency):

Cruciferous vegetables (broccoli, kale, Brussels sprouts, cauliflower, cabbage, collard greens, turnip greens, asparagus)

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Melons (cantaloupe, honeydew, watermelon, etc)

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Citrus fruits (oranges, grapefruit, lemons, etc)

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Citrus juices

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Strawberries

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Papaya, mangos, kiwi (including juices)

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Peppers (sweet green, red, yellow, hot green chili, hot red chili, jalapeno)

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Highly fortified breakfast cereals (total, all bran, 100% bran, honey buckwheat crisp, bran buds, product 19, ovaltine, maypo, instant breakfast, etc)

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

Fortified energy/fitness bars/drinks (power bars, Powerade, etc)

daily.....3-6x/wk.....1-2x/wk.....3x/month.....2x/month.....1x/month.....6-12x/yr.....less

APPENDIX I  
VITAMIN C ASSAY

**Collection, Assessment, and Calculations for Plasma Ascorbic Acid Assay  
(Omaye 1979)**

**Solutions**

1. 5% TCA (Trichloroacetic Acid)

- Weigh 5 g TCA, add up to 100 mL distilled H<sub>2</sub>O

2. 10% TCA

- Weigh 10 g TCA, add up to 100 mL distilled H<sub>2</sub>O

3. 9N H<sub>2</sub>SO<sub>4</sub>

- Measure 300 mL distilled H<sub>2</sub>O
- Slowly add 100 mL 36N H<sub>2</sub>SO<sub>4</sub> to the H<sub>2</sub>O

4. 65% H<sub>2</sub>SO<sub>4</sub>

- Measure 35 mL distilled H<sub>2</sub>O
- Slowly add 65 mL 36N H<sub>2</sub>SO<sub>4</sub> to the H<sub>2</sub>O

5. DTC

- Weigh 0.4 g Thiourea
- Weigh 0.05 g CuSO<sub>4</sub>·5H<sub>2</sub>O
- Weigh 3 g 2,4 dinitrophenyl hydrazine
- Slowly add to 100 mL 9N H<sub>2</sub>SO<sub>4</sub>

6. Stock Solution

- Weigh 5 mg Ascorbate
- Add to 25 mL 5% TCA
- Separate into 1 mL aliquots and freeze at -43°C

7. Working Solution

- Pipette 500  $\mu$ L of Stock Solution and add to 5 mL 5% TCA, using graduated test tubes to measure

**Processing Steps:**

1. Draw blood into 7 mL EDTA tube. Mix well and put on ice immediately. Spin at 2800 rpm for 10 minutes.
2. Mix 2.5 mL plasma sample with 2.5 mL cold 10% TCA and vortex for 10 seconds. Spin immediately at 3500g for 20 minutes at 0°C.
3. Aliquot supernatant into 3 aliquots at approximately 1.5 mL each. Freeze immediately at -80°C.

**Standards Preparation:**

REAGENTS IN TUBE				* CONCENTRATION	
Tube #	$\mu$ l Work Soln	$\mu$ l 5%TCA	$\mu$ l DTC	$\mu$ g AA/0.5ml	AA mg/dl
1	0	500	100	0	0
2	100	400	100	2	0.4
3	200	300	100	4	0.8
4	300	200	100	6	1.2
5	400	100	100	8	1.6
6	500	0	100	10	2
<b>SAMPLE</b>	500	0	100	sample	sample

**Assay Procedure**

1. Prepare standards and samples as above. Incubate tubes for 3 hours at 37°C.
2. Add 0.75mL (750  $\mu$ L) ICE COLD 65% H<sub>2</sub>SO<sub>4</sub> to all tubes. Vortex for a count of 5 seconds after each addition to mix.

3. Allow to stand at room temperature for approximately 30 minutes. Read at 520nm in spectrophotometer. Use tube 1 as the blank.
4. Using Excel, create a standard curve using Ascorbic Acid concentration (mg/dL) as x-axis and absorbance as y-axis. Produce a linear trend line and produce equation. By solving for  $y = mx + b$ , Ascorbic Acid concentrations can be found.

APPENDIX J  
HISTAMINE ASSAY

## Histamine Elisa Assay Steps (ALPCO, 2013)

### 1. Prepare Agents

- Dilute the 20 mL Wash Buffer Concentrate with distilled water to a volume of 1000 mL.
- Keep the Acylation Diluent at room temperature to prevent freezing.
- Each vial should be reconstituted with 1.25 mL Acylation Diluent.

### 2. Sample preparation and acylation

1. Pipette **25 µL** of **standards**, **25 µL** of **controls**, **25 µL** of **plasma samples**, **10 µL** of **urine samples**, or **50 µL** of **supernatant** from the **release test\*** into the respective wells of the **Reaction Plate**.
2. Add **25 µL** of **Acylation Buffer** to all wells.
3. Add **25 µL** of **Acylation Reagent** to all wells.
4. Incubate for **45 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
5. Add **200 µL** of **distilled water** to all wells.
6. Incubate for **15 min.** at **RT** (20-25°C) on a shaker (approx. 600 rpm). Take **25 µL** of **the prepared standards, controls, and samples** for the **Histamine ELISA**.

### 3. Histamine ELISA

1. Pipette **25 µL** of the **acylated standards, controls, and samples** into the appropriate wells of the **Histamine Microtiter Strips**.
2. Pipette **100 µL** of the **Histamine Antiserum** into all wells and cover plate with **Adhesive Foil**.
3. Incubate for **3 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm). *Alternatively: shake* the **Histamine Microtiter Strips** briefly by hand and incubate for **15 – 20 hours** at **2 – 8°C**.
4. Remove the foil. Discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
5. Pipette **100 µL** of the **Enzyme Conjugate** into all wells.

6. Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7. Discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
8. Pipette **100 µL** of the **Substrate** into all wells and incubate for **20-30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
9. Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10. **Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** with a reference wavelength between 620 nm and 650 nm.

#### **4. Calculation of results**

	<b>Concentration of the standards</b>					
<b>Standard</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
<b>Histamine (ng/mL = µg/L)</b>	<b>0</b>	<b>0.5</b>	<b>1.5</b>	<b>5</b>	<b>15</b>	<b>50</b>
<b>Histamine (nmol/L)</b>	<b>0</b>	<b>4.5</b>	<b>13.5</b>	<b>45</b>	<b>135</b>	<b>450</b>
<b>Conversion:</b>	<b>Histamine (ng/mL) x 9 = Histamine (nmol/L)</b>					

The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use a non-linear regression for curve fitting (e.g., spline, 4- parameter, akima).

#### **Plasma samples and controls:**

The concentrations of the **plasma samples** and the **controls** can be read directly from the standard curve.