

Immune Response after intake of Ascorbic Acid rich foods and supplement in treatment of Viral infection

Thesis

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Biotechnology

By

Mohit Kumar Verma

Under the supervision of

Prof. Akhand Pratap Singh
Supervisor
Dept. Of Science
Maharishi University, Lucknow

Dr. Himani Kulshrestha
Co-Supervisor
Dept. Of Science
Maharishi University, Lucknow

**Department of Biotechnology
Faculty of Science
Maharishi University
Lucknow-226013**

Declaration

I, Mohit Kumar Verma , student of Ph.D. hereby declare that the thesis titled “Immune Response after intake of Ascorbic Acid rich foods and supplement in treatment of Viral infection” which is submitted by me to the Department of Biotechnology, Faculty of Science, Maharishi University, Lucknow in partial fulfilment of the requirement for the award of the Degree of Doctor of Philosophy has not previously formed the basis for award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition. This is to declare further that I have also fulfilled the requirements of Para 8 (viii and ix) of the Ph.D. Ordinance.

Place & Date:

Mohit Kumar Verma

Certificate

On the basis of declaration submitted by Mohit Kumar Verma, student of PhD., I hereby certify that the thesis titled “Immune Response after intake of Ascorbic Acid rich foods and supplement in treatment of Viral infection” which is submitted to Department of Biotechnology , Faculty of Science, Maharishi University, Lucknow in partial fulfilment of the requirement for the award of the degree Doctor of Philosophy, is an original contribution with existing knowledge and faithful record of research carried out by him under our guidance and supervision.

To the best of our knowledge this work has not been submitted in part or full for any Degree or diploma to this University or elsewhere.

Prof. Akhand Pratap Singh
Supervisor
Dept. Of Science
Maharishi University, Lucknow

Dr. Himani Kulshrestha
Co-Supervisor
Dept. Of Science
Maharishi University, Lucknow

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Mohit Kumar Verma

I am eternally Grateful

Abbreviations

AA	Ascorbic acid
SVCT	Sodium Dependent Co Transporters
DHA	Dehydroascorbate
GLUT	Glucose Transporters
RDA	Recommended Dietary Allowance
ROS	Reactive Oxygen Species
H ₂ O ₂	Hydrogen Peroxide
NHANES	National Health and Nutrition Examination Survey
NO	Nitric Oxide
URI	Upper respiratory tract infections
IL-1	Interleukin-1
IL-6	Interleukin-6
PUFA	Polyunsaturated fatty acids
RTLF	Respiratory tract lining fluid
HSF	Histamine Suppressor Factor
REAP	Rapid Eating and Activity Assessment for Patients
BMI	Body Mass Index
LBS	Weight in Pounds
CON	Control Group
VTC	Vitamin C Group
WURSS-21	Wisconsin Upper Respiratory Symptom Survey

Units

μl	Microliter
μM	Micromolar
mg	Milligram
mM	Milli molar
ng	Nano gram
nm	Nano meter
rpm	Revolution per minute

Symbols

°C	Degree Celsius
β	Beta
±	Plus-minus sign

INTRODUCTION

In the course of a year, Humans suffer 2-5 billion colds. Generally, children get 6 to 10 colds a year, and adults get 2 to 4 colds per year (National Institute of Allergy and Infectious Diseases, 2005). Seeking treatment for colds is a major reason for doctor visits; unfortunately, doctors can do little to treat the illness, as antibiotics cannot fight viruses. In fact, misuse of antibiotics as a means to treat the common cold contributes to the rise of drug-resistant bacterial infections and additional risks for allergic reactions (Anon, 2010).

The common cold is known to be caused by over 200 different viruses, including rhinoviruses and coronaviruses (NIAD, 2001). The debilitating symptoms of the common cold can cause substantial health-related productivity losses. Adults on average lose 8.7 work hours per cold incidence (Bramley, Lerner, & Sarnes, 2002), and students lose a total of 189 million school days annually due to the cold (Fendrick, Monto, Nightengale, & Sarnes, 2003). The indirect costs of the common cold, such as missed workdays as well as direct costs of care, contribute to a significant burden on the economy. Colds cost population and their healthcare providers \$40 billion per year, putting it ahead of conditions such as congestive heart failure, osteoporosis, asthma, and migraines (Fendrick et al., 2003).

There is no cure for the common cold, however, we are not completely defenseless. The immune system utilizes various mechanisms to detect, attack and eliminate pathogens including viruses that cause the common cold. Vitamin C consumption is one dietary factor that enhances the immune system and the modulation of resistance to respiratory tract infections, reducing the risk, severity, and duration of the common cold (Wintergerst, Maggini & Hornig, 2006).

Vitamin C aids the immune system by decreasing oxidative stress (Bandyopadhyay, Das & Banerjee, 1999), enhancing production of the defense collagens that populate the pulmonary tissues (Behndig, Blomberg, Helleday, Kelly & Mudway, 2009), promoting leukocyte motility, and reducing circulating histamine concentrations (Johnston, Martin & Cai, 1992). Clinical trials have verified that vitamin C ingestion helps reduce the incidence, severity, and duration of common colds (Sasazuki et al., 2005; Elwood, Lee, Leger, Baird, & Howard, 1976; Anderson, Reid & Beaton, 1972).

In a double-blind, 3-year randomized controlled trial in a Japanese patient population, researchers found that vitamin C supplementation reduced the frequency of the common cold by 20% (Sasazuki et al., 2005). Additional studies have found consistent, statistically significant benefits using regular vitamin C prophylaxis indicating its role in respiratory defense mechanisms (Wintergerst, Maggini, & Hornig, 2006). Also, individuals exposed to severe physical exercise or extreme environmental conditions particularly benefit from vitamin C supplementation (Hemila, Chalker, & Douglas, 2007). Previous research on the relationship between vitamin C supplementation and the common cold lacks conclusiveness due to methodological limitations such as appropriate dosage, subject pool, and symptom measurement. The current research examined the impact of vitamin C supplementation (1000 mg/d in a divided dose) on cold symptom severity in a population group most at risk for vitamin C deficiency, young men, during cold and flu season (January-March) (Schleicher, Carroll, Ford, & Lacher, 2009).

The purpose of this randomized clinical trial was to examine the effect of vitamin C supplementation in young college males, aged 18-35, on reported perception of sickness and severity of common cold symptoms at a large Maharishi university. We hypothesized that

vitamin C supplementation (1000 mg/d, in a divided dose) will have no effect on plasma histamine concentrations.

Secondly, we hypothesized that there will not be a decrease in reported perception of sickness or symptom severity (magnitude of discomfort) of the common cold. When researching the effects of vitamin supplementation, consumption of the vitamin through the diet must be controlled. In this study, subjects only had to follow a fruit juice restriction. To control for any confounding dietary factors, vitamin C consumption was accounted for through two measures. First, milligrams of vitamin C consumed per week was calculated using food frequencies questionnaires. Second, a validated diet quality survey entitled Rapid Eating and Activity Assessment for Participants Short Version (REAPS) was conducted at weeks 0 and 8 (Segal-Isaacson, Wylie-Rosett & Gans, 2004). With these methods in place, we can conclude that the results of the study are due to the vitamin C supplementation and not from an increase in vitamin-C rich foods.

Definition of Terms

- Common cold: a syndrome caused by viral infection of the upper respiratory tract mucosa. Symptoms include weakness and fatigue, nasal discharge and obstruction, as well as sneezing and sore or ‘scratchy’ throat. Headache, congestion, and cough may also be present (Rabkin)
- Plasma histamine: physiological range for humans is 90 to 800 nmol/L; the optimal range of blood histamine has not been identified (Johnston, Solomon, & Corte, 1996)
- Plasma vitamin C: >0.4 mg/dL is adequate; 0.2-0.4 mg/dL is low; <0.2 mg/dL is deficient (Jacob & Sotoudeh, 2002)

- BMI: $[\text{weight (in pounds)} / \text{height (in inches)} \times \text{height (in inches)}] \times 703$; underweight is <18.5 , normal is 18.5-24.9, overweight is 25.0-29.9, obese is 30 and above (American Dietetics Association, 2011)
- Regular smoker: greater than or equal to 10 cigarettes per day (Moran, Wechsler, & Rigotti, 2004)
- Social smoker: fewer than 10 cigarettes per day [typically does not smoke daily] and smokes mainly with others in a social scene (Schane, Glantz, & Ling, 2009; Moran, Wechsler, & Rigotti, 2004)
- Training athlete: engaging in purposeful exercise 5 or more times per week

Limitations

- Subject compliance to the protocol, which includes taking the pills, restricting all juice consumption, and completing the symptom surveys daily. To reduce this limitation, we chose to use the one-page WURSS-21 and placed forms in a booklet for organization.
- The nature and severity of the common cold is a very subjective measure. We examined the perception of sickness as well as severity of cold symptoms reported.
- The time of day in which subjects filled out the WURSS-21 questionnaire was not specified. It is possible that symptoms of the cold are worse/less at different times of the day.
- The time period of the study did not overlap various seasons and only lasted for 8 weeks. It is possible that a longer time period is necessary to get valid results.

Delimitations

Subjects were healthy college men, aged 18-35, at Maharishi University. Exclusion criteria for subjects included regular smoking, BMI > 35 or use of dietary supplements and/or prescription medications. Subjects' vitamin C plasma concentrations had to be <0.79 mg, and a weekly food

frequency questionnaire was used to estimate mg of vitamin C from dietary intake. Diet quality was also assessed through a REAPS survey. Training athletes or those who engaged in purposeful exercise more than 5 times a week were excluded. The study lasted from mid- July to September.

Chapter 1

Review of Literature

1.1 Ascorbic Acid (Vitamin C) Overview

History. The history of vitamin C can be classified into three periods (Jacob, 1996). The first period took place between the sixteenth and nineteenth centuries and involved the investigation of the cause and cure of scurvy. The second period occurred between 1900 and 1980 and is defined by the chemical isolation and characterization of ascorbic acid. It was during this time that ascorbic acid was identified as being necessary to prevent scurvy. The last period, from 1980 until present, includes the exploration of the roles vitamin C can have in optimizing human health and preventing disease.

Accounts of scurvy most notably occurred during the sixteenth and eighteenth centuries. Seamen commonly complained of symptoms such as bleeding gums, bruising on extremities, swollen joints, and a red blotchy skin irritation called petechiae (Hughes, 2010). Although there is some controversy over the discovery of a cure for scurvy, James Lind is credited with conducting one of the first recorded accounts of a controlled clinical trial that labeled scurvy as a nutrient deficient illness (Bartholomew, 2002). In May 1747, Lind was working on the *HMS Salisbury* as a naval surgeon where he isolated 12 scurvy patients experiencing similar symptoms. He paired them off and prescribed various treatment remedies including vinegar, mustard and garlic purges, as well as oranges and lemons. The only pair to quickly recover from their scurvy symptoms was the two prescribed to incorporate oranges and lemons into their diet (Bartholomew, 2002). In an

excerpt from James Lind's personal documentation of the experiment, he illustrates the details of the recovery:

The consequence was that the most sudden and visible good effects were perceived from the use of the oranges and lemons; one of those who had taken them being at the end of six days fit for duty. The spots were not indeed quite off his body, nor his gums sound; but without any other medicine than a gargarism or elixir of vitriol he became quite healthy before we came into Plymouth, which was the 16th of June (Bruzeliuss, 1996).

After years of testing various hypotheses to cure the symptoms of scurvy, evidence that lack of a nutrient from citrus foods was the cause prompted the British navy to begin supplying lime juice on crew ships in order to prevent scurvy in the early 1800's (Hughes, 2010). This discovery introduced an effective treatment measure and established scurvy as a nutritional disease worldwide (Clemetson, 1989). Although a reliable treatment had been identified and deaths due to scurvy decreased, the pathophysiology was not yet understood.

The second period of vitamin C history began in 1907 when two Norwegian scientists, Axel Holst and Alfred Frohlich, were studying the development of beriberi in guinea pigs (Holst & Frohlich, 1907). Unexpectedly, symptoms of scurvy resulted. Holst and Frohlich discovered that the guinea pigs were cured of their scurvy symptoms through a diet high in fruits and vegetables. This discovery was a tremendous breakthrough for future research because it unveiled an experimental mode to study scurvy.

Almost twenty years after the identification of scurvy as a vitamin C deficiency by Casimir Funk in 1912, Albert Szent-Gyorgyi in Europe and Glenn King in the United States successfully isolated "hexuronic acid" from citrus (Szent-Gyorgyi, 1928). The substance was shown to

completely reverse the symptoms of scurvy and was later named ascorbic acid. The isolation of the compound allowed for the identification of its chemical and physical characteristics.

After these advancements in the discovery of vitamin C, research explored the proper metabolic needs for humans to prevent scurvy. Furthermore, studies were conducted to see if there were benefits of taking high doses of the supplement to improve health through roles such as immunocompetence and antioxidant protection (Jacob, 1996).

Biochemistry, Absorption & Metabolism. Vitamin C is a water-soluble vitamin that exists primarily in its reduced form in the body, ascorbic acid (AA). The active form of AA (L-ascorbic acid) is composed of a 6-carbon α -ketolactone with two enolic hydrogen atoms, which allows it to donate hydrogens and therefore reduce molecules (Rose & Bode, 1993). AA is exceptionally resilient in that it is readily recycled intracellularly from its oxidized form, dehydroascorbic acid (DHA) through chemical reduction by glutathione and enzymatic reduction (Park & Levine, 1996). The typical amount of vitamin C in the blood (35 μ M) can be completely regenerated every 3 minutes (Mendiratta, Qu & May, 1998).

Through evolution, humans, guinea pigs, fruit bats, and primate monkeys lost their ability to synthesize vitamin C from glucose. This is because they lack the enzyme, gulonolactone oxidase, which catalyzes the last enzymatic step in the synthesis of ascorbate (Chatterjee & De, 1969). Instead, the vitamin must be obtained through the diet. Vitamin C is absorbed across the brush border of the small intestine through different mechanisms, namely two different sodiumdependent cotransporters, SVCT1 and SVCT2 (Tskaguchi et al., 1999). SVCT1 is expressed on epithelial tissues of the intestine and kidney as well as inside the liver (Tskaguchi et al., 1999). SVCT2 is found in a number of tissues including neurons and bone. The distribution

of the two transporters suggests that SVCT1 is involved in the bulk of AA transport and homeostasis while SVCT2 is involved in tissue-specific uptake of AA (Hediger, 2002).

Ascorbate accounts for 80-90% of vitamin C content in food products (Vanderslice & Higgs, 1991); however, prior to absorption, AA can be oxidized to form dehydroascorbate (DHA). DHA can also be absorbed through facilitated diffusion using sodium-independent carriers with the aid of glucose transporters, especially GLUT1 and GLUT3 (Wilson, 2005). Because of its dependence on glucose transporters, extracellular glucose has an inhibitory effect on DHA absorption (Washko & Levine, 1992). Once inside the cell, DHA is rapidly reduced back to AA by glutathione or enzymatic reduction.

The absorption of ingested AA depends highly on dosage and the resulting bioavailability. Intakes of 30-180 mg of AA per day result in approximately 70- 90% absorption; however, absorption decreases to less than 50% when doses are greater than 1 gram (Institute of Medicine, 2000). Additional research further supports this dose-response relationship concluding that a 20 mg dose can reach 98% absorption, yet higher doses of 12 g only have a 16% absorption rate (Levine et al., 1996). Some studies have shown that intakes between 100 and 200 mg vitamin C elevate plasma concentrations to ~1.0 mg/dL, maximizing the body pool (Kallner, Hartmann & Hornig, 1979). However, there is evidence that a single daily dose of 100-200 mg only allows for tissue saturation, and >500 mg/day is necessary to achieve plasma saturation (Johnston & Cox, 2001). When plasma saturation is achieved, antioxidant protection can be maximized.

After absorption, AA is transported primarily as an ascorbate anion in plasma (Wilson, 2005). For a healthy adult, plasma concentrations of AA range from 0.4 to 1.7 mg/dL; less than 0.2 mg/dL is considered deficient (Jacob & Sotoudeh, 2002). Tissue concentrations of AA and DHA vary greatly depending on the tissue type. The highest concentrations of vitamin C are found in

the adrenal and pituitary glands, and smaller amounts are found in the liver, spleen, pancreas, kidney and brain (Hornig, 1975). Because vitamin C is a polar compound of relatively large molecular weight, flux in and out of cells is controlled by specific mechanisms including active transport and facilitated diffusion. Active transport of DHA is facilitated by glucose transporters (GLUT), and active transport of AA is facilitated by sodium-dependent cotransporters (SVCT) (Wilson, 2005).

Vitamin C and its metabolites are filtered by the glomerulus in the kidney, and are reabsorbed at the proximal tubule through active transport (Rose, 1986). Excess AA is excreted as urine. At an intake of 500 mg, approximately 70% of the dose is absorbed; however, about half of the absorbed dose is excreted unmetabolized in urine (Levin, Conry-Cantilena, Wang, et al, 1996). With a higher dose of 1250 mg, only 50% of it is absorbed and >85% of the absorbed dose is excreted. These findings suggest that vitamin C is efficiently regulated in the body; and thus, vitamin C is relatively nontoxic. However, a Tolerable Uptake Level (UL) has been set at 2,000 mg due to adverse effects such as nausea, osmotic diarrhea, and gastrointestinal distress (Food and Nutrition Board and Institute of Medicine, 2000). High doses of vitamin C have been shown to increase urinary oxalate excretion, which may contribute to the development of kidney stones (Massey, 2005). Therefore, individuals who are at risk for kidney stones should not supplement vitamin C over 500 mg per day.

Sources & Recommended Intake. The best dietary sources of vitamin C include a variety of vegetables, fruits and juices, some of which are listed in **Table 1**. The vitamin C content of the fruits and vegetables depends on the stability of the molecule, which can readily be influenced by oxygen, pH, heat, and metallic ions; consequently, AA can become oxidized and cannot function at its optimal level (Lopez, Krehl & Good, 1967). This means that the essential functions of AA,

such as antioxidant protection, are lost. Some cooking practices and prolonged storage can cause the degradation of vitamin C. For example, 50 to 80% of vitamin C is lost from boiling vegetables (Vanderslice & Higgons, 1991).

However, steaming vegetables in minimal amounts of water can substantially decrease the loss of vitamin C from cooking (Rumm-Kreuter & Demmel, 1990). Vitamin C is also well preserved in frozen foods suggesting the importance of purchasing frozen vegetables and frozen concentrate orange juice (Johnston & Bowling, 2002).

Table 1. Examples of vitamin C-rich foods.

Examples of Vitamin C rich foods	
Food, Standard Amount	Vitamin C Content (mg)
Red pepper, raw, 1/2 cup	95
Orange juice, 3/4 cup	93
Kiwifruit, 1 medium	71
Grapefruit juice, 3/4 cup	70
Broccoli, cooked, 1/2 cup	51
Strawberries, fresh, 1/2 cup	49

National Institutes of Health

In general, most people would assume that fresh orange juice bought at the local supermarket is packed with vitamin C; however, due to the environment, much of the molecule can be destroyed by the time it gets to the consumer. One study compared the vitamin C content of commercial orange juice brands over an eight-day period (Johnston & Hale, 2005). At day 8, the orange juice

that was refrigerated after reconstitution from frozen concentrate had significantly less bio available vitamin C compared to its baseline amount. Furthermore, vitamin C's protective property as a plasma antioxidant was diminished compared to its baseline value. This is a very influential factor in the vitamin C status of individuals worldwide since the majority of food items are transported, stored, and processed before purchased.

The resulting AA concentration in serum after absorption is not proportional to the amount ingested, which strongly affects the dietary recommendations for vitamin C (Rumsey & Levine, 1998). To prevent scurvy, adults must consume ~10 mg/d of vitamin C (Levine, Rumsey, Daruwala, et al; 1999); however, the Recommended Dietary Allowance (RDA) has been set at 90 mg/d for men and 75 mg/d for women (Levine, Wang, Padayatty et al., 2001).

These RDAs, established a decade ago, should maintain near-maximum neutrophil AA concentrations with minimal urinary excretion (Jacob & Sotoudeh, 2002).

The RDA is based primarily on the prevention of deficiency rather than the prevention of chronic disease and promotion of optimal health (National Research Council, 1989). The optimal intake of vitamin C is likely to be higher than the RDA and vary depending on the individual. Remarkably, an NHANES study conducted from 1976-1980 found that nearly 20% of Americans consume less than 60 mg of vitamin C daily and about 10% consume less than 30 mg (Koplan, Annett, Layde & Rubin, 1986). A more recent NHANES study conducted between 2003 and 2004 concluded that vitamin C status in the US population has substantially improved although deficiency in various subgroups, such as smokers and low-income persons, is still of concern (Schleicher, Carroll, Ford & Lacher, 2009). Pharmacokinetic trials in young healthy men and women have identified that leukocytes became saturated between intakes of 100 and 200

mg of vitamin C/d (Levine, Conry-Cantilena, Wang, et al., 1996). Furthermore, there is evidence that >500 mg/day is necessary to achieve plasma saturation and maximize antioxidant protection (Johnston & Cox, 2001). Additional research is needed to reexamine the recommended level of vitamin C ingestion to optimize its protective functions.

The established RDAs are based on healthy adults; however, factors such as sex, age, or clinical condition could alter the absorption and transportation of the compound. As indicated above, studies have shown that females need less vitamin C per day than males (Biard et al, 1979; Levine Conry-Cantilena, Wang, et al., 1996). This is because females on average have smaller lean body masses and maintain a higher plasma AA concentration at a given intake (Institute of Medicine, 2000). Some epidemiologic studies show that the elderly, especially those institutionalized, have a lower vitamin C status than young adults (Institute of Medicine, 2000). This is most likely due to low dietary intake, less efficient absorption, or clinical conditions such as repeated infections; however no special RDA has been established for elderly people. Smokers have an increased recommendation (125 mg/d for males and 110 mg/d for females) due to either increased AA catabolism or the debilitating oxidative stress of smoking (Higdon & Frei, 2002). Finally, those suffering from a clinical condition such as cancer, diabetes, or cardiovascular disease, may benefit from increased vitamin C consumption (Li & Schellhorn, 2007). The exact mechanisms through which vitamin C can relieve the onset and progression of degenerative disease is specific to each illness; however, its antioxidant properties are strong contributing factors in most cases (Li & Schellhorn, 2007).

Functions. Oxygen is essential for aerobic life; however, about 5% of inhaled oxygen is converted to reactive oxygen species (ROS) (Bandyopadhyay, Das & Banerjee, 1999). ROS, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$), cause

oxidative stress and deregulate cell functions leading to illnesses such as cardiovascular disease and cancer (Taniyama & Griendling, 2003). They are generated through normal physiological processes as well as environmental stressors, and can cause substantial oxidative damage to lipids, cell proteins, and nucleic acids.

Four enzymes work to defend the body from the damage of ROS: superoxide dismutase, glutathione peroxidase, heme peroxidase, and catalase (Bandyopadhyay, Das & Banerjee, 1999). A secondary defense against ROS is termed 'scavengers'. These 'scavengers,' also known as antioxidants, react with the dangerous radical compounds to produce less harmful molecules (Ogawa, Suzuki, Okutsu, Yamazaki & Shinkai, 2008). AA is a powerful antioxidant and, thus, is essential to minimize oxidative stress in the body.

Recent evidence has shown that AA may also act as a pro-oxidant, promoting DNA damage *in vitro*; however it is uncertain if this occurs *in vivo* (Carr & Frei, 1999). Pro-oxidation happens when free transition metals are reduced by AA and then react with hydrogen peroxide. These metals, such as copper and iron, lead to the synthesis of highly reactive hydroxyl radicals (Li & Schellhorn, 2007).

AA also plays a critical role as a co-antioxidant for regenerating α -tocopherol, a form of vitamin E with saturated side chains. This regeneration is very important since *in vitro* experiments have shown that the oxidized form, α -tocopheroxyl radical, can act as a prooxidant in the absence of co-antioxidants (Chappell et al., 2002). Sustaining α -tocopherol levels limits peroxidation of cells and therefore maintains cell integrity (Mandl, Szarka & Banhegyi, 2009).

Similar to its antioxidant properties, AA also functions as a cofactor by maintaining metal ions in a reduced state, which is necessary for enzyme activity (Padh, 1991). Several hydroxylases and

mono-oxygenases depend on AA. One major physiological function is that AA serves as a cofactor in collagen synthesis.

Collagen is the most prevalent protein in mammalian bodies (Spanheimer & Peterkofsky, 1984). Of the 8 enzymes that vitamin C acts as a cofactor, 3 participate in collagen hydroxylation: prolyl-4-hydroxylase, prolyl-3-hydroxylase, and lysyl-hydroxylase. With the help of AA, hydroxyl groups are added to the amino acid lysine or proline in order to make the collagen molecule more stable (Padayatty et al, 2003). The symptoms that develop due to a vitamin C deficient state are mostly related to defective collagen synthesis—bone and connective tissue disorders, tooth loss, and blood vessel fragility.

Carnitine plays a critical role in energy metabolism, specifically the transport of fatty acids across the mitochondrial membrane (Johnston, Corte, & Swan, 2006). This is essential for tissues whose primary source of energy is fatty acids such as skeletal and cardiac tissue. Ascorbic acid is a cofactor for two enzymes used in the production of carnitine, trimethyllysine dioxygenase and 4- gamma-butyrobetaine dioxygenase (Rebouche, 1991). Specifically, ascorbic acid reduces iron to its ferrous (Fe^{2+}) state, which is necessary for this enzymatic pathway to function. Fatigue and weakness are early signs of scurvy and can be linked directly to defective carnitine production (Hughes, Hurley & Jones, 1980).

In a study where guinea pigs were fed high fat diets, those that were also given high doses of AA maintained 50% more heart and skeletal muscle carnitine compared to the AA deficient group (Rebouche, 1991). Another study found that free-living participants with marginal vitamin C status oxidized 25% less fat per kg body weight during an hour long treadmill test compared to those with adequate vitamin C status (Johnston, Corte & Swan, 2006). Synthesis of specific

neurotransmitters and hormones require vitamin C to reduce copper to its cuprous (Cu¹) state in order for enzyme activity to take place.

Vitamin C acts as a cosubstrate for dopamine- β -monooxygenase, which converts the neurotransmitter dopamine to norepinephrine (Wimalasena, & Wimalasena, 1995). The results of deficient neurotransmitter conversions can explain some of the symptoms of scurvy—depression and mood swings. Vitamin C is also involved in the activation of hormones and hormone-releasing factors that involve α -amidations in the posttranslational steps (Murth, Keutmann & Eipper, 1987).

Petidylglycine α -amindating mono-oxygenase catalyzes α -amindations and is AA dependent. Without vitamin C, essential hormones such as vasopressin, oxytocin, cholecystokinin, and gastrin could not be synthesized (Oldham et al., 1992). The important role of vitamin C in steroid hormone synthesis is shown through its high concentration in the adrenal glands (Patak, Willenberg & Bornstein, 2004).

During times of high stress, the adrenal glands release stress-response hormones, most notably being cortisol. These ‘fight or flight’ hormones trigger the body to prepare for action, and can affect the body negatively, including immune function (Ebrecht, Hextall, Kirtley et al., 2004), when present for prolonged periods of time. Vitamin C may act as an antioxidant and work to reduce the release of stress hormones from the adrenal gland (Peters, 1997). Studies have shown that supplementation with antioxidant mixtures for up to two months can significantly reduce cortisol responses to prolonged exercise (Fischer et al, 2004; Vassilakopoulos et al, 2003). More research is warranted on vitamin C’s ability to decrease cortisol release during times of stress.

Role in Disease States. Supplementation of vitamin C provides a protective influence on many disease states including vascular disease, cancer, and the main purpose of this review, the

common cold (Li & Schellhorn, 2007; Jacob & Sotoudeh, 2002). Much research has investigated the therapeutic effects of vitamin C and the mechanisms by which the nutrient provides beneficial effects for chronic disease states.

Many epidemiological studies have found an inverse relationship between vitamin C intake and incidence of cardiovascular disease. Data from the National Health and Nutrition Examination Survey (NHANES) conducted from 1976-1980 revealed an 11% decrease in cardiovascular disease and stroke incidence for every 0.5 mg/dL rise in serum vitamin C (Jacob & Sotoudeh, 2002). One critical molecule of the cardiovascular system is nitric oxide (NO), which is necessary for healthy endothelial function (Carr & Frei, 1998). NO induces vasodilatation and inhibits potentially dangerous processes such as smooth muscle proliferation and platelet aggregation from occurring (Frei, 1997). AA, especially with tocopherol, may function to scavenge superoxide radicals which can readily inactivate NO (Carr & Frei, 1998). One study concluded that treatment with vitamin C (500 mg/d for 4 weeks) can improve endothelium-dependent vasodilatation in patients with coronary artery disease, angina pectoris, hypercholesterolemia, hypertension, or diabetes (Gokce, Keaney, Frei, et al., 1999). In another study, researchers found that vasodilatation was increased in participants by supplementing 1,000- 2,000 mg/d of oral AA (Duffy, Gokce & Holbrook et al, 1999). Additionally, vitamin C can decrease the prevalence of cardiovascular disease by decreasing hypertension (Duffy, Gokce & Holbrook et al, 1999; Hajjar, George & Sasse et al, 2002). In a cross-sectional study conducted in the UK with more than 500 men and women, plasma AA levels were inversely related to diastolic and systolic blood pressure (Skyrme-Jones et al., 2000). In another study where healthy people were fed a vitamin C deficient diet for 30 days followed by a vitamin C adequate diet for another 30 days, plasma ascorbate levels were inversely related to diastolic

blood pressure (Block, 2002). Other clinical trials have conflicting results indicating that more research is needed in regards to the role of supplemental vitamin C in reducing cardiovascular disease risk.

Numerous epidemiological studies provide evidence that diets high in fruits and vegetables are related to decreased risk of some cancers (Carr & Frei, 1999). Many studies suggest that the link between high fruit and vegetable diets and decreased cancer risk is due to high vitamin C content of these food items (Szeto, Tomlinson & Benzie, 2002; Rossing, Vaughan, McKnight, 1989).

Vitamin C's protective influence is stronger with cancers of the oral cavity, pharynx, esophagus, and stomach. Additionally, meta-analyses have shown an inverse relationship between high vitamin C intakes and decreased risk (20%) for breast cancer (Gandini, Merzenich, Robertson et al, 2000). A longitudinal study observed 870 men for 25 years and found that those who consumed >83 mg/d of vitamin C had a marked 64% decrease in lung cancer incidence compared to those that consumed <63 mg/d (Sandra et al., 2001).

Observational studies have linked increased dietary vitamin C to decreased risk of stomach cancer (Mirvish, 1994). Theoretically, vitamin C present in gastric juice inhibits the formation of *N*-nitroso carcinogens in the stomach. *Helicobacter pylori* is a bacterial infection that has been linked to an increased risk for stomach cancer and also lowers the vitamin C content in gastric secretions (Jarosz, Dzieniszewski, Dabrowska-Ufniaz et al, 1998). Vitamin C supplementation in addition to other therapies has been a suggested treatment option for patients suffering with this condition.

The possible mechanisms for a decreased risk of cancer with vitamin C is thought to involve its role as an antioxidant and its ability to detoxify carcinogens or block carcinogenic processes (Block, 1992; Block, Patterson, Subar, 1992; Carpenter, 1991). Although there is evidence

supporting vitamin C's ability to reduce the risk for esophageal cancer, lung cancer, and breast cancer, the trials have not been able to distinguish between the influence of vitamin C and other components of vitamin C-rich fruits and vegetables. Furthermore, some studies suggest that vitamin C may hinder anticancer therapies via its antioxidant properties, which may promote tumor cell integrity (Perrone et al., 2009). Because results for studies regarding cancer risk and vitamin C supplementation are so conflicting, more research is needed in order to offer safe, effective advice to cancer patients.

The publication of *Vitamin C and the Common Cold* by Linus Pauling in 1970 stirred up public debate about vitamin C's role in immune function. His hypothesis rested on the discovery that leukocytes had high concentrations of vitamin C, which rapidly declined during times of stress and infection (Higdon & Frei, 2002). Pauling advocated that the optimal daily intake of vitamin C for an adult consuming 2500 kcal/d should be 2300 mg/d. Numerous epidemiologic, clinical, and biochemical studies conducted since Pauling's published work have deciphered a much more moderate vitamin C level between 100 and 200 mg/d to be associated with tissue saturation and reduced risk for chronic diseases in healthy adults (Carr & Frei, 1999; Levine, Rumsey, Daruwala et al, 1999). Even though Pauling's views on vitamin C supplementation have been criticized, he is still seen as a pioneer in the health field that stimulated interest in the role of micronutrients to promote optimal health and prevent chronic diseases. The interest in vitamin C's role with the immune function and its ability to treat the symptoms of the common cold has stirred continued research in order to determine the mechanisms behind the proposed immunocompetence and the specific populations that would benefit. Current research provides evidence that Vitamin C aids the immune system through its antioxidant properties, direct

antimicrobial function, and/or effect on immune system modulators such as histamine (Jacob & Sotoudeh, 2002). Several trials have been conducted, however, there is still one question that cannot be fully answered: Is supplementations of vitamin C an effective measure in treating the common cold?

1.2 Upper Respiratory Tract Infections

Physiology. The respiratory system includes the nose and nasal cavity, mouth, pharynx (throat), larynx (voice box), trachea, bronchi, and lungs (Marieb, 2004). Its main function is to supply the body with oxygen and excrete carbon dioxide. This process, called respiration, consists of four processes. The first is pulmonary ventilation, which is the movement of air in and out of the lungs. This process ensures that gases are continually circulated and refreshed. The second step of respiration is external respiration. This is the incorporation of oxygen into the blood and the removal of carbon dioxide from the blood. Transport of respiratory gases, the third step of respiration, is the transfer of oxygen from the blood to the tissue cells of the lungs. The final step, internal or cellular respiration, involves the exchange of oxygen in the blood to tissue cells throughout the body (Marieb, 2004).

In addition to supplying oxygen to the body, the respiratory system also works to protect the body from harmful toxins that can be inhaled. The respiratory tract is lined with a mucus membrane and hair-like structures called cilia, which trap and remove particles such as pollution, smoke, or pathogens (Dugdale, 2008).

Prevalence and Transmission of URIs. Upper respiratory tract infections (URI) are the most common acute illness in the US with Americans suffering 1 billion colds per year (NIAID, 2001). On average, children endure 6 to 10 colds per year, and adults get 2 to 4 colds per year

(NIAID, 2001). Consequently, adults on average lose 8.7 work hours per cold incidence, and students lose a total of 189 million school days each year due to the common cold (Bramley, 2002). This tremendous health-related loss in productivity contributes to the significant economic burden caused by the common cold. Colds cost Americans and their healthcare providers approximately \$40 billion each year—\$17 billion from direct medical costs and \$22.5 billion from indirect costs (Fenrick et al, 2003). The economic burden of the common cold is greater than conditions such as congestive heart failure, high blood pressure, asthma, and migraines (Fenrick et al, 2003).

The rhinovirus is highly contagious. It can be transmitted through various mechanisms including aerosol, droplet, or direct hand-to-hand contact with infected secretions followed by contact with the nasal mucosa or conjunctivae (Musher, 2003). A quantitative study that examined the transfer of respiratory viruses to and from fingers of adult subjects found that 37.8% of rhinovirus could still be detected 1 hour after contamination and nearly 16% after 3 hours (Ansari, Springthorpe & Sattar et al, 1991). Furthermore, a systemic review conducted on the persistence of pathogens found that rhinovirus could be detected on dry inanimate surfaces up to 7 days after contamination (Kramer, Schwebke & Kampf, 2006). Thus, transmission occurs more commonly in crowded areas, one great example being a college dorm.

Infection & Treatment. The “common cold” refers to acute rhinosinusitis, which is caused by viral infection of the upper respiratory tract mucosa (Rabkin). Symptoms include nasal congestion and/or discharge, sneezing, sore or dry throat, and headache. The onset of symptoms occurs 1 to 3 days after exposure to a pathogen, and symptoms of the cold generally last from 1 to 2 weeks (Piccirillo, 2004; Sande & Gwaltney, 2004). Most colds are caused by four different families of viruses: rhinovirus, coronavirus, adenovirus, and respiratory syncytial virus (Cooper,

Hoffman, Bartlett et al, 2001). The infection rates of the particular virus vary by season; however, the symptoms of the four viruses are almost indistinguishable (Rabkin). Seeking treatment for colds is a major reason for doctor visits; however, doctors can do little to treat the illness since antibiotics are not effective against viruses (Anon, 2010). Although there is substantial evidence that antibiotics do not cure the common cold (Gonzales, Steiner & Maselli, 2001), almost 75% of adults with URIs are prescribed antibiotics by their doctors (Hirschman, 2002).

Astonishingly, unnecessary prescriptions for URIs and bronchitis represent 31% of total antibiotic prescriptions in the US (Rabkin) and account for \$700 million in medical costs per year (Gonzales, Steiner & Maselli, 2001). So why are doctors still prescribing antibiotics for cold symptoms?

The answer in the literature is two-fold: a) the patient may benefit from the antibiotic or b) the patient expects antibiotics. Although it is possible for a bacterial infection to coincide with a viral infection, the chances are small.

Furthermore, prescribing antibiotics without evidence of a bacterial infection can cause harm to the patient. In a cross-sectional survey, researchers found that 82% of subjects with common cold symptoms such as runny or obstructed nose, cough or sore throat were prescribed antibiotics to treat the symptoms (Kumar, Indira & Rizvi, 2008). Antibiotics were more likely to be prescribed in private than government settings, and in rural than urban settings. Additionally, it is possible that a patient expects their physician to give them a medication to treat their symptoms, which can sway a doctor's judgment.

In contrast with the notion that all patients expect antibiotics when visiting a doctor, studies have shown that a patients' satisfaction is independent of whether or not antibiotics are prescribed.

Instead, the satisfaction is related more closely to if the physician addressed the patients' concerns by providing information and giving verbal advice, even if that means explaining why antibiotics will not benefit the patient (Arrol, Kenealy & Kerse, 2002).

The use of antibiotics not only fails to cure the common cold but can contribute to the rise of drug-resistant bacterial infections and the risk for allergic reactions (Anon, 2010). Antibiotics can also destroy the healthy gut bacteria leading to gastrointestinal discomfort (Lode, 2010). Although antibiotics cannot cure the common cold, the body is not completely defenseless. The extraordinary mechanisms of the immune system can detect and eliminate pathogenic agents including viruses that cause the common cold.

The Immune System & URIs. The immune system is composed of a network for cells, tissues, proteins and organs that protect the body from invading pathogens such as viruses, bacteria, and parasites. It can detect toxic agents and distinguish them from healthy cells and tissues. When activated, lymphocytes proliferate and release inflammatory mediators such as proinflammatory cytokines (IL-1; IL-6) (Wintergerst, Maggini, & Hornig, 2006). When produced by lymphocytes, interleukin-1 (IL-1) has a paracrine effect by signaling the surrounding cells to trigger a blood clot cascade, stimulate the synthesis of other interleukins, and activate T cells and thus the adaptive immune response (Bendtzen, 1988). IL-6 works to stimulate the immune system by increasing energy metabolism. Through this process, the infection is isolated and cells are signaled to begin the healing process; however, excessive release of IL-1 can result in inflammation, fever, and tissue damage (Dinarello, 2000).

Once triggered by antigen, the immune system is primed and can tackle specific pathogens more efficiently at future infection. This process is known as “acquired immunity” and contributes to immunological memory (NIAID, *Immune System*, 2010). Generation of ROS is a mechanism for

host defense used to kill viruses or other invading pathogens intracellularly; however, ROS can also interfere with immune cell communication and damage other biomolecules such as PUFAs. Many factors contribute to the ability of the immune system to do its job, one of the most important being diet (Starnbach, 2010). Just as for any system of the body, the immune system cannot perform at its optimal level without proper nourishment. A community-based longitudinal study in rural Bangladesh examined the relationship between nutritional status, cell mediated immune response, and incidence of acute upper respiratory tract infections (Zaman, Baqui & Yunus et al, 1997). This year-long study included 696 children ages 0-59 months. Approximately three quarters of whom were below -2 z-score weight for age and height for age. The study found that incidence of upper respiratory tract infections was 16% greater in the classified malnourished children than in adequately nourished children. The study also found that anergic children (e.g., children whose immune system is unresponsive) had a 20% higher risk for URI than immunocompetent children. This evidence supports the theory that cell immunity is directly related to nutritional status.

Because diseases commonly coexist with micronutrient deficiencies, underprivileged populations in developing countries are common victims of infections such as HIV/AIDS, tuberculosis, malaria, and measles (Bhaskaram, 2002). In a randomized controlled trial of 200 Zambian children suffering from severe malnutrition and diarrhea, researchers found that children given either a routine nutritional rehabilitation diet or elemental diet were free of diarrhea after one month (Amadi, Mwiya, Chomba et al, 2005). Additionally, fecal samples were taken at the beginning and end of the study to measure pathogenic protozoa.

Results showed that host defense improved with the nutrition intervention suggesting that virulence is associated with host defenses and thus can be modulated by nutritional status

(Hughes & Kelly, 2006). The idea of “boosting” immunity through nutritional supplements has been a highly debated topic in the health field for years (Goldrosen & Straus, 2004). The theory suggests that specific nutrients and/or minerals can enhance the efficiency and effectiveness of the immune system and thus decrease incidences of illnesses caused by pathogens. Nutrient deficiencies may impair the proper functioning of various immune processes, which are critical for host defense. Antioxidants (Bandyopadhyay, Das & Banerjee, 1999), especially vitamin C (Beetens & Herman, 1983), could play a major role in preventing tissue damage from immune-system-generated ROS, especially in populations with poor dietary habits. Research investigating the topic of immunity is immense, however there is still much that is inconclusive.

1.3 Ascorbic acid (Vitamin C) and Upper Respiratory Tract Infections

Vitamin C is a major nutrient that has been linked to immunity, specifically decreasing upper respiratory track infections. There are many mechanisms by which vitamin C aids the immune system including decreasing oxidative stress (Bandyopadhyay, Das, & Banerjee, 1999), enhancing production of the defense collagens that populate the pulmonary tissues (Behndig, Blomberg, Helleday, Kelly, & Mudway, 2009), promoting leukocyte motility, and reducing circulating histamine concentrations (Johnston, Martin, & Cai, 1992). These properties are the foundation for the hypothesis that vitamin C supplementation can decrease the incidence and severity of the common cold.

Decreasing Oxidative Stress. Oxidative stress is the result of reactive oxygen species (ROS), which cause damage to cells and tissues in the body. It contributes to the process of aging and can lead to the development of disease. ROS, such as superoxide (O_2^-) and the very reactive hydroxyl radical ($\cdot OH$), are byproducts of oxygen reduction in the body (Bandyopadhyay, Das,

& Banerjee, 1999). Because ROS are molecules with unpaired electrons, they can damage vital cell components such as proteins, polyunsaturated fatty acids, and nucleic acids. These reactions can alter membrane fluidity, protein synthesis, and can cause DNA damage. Ultimately, ROS can lead to cell death. AA reduces ROS activity by donating electrons to neutralize these molecules (Rose & Bode, 1993). Oxidized AA, DHA, is then readily reduced back to AA through glutathione or enzymatic reduction making it an efficient combater of oxidative stress (Rumsey & Levine, 1998).

Lipid peroxidation, a result of ROS damage, occurs in polyunsaturated fatty acids (PUFA) and effects cell membrane fluidity, permeability, and cellular metabolic functions (Bandyopadhyay, Das, & Banerjee, 1999). Chemically, the methylene C-H bonds of PUFAs are prone to hydrogen abstraction because of the adjacent double bond. A randomized controlled trial investigating the ability of AA to decrease lipid peroxidation in relation to the pathogenesis of atherosclerosis found that supplementation of 500 mg vitamin C/d for 2 months resulted in a ~10% reduction in lipid peroxidation (Huang, Appel, Croft et al., 2002). The antioxidant properties of AA reduce the amount of oxidative stress in the body allowing physiological systems, including the immune system, to operate more efficiently.

Promoting Leukocyte Mobility. Leukocytes are white blood cells that play a major role in the immune system by protecting the body from infectious pathogens. Vitamin C is highly concentrated in leukocytes, and the recycling process of AA is especially efficient in leukocytes suggesting its importance to the immune system (Rumsey & Levine, 1998). Exposure of neutrophils to microbial pathogens can actually increase AA recycling 30-fold (Jacob & Sotoudeh, 2002). Neutrophil chemotaxis is an important part of immune response.

Through this process, chemotactic agents signal neutrophils and other serum factors to the site of infection in order to contain and destroy pathogens. Although more evidence is needed, studies suggest that vitamin C, through its ability to suppress histamine, may indirectly promote chemotaxis and therefore promote immune function (Johnston, Martin, Cai, 1992; Anderson, 1981).

Enhancing Production of Defense Collagens in Pulmonary Tissues. The lungs are particularly susceptible to oxidative damage due to exposure to toxins present in inhaled air. Many pollutants, including bacteria and viruses, are powerful oxidants and could potentially cause significant damage to lung tissue.

However, the pulmonary epithelium is lined with respiratory tract lining fluid (RTLFL), which is heavily populated with antioxidants such as AA and glutathione (van der Vliet et al., 1999). It has been proposed that these antioxidants in the RTLFL may help neutralize and eliminate inhaled toxins and decrease tissue damage by activating defense collagens on neutrophils in the airways (van der Vliet et al., 1999). Neutrophils can then contain and destroy invading pathogens that may have been inhaled. In vitro studies have shown that AA is directly oxidized by free radical gases, ozone and nitrogen dioxide (Kelly & Tetley, 1997). In vivo studies have also shown that AA is oxidized in the RTLFL when exposed to nitrogen dioxide (Kelly et al., 1996). These observations emphasize the importance of AA to protect the lung lining and suggest that protection against air toxins, including pathogens, may be increased by augmenting RTLFL AA concentrations.

In a randomized controlled trial, researchers set out to see if augmentation of RTLFL ascorbate concentrations could protect the lung tissue from air toxins and airway inflammatory episodes (Behndig, Blomberg, Helleday et al., 2009). 24 subjects with low plasma ascorbate

concentrations were recruited and the experimental group received 60 mg/day of vitamin C for two weeks. Dosage increased in increments of 250, 500, and 1000 mg of vitamin C throughout the time period of the experiment. The results illustrated that acute vitamin C supplementation (1g) has a rapid, but transient, increase in AA concentrations in RTLF. Ascorbate in nasal RTLF samples more than doubled after just 2 hours after supplementation (1.1 μ M at baseline to 2.6 μ M), more than tripled after 4 hours (3.6 μ M), and returned to pre-supplementation levels around 24 hours. This instant antioxidant protection may provide protection to lung tissue as well as decrease oxidative stress and inflammation in airways.

Although the exact mechanism is unknown, it is believed that RTFL ascorbate concentrations are a result of passive paracellular transport from the plasma to the airway lining; therefore, increasing plasma AA concentrations will increase antioxidant protection in the airways (Behndig, Blomberg, Helleday et al., 2009). Through redox reactions, AA can neutralize potential free radical damage from inhaled toxins (Kelly et al, 1996). The protective influence of vitamin C in the respiratory tract may decrease the incidence of the cold by eliminating infections caused by inhaled pathogenic toxins.

Histamine. Histamine is involved in numerous physiological processes including vasoconstriction and vasodilation, allergic reactions, and neurotransmission (Falus & meretey, 1992). It is also a critical molecule in the initial stages of an immune response by providing a signal of “alertness”. When the body identifies a foreign invader, histamine increases capillary permeability and smooth muscle contraction (Plaut & Lichtenstein, 1982). This mechanism of immunoresponsiveness triggers the flow of immune factors to the inflammatory site. Shortly after, histamine suppresses the immune cells by activating T suppressor cells and the Histamine

Suppressor Factor (HSF). This is possibly to contain the inflammatory effect (Busse & Sosman, 1976).

Vitamin C acts as an antihistamine and can inhibit the immunosuppressive actions of histamine. In vitro studies have illustrated that AA breaks the imizadole ring structure of histamine, inhibiting its ability to function (Uchida, Mitsui & Kawakishi, 1989). Additionally, vitamin C may compete with histamine for the H1 receptor site on cells in the respiratory tract and blood vessels (Woo, 2008). Because histamine is a mediator for symptoms of URIs, it is possible that vitamin C may function to reduce cold symptom severity by reducing concentrations or actions of histamine. Possibly, AA would inhibit the inflammatory process and decrease cold symptoms such as congestion.

A randomized controlled trial analyzing 437 human blood samples found that when plasma AA fell below 1 mg/100 ml, blood histamine levels increased exponentially (Clemetson, 1978). However, 1 g of vitamin C per day for 3 days resulted in the reduction of histamine in all 11 selected volunteers. Another study involving healthy men and women, ages 19-47, found that ingestions of 2 grams of vitamin C per day resulted in a 38% decrease in histamine levels in just 2 weeks (Johnston, Martin & Cai, 1992). In fact, histamine degradation may be able to provide a valid measure to detect subnormal, non-scorbutic vitamin C status before scurvy develops (Johnston, Solomon & Corte, 1996).

1.4 Current Evidence:

When investigating the effect of vitamin C supplementation on treating the common cold, it is important to account for is the baseline AA status of study participants. Participants with adequate AA tissue saturation would be less likely to show a benefit with vitamin C

supplementation as compared to participants with AA deficiency. Because of this, recruiting individuals with low to marginal plasma vitamin C would be most useful. Several studies have shown that subjects in the UK have particularly low plasma AA levels, most likely due to diet (Hemila, 1997). A randomized controlled trial with 1524 British men and women sought to see if 10 g of AA taken the first 3 days of cold symptom onset would decrease the severity of symptoms reported.

Researchers concluded that AA supplementation is “of no value in the treatment of the common cold” (Tyrrell, Wallace, Meade & White, 1977). Another important aspect of research that must be accounted for to strengthen internal validity of a study is dietary intake of vitamin C. Completely restricting vitamin C-rich foods from the diet would ensure that the results are due to the supplemented vitamin C, however it could cause the development of deficiency symptoms in the participants. To avoid harming the subjects, diet can instead be accounted for through diet assessment measures. The Rapid Eating and Activity Assessment for Patients (REAP) survey has been validated against the Healthy Eating Index and includes questions to assess intake of whole grains, calcium-rich foods, fruits and vegetables, fat, sugary beverages and foods, sodium and alcohol. It is used to evaluate the eating behaviors of subjects and provides assessment of the diet related to the US Dietary Guidelines (Gans, Risica, Wylie-Rosett, et al., 2005). The shortened version of the survey has been validated and scores the eating behavior questions as follows: “usually/often” = 1, “sometimes” = 2, and “rarely/often” = 3 (Segal-Isaacson, Wylie-Rosett & Gans, 2004). The higher the score out of 39 points, the higher the diet quality based on US Dietary Guidelines. Food frequency questionnaires, 24-hour recalls, and diet records are other diet assessment tools that can be used to collect information on food intake and convert to estimates of nutrient intake.

Extensive research has been conducted to investigate the efficacy of vitamin C supplementation in preventing and treating the common cold. Pauling conducted a meta-analysis of four placebo-controlled studies and concluded that vitamin C supplementation of at least 100 mg per day decreased the incidence of common cold episodes by 45% (Pauling, 1971). However, since this finding, other studies have found no substantial effect between the experimental and placebo groups (Hemila, Chalker & Douglas, 2010). In a double blind trial with 1000 subjects taking 4000 mg/d at the onset of a cold, researchers sought to reevaluate Pauling's findings (Anderson, Reid & Beaton, 1972). They found that the average number of colds and days of sickness was less for the vitamin C group than the placebo group, but the differences were small and not statistically significant. However, they did find a significant difference between groups for the number of participants who did not contract any illness during the study: 26% in the vitamin C group as compared to 18% of the control group. This could suggest that vitamin C may be an effective prevention measure in terms of the common cold.

Another randomized, controlled trial found an 18% decrease in 'chest colds' (cough, difficulty breathing) but no effect in the incidence of 'simple colds' (runny nose or sneezing) (Elwood, Lee, Leger, Baird, & Howard, 1976). A similar study found a 21% decrease in 'throat colds' but no effect in 'nose colds' (Anderson, Reid & Beaton, 1972). These findings suggest that vitamin C supplementation may be more effective in treating symptoms of certain types of cold infections.

A recent meta-analysis of 29 placebo-controlled trial comparisons with over 11,000 subjects found that regular supplementation of vitamin C had no effect on the incidence of the common cold in the general population (Hemila, Chalker, & Douglas, 2007). However, there were modest decreases duration and severity of cold symptoms in those people under high physical stress,

such as a marathon runner or skier. Those who supplemented vitamin C had a 50% less risk of contracting the common cold. A recent study that surveyed 37 triathletes found that 97% supplemented vitamin C and 48% did so to prevent or reduce common cold symptoms (Knez & Peake, 2010). This finding is supported by a randomized controlled trial with 92 runners who took 600 mg of vitamin C daily (Peters, Goetzsche, Grobbelar & Noakes, 1993). After a competitive ultramarathon race (>42 km), subjects were monitored for cold symptoms. 68% of runners in the placebo group reported symptoms of a URI in comparison to 33% in the vitamin C group. It may be that vitamin C supplementation is beneficial for specific subpopulations, such as extreme athletes, in fighting the common cold.

Another very interesting study aimed to investigate dietary vitamin C's relationship with self-reported respiratory symptoms in 4300 young smokers in Norway (Omenaas, Fluge, Buist, et al., 2003). Researcher's concluded that dietary vitamin C was inversely related to "morning cough," "chronic cough," "wheeze," and "wheeze ever." This finding suggests that high dietary vitamin C intake may decrease the prevalence of smoking-related respiratory symptoms by acting as an antioxidant and reducing cough and wheeze in smokers with high oxidant stress (Block, 1996). One major trial investigating the effect of vitamin C on the common cold monitored subjects for 3.5 years (Sasazuki, 2006). The long time span of the trial is highly beneficial because it was able to cover multiple seasons, giving the results credibility. In the trial, there was a low dose group (50 mg VC/d) and a high dose group (500 mg VC/d). Interestingly enough, researchers found that the high dose group about 3 times less incidence of the common cold compared to the low-dose group; however, there was no observed effect on duration or severity which may be due to methodological errors. Because the mixed findings in this study and others, much more

valid and reliable research is necessary to determine if vitamin C supplementation is effective in treating the common cold.

Chapter 2

Aims and Objectives

Aim of the present study was to analyze the plasma histamine, sickness and symptoms of viral infection after taking of ascorbic acid (Vitamin C).

The objectives of the study are as follows:

1. Effect of ascorbic acid supplement on blood plasma histamine level in viral infection (common cold) .
2. Effect of ascorbic acid supplement on sickness of and symptoms severity of viral infection (common cold).

Chapter 3

Materials and Methods

3.1 Ethical Statement

The study was approved by Institutional research and Ethics committee of Maharishi University Lucknow. The experiments were undertaken with the understanding and written consent of each subject, and the study confirms with The Code of Ethics of the world Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

3.2 Study Subjects and specimen collection:

Healthy college men, aged 18-35, were recruited through flyer distribution and college department List Serves on the Maharishi University for this two-treatment parallel-design study. Initial contact by phone call (Appendix A) was used to determine if those interested met medical history, diet, and physical activity requirements for the study.

Eligible individuals were scheduled for a screening visit (study visit 1). Exclusion criteria for subjects included regular smoking >10 times a day several times a week (Moran, Wechsler, & Rigotti, 2004), BMI >35, daily use of dietary supplements containing >60 mg of vitamin C, and/or use of prescription medications for chronic conditions. Subjects' vitamin C plasma concentrations from the screening visit had to be below 0.80 mg/dL in order for them to participate in the 8-week trial. Training athletes or those who were engaged in purposeful, vigorous exercise more than five times a week were also excluded.

This randomized, double-blind, parallel arm study was approved by the Institutional Research committee Maharishi University, Lucknow . A power analysis was calculated using a probability of 0.05 and a power of 0.8. Based on previous studies measuring plasma vitamin C concentrations, a standard deviation of 0.2 was used to determine sample size. We anticipate a change in plasma vitamin C to be 0.2 mg/dL. Using a verified sample size calculator, 34 participants were deemed adequate. A total of 45 subjects were recruited, with 30 attending the screening and becoming enrolled in the study.

This allowed for 15 subjects per group. One subject was withdrawn from the study due to issues with compliance. Additionally, one subject was excluded because his results from the WURSS-21 survey were greater than 3 standard deviations from the mean. In all, 15 subjects were in the VC group and 13 were in the placebo group.

After providing written consent (Appendix), BMI, weight/height, postprandial blood sample (no food or drink with the exception of water for 5 hours), Rapid Eating Assessment for Participants Questionnaire (REAPS), 24- hour food recall, and a Medical History Questionnaire were completed at the first study visit (Appendix). Participants who met the inclusion criteria were stratified by age, BMI, and plasma vitamin C concentrations and randomly assigned to either the experimental or the control group. The experimental group (VTC) was instructed to ingest the capsule (500 mg vitamin C) twice daily, once in the morning and once in the evening. The control group (CON) was instructed to ingest the capsule (a flour placebo identical in appearance to the vitamin C capsule) twice daily, once in the morning and once in the evening. Subjects were requested not to drink any fruit drinks or juices during the study. They were told to consume the pill with food if there was any sign of gastrointestinal distress.

At the study baseline (study visit 2), all subjects were given a booklet, which contained copies of the Wisconsin Upper Respiratory Symptom Survey-21 (Appendix) to record their daily symptom status and the use of any medications, herbal supplements, or lozenges taken to relieve respiratory symptoms. The booklet also included a food frequency questionnaire (Appendix) to be completed weekly to account for dietary vitamin C consumption during the course of the study. Survey booklet compliance was checked at week 1 (study visit 3) and the booklets were turned in at weeks 4 and 8 (study visits 4 and 5).

Fasting blood samples (no food or drink with the exception of water for 8 hours) for plasma vitamin C were drawn again at weeks 4 and 8. Fasting blood samples for histamine analyses were drawn again at week 8. A second REAPS was also completed at week 8 (study visit 5). At these visits, subjects also completed several measuring tools that were used for a separate study (POMs and Godin- Leisure Time Exercise Questionnaire).

3.3 Blood Analyses:

Plasma vitamin C was determined using the 2,4- dinitrophenylhydrazine method of Omaye et al. A 1 ml aliquot of plasma was mixed with 1 ml ice-cold 10% trichloroacetic acid. Following centrifugation (3500 x g, 0°C), the supernatant was frozen (-80°C) and vitamin C analysis was performed on the sample in 7 days (Johnston & Martin, 1992). The histamine was quantitatively acylated and analyzed using an ELISA kit with the microtiter plate format. (ALPCO Diagnostics, 2010)

3.4 Histamine ELISA Test Procedure:

Allow all reagents and samples to reach room temperature prior to use. Measurement in duplicates is recommended.

1. Preparation of reagents

Wash Buffer: Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1,000 mL. Store the diluted Wash Buffer Concentrate (Wash Buffer) at 2 – 8°C. *Shelf life:* please refer to expiry date indicated on the kit.

Acylation Diluent: The Acylation Diluent has a freezing point of 18.5°C. To ensure that the Acylation Diluent is liquid when being used, it must be ensured that the Acylation Diluent has reached room temperature and forms a homogenous, crystal-free solution before being used. Alternative the Acylation Diluent can be stored at room temperature (20 - 25°C) separate from the other kit components.

Acylation Reagent: Reconstitute each vial with 1.25 mL Acylation Diluent. *The Acylation Reagent has to be newly prepared prior to the assay (not longer than 1 hour in advance). If more than 1.25 mL is needed, pool the contents of 2 or 3 vials and mix thoroughly.*

2. Sample preparation and acylation

- Pipette 25 µL of standards, 25 µL of controls, 25 µL of plasma samples or 50 µL of supernatant from the release test* into the respective wells of the Reaction Plate.
- Add 25 µL of Acylation Buffer to all wells.
- Add 25 µL of Acylation Reagent to all wells.
- Incubate for 45 min at RT (20 -25°C) on a shaker (approx. 600 rpm).
- Add 200 µL of distilled water to all wells.
- Incubate for 15 min at RT (20 -25°C) on a shaker (approx. 600 rpm).

* For the release test the Histamine Release supplementary kit (available for purchase separately. Cat. No. BA E-1100) has to be used.

3. Histamine ELISA

- Pipette 25 μ L of the acylated standards, controls, and the samples into the appropriate wells of the Histamine Microtiter Strips.

- Pipette 100 μ L of the Histamine Antiserum into all wells and cover the plate with Adhesive Foil.

- 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.

- Remove the foil. Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 μ L Wash Buffer. Blot dry by tapping the inverted plate on absorbent material.

- Pipette 100 μ L of the Enzyme Conjugate into all wells.

- Cover plate with Adhesive Foil and incubate for 30 min at RT (20 - 25°C) on a shaker (approx. 600 rpm).

- Remove the foil. Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 μ L Wash Buffer. Blot dry by tapping the inverted plate on absorbent material.

- Pipette 100 μ L 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!*

- Add 100 μ L of the Stop Solution to each well and shake the microtiter plate to ensure a homogenous distribution of the solution.

- 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

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.

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3.5 Wisconsin Upper Respiratory Symptom Survey—21:

The Wisconsin Upper Respiratory Symptom Survey (WURSS-21) is a standardized measure for evaluating the signs and symptoms of the common cold (Appendix C). It consists of 21 questions: a global severity indicator, 19 symptom-severity items using 7- point severity scales, and one comparative question referring to symptom severity the previous day. The WURSS-21 has been tested and is a reliable and valid method for examining the incidence, severity, and duration of the common cold (Barrett et al, 2009). The survey assessed symptom severity and functional impairment, with a score of 1 considered to be very mild; 3, mild; 5, moderate; and 7, severe. In this study, summing the scores of the first 10 questions provided a measure of symptom severity, summing the scores of the second 9 questions provided a measure of functional impairment, and summing all 19 items provided a global measure of illness severity. Perception of sickness was indicated by a question that asked, “How sick are you today?” Again,

results were recorded on a magnitude scale with 1 considered to be very mild; 3, mild; 5, moderate; and 7, severe.

3.6 Statistical Analysis:

Data were reported as means \pm SE. Data were analyzed using repeated measures through analysis of variance (ANOVA). Statistical analysis was performed using the SPSS Statistical Analysis System 19.0. Data were tested for normality and transformed if necessary. Outliers of more than 3 standard deviations from the mean were excluded from the results. Nonparametric measures were used when data were not normally distributed. Within- and between-group differences were expressed as mean percent difference (95% CI). Differences were considered significant at $p \leq 0.05$.

Chapter 4

Results

4.1 Baseline Characteristics

Recruitment took place from July to September. Of the 43 participants screened, 30 were enrolled, stratified by age, BMI, and plasma vitamin C concentrations, and randomly assigned to either the control (CON) group or the vitamin C group (VTC). This allowed for 15 subjects per group. One subject was withdrawn from the study due to issues with compliance. Additionally, one subject was excluded because his results from the WURSS-21 survey were greater than 3 standard deviations from the mean. Hence for data analysis, 15 subjects were in the VTC group and 13 were in the CON group. Age ranged from 18 to 32 years, though the mean age was similar in both groups (23.00 ± 0.79 in VTC and 23.36 ± 1.12 in CON). Baseline height, weight, BMI, percent body fat, and REAPS scores were also similar between the VTC and CON groups indicating proper stratification for the study (**Table 2.**). Baseline plasma histamine was significantly different between the CON and VTC groups, 0.356 ± 0.247 and 0.667 ± 0.628 respectively ($p=0.043$). Baseline plasma vitamin C was below US norms for this age group in both groups (0.52 ± 0.03) (Schleicher, Carroll, Ford & Lacher, 2009). Additionally, baseline plasma vitamin C status of the participants was inversely related to body weight ($r=-0.579$, $p=0.001$; **Figure** as well as BMI, and percent body fat.

Table 2. Baseline Characteristics of Participants^{1,2}

	All (<i>n</i> =28)	VTC (<i>n</i> =15)	CON (<i>n</i> =13)	<i>P</i> -value ³
Age (y)	23.0±0.7	23.0±0.8	23.2±1.2	0.913
Height (in)	71.0±0.4	71.9±0.5	69.0±0.7	0.026
Weight (lb)	181.1±4.0	182.5±5.2	179.6±6.3	0.720
BMI (kg/m ²)	25.2±0.7	24.6±1.0	26.0±1.1	0.348
Body fat (%)	19.0±1.2	17.5±1.7	20.7±1.8	0.201
Plasma VC (mg/dL)	0.523±0.029	0.532±0.039	0.513±0.047	0.744
Plasma histamine (ng/mL)	0.522±0.507	0.667±0.628	0.356±0.247	0.043
REAPS	31±0.6	31±0.8	31±0.9	0.133
¹ VTC - vitamin C group; CON - placebo group; REAPS - diet quality analysis at weeks 0 & 8; Plasma VC was collected 5-hours post meal ² Values reported as means ± SE. ³ Analysis by independent t-test				

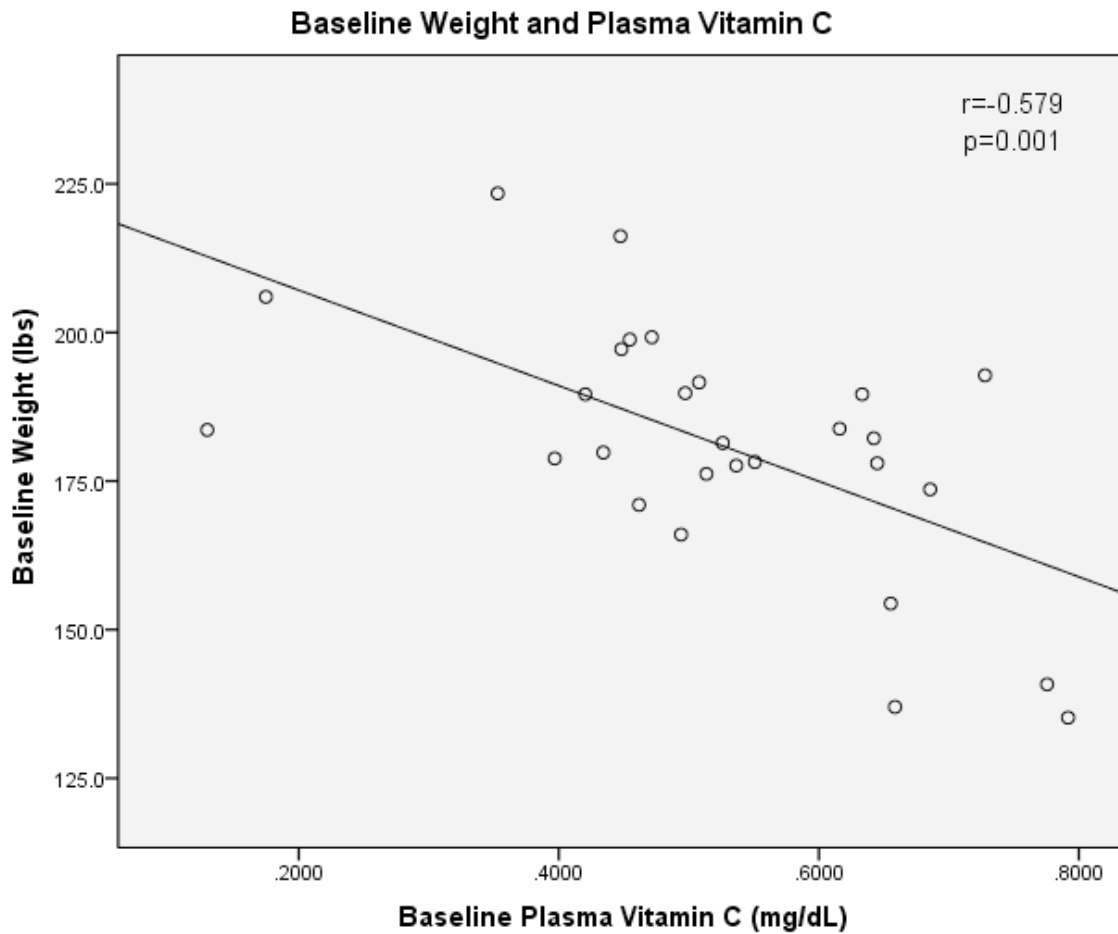


Figure 1. Relationship between baseline body weight and plasma vitamin C status

4.2 Plasma Indices & Dietary Data

Plasma vitamin C was significantly higher in the VTC group compared to the CON group at week 4 of the study, 0.726 ± 0.049 and 0.541 ± 0.056 respectively ($p = 0.019$) (**Table 3.**). There was also a significant difference between the groups at week 8, (VTC was 0.742 ± 0.47 and CON was

0.597±0.53; $p=0.051$). Plasma histamine fluctuated only slightly over the 8-week trial, -4.2% in the VTC group and +17.4% in the CON group ($p=0.562$) (**Figure 2**). There was a 94.5% compliance rate for subjects taking the pills, which was determined by the number of pills returned at weeks 4 and 8. Because the only diet restriction placed on the subjects was to avoid fruit juices, a weekly Food Frequency Questionnaire (FFQ) was conducted to account for milligrams of vitamin C consumed by the participants. Dietary vitamin C intakes (mg/d) determined from weekly FFQ analyses averaged over trial weeks 1-4 and trial weeks 5-8 did not differ by group ($p=0.107$ and 0.958 respectively). Additionally, diet quality was assessed using the Rapid Eating Assessment for Participants Questionnaire at weeks 0 and 8. There was no change in diet quality by group over the study period ($p=0.133$).

Table 3. Plasma Indices & Dietary Data at Study Weeks 4 & 8, 2				
	Week 0	Week 4	Week 8	<i>P</i> -value ³
Plasma histamine (ng/mL)				0.562
VTC	0.667±0.628	ND	0.639±0.277	
CON	0.356±0.247	ND	0.431±0.298	
REAPS				0.133
VTC	31±0.8	ND	32±0.9	

CON 31±0.9 ND 30±1.3				
Fasting Plasma				
VC (mg/dL)				
VTC	ND	0.726±0.049	0.742±0.047	0.019 (wk4)
CON	ND	0.542±0.056	0.597±0.053	0.051 (wk8)
FFQ (mg VC)				
VTC	ND	85±10	101±11	0.107 (wk4)
CON	ND	113±14	102±19	0.958 (wk8)
<p>1 VTC - vitamin C group (<i>n</i>=15); CON - placebo group (<i>n</i>=13); REAPS - diet quality analysis at weeks 0 & 8; ND- No data</p> <p>2 Values reported as means ± SE.</p> <p>3 Between group comparisons: repeated measures ANOVA for Plasma Histamine & REAPS; Independent t-test for Plasma VC & FFQ</p> <p>4 FFQ recorded as average of weeks 1-4 and average of weeks 5-8.</p>				

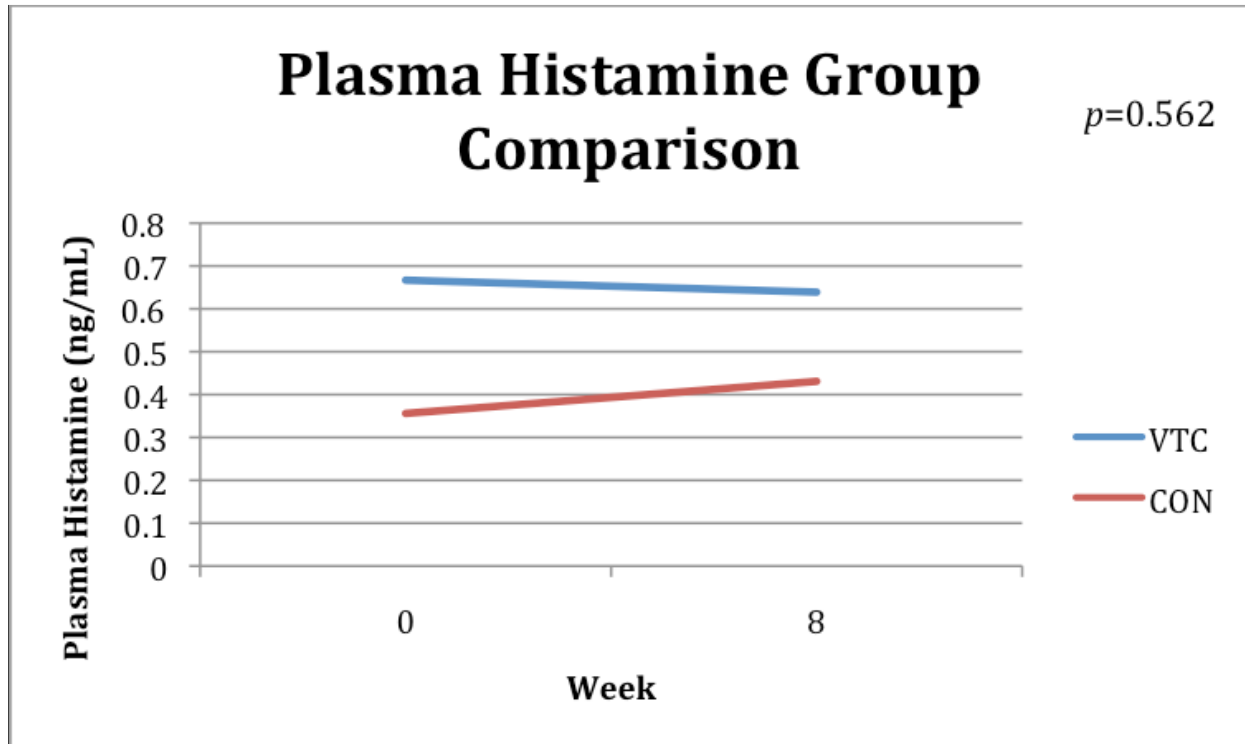


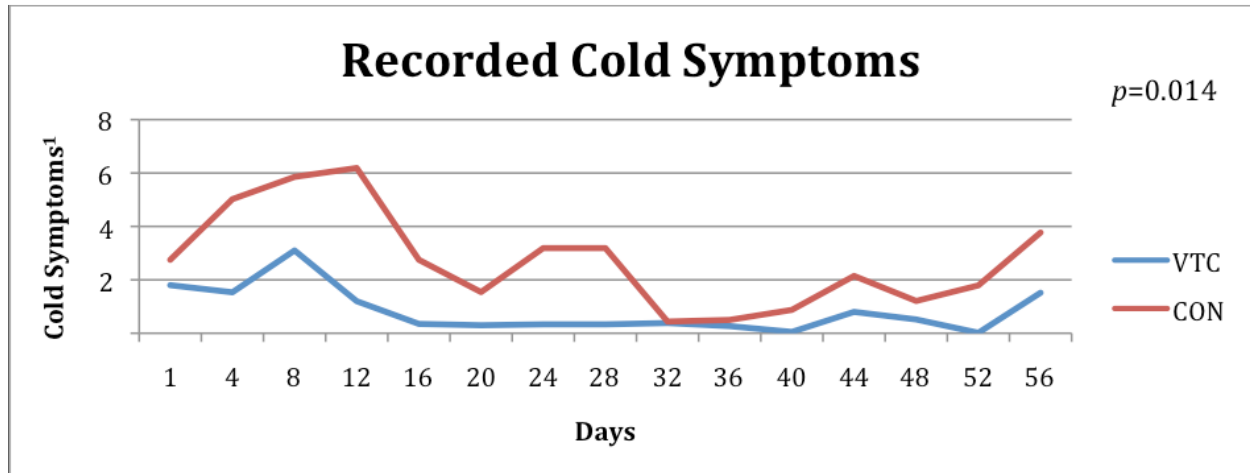
Figure 2. Plasma histamine group comparison over 8-weeks

4.3 Cold Symptom Severity & Incidence of Sickness

The Wisconsin Upper Respiratory Symptom Survey-21 was used to track common cold symptoms during the 8-week study (**Table 4.**). Scores are arbitrary units that represent the magnitude of symptoms. The average total symptoms reported for the entire 8 weeks for the VTC group was 43 ± 15 compared to 148 ± 36 in the CON group—a 244% difference in symptoms ($p=0.014$) (**Figure 3**). Looking at only the first 10 questions of the survey gives insight into symptom severity reported. Symptoms assessed included “runny nose”, “plugged nose”, “sore throat”, “cough”, and “chest congestion.” The VTC group reported significantly less severity of symptoms compared to the CON group, 31 ± 10 and 101 ± 25 respectively ($p=0.031$). The second 9 questions of the survey focuses on functional impairment such as decreased ability to sleep well,

work outside the home, and interact with others. There was a greater amount of functional impairment related to cold symptoms reported by the CON group (46 ± 15) compared to the VTC group (18 ± 10); however, the results did not attain statistical significance ($p=0.058$). Overall, there was a 65% lower reported perception of sickness in the VTC group than the CON group ($p=0.022$) (**Figure 4**).

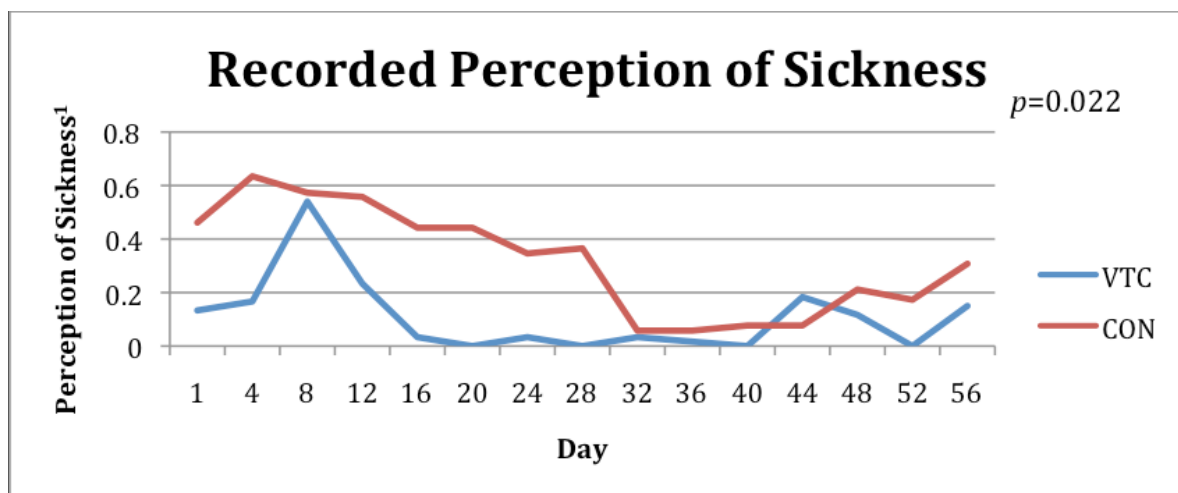
Table 4. Cold Symptom Analysis of WURSS-21 Survey over 8-Weeks ^{1,2}				
	All (<i>n</i> =28)	VTC (<i>n</i> =15)	CON (<i>n</i> =13)	<i>P</i> -value ³
Average Total Symptoms	92 \pm 109	43 \pm 15	148 \pm 36	0.014
Symptom Severity	63 \pm 76	31 \pm 10	101 \pm 25	0.031
Functional Impairment	31 \pm 48	18 \pm 10	46 \pm 15	0.058
Perception of Sickness	11 \pm 2	6 \pm 2	17 \pm 4	0.022
¹ VTC - vitamin C group; CON - placebo group; WURSS-21 - Cold Symptom Survey ² Values reported as means \pm SE; Units are an arbitrary measure of magnitude ³ Analysis by nonparametric independent samples test (Mann-Whitney U)				



¹Units are an arbitrary measure of magnitude. Symptoms are reported over 56 days, in 4-day increments. (1=very mild; 3=mild; 5=moderate, 7=severe)

²One subject was excluded because initial day was an extreme outlier value ($n=27$).

Figure 3. Comparison of cold symptoms reported by the VTC and CON groups over the course of the study



¹Units are an arbitrary measure of magnitude. Symptoms are reported over

56 days, in 4- day increments. (1=very mild; 3=mild; 5=moderate,7=severe)

Figure 4. Comparison of perception of sickness reported by the VTC and CON groups over the course of the study

To control for the possibility that allergy symptoms may have had some influence on the results, any report of allergies or use of allergy medications on the medical history questionnaire as well as the WURSS-21 survey was taken into account. Only one participant reported using an allergy medication daily.

Significance of the results were maintained when controlling for this subject ($p<0.05$).

Chapter 5

Discussion

In this double-blind, randomized controlled trial, ascorbic acid (vitamin C) supplementation reduced the perception of sickness and reported cold symptom severity compared to placebo. These results support the daily supplementation of 1000 mg vitamin C by college men to reduce cold symptoms such as cough, congestion, and sore throat. These findings are consistent with other studies that reported decreased severity of specific cold symptoms with vitamin C supplementation (Elwood, Lee, Leger, et al., 1976; Anderson, Reid & Beaton, 1972). Anderson et al. conducted a randomized controlled trial with 818 subjects and found that those taking 4000 mg of vitamin C per day had a 21% decrease in 'throat colds' but no effect in 'nose colds' compared to the placebo group. A similar study conducted by Elwood et al. observed 688 women for 100 days. They found that those supplementing 1000 mg of vitamin C per day had an 18% decrease in 'chest colds' (cough, difficulty breathing) but no effect in 'simple colds' (runny nose or sneezing) compared to the placebo group. It is important to note that the participants began this study with low adequate vitamin C status and that these results are likely limited to this population. Baird *et al.* concluded that males benefited more so from vitamin C supplementation than females in terms of total number of symptoms reported and especially in duration of cold symptoms (1979). Another double-blind survey also found that males had less reported colds than females when taking AA supplements for 15 weeks (Clegg, 1974). This is

most likely due to the fact that young males generally have lower plasma AA than females (Schleicher, Carroll, Ford & Lacher, 2009) because females on average have smaller lean body masses and maintain higher AA concentrations at a given intake (Institute of Medicine, 2000). Therefore, compared to females, males are more likely to have lowadequate AA status and benefit from the vitamin C supplementation. There are multiple functions of vitamin C that can account for its ability to decrease common cold symptom severity. Through vitamin C's function as an antioxidant, it is able to react with dangerous radical compounds known as reactive oxygen species (ROS) and produce less harmful molecules (Ogawa, Suzuki, Okutsu, Yamazaki & Shinkai, 2008). ROS have unpaired electrons and can cause substantial damage to vital cell components such as proteins, polyunsaturated fatty acids, and nucleic acids effecting membrane fluidity, protein synthesis and DNA synthesis (Rose & Bode, 1993). This function of vitamin C reduces the amount of oxidative stress in the body and therefore allows physiological systems, including the immune system, to operate more efficiently. Of particular interest is the ability of AA to reduce harmful pathogens inhaled through the lungs (Behndig, Blomberg, Helleday et al., 2009) since inhalation is a mode of virus transmission (Musher, 2003). With a strong immune system, the body can be more effective at fighting off the virus that causes the common cold.

Ascorbic acid (Vitamin C) has also been linked to promoting leukocyte mobility, an essential aspect of the immune system to protect the body from invading pathogens (Rumsey & Levine, 1998). Although more evidence is needed, some studies suggest that vitamin C may indirectly promote leukocyte mobility through its ability to suppress histamine (Johnston, Martin & Cai, 1992; Anderson, 1981). By promoting quick action when there is an invading pathogen, cold symptom severity may be decreased by vitamin C. Because of its role in the immune system,

histamine is a mediator of cold symptoms (teary eyes, stuff nose, sneezing, etc.) (Busse & Sosman, 1976). It is possible that the benefit of vitamin C supplementation for cold symptom relief is related to its antihistamine function. However, in our study, plasma vitamin C concentrations did not reach levels necessary to observe an anti-histamine effect.

Previous research shows that the antihistamine effect of vitamin C is not observed until plasma vitamin C levels are above 1.0mg/100ml (Johnston, Retrum & Srilakshmi, 1992). Because the subjects in this study had plasma vitamin C levels below 1.0mg/100ml, the reduction in cold symptoms noted herein cannot be explained by the antihistamine role of vitamin C. Rather, it is likely related to the protective effects that vitamin C has in lung tissues and/or promotive effects vitamin C has on the immune system or antioxidant properties that may lessen damage and inflammation.

It is especially interesting to see that the rise and fall of recorded cold symptoms (**Figure 3.**) over the 8-week period is similar between the VTC and the CON group; however, the VTC group had significantly lower cold symptoms even at peaks of apparent illness. This relationship was emulated in a similar study. A double-blind, randomized controlled trial conducted over 3 years with 244 subjects also found nearly identical peaks of cold incidence for the ‘highdose’ group (500 mg vitamin C/day) and the ‘low-dose’ group (50 mg vitamin C/day), however the peaks were consistently lower for the ‘high-dose’ group (Sasazuki et al., 2006). These results, however, conflict with previous research that has concluded vitamin C supplementation has no effect on the incidence of the common cold (Hemila, Chalker, & Douglas, 2010).

The WURSS-21 is a valid measurement for assessing magnitude of cold symptoms and performs as an “illness-specific quality-of-life evaluate outcome instrument” (Barrett et al, 2009). To the author’s knowledge, there is only one other study that has used the WURSS-21 survey for

research purposes (Barrett, Brown, Rakel, et al., 2010). Because there is no gold standard for identifying, classifying, or assessing colds, it can be difficult to compare results of similarly structured studies. It is important to note that this study analyzed ‘perception of sickness’ which could include other ailments other than common cold symptoms.

For more comparable results, future studies using the WURSS-21 survey should explain that questions about ‘sickness’ pertain to cold symptoms only. To maintain internal validity of the study, dietary assessment measures were included in the methodology. The shortened REAPS survey was used at weeks 0 & 8 to investigate dietary behaviors of subjects and determine diet quality scores in terms of US Dietary Guidelines. Through this validated survey (Segal-Isaacson, Wylie-Rosett & Gans, 2004), diet quality scores are calculated out of a total of 39 possible points where “usually often” = 1, “sometimes” = 2, and “rarely/often” = 3. The higher the score, the higher the diet quality. The VTC group had average scores of 31 ± 0.8 at week 0 and 32 ± 0.9 ; CON had 31 ± 0.9 at week 0 and 30 ± 1.3 at week 8. In this study, REAPS scores in both the VTC and CON groups did not significantly change over time ($p=0.133$). Supported by insignificant calculations of milligrams of vitamin C consumed by the VTC group compared to the CON group ($p=0.107$ at week 4 and $p=0.958$ at week 8) throughout the course of the study, we can conclude that changes in dietary vitamin C did not confound these results.

An interesting observation showed that baseline vitamin C status was inversely related to body weight. This finding is consistent with other literature (Johnston, Beezhold, Mostow & Swan, 2007; Canoy, Wareham, Welch et al, 2005) although the exact mechanism is unknown. More research is necessary to determine if vitamin C supplementation plays a part in weight management.

Limitations. This study has limitations. First of all, the 8-week duration is fairly short compared to other studies, especially since having a cold was not a specified inclusion criteria. Thus, a participant could have complied with instructions but simply was not exposed to a cold virus during the short time period of observation. A longer period of observation over various seasons would provide a more complete assessment. Also, although the power analysis suggested 34 participants, there were only 28 subjects in this study due to recruitment restrictions and compliance issues. Another factor to consider is that sickness and severity of symptoms is a subjective measure. What one person considers a ‘severe cough’ may not agree with another person’s perception. Also, compliance was not strictly recorded but was assessed by pill return counts. Even though pill counts indicated a 96% compliance, it may be that compliance for capsule consumption twice daily as a divided dose was not high.

Strengths. One study strength is that all participants were young males, the sample most likely to have the lowest vitamin C status compared to the population at large (Schleicher, Carrol, Ford & Lacher, 2009). Hence, on average, participants began the study with low-adequate vitamin C status and tissues were not vitamin C saturated. Results showed significance in fasting plasma vitamin C between the VTC and CON groups at weeks 1-4 ($p=0.019$) and at weeks 5-8 ($p=0.051$). Also, subjects were not regular smokers or training athletes, both of which call for an increase in vitamin C intake due to the physiological stress placed on the body (Jacob & Sotoudeh, 2002; Schleicher, Carrol, Ford & Lacher, 2009). Since diet quality and weekly ingestion of vitamin C rich foods was monitored through the REAPS survey and weekly FFQs we were able to eliminate diet as a confounding factor in the results. Restriction of fruit juices contributed to minimizing the impact of vitamin C consumption through the diet. Additionally,

medical history surveys and reported medications taken during the study were examined in order to prevent confusion between allergy symptoms and cold symptoms.

Summary and Conclusion:

In summary, this randomized controlled trial suggests that perception of sickness and reported symptom severity were reduced with ascorbic acid (vitamin C) supplementation (500 mg/twice in a day) compared to placebo. These results support the ability of vitamin C to decrease the physiological burden of the common cold.

Future research should investigate vitamin C's effect on the duration of the common cold and plasma histamine levels over a longer period of time.

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Appendix

PHONE SCRIPT

Nutrient Supplementation and Health Parameters

Phone Script

Name: 1st Contact (Date, Time):

2nd Contact (Date, Time):

3rd Contact (Date, Time):

Hello ,

Thank you for your interest in the Nutrient Supplementation and Health Parameters research study. Do you have time to answer 6 short questions to see if you qualify for the trial?

If no: When would be a better time?

If yes:

1. First of all, how old are you? years

2. Do you currently smoke cigarettes? *Yes or No*

a. *If yes:* How often?

b. *If “a” answered, ask:* Approximately how many cigarettes do you smoke [*refer to period of time that “a” was answered in*]?

c. *If “a” and/or “b” answered, ask:* Do you usually smoke with others in a social scene or alone?

3. Do you currently take vitamin and mineral supplements?

a. *If yes:* Which supplements do you take?

4. Do you currently take any prescriptions?

a. *If yes:* Which prescriptions do you take?

5. Are you currently seeing a doctor for a health condition?

a. *If yes:* What condition are you being seen for?

6. Do you have any limitations regarding physical activity and exercise?

→ *If any of the answers do not meet the requirements for the study, the individual will be excluded.*

If meets requirements:

Based on this information, I would like to invite you to be a participant in the Nutrient Supplementation and Health Parameters study. We will be contacting you shortly about the first study visit. Thank you for your time. Have a great day!

If does not meet requirements:

Unfortunately, we will not be able to accept you into the study. We appreciate your interest and time. Have a great day! Thank you, .

INFORMED CONSENT

CONSENT FORM

Nutrient Supplementation and Health Parameters

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

Prof. Akhand Pratap Singh and Dr. Himani kulshreshtha, Department of Science, Maharishi University, Lucknow , as well as Mohit Kumar Verma , Deparetment of Biotechnology, have invited your participation in a research study.

STUDY PURPOSE

The purpose of the research is to examine the effect of nutrient supplementation in young college males, aged 18-40, on immune function and psychological status.

DESCRIPTION OF RESEARCH STUDY

If you decide to participate, then as a study participant you will join a study to evaluate the effect of ingestion of a nutrient supplement twice daily for 8 weeks on health markers. You will be instructed to complete a one-page questionnaire daily regarding illness and three one-page questionnaires each week regarding physical activity and diet. If you are interested in joining the

study, you will be asked to come to an initial screening where a fasting blood sample (no food or drink for 5 hours with the exception of water) will be drawn, your body weight and height will be measured, and you will complete health history, diet, and mood questionnaires. If you are eligible for the study, you and the other participants will be randomly placed in either the control (placebo) or experimental (nutrient supplement) group. Subjects will be asked to visit the research site on 7 occasions at 0, 1, 2, 4, 7 and 8 weeks. At weeks 0, 4, and 8, a blood sample (requiring overnight fasting for 8 hours) will be drawn, you will be weighed, and you will complete mood and physical activity questionnaires. At each blood sampling approximately 10 ml of blood will be collected. At the study visits, you will need to bring in the questionnaire booklet on diet, illness, and physical activity. We will also ask you to wear a pedometer at your waist for 2 three-day periods at the start and end of the study.

If you say YES, then your participation will last for 8 weeks at Maharishi University. Approximately 40 of subjects will be participating in this study locally.

RISKS

There may be a slight chance of gastrointestinal distress when the supplement is taken on an empty stomach. This risk is reduced if you ingest the nutrient supplement with a meal. Blood draws may cause light-headedness or temporary bruising. A nurse or trained phlebotomist will be performing the blood draws.

BENEFITS

Although there may be no direct benefits to you, the possible benefits of your participation in the research is that you will be able to experience what it is like to be a part of a research study that may provide new evidence for health promotion for many college students.

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. Your name will not be associated with any data pertaining to the study. In order to maintain confidentiality of your records, Dr. Himani Kulshreshtha will assign you a subject number which will be used to identify you throughout the entire course of the study.

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 grades or any relationship with Maharishi 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. In order to help defray your costs you will receive two gift voucher at the 0 and 4 week visits and a gift voucher at week 8.

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 Prof. A.P Singh, and Dr. Himani Kulshrestha, Department of Science, MUIT, Lucknow.

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 given (offered) to you.

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

Subject's Signature Printed Name Date

Preferred contact: phone or email: _____

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 Maharishi University, Lucknow. I have provided (offered) the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

SUBJECT QUESTIONNAIRES

REAPS (Rapid Eating Assessment for Participants - Shortened Version)

CJSegal-Isaacson, EdD RD, Judy-Wyllie-Rosett, EdD RD, Kim Gans, PhD, MPH

In an average week, how often do you:	Usually/ Often	Sometimes	Rarely/ Never	Does not apply to me	
1. Skip breakfast?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
2. Eat <u>4 or more</u> meals from sit-down or take out restaurants?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
3. Eat <u>less than 2 servings</u> of whole grain products or high fiber starches a day? Serving = 1 slice of 100% whole grain bread; 1 cup whole grain cereal like Shredded Wheat, Wheaties, Grape Nuts, high fiber cereals, oatmeal, 3-4 whole grain crackers, ½ cup brown rice or whole wheat pasta, boiled or baked potatoes, yuca, yams or plantain.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
4. Eat <u>less than 2 servings</u> of fruit a day? Serving = ½ cup or 1 med. fruit or ¼ cup 100% fruit juice.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
5. Eat <u>less than 2 servings</u> of vegetables a day? Serving = ½ cup vegetables, or 1 cup leafy raw vegetables.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
6. Eat or drink <u>less than 2 servings</u> of milk, yogurt, or cheese a day? Serving = 1 cup milk or yogurt; 1½ - 2 ounces cheese.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
7. Eat <u>more than 8 ounces</u> (see sizes below) of meat, chicken, turkey or fish <u>per day</u> ? Note: 3 ounces of meat or chicken is the size of a deck of cards or ONE of the following: 1 regular hamburger, 1 chicken breast or leg (thigh and drumstick), or 1 pork chop.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	Rarely eat meat, chicken, turkey or fish <input type="radio"/>	
8. Use <u>regular processed meats</u> (like bologna, salami, corned beef, hotdogs, sausage or bacon) instead of low fat processed meats (like roast beef, turkey, lean ham; low-fat cold cuts/hotdogs)?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	Rarely eat processed meats <input type="radio"/>	
9. Eat <u>fried foods</u> such as fried chicken, fried fish, French fries, fried plantains, tostones or fried yuca?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
10. Eat <u>regular potato chips, nacho chips, corn chips, crackers, regular popcorn, nuts</u> instead of pretzels, low-fat chips or low-fat crackers, air-popped popcorn?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	Rarely eat these snack foods <input type="radio"/>	
11. <u>Add butter, margarine or oil</u> to bread, potatoes, rice or vegetables at the table?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
12. Eat <u>sweets</u> like cake, cookies, pastries, donuts, muffins, chocolate and candies more than 2 times per day.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
13. <u>Drink 16 ounces or more</u> of non-diet soda, fruit drink/punch or Kool-Aid a day? Note: 1 can of soda = 12 ounces	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		
	YES			NO	
14. You or a member of your family usually shops and cooks rather than eating sit-down or take-out restaurant food?	<input type="radio"/>			<input type="radio"/>	
15. Usually feel well enough to shop or cook.	<input type="radio"/>			<input type="radio"/>	
16. How willing are you to make changes in your eating habits in order to be healthier?	1 Very willing	2	3	4	5 Not at all willing

HEALTH /HISTORY QUESTIONNAIRE ID#_____

1. Gender: M F

2. Age: _____

3. Have you lost or gained more than 5 lbs in the last 12 months? Yes No

If yes, how much lost or gained? _____ How long ago? _____

4. College Status (please circle) Fresher. Staff, . Graduate, Post graduate.

5. Do you smoke? No, never _____

Yes _____ # Cigarettes per day = _____

I used to, but I quit _____ months/years (circle) ago

6. Do you take any medications regularly? Yes No *If yes, list type and*

frequency:

Medication Dosage

Frequency

7. Do you currently take supplements (vitamins, minerals, herbs, etc.) ? Yes No

If yes, list type and frequency:

Supplement Dosage

Frequency

8. Have you ever been hospitalized? _____

If yes, for what? _____

9. Please ANSWER (YES/NO) if **you currently have** or if **you have ever** been diagnosed with any of the following diseases or symptoms:

	YES	NO		YES	NO
Coronary Heart Disease			High Blood Pressure		
Chest Pain			Shortness of Breath		
Heart Murmur			Heart Palpitations		
Rheumatic Fever			Any Heart Problems		
Irregular Heart Beat			Coughing of Blood		
Varicose Veins			Feeling Faint or Dizzy		
Stroke			Lung Disease		
Diabetes			Liver Disease		
Low Blood Sugar			Kidney Disease		
Bronchial Asthma			Thyroid Disease		
Hay Fever			Anemia		
Leg or Ankle Swelling			Hormone Imbalances		
Eating Disorders			Emotional Problems		

Please elaborate on any condition listed

above. _____

10. How would you rate your lifestyle?

Not active _____ Active _____

Somewhat active _____ Very Active _____

11. Please circle the total time you spend in each category for an average week.

Light activities such as:

Slow walking, slow cycling, easy swimming, gardening

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

Moderate activities such as:

Mod. Walking, mod. cycling, singles tennis, mod. swimming,
mod. weight lifting

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

Vigorous activities such as:

Fast walking/jogging, fast cycling, court sports, cricket, fast swimming,
heavy/intense
weight lifting

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

12. How much alcohol do you drink? (average drinks per day) _____

13. Do you have any food allergies? Yes No If yes,

explain: _____

14. Do you follow a special diet? (weight gain/loss, vegetarian, low-fat, etc.) Yes/No

If yes, explain:

Please indicate how many times you consumed the following foods THIS WEEK:

- **Citrus fruits and/or juices** (oranges, grapefruit, lemons, etc) ☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

- **Apples, pears and/or plums** ☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

- **Strawberries and/or other berries** ☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

- **Bananas** ☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

- **Melons** (cantaloupe, honeydew, watermelon, etc) ☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

- **Papaya, mangos, kiwi** (including juices) ☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

- **Cruciferous vegetables** (broccoli, kale, Brussels sprouts, cauliflower, cabbage, asparagus)

☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

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

☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

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

☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

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

☐ *small* ☐ *medium* ☐ *large*

☐ 0 ☐ 1-3x/wk ☐ 4-6x/wk ☐ 7-9x/wk ☐ 10-12x/wk ☐ 13-15x/wk ☐ 15 or more

Wisconsin Upper Respiratory Symptom Survey – 21 --- Daily Symptom Report

Day:	Date:	Time:	ID:
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Please fill in one circle for each of the following items:

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

Compared to yesterday, I feel that my cold is ...

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

Name	Dosage	Time(s) taken

METHODOLOGYTIMELINE

Methodology Timeline

Initial Contact

- Age
- Phone Questionnaire
 - Smoking habits, supplement/prscription medical history, physical activity limitations

Screening *Study Visit 1*

- Informed Consent
- Weight/Height
- Tanita (BMI)
- Fasting Blood Sample (5-hour fast)
- Medical History Questionnaire
- REAPS-Diet Quality Questionnaire
- 24-hr recall

----- Randomization -----

Baseline *Study Visit 2*

- POMS
- Survey booklets and capsule distribution (Baseline – Week 4 materials)
- Godin Leisure-Time Exercise Survey

Week 1 *Study Visit 3*

- Survey booklet compliance check

Week 4 *Study Visit 4*

- Fasting Blood Sample (8-hour fast)
- Weight
- POMS
- Survey booklets and capsule distribution (Week 5 – Week 8 materials)

Week 8 *Study Visit 5*

- Fasting Blood Sample (8-hour fast)
- Weight
- POMS
- REAPS-Diet Quality Questionnaire