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Antioxidant and Anti-inflammatory Nutrients on Lung Function in Chronic Obstructive Pulmonary Disease

Jessica M. Hinds
University of Nebraska Medical Center

Antioxidant and Anti-inflammatory Nutrients on Lung Function in Chronic Obstructive Pulmonary Disease

By

Jessica Hinds, RD, LMNT

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Dr. Corrine Hanson, Dr. Sarah Keim, and Elizabeth Lyden

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ABSTRACT

Introduction Veterans are three times more likely to develop chronic obstructive pulmonary disease (COPD) than the general population. This is likely due to increased use of tobacco and exposure to environmental risk factors. Nutritional intake has been linked to COPD, and veterans report unique barriers to improving their eating habits.

Purpose The primary outcome of this study was to examine the relationship between antioxidant and anti-inflammatory nutrient intake and lung function in veterans with COPD. It was hypothesized that a higher intake of lutein + zeaxanthin, ß-cryptoxanthin, lycopene, retinol, α-carotene, ß-carotene, vitamin C, vitamin D, α-tocopherol, omega-3, zinc and selenium would have a positive association on lung function in veterans with this disease. It was further hypothesized that intakes of ɣ-tocopherol and omega-6 would have an inverse association with lung function.

Methods A total of 42 veterans participated in this prospective cohort study. All participants filled out a food frequency questionnaire (FFQ) and lung function data were also gathered. A micronutrient adequacy score was calculated from the results of the FFQ based on 2/3 recommended dietary allowance (RDA). Veteran's smoking status and use of nutritional supplements was also included in the data analysis. Significant findings were adjusted for possible confounders of age and smoking.

Results Participants had a very low intake of omega-3 as only 21% of veterans met the AI (1.6g for men) with a mean consumption of 0.3g. Participants also consumed less of the anti-inflammatory nutrient α-tocopherol (mean intake of 8mg) compared to the pro-inflammatory nutrient ɣ-tocopherol (mean intake of 11mg). Thirty participants (71%) had a micronutrient adequacy score of 4 out of 7. However, when the nutrient consumption was evaluated separately, a low percentage of participants met the recommended intake for vitamin D (19%), α-tocopherol

(5%), omega-3 (21% of males, 0% of females), zinc (25% of males, 0% of females) and selenium (0%). Veterans had the highest consumption of retinol activity equivalents (RAEs) and vitamin C. After adjusting for confounders, no significant relationship was found between nutrient intake and lung function.

Conclusion From the results of this study, it cannot be concluded that there is an association between the intakes of antioxidant and anti-inflammatory nutrients with lung function. However, there are conflicting results in the literature and more research is needed in this area.

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KEYWORDS

Chronic Obstructive Pulmonary Disease	COPD
Food Frequency Questionnaire	FFQ
Recommended Dietary Allowance	RDA
Retinol Activity Equivalents	RAEs
Forced Expiratory Volume in One Second	FEV_1
Forced Volume Capacity	FVC
Dietary Recommended Intake	DRI
Estimated Average Requirement	EAR
Adequate Intake	AI
Upper Limit	UL

INTRODUCTION

Veterans are three times more likely to develop COPD than the general population. Higher tobacco usage is found in military personnel compared to civilians, and 75% of COPD cases are tied to smoking. Not only are veterans more likely to smoke, but they are also more exposed to environmental factors including dust from sand, smoke, or aerosolized metals and chemicals[1].

Nutrition and diet can affect the development of several chronic diseases. In most recent years, lung diseases have become more of a concern including COPD[2]. COPD is a major cause of morbidity and mortality worldwide and is the third leading cause of death in the United States. The prevalence of COPD is 3-4% in the United States and 10% worldwide[3, 4]. The increase in prevalence has caused a rising concern within our healthcare system.

The increased concern of lung diseases has made maintaining or improving lung function a priority. Many lung diseases, including COPD, involve oxidative stress and inflammation. Therefore researching nutrients with antioxidant and anti-inflammatory properties may be useful. These nutrients can be observed for prevention or treatments of lung diseases including COPD[2]. Research looking at diet and COPD in veterans is unfortunately lacking. This is an important area as research studies have shown there is an association between antioxidant and anti-inflammatory nutrients with lung function in the general population. Studies have also shown that veterans utilize health care more than civilians[5]. They also have documented barriers, such as post-traumatic stress disorder, that impair their ability to improve their eating habits[6, 7].

The purpose of this study is to determine if there is an association between intakes of antioxidant and anti-inflammatory nutrients with lung function in veterans with COPD. A micronutrient adequacy score was also created and analyzed to help identify the effects of diets higher in antioxidants and anti-inflammatory nutrients. The effects of smoking status and

supplementation of these nutrients were analyzed. It was hypothesized that a higher intake of lutein + zeaxanthin, ß-cryptoxanthin, lycopene, retinol, α-carotene, ß-carotene, vitamin C, vitamin D, α-tocopherol, omega-3, zinc and selenium would have a positive association on lung function in subjects with COPD. It was further hypothesized that intakes of ɣ-tocopherol and omega-6 would have an inverse association with lung function.

REVIEW OF THE LITERATURE

COPD Overview

COPD is characterized by slow, progressive obstruction of the airways. COPD consists of two categories which are emphysema and chronic bronchitis. Emphysema is characterized by abnormal, permanent enlargement, and destruction of the fragile walls and elastic fibers of the alveoli. During exhalation, small airways collapse which impairs airflow out of the lungs[8, 9]. Chronic bronchitis usually involves a productive cough with inflammation of the bronchi and other lung changes. The lungs produce more mucus which further blocks the narrowed tubes[10].

Oxidative Stress and Inflammatory Mechanisms of COPD

Oxidative stress is the production of free radicals and the body's inability to counteract the harmful impact. There is an oxidant-antioxidant imbalance in favor of oxidants, which can cause damage by free radicals to lung tissue. Glutathione, a vital intra- and extracellular protective antioxidant, works against oxidative stress, which plays a key role in the control of pro-inflammatory processes in the lungs[11]. In a study by Ochs-Balcom *et al.* glutathione was observed as an oxidative stress biomarker. Data was gathered in western New York State including 2,346 participants from 1995 to 2000. A higher concentration of glutathione was found to increase an individual's forced expiratory volume in one second (FEV_1) (mean=93.4 FEV_1); 95% CI 91.6-95.3 p-trend <0.04)[12].

The chronic inflammatory process in COPD is characterized by infiltration of the airways by neutrophils, macrophages, and CD8+ T cells. Such features of inflammation in COPD are likely driven by various cellular pathways, including pro-inflammatory cytokines and mediators of oxidative stress released locally or systemically. More than airway inflammation, a systemic inflammation has also been observed in COPD, with the detection of increased levels of

cytokines and inflammatory mediators, particularly from the endothelium, that can cause lung and airway injuries[4, 13].

The early decline in lung function in COPD is correlated with inflammatory changes in the peripheral airways. Inflammation leads to consistent and repetitive injuries to the peripheral airways caused by cigarette smoking which affects lung repair mechanisms[14]. Inflammatory changes such as airway edema and mucus hypersecretion also contribute to airway narrowing in COPD. Inflammation of the submucosal glands and hyperplasia of goblet cells may contribute to symptoms, such as chronic sputum production. Fibrosis of airways plays the largest role in inflammation of the air passages. Bronchiolar abnormalities contribute more to mild-moderate chronic airflow limitation[15].

COPD Diagnosis and GOLD Criterion

COPD requires close management as it progresses through its four stages. The GOLD classification is the main method physicians use to describe the severity of COPD. GOLD stands for Global Initiative for Chronic Obstructive Lung Disease. This staging system was created between the National Institutes of Health and the World Health Organization. FEV_1/forced volume capacity (FVC) and FEV_1 are used in conjunction to diagnose COPD and its severity[16, 17]. Individuals with COPD take longer to exhale air from their lungs which can be diagnosed by a FEV_1/FVC of <70%. The GOLD criterion is used to describe the severity of the airflow. As the severity of COPD progresses FEV_1 declines. A summarization of the GOLD criteria can be seen in Table 1.

Table 1: COPD Severity by GOLD Criterion

COPD Stage	FEV_1% predicted	Post bronchodilator FEV_1/FVC
I. Mild	$FEV_1 \geq 80\%$	<0.7
II. Moderate	$50\% \leq FEV_1 < 80\%$	<0.7
III. Severe	$30\% \leq FEV_1 \leq 50\%$	<0.7
IV. Very Severe	<30% FEV_1	<0.7

There are a few factors that increase a patient's risk of COPD. Tobacco smoking or continual contact with second-hand smoke is the primary causative factor[14]. Smoking decreases glutathione and also causes a fall in the plasma antioxidant capacity, a global measure of systemic oxidative stress[11]. Environmental air pollution and genetic susceptibility are other possible causes[14]. Age is also a factor and COPD develops slowly over the years and most individuals are at least 35 to 40 years old when symptoms begin[10]. There are several symptoms that someone with COPD can experience which makes it difficult to diagnose. Some of the most common are cough, dyspnea, and fatigue[18].

COPD is commonly misdiagnosed as asthma or shortness of breath. However, there are tests that can be completed in order to make an accurate diagnosis. Some of the most frequently used are pulmonary function tests, but chest x-rays and CT scans can also be used. An arterial blood gas analysis can measure how well lungs bring oxygen into the blood and remove carbon dioxide[8]. Pulse oximetry can be used to measure oxygen saturation of the arterial blood. The pulse oximeter uses light waves to measure oxygen saturation which normally is 95 to 99 percent[11].

Lung Function Tests

Various tests are used to diagnose or monitor the status of lung disease. Most are designed to measure the ability of the respiratory system to exchange oxygen and carbon dioxide. The major function of the pulmonary system is gas exchange. Spirometry is what is used to measure lung function. A participant breathes into a spirometer which gives information on lung volume and the rate at which air can be inhaled and exhaled[19]. FVC is the volume delivered during an expiration made as forcefully and completely as possible, starting at full inspiration. FEV_1 is the volume delivered in the first second of the FVC maneuver[17].

Interventions

There are many ways to improve symptoms and keep the disease from progressing. However, even though there are interventions there is still no cure. For earlier stages of COPD, inhalers and steroids can help. Inhalers, also known as bronchodilators, help open the airways while steroids help reduce lung inflammation. Anti-inflammatory drugs can also be used to reduce swelling in the airways.

COPD exacerbations likely need a trip to the emergency room or a hospital stay. In the hospital, patients still receive steroids, bronchodilators through a nebulizer, oxygen, or assistance breathing from a machine. Some of these machines include bi-level positive airway pressure (BiPAP) or an endotracheal tube. Surgery might also be an option for some people with severe emphysema. This includes lung volume reduction surgery, in which small damaged lung tissue is removed, or some require a lung transplant[20].

Nutritional Intake of Veterans

Eber *et al.* conducted a study to compare the health status between deployed and non-deployed veterans in the same time frame. This study involved 20, 563 veterans who were

surveyed periodically over a ten year period. Veterans filled out a survey including questions regarding health status, deployment related exposures, and other relative questions. Medical records were compiled to validate the questions from the survey such as reason for a doctor visit or overnight hospital stay within the past year. Of the conditions mentioned in the survey, 89.3% of veterans had a confirmed medical condition that needed attention in the past year. It was discovered that veterans were 29% more likely to develop a respiratory disease, such as asthma, bronchitis, or sinusitis compared to non-deployed veterans. Deployed veterans also tend to utilize health care more often than civilians likely due to their higher susceptibility of chronic lung diseases, like COPD, and other medical conditions due to the increased exposure to environmental risk factors and increased rate of tobacco consumption.

Nosova *et al* reviewed the nutrient intake of 88 veterans with peripheral arterial disease. A validated questionnaire was used to estimate the subject's average intake of nutrients from a variety of foods. The values collected were then compared to guidelines set for the general population from the American Heart Association. It was found that 59% of veterans consumed the daily recommended amount of omega-3 for people diagnosed with cardiovascular disease. Of these participants, 28% reported taking an omega-3 supplement daily. Veterans were also found to have a higher intake of cholesterol, sodium, and total fat. Unfortunately, they were found to have an inadequate intake in magnesium, calcium, and soluble fiber. It was concluded that veterans had a poor nutritional intake when compared to recommended guidelines for the general American population[6].

Another study looked at dietary intake in veterans with post-traumatic stress disorder (PTSD) compared to those without a mental health disorder. Many veterans in the overall sample reported poor dietary habits, including making poor food choices and eating too much. Habits such as overeating, eating rapidly, and hoarding high-calorie foods were reported. Food preoccupation can persist after veterans return home from experiencing food deprivation or

insecurity during deployment. Interestingly, over 50% of veterans with PTSD and over 21% of veterans with no mental health disorder report that stress or depression is their biggest barrier in improving their eating habits[7].

COPD and Nutrient Needs

Malnutrition affects the structure, elasticity, and function of the lungs. Malnourished patients with COPD have a poorer prognosis than nourished patients. Protein and iron deficiencies result in low hemoglobin levels which diminish the oxygen-carrying capacity of the blood[21]. Hypoproteinemia contributes to the development of pulmonary edema, allowing body fluids to move into interstitial space. Low levels of calcium, magnesium, phosphorus, and potassium compromise respiratory muscle function at the cellular level[22]. Normal airway mucus is a substance consisting of water, glycoproteins, and electrolytes, and thus requires adequate nutritional intake. Energy requirements are increased since weight loss is common in those with COPD. Weight loss is also associated with a poor prognosis in people with pulmonary disease. This disease also affects the absorption and metabolism of most nutrients[19, 23].

Energy expenditure is elevated because of airflow obstruction, thus increasing the energy needs from the increased work of breathing. Many different aspects of COPD play a role in energy expenditure including gas diffusing capacity, CO_2 retention, respiratory inflammation, and biochemical mediators, such as hormones and cytokines[4]. Indirect calorimetry is the "gold standard" to determine the correct amount of energy needed for an individual. Repletion but not overfeeding is particularly critical in patients with compromised ability to exchange gases as excess feeding of calories result in CO_2 that must be expelled. Fluid, protein, fat and carbohydrate needs are determined by the severity of the lung disease, oxygen therapy, medications, weight status and fluid fluctuations in COPD patients[4]. Adequate nutrition is

necessary to maintain or restore lung and muscle strength, as well as to promote immune function[19].

Nutrients and Lung Function

The increased concern of mortality from lung diseases has made maintaining or improving lung function a priority. Many lung diseases involve oxidative stress and inflammation. Therefore, nutrients with antioxidant and anti-inflammatory properties may be useful. These nutrients can be observed for prevention or treatment of lung diseases including COPD[2]. It can be beneficial to review nutrient intake and serum levels as they provide pertinent nutritional information.

Patients with COPD have been shown to have an increase in the level of systemic inflammatory markers, suggesting that inflammation is important in exacerbations of COPD. A potential way of reducing lung function decline is looking into reducing the level of circulatory inflammatory markers[4]. In Tsiligianni and Molen's systematic review, a low intake of vitamins C, D, E, A, α-carotene, and ß-carotene, measured by FFQs and serum levels was reported to reduce antioxidant defenses and increase the possibility of airway inflammation[24].

Some studies have focused on antioxidants and the presence of lung function and respiratory symptoms. Grievink *et al* analyzed a sample of 6,555 adults from 1994 to 1995 who were given a questionnaire regarding their food consumption and respiratory symptoms. Vitamin C intake was inversely related to cough (OR 0.66; 95% CI 0.50-0.87). It was also found that vitamin E intake had no relationship with most symptoms and lung function, but had a positive association with productive cough (OR 1.26; 95% CI 1.02-1.56). ß-carotene was not associated with most respiratory symptoms but was positively associated with wheeze (OR 1.27; 95% CI 1.04-1.55)[9].

The National Health and Nutrition Examination Surveys (NHANES) have demonstrated the positive associations that antioxidant nutrients and anti-inflammatory nutrients have on lung function. NHANES III was conducted throughout the United States with a sample size of 18,162 adults over the age of seventeen. Participants were asked to fill out a 24 hour dietary recall and their history of smoking or non-smoking. When nutrients were looked at separately and adjusted for caloric intake, vitamin C (9.5 ml FEV_1%; 95% CI -0.2 to 19.2), vitamin E (16.4ml FEV_1%; 95% CI 5.5 to 27.4), and total carotene (18.2ml FEV_1%; 95% CI 8.7 to 27.6) all were positively associated with FEV_1% predicted. The association of each nutrient with FEV_1 was found to vary somewhat based on smoking status, but these differences were generally not statistically significant. However, for total carotene, the association of dietary intake and FEV_1 differed by smoking status ($p=0.07$)[25].

Serum 25-hydroxy vitamin D levels have also showed a positive influence on lung function. Data was collected from NHANES using a cross-sectional survey involving 14,091 participants who were at least 20 years of age. All participants who were interviewed, had spirometry tests and serum 25(OH) D levels completed. After adjusting for confounders, the mean FEV_1 and FVC was 126ml and 172ml respectively greater for the highest quintile of serum 25(OH) vitamin D levels (85.7nmol/L) compared to the lowest quintile (<40.4nmol/L; $p<0.0001$)[26]. Shaheen *et al.* found that total vitamin D intake was positively associated with FEV_1 ($p=0.007$) and FEV_1/FVC ($p=0.02$) was associated negatively with COPD (OR 0.57; 95% CI, 0.38 – 0.87; $p=0.02$)[27].

It was also discovered in a cross-sectional study of 414 ex-smokers who had COPD that 25(OH) D levels were positively associated with FEV_1 ($p<0.0001$). A greater effect of pack-years of smoking on FEV_1 decline was significantly different in those with vitamin D deficiency ($p=0.01$)[28]. In a study involving 626 men from the Normative Aging Study, Lange *et al* reported that the median vitamin D intake was roughly half of the recommended amount according to

other research studies[29]. This is a concern putting COPD patients at high risk for vitamin D deficiency. Evidence suggests that a significant proportion of COPD patients are vitamin D deficient and careful monitoring of this nutrient in this population is recommended[30].

More studies are needed to determine if supplementation of anti-inflammatory nutrients would be beneficial. Black *et al.* noted a strong relationship between serum concentrations of 25(OH) D, FEV_1, and FVC ($p<0.0001$) in an analysis using NHANES III data[26]. Vitamin D is thought to protect the body against viral and bacterial infections that can trigger COPD attacks. In a meta-analysis of nutritional supplementation, participants with <10ng/ml of 25 (OH) D reduced the risk of COPD exacerbations following vitamin D supplementation[25]. Other studies seem to doubt that vitamin D and lung function are correlated. In the Lung Health Study, there were 196 COPD participants which found no relationship between vitamin D levels and rate of change in FEV_1 ($p=0.15$)[32]. Also, an analysis of 973 participants with COPD in a 1-year study showed no relation between vitamin D status at baseline and risk of acute exacerbations of COPD ($p=0.82$)[33].

Polyunsaturated fatty acids, specifically omega-3 and omega-6, have been found to play a role in lung function. In the Honolulu Heart Study, men of Japanese descent who smoked 30 or less cigarettes per day and ate fish twice or more a week had a 50ml (95% CI 17-87ml) increase in their FEV_1. In a cross-sectional analysis using data from The First National Health and Nutrition Examination Survey reported that high fish consumption was associated with an ~80ml (95% CI: 62, 227) increase in FEV_1 in smokers and non-smokers[34]. J. de Battle *et al.* discovered that a-linolenic acid (omega-3) intake was associated with a significantly lower TNFa serum concentration (adjusted OR= 0.46 p=0.049) whereas higher arachidonic acid (omega-6) intake was associated with a higher serum concentration of IL-6 (OR=1.96 p=0.034)[3]. The MORGEN-EPIC study conducted in the Netherlands involving 13, 820 participants, ages 20 to 59 years, discovered something contradicting de Battle's findings. Increased intake of docosahexaenoic

acid (DHA) was significantly associated with a lower FEV_1. The highest quintile of intake was associated with a 39.3ml (95% CI -64.8 to -13.8) lower FEV_1 compared with the lowest quintile intake. Higher levels of individual intake of omega-6 fatty acids (i.e. linoleic acid, arachidonic acid) were associated with lower FEV_1[21]. Omega-6 fatty acids and pro-inflammatory mediators are rather complex and not easily predictable. Some studies suggest that omega-6 fatty acids pro-inflammatory activities occur only when the intake of omega-3 fatty acids are low. This may be due to omega-3 fatty acids potentially inhibiting arachidonic acid metabolism as α-linolenic acid converts into eicosapentaenoic acid (EPA) or DHA[4].

Studies involving vitamin E seem to be inconsistent. However, the different isoforms of this vitamin may explain these conflicting results. Vitamin E is made up of different isoforms including δ, ß, α, and ɣ-tocopherol. α-tocopherol and ɣ-tocopherol are the most abundantly found in the American diet. There is a 10-fold higher tissue concentration of α-tocopherol when compared to ɣ-tocopherol. Clinical studies indicate that higher intakes of α-tocopherol may show a protective effect on adult-asthma and beneficial effect on FEV_1 in studies in Finland and Italy but not the United States or the Netherlands[35]. Differences in outcomes from clinical reports on the association between vitamin E and asthma may be due to the different intakes and serum levels of α-tocopherol and ɣ-tocopherol due to their opposing effects. In the U.S., diets are rich in soy oil which is high in ɣ-tocopherol compared to European diets which are rich in olive oil and low in ɣ-tocopherol, which has led some researchers to speculate this may be a partial explanation of the high rates of asthma in the United States when compared to other countries[35].

The Coronary Artery Risk Development in Young Adults (CARDIA) study analyzed spirometry and serum tocopherol data for over 20 years between white and black adults. In cross-sectional regression analysis at year 0, higher serum ɣ-tocopherol was associated with a lower FEV_1 ($p=0.03$ in blacks and $p=0.01$ in all participants) and FVC ($p=0.01$ in blacks, $p=0.05$ in whites and $p=0.01$ in all participants). Whereas higher serum α-tocopherol was associated with

higher FVC (p=0.04 in blacks and whites and p=0.01 in all participants). In the lowest quartile of α-tocopherol serum levels, higher serum ɣ-tocopherol was associated with a lower FEV_1 (p=0.05 in blacks and p=0.02 in all participants). In contrast, in the lowest quartile of ɣ-tocopherol serum levels, higher serum α-tocopherol was associated with a higher FEV_1 (p=0.03 in blacks). Serum ɣ-tocopherol >10uM was associated with a 175-545 ml lower FEV_1 and FVC at ages 21 to 55 years. In the tocopherol isoform quartile analysis, the association of tocopherols with FEV_1 were most evident when the concentration of the opposing tocopherol was low, causing the least competing opposing effect[13].

Similar results were seen in a cohort study involving a random sample of 580 men from the Veterans Administration Normative Aging Study. Men between the ages of 21 and 80 years old participated in the study. This study looked at serum levels of α, ɣ, and δ tocopherol, intake of vitamin E and effect of vitamin E supplements, as well as measurements of lung function (i.e. FEV_1, FVC, and FEV_1/FVC). An inverse association was seen between serum ɣ-tocopherol and FEV_1 (r= -0.12, p=0.003) and FVC (r= -0.10, p=0.01). Serum levels of α- and δ-tocopherol were not associated with any lung parameters. After adjusting for confounders, serum ɣ-tocopherol maintained a significant inverse association with FVC (B= -0.10, p=0.05)[2]. This study also analyzed the opposing tocopherol actions on lung function. For this analysis, subjects were divided into above or below serum median levels of both α-tocopherol and ɣ-tocopherol. When looking at serum ɣ-tocopherol levels above and below the median, no significant associations were found between α-tocopherol and lung function. However, when analyzing serum α-tocopherol levels below or above the median, ɣ-tocopherol had a significant inverse relationship with lung function measures[2]. The nutrient intake and supplementation of vitamin E was also reviewed in this cohort study. After adjustment for confounders, vitamin E intake still had a positive association with both FEV_1 (ß- 0.03, p=0.02) and FVC (ß=0.03, p=0.01). Vitamin E supplementation was associated with an increase in FEV_1 (ß= 0.05, p=0.03) and FVC (ß= 0.06, p=0.02) after confounders were adjusted. Supplementation increased α-tocopherol levels

($p<0.0001$) and decreased ɣ-tocopherol levels ($p<0.0001$). When looking at vitamin E intake without supplementation, the relationship between vitamin E intake and lung function was no longer significant[2].

A summary of nutrients and their antioxidant or anti-inflammatory functions are shown in Table 2.

Table 2: Summary of Nutrients and their Antioxidant and Anti-inflammatory Function

Nutrients	Antioxidant/Anti-inflammatory Functions
Lutein + Zeaxanthin/Lycopene	Reduce free radicals[8, 25]
α-carotene ß-carotene	Scavenge oxygen and reduce lipid peroxidation[45]
Retinol	Shown to protect against respiratory illnesses, such as lung cancer.[30, 46]
ß-cryptoxanthin	Prevents free radical damage to cells and repair damage done.[46]
Vitamin C	Maintains antioxidant capacity in the aqueous phase of the cell.[46] Functions as cofactor for enzymes and neutralizes free radicals.[46]
Vitamin E	Primary defense of cellular membranes against oxidative damage.[35]
α-tocopherol	Major scavenger of free radicals in the lipid phase, such as in the cell membrane. Blocks airway hyperactivity.[13,35]
ɣ-tocopherol	Pro-inflammatory properties. Increases hyperactivity in the lungs.[35]
Vitamin D	Regulates expression of genes in bronchial smooth muscle cells. Deficiency may increase inflammation and cause changes to lung function and structure.[30]
Omega-3	Lowers inflammatory markers (i.e. CRP, IL-6, and TNFa).[3]
Omega-6	Initiates inflammatory responses in cells.[3]
Zinc	Catalytic agent in enzymatic reactions.[25]
Selenium	Cofactor for glutathione peroxidase (Important antioxidant enzyme).[25]

Dietary Reference Intakes

Dietary reference intakes (DRIs) are a common set of reference values based on indicators of nutritional adequacy as well as prevention of chronic disease in healthy populations. DRIs are used widely in the United States and include four nutrient-based reference values: estimated average requirement (EAR), RDA, adequate intake (AI), and upper limit (UL). These references are used for vitamins, minerals, macronutrients, and energy and were created by the Food and Nutrition Board of the Institute of Medicine.

The study which included veterans with COPD focused on the use of the RDA and AI for antioxidants and anti-inflammatory nutrients. By definition the RDA is "the average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all healthy individuals in a particular life stage and gender group." When an RDA cannot be established due to lack of scientific evidence an AI is developed. It is developed more on judgement and is expected to meet or exceed the needs of most healthy individuals and is assumed to be adequate. The use of an AI usually indicates that more research is needed to determine the requirements for a specific nutrient.

The DRIs are not suggested to be used in people with chronic diseases. Meeting the recommended intakes for the nutrients would not necessarily provide enough for individuals who are already malnourished, nor would they be adequate for certain disease states marked by increase nutrient requirements. The DRIs are intended to help practitioners arrive at a reasonable estimate of the adequate nutrient level required to prevent adverse effects of excess intake. The DRIs, RDA, and AI, were used in this study based on their usage in previous literature[23].

Dietary Patterns

Looking at overall dietary patterns is becoming more popular in recent research[1]. Dietary patterns focus on a broader representation of dietary intake. This may be more beneficial than

looking at specific nutrients, as dietary patterns may give a better overview of the complicated interactions among nutrients. Dietary patterns can help classify participants as low or high antioxidant consumers and help recommend a specific-type diet[35, 36, 37].

There are few studies that use the micronutrient adequacy score to look at the quality of a diet. For example, one study looked at a diet score based on the amount of antioxidants in the diet of post-menopausal women over the age of 45. All women had to have a diagnosis of osteoporosis to be eligible. All participants conducted a 3-day food diary. The antioxidant nutrients observed were designated a score of zero or one based on their intake above or below 2/3rd EAR. The EAR was used in this study because it is the cut-off recognized by the Institute of Medicine. Majority of participants (90%) were found to have an intake below 2/3rd EAR in vitamin E. However, almost all women had an intake above 2/3rd EAR for zinc and selenium. It was found using ANOVA that there was a significant association between vitamin A and bone mass density of the lumbar spine when participant's vitamin A intake was lower ($p=0.013$)[37].

A micronutrient adequacy score was assigned to participant's nutritional intake using a Mediterranean type diet. Tur, J. *et al.* used cross-sectional data from 24 hour dietary recalls of a sample of 1,200 participants between 16 and 65 years old from the Balearic Islands from 1999 to 2000. The dietary questionnaires used were two 24 hour dietary recalls. The nutrients selenium, zinc, ß-carotene, vitamin C, and vitamin E were assigned a score of either zero or one based on the criteria below or above 2/3rd recommended dietary intake. Zinc, ß-carotene, and vitamin E were assigned a score of zero. Selenium and vitamin C intake were >2/3rd RDA and were assigned a score of one. It was also discovered that smoking (OR 1.00; p-value 0.01), a sedentary lifestyle (OR 1.79; p-value 0.0001), and having a higher BMI (OR 1.00; p-value 0.0001) increased the risk of having a lower antioxidant intake[36].

Another similar study evaluated diet quality using the RDIs. Aranceta *et al.* studied 10,208 participants aged 25 to 60 years old from 1990 to 1998. Repeated 24 hour dietary recalls

were used to assign a diet quality score (DQS) to folate and vitamins A, C, and E. The score was based on the nutrient's RDI. More than 35% of the sample was classified as having either a poor or very poor DQS. The poor and very poor DQS were among 47.9% of low educated participants, 49.6% of low socio-economic participants, 51.9% of those living in rural areas, and 49.9% of older adults. The rate for smokers (49.9%) and obese (88.5%) participants was higher than that for non-smokers (41.1%) and non-obese (11.5%) participants. Overall, dietary patterns in people with a good diet score had higher consumption of fruits, vegetables, fish, dairy products, eggs, and nuts[38].

A study evaluating the Alternate Healthy Eating Index 2010 investigated the association between diet quality and risk of COPD among US men and women. This Alternative Healthy Eating Index includes The Nurses' Health Study and The Health Professionals Follow-Up Study. The Nurses' Health study included female nurses ages 30 to 55 which began in 1976 and involved 73, 228 participants. The Health Professionals Follow-Up Study began in 1986 involving 47, 026 men ages 40 to 75 years old. All participants were mailed a health questionnaire. This questionnaire was sent every two years regarding information on risk factors such as smoking habits, weight, and physical activity. Participants in both studies filled out a FFQ at baseline and every two to four years thereafter. Scoring criteria was based on eleven components. These included vegetables, fruits, whole grains, nuts and legumes, omega-3 fatty acids, polyunsaturated fatty acids, alcohol, sugar sweetened drinks and fruit juice, red and processed meat, trans fat, and sodium. Each component was given a minimal score of 0 and a maximal score of 10 based on nutrient intake. The scores were then summed to obtain a total score ranging from 0 to 110 with the higher score representing a healthier diet. After control for potential confounders, the risk of newly diagnosed COPD was lower in participants who ate the healthiest diet compared to those who ate the least healthy diet HR 0.67 (0.53 to 0.8; p=0.63). This inverse association was seen in both ex-smokers HR 0.50 (0.33 to 0.75; p=0.72) and current

smokers HR 0.69 (0.49 to 0.98; p= 0.86). Among both men and women, those with the highest score and therefore healthiest diet, were more physically active with 28.4 $\pm$ 35.7 metabolic equivalents per week, less often obese with a mean BMI 24.3kg/m^2 $\pm$ 5.0, and less likely to be current smokers (4.4% of participants). The high scores of whole grains and fruits were associated with a lower risk of COPD 30% (significant) and 19% (borderline significant) respectively. A low dietary intake of sugar sweetened drinks and fruit juices was associated with a 21% lower risk of developing COPD (p for trend 0.02). A 53% lower risk of diagnosed COPD was found with a low intake of red and processed meat (p for trend 0.03). Vegetables, nuts, trans-saturated fat, polyunsaturated fatty acids, long chain fats, sodium, and alcohol were found to have no significant association with COPD risk[39].

Nutrient Supplementation in COPD

Vitamin supplementation and COPD outcomes were gathered in a systemic review. Vitamins C, E, A, and omega-3 supplementation showed conflicting results. There is no clear evidence of the benefit of supplementation of these antioxidant and anti-inflammatory nutrients. Most studies regarding supplementation of a multivitamin showed no benefit in symptoms, spirometry function, or hospitalization for COPD[24].

Agacdiken *et al* researched vitamin E (200IU/day) and vitamin C supplementation (500mg/day) in 21 COPD participants and 10 controls for one month. Supplementation was compared to lung function using a treadmill exercise test. The exercise time of these participants increased significantly in those who were supplemented with vitamins E and C (6.4+1.8 vs 8.8+2.1minutes p=0.01)[37].

Contrary to these findings, 35 COPD participants were randomly given either a placebo, 400mg/day of vitamin E, 200mg/day of vitamin E, or 250mg/day of vitamin C for 12 weeks. There was no improvement of lung function in the supplemented participants (p>0.05)[38]. Daga *et al* studied 30 COPD participants who were supplemented with 400IU of vitamin E daily for 12

weeks. Between day one and week 12 there was no significance found in FEV_1 ($p=0.801$) or FEV_1/FVC ($p=0.845$)[39].

Vitamin A supplementation has also been researched in lung function. Vitamin A supplementation was provided for 30 days to participants who had and did not have COPD including those who smoked and did not smoke. The mean FEV_1 increased by 22.9% in the COPD vitamin A supplemented group ($p=0.004$)[40].

Research on fish oil supplementation and lung function is limited. Shahar *et al* noted that COPD was strongly and inversely related to the intake of omega-3 through fish consumption in a dose-response fashion ($p<0.001$). This means that a higher intake of omega-3 decreases the risk of COPD[44]. Schwartz *et al* mentioned that supplementation of omega-3 fatty acids much higher than average dietary intake may have health benefits[34].

A small study used fish oil supplementation to see its effects on neutrophils and monocytes. A group of seven men ages 22 to 53 years were provided supplementation. The supplement provided 3.2g of EPA, and 2.2g of DHA. Blood was withdrawn before supplementation, after three weeks, and after six weeks of supplementation. It was concluded that dietary supplementation of fish oil for six weeks resulted in the impairment of leukotriene B4. This may stimulate mucus secretions in the airways of the lungs[42]. This demonstrates that fish oil supplementation perhaps decreases the mucus in the lungs allowing COPD patients to be able to breathe better.

PARTICIPANTS AND METHODS

Participants and Study Design

This study was a secondary analysis of patients enrolled in the AgCOPD study, a larger study of COPD patients and controls, agricultural exposure, and genotype conducted through the Veterans Affairs (VA) Medical Center. After approval was obtained through the VA Institutional Review Board, the AgCOPD database was used to identify participants with COPD, as defined by an FEV_1/FVC ratio of less than 70%. Exclusion criteria included participants without a diagnosis of COPD or anyone who did not fill out a FFQ.

Data Collection

The Harvard FFQ was used to gather the nutritional data, including the absolute values of nutrient intakes via food and supplementation. FFQs were mailed out to 168 participants with COPD to obtain their nutritional intake. Forty-two FFQs were returned and then sent to Harvard for analysis. Anthropometric data were gathered from the participant's VA records collected as part of the larger study. Demographic information was also collected for all participants including age, gender, and race. According to the American Thoracic Society, post bronchodilator lung function is what is used to diagnose COPD[44]. Post-bronchodilator $FEV_{1,}$ FVC, and FEV_1/FVC was used to determined lung function. Smoking status was also collected and categorized as current, former, or never. Vitamins A, C, D, and E, zinc, ß-carotene, omega-3, and lycopene were also analyzed to see if supplementation of these nutrients did or did not improve lung function.

Fifteen specific nutrients were chosen for inclusion in the analysis based on their antioxidant or anti-inflammatory properties that have previously been associated with lung disease in the literature. Lutein + zeaxanthin and lycopene reduce free radicals, whereas α-carotene and ß- carotene deficiency in fetuses can cause malformation of the lungs, and functional defects in adults[8,25,45]. Retinol has been shown to protect against respiratory illnesses,

such as lung cancer and ß-cryptoxanthin prevents free radical damage to cells and helps repair damage done[30, 46]. Vitamin C helps maintain antioxidant capacity in the aqueous phase of the cell and also functions as a cofactor for enzymes and neutralizes free radicals[46].

Other nutrients evaluated include vitamin E, vitamin D, omega 3 and omega 6, zinc and selenium. Vitamin E is the primary defense of cellular membranes against oxidative damage and it has been associated with lung disease in the literature. Vitamin E consists of four natural isoforms which consist of α, ß, ɣ, and δ tocopherol. α-tocopherol and ɣ-tocopherol are the most abundant in the diet and tissues and have been associated with lung function in the literature[35]. α-tocopherol has been studied the most which blocks airway hyperactivity whereas ɣ-tocopherol is pro-inflammatory and increases hyperactivity[13, 35]. Vitamin D regulates expression of genes in bronchial smooth muscle cells and deficiency may increase inflammation and cause changes to lung function and structure[30]. Like Vitamin D, omega-3 fatty acids are anti-inflammatory and lowers inflammatory markers such as CRP, IL-6, and TNFa. Omega-6 has the opposing affect and initiates inflammatory responses in cells[3]. Zinc is a catalytic agent in enzymatic reactions and selenium is a cofactor for glutathione peroxidase, which is an important antioxidant enzyme[25].

A micronutrient adequacy score was created using the individual nutrient intake of each participant. This score included the consumption of RAEs, vitamin C, vitamin D, α-tocopherol, sodium, selenium, and zinc. A score of 0 was given if the participant's specific nutrient intake was $<2/3^{rd}$ the RDA. A score of 1 was assigned if the participant's individual nutrient intake was $\geq 2/3^{rd}$ the RDA. A total score was assigned to each participant and could range from 0-7 with 7 being considered the diet with the highest amount of antioxidant and anti-inflammatory nutrients. According to the Food and Nutrition Board of the Institute of Medicine, the RDA is the daily level of intake sufficient to meet the nutrient requirements of nearly all healthy people. The fraction $2/3^{rd}$ RDA was chosen based on its previous use in studies using an adequacy score[36, 38].

All data was obtained from an investigator familiar with VA participants or a trained dietitian. This data was all compiled in an excel spreadsheet and patient identifiers were not recorded. All available information on each participant was included in the analysis.

Data Analysis

Descriptive statistics were calculated for all variables and continuous data are represented as mean and standard deviation. The mean and standard deviation were calculated for age and lung function (i.e. FEV_1, FVC, and FEV_1/FVC). Categorical data are expressed as counts and percentages which were used for gender, BMI, race, smoking status, and GOLD stages.

The mean intake and proportion of participants who met either $2/3^{rd}$ RDA or RDA was calculated for each nutrient. Nutrients that are assigned an AI or non-established DRI also had mean intake and proportion of $2/3^{rd}$ AI or AI met calculated. Since the data was normally distributed mean was used to determine the average of each nutrient. Determining the proportion of $2/3^{rd}$ RDA or $2/3^{rd}$ AI met for each nutrient helped create the micronutrient adequacy score.

The purpose of this study is to determine if there is an association between intakes of antioxidants and anti-inflammatory nutrients with lung function in veterans with COPD. Spearman's correlation coefficient was calculated to determine if there is an association between nutrient intake and lung function. Spearman's correlation coefficient was also used to determine the association between the micronutrient adequacy score and lung function. Variables significant in the univariate analysis were included in a multivariate regression model that adjusted for the possible confounders of age and smoking. A decision was made to dichotomize the micronutrient adequacy score using the median of 4. Comparisons using the dichotomized micronutrient adequate score and median lung function were made using a Mann-Whitney test. Median nutrient intakes were compared between smoking statuses using a Kruskal-Wallis test. Median lung function and median nutrient intake were gathered in these last two statistical tests

due to the non-normally distributed data.

The median micronutrient adequacy score was also compared to COPD stage 1 vs. stages 2-4 to see if those who had worse COPD had a lower median micronutrient score. Although COPD has four stages, a decision was made to divide the four stages into two groups based on the distribution of the data. A Mann-Whitney test was performed due to the small sample size of each individual group and normality could not be assumed. This test was also used when looking at median lung function compared to those who used supplements versus those who did not. Supplementation was represented categorically (Y/N). All statistical analysis was performed using SPSS and adjustments for confounders included age and smoking. Statistical significance was set at a p-value < 0.05.

RESULTS

A summary of the descriptive statistics can be seen in Table 3. Among the 42 participants 41 (98%) were males with the mean age of 66.4 ± 7.8 years. All participants were white with a mean BMI of 29.3kg/m^2 ± 7.5. The lowest BMI was 16.5kg/m^2 with the highest BMI being 58.9kg/m^2. The mean lung function data was also collected. Mean FEV_1 was 2.4L ± 0.9, mean FVC was 4.1L ± 1.0, and mean FEV_1/FVC was 0.6L ± 0.1.

Table 3: Descriptive Statistics for Participants with COPD

	N	**Mean (SD)**
Age (years)	42	66.4 (7.8)
Lung Function (L)		
FEV_1	35	2.4 (0.9)
FVC	35	4.1 (1.0)
FEV_1/FVC	35	0.6 (0.1)
	N	**% (Percentage)**
Gender		
Male	41	98
Female	1	2
BMI (kg/m^2)		
18.5-24.0	9	21
25.0-29.9	18	43
30.0-34.9	9	21
35.0-39.9	2	5
>40	4	10
Race		
White	42	100
African American	0	
Native American	0	
Asian	0	
Other	0	
Smoking Status Current/Former/Never	7 /29 /6	17/29/14
COPD GOLD Stages 1, 2, 3/4	11/ 16/ 8	26/38/19

The mean intake of each nutrient observed can be seen in Tables 4, 5, and 6. The proportion of participants whose intake was above 2/3rd RDA/AI and RDA/AI is included in

Tables 4 and 5. Seventy-nine percent met 2/3rd of the RDA for RAEs and vitamin C. Twenty-one percent of participants met 2/3rd of the AI for omega-3 and over half (55%) met 2/3rd the AI for omega-6. None of the participants met either 2/3rd the RDA or the RDA for selenium, however all 42 participants met 2/3rd of the AI for sodium. Participants also consumed more of the pro-inflammatory nutrient ɣ-tocopherol (11mg) than the anti-inflammatory nutrient of α-tocopherol (8mg). Regarding the micronutrient adequacy score, 30 participants (71%) had a score of 4 or greater which showed a higher intake of antioxidants and anti-inflammatory nutrients (Table 6). However, when the nutrient consumption was evaluated separately, a low percentage of participants met the RDA or AI for vitamin D, α-tocopherol, omega-3, omega-6, zinc, and selenium.

Table 4: Mean Intake and Proportion of Participants who met 2/3 RDA and RDA

Nutrient	Mean Intake	Recommended Dietary Allowance (RDA)	Percent who met 2/3 RDA	Percent who met RDA
RAE (ug)*	1400	900ug men 700ug women	79 2	74 2
Vitamin C (mg)	166	90mg men 70mg women	79 2	67 2
Vitamin D (mg)	11	20mg	26	19
α-tocopherol (mg)**	8	15mg	14	5
Zinc (mg)	16	11mg men 8mg women	34 2	25 0
Selenium (ug)	2.9	55ug	0	0

*Vitamin A RDA is based on RAEs which consists of retinol, ß-carotene, α-carotene, and ß-cryptoxanthin
**Vitamin E RDA is based on α-tocopherol

Table 5: Mean Intake and Proportion of Participants who met 2/3 AI

Nutrient	Mean Intake	Adequate Intake (AI)	Percent who met 2/3 AI	Percent who met AI
Omega-3 (g)***	0.3	1.6g men 1.1g women	21 0	21 0
Omega-6 (g)***	14	14g men 8g women	55 2	16 0
Sodium (mg)***	2199	1200mg	100	90

***AI used because not enough scientific evidence to determine an EAR and therefore an RDA

Table 6: Mean Intake of Nutrients with a Non-established DRI

Nutrient	Mean Intake
Retinol (ug)	842
α-carotene (ug)	721
ß-carotene (ug)	3987
ß-cryptoxanthin (ug)	133
Lutein+Zeaxanthin (ug)	2195
Lycopene (ug)	4634
ɣ-tocopherol (mg)	11
Micronutrient Adequacy Score	4

Spearman's correlation coefficient revealed four significant relationships between nutrient intake and lung function. Omega-3 was found to have an inverse relationship with FEV_1 (r= -0.357, p= 0.03) and FEV_1/FVC (r= -0.336, p=0.048). ɣ-tocopherol (r= -0.336 and p= 0.048) and omega-6 (r= -0.422 and p= 0.012) had an inverse relationship with FEV_1/FVC as well. All correlations can be found in Table 7 and significant findings were depicted using scatterplots (Figures 1-4). No significant relationships were discovered between nutrient intake and FVC. The four significant relationships between nutrient intake and lung function were adjusted for confounders. Due to the small sample size our adjustments were limited to age and smoking. The results of these adjustments can be seen in Table 7. The relationship between omega-3, omega-6, and ɣ-tocopherol with lung function were no longer significant after adjusting for confounders.

Table 7: Results of correlation and regression models for the relationship between nutrient intake and lung function (adjusted for age and smoking)intake and lung function (adjusted for age and smoking)

	Univariate Analysis						Multivariate Analysis					
	FEV_1		FVC		FEV_1/FVC		FEV_1		FVC		FEV_1/FVC	
Nutrient	r	p	r	p	r	p	ß	p	ß	p	ß	p
Vitamin C	0.196	0.259	0.260	0.131	0.132	0.450						
Vitamin D	0.138	0.428	0.181	0.298	0.073	0.676						
Retinol	-0.027	0.876	-0.006	0.973	-0.020	0.908						
α-carotene	-0.152	0.348	0.066	0.705	-0.223	0.197						
ß-carotene	-0.13	0.455	0.007	0.966	-0.090	0.607						
α-tocopherol	0.054	0.756	0.276	0.109	-0.128	0.465						
δ-tocopherol	-0.032	0.833	0.238	0.168	-0.294	0.087						
ɣ-tocopherol	-0.128	0.465	0.189	0.276	**-0.336**	**0.048**					**-0.147**	**0.40**
Omega-3	**-0.357**	**0.035**	-0.046	0.791	**-0.430**	**0.010**	**-0.21**	**0.22**			**-0.27**	**0.11**
Omega-6	-0.236	0.172	0.090	0.608	**-0.422**	**0.012**					**-0.263**	**0.12**
Zinc	-0.013	0.940	0.003	0.985	0.050	0.774						
Selenium	-0.037	0.833	-0.014	0.937	0.085	0.629						
Sodium	0.054	0.757	0.145	0.407	-0.125	0.473						
ß-cryptoxanthin	0.067	0.701	0.102	0.560	0.021	0.906						
Lutein + Zeaxanthin	-0.046	0.795	0.078	0.656	-0.017	0.924						
Lycopene	0.047	0.790	-0.012	0.946	0.140	0.423						

Figure 1: Association between Omega-3 and FEV_1

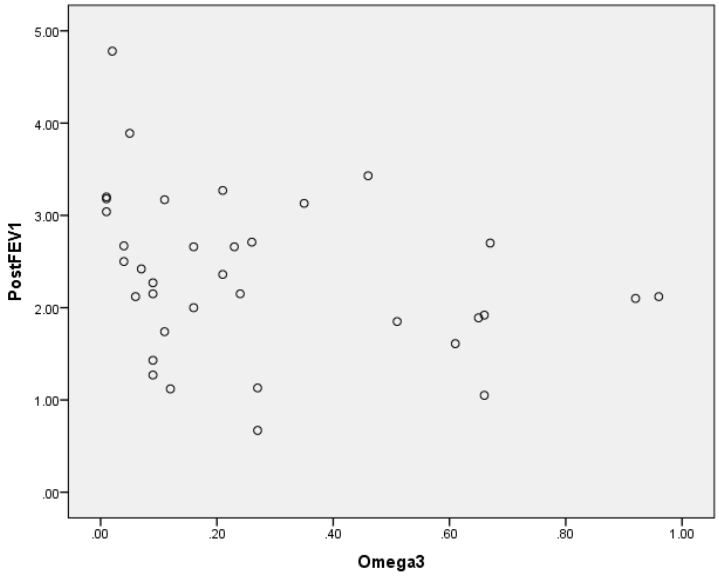

Figure 1: There was a negative relationship between Omega-3 intake and FEV_1 (r= -0.357, p= 0.04)

Figure 2: Association between Omega-3 and FEV_1/FVC

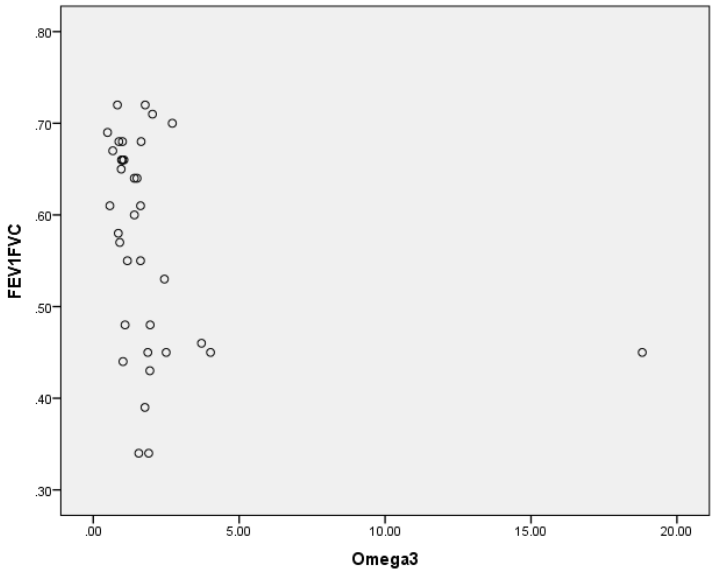

Figure 2: There was a negative association between Omega-3 intake and FEV_1/FVC (r= -0.43, p=0.01)

Figure 3: Association between ɣ-tocopherol and FEV_1/FVC

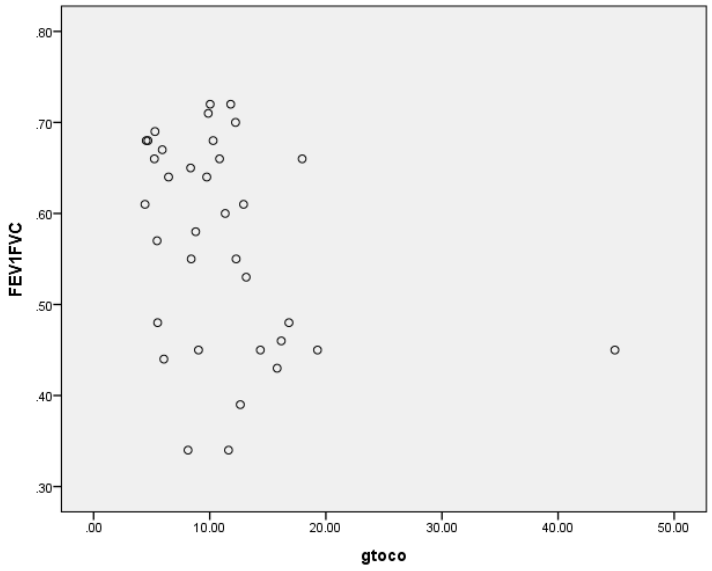

Figure 3: There was a negative association between ɣ-tocopherol intake and $FEV_{1/}FVC$ (r=0.34,p=0.05)

Figure 4: Association between Omega-6 and FEV_1/FVC

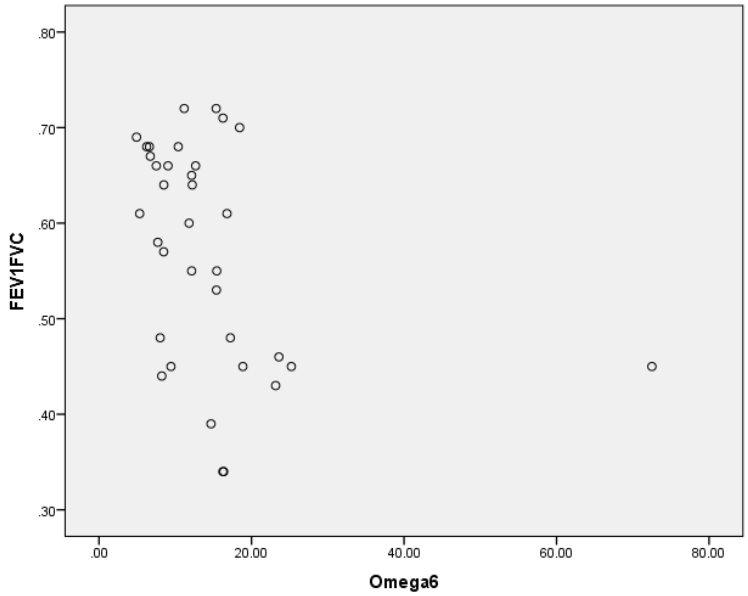

Figure 4: There was a negative relationship between Omega-6 intake and $FEV_{1/}FVC$ (r= -0.42, p=0.01)

The Spearman correlation coefficient showed no significant relationship between the micronutrient adequacy score and FEV_1, FVC, and FEV_1/FVC. No significant associations were found when the micronutrient adequacy score was dichotomized and compared to the median lung function using a Mann-Whitney test. A summary of the data is described in Tables 8 and 9. The median micronutrient adequacy score was also compared between COPD stages. Due to the distribution, the COPD stages were dichotomized and a Mann-Whitney test was performed to determine if participants with worse COPD would have a lower micronutrient adequacy score (Data not shown, p-value 0.828).

Table 8: Micronutrient Adequacy Score compared to Lung Function

	FEV_1		**FVC**		**FEV_1/FVC**	
	Spearman's Correlation	P-value	Spearman's Correlation	P-value	Spearman's Correlation	P-value
Micronutrient Adequacy Score	0.092	0.599	0.121	0.488	0.100	0.567

Table 9: Dichotomize Micronutrient Adequacy Score compared to Lung Function

Micronutrient Adequacy Score	**Number of Participants**	**FEV_1 Median (Min, Max)**	**FVC Median (Min, Max)**	**FEV_1/FVC Median (Min, Max)**
<4 (n=0)	11	2.29 (1.43, 3.20)	4.37 (3.18, 5.63)	0.59 (0.43, 0.69)
≥4 (n=1)	24	2.27 (0.67, 4.78)	4.00 (1.98, 6.63)	0.60 (0.34, 0.72)
P-value	-	0.756	0.841	0.971

There was no significance between smoking status and the median nutrient intake using a Kruskal-Wallis test depicted in Table 10.

Table 10: Median Nutrient Intake among Current, Former, and Never Smokers

Nutrient	Smoking Status			p-value
	Current Median (Min, Max)	Former Median (Min, Max)	Never Median (Min, Max)	
Vitamin C	86 (15, 318)	118 (31, 730)	143 (48, 1270)	0.549
Vitamin D	295 (67, 1445)	335 (39, 1663)	282 (66, 906)	0.351
Retinol	2303 (605, 5879)	2396 (357, 9918)	1645 (474, 4747)	0.483
α-carotene	434 (264, 858)	368 (0, 3620)	492 (88, 1319)	0.895
ß-carotene	3223 (1267, 16278)	2312 (2, 13408)	4288 (1860, 6365)	0.588
α-tocopherol	6 (3, 13)	7 (3, 27)	6 (3, 11)	0.451
ɣ-tocopherol	10 (6,16)	10 (4, 45)	7 (5, 12)	0.106
d-tocopherol	2 (1, 3)	2 (1, 5)	2 (1, 3)	0.282
Omega-3	0.3 (0.0, 1)	0.1 (0, 1)	0.2 (0, 0.4)	0.369
Omega-6	13 (8, 24)	12 (5, 73)	10 (6, 27)	0.205
Zinc	11 (6, 22)	14 (5, 74)	13 (6, 27)	0.798
Selenium	0 (0, 10)	0 (0, 20)	0 (0, 20)	0.528
Sodium	1921 (1028, 3070)	2046 (1028, 5452)	2244 (1197, 3478)	0.871
ß-cryptoxanthin	69 (5, 193)	116 (0, 519)	163 (22, 215)	0.939
Lutein + Zeaxanthin	1384 (833, 3545)	1763 (8, 11435)	2377 (1768, 4055)	0.293
Lycopene	5787 (1840, 21769)	2709 (0, 16880)	3811 (0, 10030)	0.201

A Mann-Whitney test was used when looking at median lung function compared to those who used supplements versus those who did not. Supplementation of vitamins A, C, D, and E, Zinc, Fish Oil (Omega-3), ß-carotene, and lycopene were included, but no statistical significance was found (Tables 11-13).

Table 11: Supplementation of Nutrients and FEV_1

Supplement	FEV_1 Median (Min, Max)	p-value
Vitamin A		
NO (n=23)	2.32 (0.67, 3.89)	0.831
YES (n=19)	2.15 (1.05, 4.78)	
Vitamin C		
NO (n=23)	2.32 (0.67, 3.89)	0.616
YES (n=19)	2.15 (1.05, 4.78)	
Vitamin E		
NO (n=25)	2.27 (0.67, 3.43)	0.934
YES (n=17)	2.26 (1.05, 4.78)	
Vitamin D		
NO (n=24)	2.47 (1.12, 3.43)	0.756
YES (n=18)	2.15 (0.67, 4.78)	
Zinc		
NO (n=14)	2.39 (0.67, 3.89)	0.687
YES (n=28)	2.10 (1.05, 4.78)	
ß-carotene		
NO (n=19)	2.32 (0.67, 3.89)	0.831
YES (n=23)	2.15 (1.05, 4.78)	
Omega-3		
NO (n=10)	2.42 (0.67, 4.78)	0.093
YES (n=32)	1.91 (1.05, 2.7)	
Lycopene		
NO (n=13)	2.32 (0.67, 4.78)	0.725
YES (n=29)	2.15 (1.05, 3.20)	

Table 12: Supplementation of Nutrients and FVC

Supplement	FVC Median (Min, Max)	p-value
Vitamin A		
NO (n=23)	3.89 (1.98, 5.88)	0.564
YES (n=19)	4.13 (2.2, 6.63)	
Vitamin C		
NO (n=23)	3.89 (1.98, 5.88)	0.769
YES (n=19)	4.13 (2.2, 6.63)	
Vitamin E		
NO (n=25)	3.77 (1.98, 4.90)	0.364
YES (n=17)	4.08 (2.2, 6.63)	
Vitamin D		
NO (n=24)	3.89 (2.84, 4.90)	0.589
YES (n=18)	4.13 (1.98, 6.63)	
Zinc		
NO (n=14)	4.10 (1.98, 6.63)	0.494
YES (n=28)	4.03 (2.2, 4.93)	
ß-carotene		
NO (n=19)	3.89 (1.98, 5.89)	0.564
YES (n=23)	4.13 (2.2, 6.63)	
Omega-3		
NO (n=10)	4.13 (1.98, 6.63)	0.334
YES (n=32)	3.80 (2.27, 4.72)	
Lycopene		
NO (n=13)	3.83 (1.98, 5.89)	0.342
YES (n=29)	4.30 (2.2, 6.63)	

Table 13: Supplementation of Nutrients and FEV_1/FVC

Supplement	FEV_1/FVC Median (Min, Max)	p-value
Vitamin A		
NO (n=23)	0.61 (0.34, 0.72)	0.681
YES (n=19)	0.58 (0.43, 0.72)	
Vitamin C		
NO (n=23)	0.61 (0.34, 0.72)	0.616
YES (n=19)	0.58 (0.43, 0.72)	
Vitamin E		
NO (n=25)	0.60 (0.34, 0.72)	0.987
YES (n=17)	0.60 (0.43, 0.72)	
Vitamin D		
NO (n=24)	0.61 (0.34, 0.72)	0.567
YES (n=18)	0.58 (0.34, 0.72)	
Zinc		
NO (n=14)	0.61 (0.34, 0.72)	0.740
YES (n=28)	0.58 (0.45, 0.72)	
ß-carotene		
NO (n=19)	0.61 (0.34, 0.72)	0.681
YES (n=23)	0.58 (0.43, 0.72)	
Omega-3		
NO (n=10)	0.61 (0.34, 0.72)	0.292
YES (n=32)	0.46 (0.45, 0.68)	
Lycopene		
NO (n=13)	0.59 (0.34, 0.72)	0.897
YES (n=29)	0.64 (0.43, 0.72)	

DISCUSSION

Nutrient Intake and DRI

The nutrient intake of antioxidants and anti-inflammatory nutrients was analyzed in this study with participants from the VA. Seventy-nine percent of participants met 2/3rd RDA for RAEs and vitamin C as seen in Table 4. This is likely due to the American diet being abundant in vitamins A and C[47, 48]. The mean omega-3 intake (0.3g) was very low considering the AI is between 1.1 to 1.6g depending on gender. Only 21% of participants met 2/3rd AI and AI for this nutrient. This may explain why a significant negative association was found between omega-3 with FEV_1 and FEV_1/FVC before adjusting for confounders. The mean intake of omega-6 was 14g which is the AI for this nutrient. Over half (55%) of participants met 2/3rd AI of omega-6 which was found to have a significant negative association with lung function before adjusting for confounders. The mean intake of selenium was below the RDA, and no participants even met the nutrient's 2/3rd RDA. This may have affected lung function in a negative way as selenium is an antioxidant. Participants consumed more of the pro-inflammatory nutrient ɣ-tocopherol (mean intake of 11mg) than the anti-inflammatory nutrient of α-tocopherol (mean intake of 8mg). Only 14% met 2/3rd RDA for α-tocopherol meaning that participants had a much lower intake of this anti-inflammatory nutrient.

Nutrient Intake and Lung Function

Four significant relationships between nutrient intake and lung function were found in this study prior to adjusting for confounders. Omega-3 was found to have an inverse relationship with FEV_1 and FEV_1/FVC before adjusting for age and smoking. This was unexpected as omega-3 has anti-inflammatory properties and has been found in the literature to increase these lung function values[3, 34]. In the Honolulu Heart Study, men that ate fish at least twice a week had a 50ml increase in their FEV_1. High fish consumption contributed to approximately 80ml increase

in FEV_1 in the first NHANES study[34]. Whereas the Morgen-Epic study discovered an increase intake of DHA was significantly associated with a lower FEV_1[9]. The inverse relationship found prior to adjusting for confounders in the COPD study involving participants from the VA was likely due to the poor intake of this nutrient, as only 21% met 2/3rd AI. Veterans in this study had a mean omega-3 intake of 0.3g which is way below the AI of 1.6g for men. Food items containing omega-3 in the FFQ included fish, walnuts, flaxseed, and canola and soybean oil. Based on these results, veterans should be encouraged to eat more omega-3 dense foods such as those listed on the FFQ. Before adjusting for confounders, omega-6 was observed to have a significant inverse relationship with FEV_1/FVC which was expected due to its pro-inflammatory properties. Previous research studies have also found similar results of an inverse relationship[3, 21]. The Morgen-Epic study found that higher levels of individual intake of omega-6 fatty acids (i.e. linoleic acid, arachidonic acid) were associated with a lower FEV_1[21]. Also, over half of the participants were found to have met 2/3rd AI of omega-6. Some studies suggest that omega-6 fatty acids' pro-inflammatory activities occur only when the intake of omega-3 fatty acids are low[3]. This may also explain why omega-6 had an inverse relationship before adjusting for age and smoking, since more participants met 2/3rd AI for omega-6 than for omega-3.

Before adjusting for confounders, ɣ-tocopherol was also found to have a significant inverse relationship with FEV_1/FVC. More participants consumed this pro-inflammatory nutrient than α-tocopherol, which has been found to improve lung function[13, 35]. This result was predicted based on previous research studies. It is also not surprising that participants had a higher intake of ɣ-tocopherol as the United States has a high consumption of soy oil, which is high in this pro-inflammatory nutrient[35]. In the Coronary Artery Risk Development in Young Adults study, higher serum ɣ-tocopherol was associated with a lower FEV_1 and FVC[13]. Hanson *et al.* also found a significant inverse relationship between serum ɣ-tocopherol and FVC after adjusting for confounders ($B= -0.10$, $p=0.05$)[2].

Possible confounders for this study would be age, height, weight, BMI, smoking, vitamin supplementation, and caloric intake. Based on the small sample size, age and smoking were selected to be adjusted for confounders. After omega-3 was adjusted for age and smoking, no significant relationship was found between FEV_1 and FEV_1/FVC. After adjusting for confounders, omega-3 would have been expected to have a positive association with lung function based on previous literature. However, the very poor intake of this nutrient may explain why a positive relationship was not found. No relationship was found between ɣ-tocopherol and omega-6 with FEV_1/FVC after adjusting for confounders. This was unexpected as current literature explains that these nutrients have a negative association with lung function. Also, the nutrient intake of ɣ-tocopherol and omega-6 were higher than anti-inflammatory nutrients that were analyzed.

Based on previous studies, there is a relationship between nutrient intake of antioxidants and anti-inflammatory nutrients with lung function. This VA study likely did not find a significant relationship between nutrient intake and lung function due to the small sample size and very poor nutrient intake of the participants. For instance, the Healthy Eating Index 2010 found a significant relationship between nutrient intake and lung function after adjusting for confounders. This is likely due to the large sample size and better consumption of antioxidant and anti-inflammatory nutrients.

Micronutrient Adequacy Score

No significant relationships between the micronutrient adequacy score and lung function were found. Even when the COPD stages were dichotomized to above or below the median micronutrient adequacy score, no significant associations were found between lung function. A small sample size and low nutrient intakes are likely the causes of these findings. Based on the results of the VA study, it is unable to conclude that having a diet higher in antioxidants and anti-

inflammatory nutrients will decrease the risk of having COPD. An association may have been found with a larger sample size and higher consumption of antioxidant and anti-inflammatory nutrients. In the study evaluating the Alternative Healthy Eating Index 2010, the risk of newly diagnosed COPD was lower in participants who ate the healthiest diet compared to those who ate the least healthy diet[39]. This study involving the Alternative Healthy Eating Index 2010 also used FFQs to gather data, however it involved more participants and was conducted over a longer period of time. It was also discovered that those who have a higher score, and therefore healthier diet, tend to be non-smokers and non-obese[38, 39].

Nutrient Intake and Smoking Status

There was no significance found between median nutrient intake and smoking status using a Kruskal-Wallis test. There were more former (n=29) and current (n=7) smokers compared to never (n=6) smokers. Perhaps if there was a larger sample size and normal distribution among the three smoking status groups a significance between median nutrient intake and smoking status may have been found.

Nutrient Supplementation and Lung Function

No significant findings were found between median FEV_1, FVC, and FEV_1/FVC with those who did and did not use supplementation of vitamins A, C, D, and E, zinc, omega-3, ß-carotene, and lycopene. Previous research observing supplementation is limited and some findings are contradictory. In a previous study, vitamin A supplementation was provided to COPD participants for 30 days. The mean FEV_1 increased by 22.9% in those who took a vitamin A supplement[41]. In a study looking at the impact of vitamins E and C supplementation on exercise, those that used supplementation had a significant increase in their exercise time[49]. However, vitamins E and C supplementation was found to not improve lung function in two studies involving COPD participants[40, 50].

Schwartz *et al.* noticed that supplementation of omega-3 fatty acids in high dosages may have some health benefits[34]. Supplementation of fish oil for 6 weeks also was shown to decrease mucus in lungs, allowing COPD participants better ability to breathe. Omega-3's poor nutrient intake or perhaps the supplementation dosages of omega-3 were not high enough in the VA study to make a difference. Currently the scientific evidence to set an appropriate range for supplementation of certain antioxidants or anti-inflammatory nutrients is unclear. Since vitamins A, C, D, and E, zinc, omega-3, ß-carotene, and lycopene all have antioxidant and anti-inflammatory affects, it was predicted that supplementing these nutrients would improve lung function. The research on these nutrients and supplementation to improve lung function is contradictory and unclear at this point.

Limitations

There are several limitations to this research study. First, seven of the forty-two participants did not have spirometry test results available. However, all 42 participants filled out a FFQ. The nutrient intakes of these participants with and without lung function are valuable. Therefore, all 42 participants were included in this study. FFQs at best reflect a short-term intake which can vary day-to-day. It is hard to measure dietary intake accurately even with detailed questionnaires regarding food portions[25].

Sample size may also be a source of bias as a larger sample size may have strengthened associations. Gender differences were also a limitation as only one female participated in this study. The DRIs are also not suggested to be used in people with chronic diseases such as COPD. The recommended intakes for the nutrients would not necessarily provide enough nutrition for individuals who are already malnourished, nor would they be adequate for certain disease states marked by increase nutrient requirements.

Serum levels were not included in this study but they are more likely to reflect long-term

nutrient status and nutrient bioavailability[25]. Supplementation used as a categorical variable is another limitation to this study. It would have been useful to compare dosages of the supplementation used since there were no significant findings between supplementation and lung function.

Strengths

A strength of this study was focusing on lung function specifically in participants with COPD. Research regarding nutrient intake and COPD is currently conflicting and more evidence is needed in this area. This research study can contribute to helping people delay or treat this disease by making veterans aware of nutritional deficiencies and their potential negative impacts. Also, using the Harvard FFQ for analysis provides more accurate information on nutrient intake compared to dietary records or recalls.

Applications for Clinical Practice

Interest in this study developed due to the lack of medical nutrition therapy emphasis in lung diseases. In the VA study, no significant association was found between antioxidant and anti-inflammatory nutrients with lung function after adjusting for confounders. However, participants were low in vitamin D, α-tocopherol, omega-3, and selenium which could have negatively impacted the results of the study. A median micronutrient adequacy score of 4 and a poor dietary pattern was found in the VA study. Knowing that many of the participants had a low intake of these important nutrients is a starting point for a dietitian. Increasing the intake of these antioxidants and anti-inflammatory nutrients should be a priority in this patient population. A registered dietitian would be able to improve the nutrient intake by dietary modifications and lifestyle changes.

CONCLUSION

After adjusting significant findings for confounders, it cannot be concluded that there is an association between antioxidant and anti-inflammatory nutrients. From the review of the literature, a majority of these nutrients have a positive association with lung function. However, there are conflicting results in the literature and more research is needed in this area.

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