Serum Level of Nutritional Antioxidants are Decreased in Veteran Smokers with COPD

Corrine Hanson, Elizabeth Lyden, Lisa Weissenburger-Moser, Jeremy Furtado, Debra J. Romberger and Tricia D. LeVan

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the U.S. and worldwide,[1-4] resulting in an economic and social burden that is both substantial and increasing. Veterans are a group of people who are disproportionately affected by COPD. COPD affects over 40% of the adult veteran population in the urban Midwest, which is five times greater than the general U.S. population.[5] Cigarette smoking is the main etiologic agent for COPD risk and is common among VA healthcare users, hence the prevalence of COPD is expected to continue and increase in upcoming decades.[5,6] Veterans with COPD suffer high morbidity, including poor quality of life, activity limitation and exacerbations leading to emergency department visits and hospitalisations.[7] Therefore, a better understanding of contributing factors to the development, progression and burden of this pervasive disease among veterans is critical in order to design effective interventions.

Epidemiological studies have shown a positive association between intake or serum levels of certain micronutrients, especially those with anti-oxidant or anti-inflammatory functions, and lung function. For example, the National Health and Nutrition Examination Surveys (NHANES) of the U.S. population have repeatedly demonstrated that increased intake or serum levels of some micronutrients, including vitamins E, D, C, A, fibre , and omega-3 fatty acids are positively associated with measures of lung function.[8-19] These results suggest that diet and nutrition may impact lung function and that nutrition interventions could be useful in those with lung disease. However, there is little data available on the intake of these nutrients, or the serum levels of nutrients with anti-oxidant properties in veterans. Thus, the purpose of this analysis was to examine the intake and serum levels of nutrients which have previously been associated with lung health in a population of veterans with COPD.

Materials and Methods

This was a secondary analysis of data obtained from a cross-sectional study of agricultural exposures and COPD in veterans seeking health care at the General Medicine clinics of the Omaha Veterans Affairs (VA) Medical Center.[20] Briefly, all subjects had greater than 2 years' experience working on a farm. Subject demographics and smoking habits were obtained by in-person and telephone interviews. A participant was considered to be a smoker if they had smoked more than 100 cigarettes in their lifetime. All veterans underwent spirometry and if they had a $FEV_1/FVC < 0.70$, then post-bronchodilator spirometry with 0.083% albuterol was performed. COPD status was ascertained for each participant using the Global Initiative for Chronic Obstructive Lung Disease (GOLD) definition of $FEV_1/FVC < 0.70$[21] or by clinical assessment. FEV_1 and FVC were adjusted for height, weight, age, gender and ethnicity based on NHANESIII reference equations.[22] All participants signed a written informed consent document at study enrolment. This study was approved by the VA Nebraska Western Iowa Healthcare Systems Institutional Review Board.

Nutrient Intake. A Food Frequency Questionnaire (FFQ) was mailed to the home address of the participant, with a follow up phone call in 2 weeks if the FFQ had not been returned. FFQs were analysed by trained personnel in the Harvard University Department of Nutrition. The FFQ administered was the Willet FFQ, which has been validated in adults of all ages and sexes and among a variety of socio-economic groups.[23-28] The FFQ allows for analysis of absolute nutrient intake values from foods and supplements. In addition, studies show that after adjustment for absolute energy intake, the Willett FFQ is robust in validity and reliability in comparison to other validated FFQs.[29-31]

Nutrient intake values calculated from the completed FFQs were compared to the appropriate Dietary Recommended Intake (DRI) values for the appropriate

gender and life stage. The Recommended Dietary Allowance (RDA) was used when a nutrient level had been established; if no RDA had been established, the Adequate Intake level (AI) was used. The RDA and the AI may both be used as goals for individual intake.[32-35] Recommended Dietary Allowances are set to meet the needs of almost all (97 to 98 percent) individuals in a group, while the AI for life stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data prevents the ability y to specify with confidence the percentage of individuals covered by this intake. Specific nutrients of interest were chosen based on prior associations with COPD as reported in the literature and included vitamin A, vitamin D, fibre , vitamin E, vitamin C, carotenes and other vitamin A-related compounds, omega-3 and omega-6 fatty acids. For intake of omega 3 and omega-6 fatty acid, the Adequate Macronutrient Distribution Range (AMDR) was used. While no defined intake level at which potential adverse effects of n-6 polyunsaturated fatty acids was identified, the upper end of the AMDR is based on the lack of evidence that demonstrates long-term safety and human *in vitro* studies which show increased free radical formation and lipid peroxidation with higher amounts of omega-6 fatty acids.[32] The upper end of AMDR for omega-3 fatty acids is based on maintaining the appropriate balance with omega-6 fatty acids, as this has been implicated in the risk for chronic disease.[36]

Serum Nutrient Level. Serum nutrient level was measured at the Biomarker Research Laboratory at the Harvard School of Public Health. Concentrations of lutein+zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene, retinol, γ-tocopherol, and α-tocopherol in serum samples were measured using the method described by Hess et al. with some modifications.[37] Serum samples were mixed with ethanol containing rac-Tocopherol (Tocol) as an internal standard, extracted with hexane, evaporated to dryness under nitrogen, and reconstituted in ethanol, dioxane and acetonitrile. Samples were quantitated by high-performance liquid chromatography (HPLC) on a Restek Ultra C18 150mm X 4.6mm column, 3µm particle size encased in a Hitachi L-2350 column oven to prevent temperature fluctuations, and equipped with a trident guard cartridge system (Restek, Corp. Bellefonte, PA). A mixture of acetonitrile, tetrahydrofuran, methanol, and a 1% ammonium acetate solution (68:22:7:3) was used as mobile phase, flow rate 1.1 ml/min, using a Hitachi Elite LaChrom HPLC system comprised of an L-2130 pump in isocratic mode, an L-2455 Diode Array Detector (monitoring at 300nm and 445nm), and a programmable AS-2200 auto-sampler with chilled sample tray. The system manager software (D-7000, Version 3.0) was used for peak integration and data acquisition (Hitachi, San Jose, CA). Every run included two replicates each of a two-level serum pool sample set. For external quality control, the laboratory participates in the standardisation program for carotenoid analysis from the National Institute of Standards and Technology U.S.A.

Table 1: Demographic characteristics of veterans with COPD (n=41)

	Mean (SD)
Age	66.5 (7.8)
Energy Intake, kcals	1873.7 (589.3)
BMI, kg/m^2	29.2 (7.6)
FEV$_1$ (L)	2.4 (0.9)
FEV$_1$, % predicted	68.6 (24.1)
FVC (L)	4.1 (1.0)
FVC, % predicted	87.9 (20.3)
FEV$_1$/FVC, mean	0.6 (0.1)
	N (%)
Male Gender	41/100
White Race	41/100
Smoking Status	
Current	7 (17)
Former	29 (71)
Never	5 (12)
COPD GOLD Stage*	
1 (Mild)	13 (32)
2 (Moderate)	16 (38)
3-4 (Severe/Very Severe)	12 (30)

COPD was defined as FEV1/FVC ≤ 0.7 or a decision by a pulmonologist

Statistical Analysis. Descriptive statistics (counts, percentages, means, and standard deviations) were calculated for all variables. The Spearman correlation coefficient was used to look at associations between lung function measures (Forced Expiratory Volume in One Second, FEV$_1$; Forced Vital Capacity, FVC; FEV$_1$/FVC ratio) and intake and serum level of nutrients. Multivariable regression models adjusted for the possible confounding of age, smoking, body mass index (BMI), energy intake (for models containing intake of nutrients) and serum cholesterol (for models containing the serum tocopherols). Intake and serum level of nutrients were log-transformed for normality and used in the regression models. The Mann-Whitney test was used to compare continuous data between 2 groups. The Kruskal-Wallis test

Table 2: Nutrition Intake of Veterans with COPD

Nutrient	Mean (SD) N = 41	RDA/AI
Vitamin C (mg)	169.3 (214.2)	**90 mg***
Vitamin D (IU)	468.0 (407.2)	**600 IU 51-70 years** **800 IU 70+ years**
Vitamin A (Retinol Activity Equivalents)	1,411.4 (1,311.6)	**900 retinol activity equivalents**
Vitamin E (mg)	16.4 (28.6)	**15 mg**
Fiber (g)	20.1 (9.3)	30**
Omega-3 fatty acids (gm)	0.29 (0.30)	1.6** (AMDR 0.6-1.2)***
Omega-6 fatty acids (gm)	14.2 (10.7)	14** (AMDR 5-10)***
α- carotene (mcg)	736.0 (810.1)	n/a
β-carotene (mcg)	3,986.4 (3,621.1)	n/a
β-cryptoxanthin (mcg)	135.9 (114.2)	n/a
Lycopene (mcg)	4,729.0 (4,942.1)	n/a
Lutein + Zeaxanthin (mcg)	2,190.6 (2,125.3)	n/a

*Individuals who smoke require 35 mg/day more Vitamin C than nonsmokers

Recommended Dietary Allowances (RDAs) in bold type, Adequate Intakes (AIs) in ordinary type followed by an asterisk (**)

*** Acceptable Macronutrient Distribution Range (AMDR) is the range of intake for a particular energy source that is associated with reduced risk of chronic disease while providing intakes of essential nutrients.

was used to compare continuous data between >2 groups and p-values for post-hoc pairwise comparisons were adjusted using a nonparametric multiple-comparison procedure. All statistical tests were two-sided and a p-value < 0.05 was considered statistically significant. Analyses were done using SAS Version 9.4.

Results

A total of 123 FFQs were mailed out with a response rate of 33%. There were no differences between responders and non-responders with respect to demographics including age, sex, race, and FEV_1/FVC ratio. All participants were white males with a mean age of 66.5 years and a mean BMI of 29.2kg/m^2. Baseline characteristics of the respondent population are summarised in Table 1.

Nutrient Intakes. A summary of the intake of anti-oxidant and anti-inflammatory nutrients assessed by the FFQ are compared to existing recommendations as shown in Table 2. Veterans with COPD had suboptimal intakes of vitamin D, fibre, and omega-3 fatty acids, with an intake of omega-6 fatty acids above the recommended level. No DRIs for carotenes, β-cryptoxanthin, lycopene or lutein+zeaxanthin have been established; however, intake values are reported due to prior associations of these nutrients with lung disease.

Intake of nutrients and lung function. In the univariate analysis, we found intake of omega-3 fatty acids were inversely associated with FEV_1 (r= -0.39, p=0.02) and FEV_1 % predicted (r= -0.42, p=0.01). After adjustment for the covariates energy intake, age, BMI, and smoking status, the correlation between omega-3 fatty acid intake and lung function was attenuated (FEV_1, β= -0.21, p=0.07; FEV_1% predicted, β=-5.5, p=0.07). Intake of omega-3 fatty acids was not associated with FVC or FEV_1/FVC ratio (data not shown). None of the other nutrients were significantly associated with FEV_1, FVC and FEV_1/FVC ratio (data not shown).

Serum Nutrient Levels. Serum antioxidant nutrient levels of the cohort are summarised in Table 3. When we evaluated the correlation between dietary intake and serum levels, β-cryptoxanthin intake was significantly associated with serum levels of β-cryptoxanthin (r=0.35, p=0.03).

Serum nutrient levels and lung function. In the univariate analysis, we found statistically significant correlations between serum levels of trans-β carotene and FVC % predicted (r=0.39, p=0.02) and cis-β carotene and FVC % predicted (r=0.39, p=0.02). After adjustment for age, BMI, and smoking status in the multivariable model, these relationships were no longer statistically significant. The remaining

Table 3: Serum Levels of Antioxidant Nutrients

Serum Nutrient (µg/L)	Mean (SD) N = 39
α-carotene	29.7 (28.8)
Trans β-carotene	133.2 (159.1)
Cis β-carotene	9.6 (11.3)
Total β-carotene	142.1 (17.05)
β-crytoxanthin	56.2 (48.7)
Trans lycopene	148.2 (72.3)
Cis lycopene	138.3 (68.7)
Total lycopene	286.4 (139.3)
Lutein+Zeaxanthin	114.9 (51.9)
Retinol	564.3 (112.6)
γ- tocopherol	1317.2 (720.6)
α- tocopherol	9613.9 (9014.2)

Table 4: Serum Antioxidant Nutrients and Smoking Status (n=39)

Serum Nutrient (µg/L)	Smoking status	Serum Level	p-value
α-carotene	Current	12.39	0.004
	Former	26.06	
	Never	70.63	
Trans β-carotene	Current	61.25	0.005
	Former	105.69	
	Never	373.60	
Cis β-carotene	Current	5.27	0.005
	Former	7.44	
	Never	26.75	
Total β-carotene	Current	65.14	0.005
	Former	112.49	
	Never	400.34	
β-cryptoxanthin	Current	36.85	0.03
	Former	48.13	
	Never	124.76	

nutrients assayed in serum were not associated with lung function (data not shown). There was no association between serum nutrient levels and COPD stage.

Nutrient levels and smoking status. There was no association between intake of nutrients (listed in Table 2) and smoking status (current, former, never) (data not shown). Importantly, serum levels of anti-oxidant nutrients (α-carotene, trans β-carotene, cis β-carotene, total β-carotene, β-cryptoxanthin) differed by smoking status (Table 4). There was a dose response of these serum nutrients with smoking status, where current smokers had fewer of these serum nutrients than never- smokers.

Discussion

Participants in our study did not meet the minimum guidelines for intake of three important nutrients that typically have been associated with improved lung health: vitamin D, fibre, and omega-3 fatty acids. Additionally, intake of omega-6 fatty acids, which may have pro-inflammatory properties, were above the Acceptable Macronutrient Distribution Range.

Intakes of nutrients previously associated with lung function Our results are consistent with other studies that show vitamin D intake in populations of patients with COPD are below current recommendations.[38] Because COPD patients spend less time outdoors,[39] they would be expected to have decreased sun exposure, which places them at high risk for vitamin D deficiency. Intake of vitamin D has been shown to be associated positively with FEV_1 (difference in FEV_1 between top and bottom quintiles of intake, 0.079 L; 95% CI, 0.02–0.14; p for trend= 0.007), ratio of FEV_1 to FVC (p=0.008), and associated negatively with COPD (odds ratio [OR] comparing top and bottom quintiles, 0.57; 95% CI, 0.38–0.87; p=0.02).[40] Intake of dairy products, but not specifically vitamin D intake, has been associated with less severe measures of emphysema (defined by computed tomographic lung density) in 3,271 subjects enrolled in the Multi- Ethnic Study of Atherosclerosis (MESA) study (p=0.02 and p=0.01 for alpha, a measure of emphysema, and apical vs basilar distribution of emphysema, respectively).[41] This association was observed primarily for low-fat dairy intake, not high-fat dairy intake, and may have been confounded by other lifestyle factors, because those who choose low-fat dairy products may make different lifestyle choices compared with those who choose high-fat dairy products. The usefulness of evaluating the effect of vitamin D intake from diet may have limited value, given the overall minor contribution of dietary intake to vitamin D status, because the major source for vitamin D is outdoor sun exposure.

The mean intake of fibre in our population was 33% below current recommendations. In large prospective studies, high fibre diets reduced mortality from infectious disease, respiratory disease, smoking-related cancers and cardiovascular disease.[42,43] With regard to lung function, four large prospective studies have shown diets high in fibre are associated with

better lung function and decreased risk of COPD.[16,44-46] A recent study using mouse models demonstrated high amounts of dietary fibre regulated the immune system in the lungs, which included an increase in the haematopoiesis of dendritic cells that could not reactivate T helper type 2 effector cells, resulting in lower airway inflammation.[47] These results provide evidence that dietary fibre can shape the immunological environment in the lung. One proposed mechanism underlying this beneficial effect comes from studies showing that fibre stimulates the growth of beneficial bacteria in the gut, which synthesse, through fermentation, high quantities of Short Chain Fatty Acids (SCFAs).[47-53] SCFAs have been detected in the portal circulation where they are active in the liver and bone marrow to decrease innate immune activation.[47,50,52-56]

Intake of omega-3 fatty acids was extremely low and omega-6 fatty acids was notably high in our cohort. A balance between omega-6 and omega-3 fatty acids is thought to be important in the prevention and treatment of coronary artery disease, diabetes, osteoporosis and other inflammatory diseases.[36,57] In patients with asthma, an omega 6:3 ratio of 5:1 decreased leukotriene production and improved respiratory symptoms.[58] In the secondary prevention of cardiovascular disease, a leading cause of death in patients with COPD, a ratio of 4:1 has been associated with a 70% decrease in total mortality.[36] Our cohort was from a Midwestern state where fish intake is commonly low, and this may have contributed to the poor intake of omega-3 fatty acids resulting in an extremely high omega 6:3 ratio of over 100:1. Fish and seafood are the main source of omega-3 fatty acids, contributing up to 71% of total intake.[59] Fats and oils, meat and poultry, and cereal-based products and cereals, are the primary sources of omega-6 fatty acids in a Westernised diet,[59] therefore it may be difficult for even populations living in an area with an ample seafood supply to achieve a desired ratio.

In the univariate analysis, we found an inverse association between omega-3 fatty acid intake and lung function. However, other studies conflict with this finding. A recent systemic review identified 11 observational studies evaluating the relationship between COPD and intake or serum levels of omega-3-fatty acids. Of these 11 studies 6 found a significant relationship and 5 of those relationships were inverse (protective against COPD).[60] The remaining 5 studies found no relationship.[60] Fish is one of the primary sources of omega-3 fatty acids in the diet, and dietary intake patterns with an increased consumption of fish have been associated with a decreased development of COPD in smokers and nonsmokers, increased FEV^1 and decreased long-term COPD mortality.[61-63] Analysis of the Seven Countries Study, a population-based cohort of 12,763 men, calculated that fruit and fish intake together explained about 67% of the variation in COPD mortality rates after 25 years.[61] Our study did not find such an association between omega-3 fatty acid intake and lung function, however it has been shown that the omega 6:3 ratio is critical in achieving respiratory benefits,[58] and given the high ratio present in our cohort, any findings may have been obscured.

Serum levels of anti-oxidant nutrients A large body of observational epidemiological evidence suggests that higher serum concentrations of β-carotene and other carotenoids obtained from foods are associated with lower risk of several chronic diseases,[35] and serum level of carotenes have been associated with improved lung function in other studies.[12-14,64,65] Although our study did not find this association, we did find a correlation between dietary beta-cryptoxanthin and the serum level of this compound, which suggest that diet is an effective intervention for increasing serum levels of beta-cryptoxanthin in subjects with COPD. This may be especially important in smokers, who exhibited decreased levels compared to non-smokers. Blood concentrations of carotenoids are the best biological markers for consumption of fruits and vegetables.[35] Although no DRIs are proposed for β-carotene or other carotenoids at the present time, existing recommendations for increased consumption of carotenoid-rich fruits and vegetables in patients with COPD would appear to be supported.

Our study also documents lower serum level of β-cryptoxanthin and carotenes in smokers. A dose-response appeared to be present, with serum levels decreasing as the level of smoking increased. Several studies have reported that smokers have lower plasma carotenoid concentrations compared to non-smokers,[66,67] and higher numbers of cigarettes per day have been shown to lead to a corresponding decrease in serum carotenoids in a dose-dependent manner.[66] Whether this is due completely to a decreased intake of carotenoids is unknown; tobacco smoke is highly oxidative, and has been shown to destroy beta carotene and other carotenoids in human plasma *in vitro*,[68] which may contribute to a reduction in serum levels. The association of serum β- carotene and FEV_1 has been shown to be weaker in smokers when compared to non-smokers, and the strength of the relationship decreased as the amount of smoking increased.[8]

Supplementation of micronutrients Should clinicians consider supplementation of micronutrients in their

veteran patients who smoke? In one study, adults at high risk for cardiovascular events received antioxidant vitamins for 5 years (600 mg vitamin E, 250 mg vitamin C, 20 mg beta-carotene). That study failed to identify any effect on lung function, although it was measured only at the last visit, or hospitalisation for non-neoplastic respiratory causes. Individuals with severe COPD, however, were excluded from that study.[69] Another large trial showed a significantly higher incidence of lung cancer in current smokers supplemented with alpha tocopherol and beta-carotene for 5-8 years.[70] However, a high intake of beta carotene and vitamin E from food was associated with an improvement in respiratory symptoms.[71] The latter study seems to suggest that prevention campaigns should stress the importance on nutrient intake from food, especially in smokers.

Limitations Our study has several limitations. Our sample size was small, and it is always difficult to separate the direct effect of a nutrient on an outcome from the overall effects of associated lifestyle factors. Our population is a Midwestern population with agriculture backgrounds, which may limit generalisability to other populations. Some significant results were attenuated after adjustment for confounders, and some potential covariates, such as corticosteroid use, for which no adjustment was made.. We assessed nutrient intake using FFQ methodology, which is subject to various limitations, including measurement error in the estimated portion sizes of foods, and inaccuracies that result from incomplete listings of all possible foods. However, we do provide both serum levels and nutrient intake levels, which are often not studied together. We do not, however, report serum levels of vitamin D, which would be relevant in this population. In addition, very little data exists on nutrients that may be of concern to a population of veterans who may be more likely than the general population to be current or former smokers.

Conclusion

Our study shows that veterans with COPD have low intakes of nutrients associated with lung function and COPD, and that smokers have lower serum levels of anti-oxidant nutrients than non-smokers. Given the importance of inflammation and oxidant stress in lung function, a direct effect of nutrients that have anti-oxidant and anti-inflammatory properties is readily plausible. As measures of lung function are an independent predictor of mortality in the general population as well as in people with lung disease,[72,73] public health initiatives to improve lung function through dietary interventions in veterans could potentially have a profound impact. In the future, larger and well-designed interventional trials are needed to confirm these associations, and to establish whether dietary interventions are effective in the prevention or treatment of lung impairment. Populations of subjects susceptible to a decline in lung function, such as smokers, may provide a target population that would incur the most benefit from the results of such trials. The low cost and safety of dietary interventions such as counselling to increase fruit and vegetable intake to maintain or improve lung function make this a very attractive intervention for researchers, as well as clinicians working with populations of patients either with, or at risk for, lung impairment.

Corresponding author: Corrine Hanson
ckhanson@unmc.edu
Authors: C Hanson[1], E Lyden[1], L Weissenburger-moser[1], J Furtado[2], D Romberger[3,1], T LeVan[1,3]
Author Affiliations:
1 University of Nebraska Medical Center - Medical Nutrition Education
2 Harvard University Ringgold standard institution - School of Public Health Department of Nutrition
3 Veterans Affairs Medical Center, Omaha, Nebraska

References

1. Minino AM. Death in the United States, 2011. NCHS Data Brief. 2013;(115)(115):1-8.
2. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study . Lancet. 1997;349(9064):1498-1504.
3. Mannino DM, Buist AS. Global burden of COPD: Risk factors, prevalence, and future trends. Lancet. 2007;370(9589):765-773.
4. **The top 10 causes of death.** World Health Organization Web site. http://www.who.int/mediacentre/factsheets/fs310/en/. Updated 2014. Accessed 3/25, 2015.
5. Calderon-Larranaga A, Carney L, Soljak, M. et al. Association of population and primary healthcare factors with hospital admission rates for chronic obstructive pulmonary disease in England: National cross-sectional study. Thorax. 2011;66(3):191-196.
6. Akinbami LJ, Liu X. Chronic obstructive pulmonary disease among adults aged 18 and over in the United States, 1998-2009. NCHS Data Brief. 2011;(63)(63):1-8.

7. Darnell K, Dwivedi AK, Weng Z et al.. Disproportionate utilization of healthcare resources among veterans with COPD: A retrospective analysis of factors associated with COPD healthcare cost. Cost Eff Resour Alloc. 2013;11:13-7547-11-13. eCollection 2013.
8. Hu G, Cassano PA. Antioxidant nutrients and pulmonary function: The Tthird National Health and Nutrition Examination Survey (NHANES III). Am J Epidemiol. 2000;151(10):975-981.
9. Bentley AR, Kritchevsky SB, Harris TB, et al. Dietary antioxidants and forced expiratory volume in 1 s decline: The health, aging and body composition study. Eur Respir J. 2012;39(4):979-984.
10. McKeever TM, Lewis SA, Cassano PA, et al. Patterns of dietary intake and relation to respiratory disease, forced expiratory volume in 1 s, and decline in 5-y forced expiratory volume. Am J Clin Nutr. 2010;92(2):408-415.
11. McKeever TM, Lewis SA, Cassano PA, et al. The relation between dietary intake of individual fatty acids, FEV1 and respiratory disease in Dutch adults. Thorax. 2008;63(3):208-214.
12. McKeever TM, Lewis SA, Smit HA et al.. A multivariate analysis of serum nutrient levels and lung function. Respir Res. 2008;9:67.
13. Grievink L, de Waart FG, Schouten EG et al.. Serum carotenoids, alpha-tocopherol, and lung function among Dutch elderly. Am J Respir Crit Care Med. 2000;161(3 Pt 1):790-795.
14. Grievink L, Smit HA, Veer P, et al. Plasma concentrations of the antioxidants beta-carotene and alpha-tocopherol in relation to lung function. Eur J Clin Nutr. 1999;53(10):813-817.
15. Grievink L, Smit HA, Ocke MC, et al. Dietary intake of antioxidant (pro)-vitamins, respiratory symptoms and pulmonary function: The MORGEN study. Thorax. 1998;53(3):166-171.
16. Hanson C, Lyden E, Rennard S, et al. The relationship between dietary fiber intake and lung function in NHANES. Ann Am Thorac Soc. 2016, May;13(5):643-50
17. Hanson C, Sayles H, Rutten E et al. The association between dietary intake and the phenotypical characteristics of COPD in the ECLIPSE cohort. Chronic Obstructive Pulmonary Diseases: The Journal of the COPD Foundation. 2014;1(1):115-1247.
18. Hanson C, Rutten EP, Wouters EF et al. Diet and vitamin D as risk factors for lung impairment and COPD. Transl Res. 2013;162(4):219-236.
19. Schwartz J, Weiss ST. The relationship of dietary fish intake to level of pulmonary function in the first National Health and Nutrition Survey (NHANES I). Eur Respir J. 1994;7(10):1821-1824.
20. Weissenburger-Moser L, Meza J Yu F et al. A principal factor analysis to characterize agricultural exposures among Nebraska veterans. J Expo Sci Environ Epidemiol. 2016.
21. Vestbo J, Hurd SS, Agusti AG, et al. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease, GOLD executive summary. Am J Respir Crit Care Med. 2012.
22. Brazzale DJ, Hall GL, Pretto JJ. Effects of adopting the new global lung function initiative 2012 reference equations on the interpretation of spirometry. Respiration. 2013;86(3):183-189.
23. Willett WC, Sampson L, Browne ML, et al. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol. 1988;127(1):188-199.
24. Longnecker MP, Lissner L, Holden JM, et al. The reproducibility and validity of a self-administered semiquantitative food frequency questionnaire in subjects from South dakota and Wyoming. Epidemiology. 1993;4(4):356-365.
25. Hunter DJ, Rimm EB, Sacks FM, et al. Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. Am J Epidemiol. 1992;135(4):418-427.
26. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol. 1992;135(10):1114-26; discussion 1127-36.
27. Eck LH, Klesges RC, Hanson CL, et al. Measuring short-term dietary intake: Development and testing of a 1-week food frequency questionnaire. J Am Diet Assoc. 1991;91(8):940-945.
28. Stryker WS, Salvini S, Stampfer MJ et al. Contributions of specific foods to absolute intake and between-person variation of nutrient consumption. J Am Diet Assoc. 1991;91(2):172-178.
29. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the block, willett, and national cancer institute food frequency questionnaires : The Eating at America's Table study. Am J Epidemiol.

2001;154(12):1089-1099.

30. Caan B, Hiatt RA, Owen AM. Mailed dietary surveys: Response rates, error rates, and the effect of omitted food items on nutrient values. Epidemiology. 1991;2(6):430-436.
31. Jain M, Howe GR, Rohan T. Dietary assessment in epidemiology: Comparison on food frequency and a diet history questionnaire with a 7-day food record. Am J Epidemiol. 1996;143(9):953-960.
32. Food and Nutrition Board, Institute of Medicine of the National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: National Academies Press; 2005.
33. Food and Nutrition Board, Institute of Medicine of the National Academies. Dietary, functional, and total fiber. In: National Academy Press, Washington, DC, ed. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids.. ; 2005:339-421.
34. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, manganese, molybdenum, nickel, silion, vandadium, zinc. Washington D.C.: National Academy Press; 2001.
35. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington D.C.: National Academy Press; 2000.
36. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood). 2008;233(6):674-688.
37. Hess D, Keller HE, Oberlin B et al.. Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. Int J Vitam Nutr Res. 1991;61(3):232-238.
38. Andersson I, Gronberg A, Slinde F et al.. Vitamin and mineral status in elderly patients with chronic obstructive pulmonary disease. Clin Respir J. 2007;1(1):23-29.
39. Donaldson GC, Goldring JJ, Wedzicha JA. Influence of season on exacerbation characteristics in patients with COPD. Chest. 2012;141(1):94-100.
40. Shaheen SO, Jameson KA, Robinson SM, et al. Relationship of vitamin D status to adult lung function and COPD. Thorax. 2011;66(8):692-698.
41. Jiang R, Jacobs DR, He K, et al. Associations of dairy intake with CT lung density and lung function. J Am Coll Nutr. 2010;29(5):494-502.
42. Young RP, Hopkins RJ, Marsland B. The gut-liver-lung axis. modulation of the innate immune response and its possible role in chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2016;54(2):161-169.
43. Chuang SC, Norat T, Murphy N, et al. Fiber intake and total and cause-specific mortality in the European Prospective Investigation into Cancer and Nutrition cohort. Am J Clin Nutr. 2012;96(1):164-174.
44. Varraso R, Willett WC, Camargo CA,Jr. Prospective study of dietary fiber and risk of chronic obstructive pulmonary disease among US women and men. Am J Epidemiol. 2010;171(7):776-784.
45. Butler LM, Koh WP, Lee HP et al. Dietary fiber and reduced cough with phlegm: A cohort study insSingapore. Am J Respir Crit Care Med. 2004;170(3):279-287.
46. Kan H, Stevens J, Heiss G et al. Dietary fiber, lung function, and chronic obstructive pulmonary disease in the Atherosclerosis Risk in Communities study. Am J Epidemiol. 2008;167(5):570-578.
47. Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med. 2014;20(2):159-166.
48. Kuo SM. The interplay between fiber and the intestinal microbiome in the inflammatory response. Adv Nutr. 2013;4(1):16-28.
49. Young RP, Hopkins RJ. A review of the hispanic paradox: Time to spill the beans? Eur Respir Rev. 2014;23(134):439-449.
50. Meijer K, de Vos P, Priebe MG. Butyrate and other short-chain fatty acids as modulators of immunity: What relevance for health? Curr Opin Clin Nutr Metab Care. 2010;13(6):715-721.
51. Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461(7268):1282-1286.

52. Kau AL, Ahern PP, Griffin NW, et al. Human nutrition, the gut microbiome and the immune system. Nature. 2011;474(7351):327-336.

53. Marsland BJ. Regulation of inflammatory responses by the commensal microbiota. Thorax. 2012;67(1):93-94.

54. Huffnagle GB. Increase in dietary fiber dampens allergic responses in the lung. Nat Med. 2014;20(2):120-121.

55. Tomodo K, Yamamoto Y, Kamura H. Alternations in gut environment accelerates emphysematous lesions by cigarette smoke in rats discontinuouly fed with a fibre-free diet. Am J Respir Crit Care Med. 2014;189(A3000).

56. Macia L, Thorburn AN, Binge LC, et al. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. Immunol Rev. 2012;245(1):164-176.

57. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. J Am Coll Nutr. 2002;21(6):495-505.

58. Broughton KS, Johnson CS, Pace BK et al. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr. 1997;65(4):1011-1017.

59. Meyer BJ, Mann NJ, Lewis JL et al. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids. 2003;38(4):391-398.

60. Fulton AS, Hill AM, Williams MT et al. Paucity of evidence for a relationship between long-chain omega-3 fatty acid intake and chronic obstructive pulmonary disease: A systematic review. Nutr Rev. 2015;73(9):612-623.

61. Tabak C, Feskens EJ, Heederik D et al. Fruit and fish consumption: A possible explanation for population differences in COPD mortality (the Seven Countries Study). Eur J Clin Nutr. 1998;52(11):819-825.

62. Shaheen SO, Jameson KA, Syddall HE, et al. The relationship of dietary patterns with adult lung function and COPD. Eur Respir J. 2010;36(2):277-284.

63. Varraso R, Fung TT, Barr RG, et al. Prospective study of dietary patterns and chronic obstructive pulmonary disease among US women. Am J Clin Nutr. 2007;86(2):488-495.

64. Schunemann HJ, Grant BJ, Freudenheim JL, et al. The relation of serum levels of antioxidant vitamins C and E, retinol and carotenoids with pulmonary function in the general population. Am J Respir Crit Care Med. 2001;163(5):1246-1255.

65. Morabia A, Menkes MJ, Comstock GW et al. Serum retinol and airway obstruction. Am J Epidemiol. 1990;132(1):77-82.

66. Fukao A, Tsubono Y, Kawamura M, et al. The independent association of smoking and drinking with serum beta-carotene levels among males in Miyagi, Japan. Int J Epidemiol. 1996;25(2):300-306.

67. Brady WE, Mares-Perlman JA, Bowen P et al. Human serum carotenoid concentrations are related to physiologic and lifestyle factors. J Nutr. 1996;126(1):129-137.

68. Baker DL, Krol ES, Jacobsen N et al. Reactions of beta-carotene with cigarette smoke oxidants. identification of carotenoid oxidation products and evaluation of the prooxidant/antioxidant effect. Chem Res Toxicol. 1999;12(6):535-543.

69. Heart Protection Study Collaborative Group. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20,536 high-risk individuals: A randomised placebo-controlled trial. Lancet. 2002;360(9326):23-33.

70. Albanes D, Heinonen OP, Huttunen JK, et al. Effects of alpha-tocopherol and beta-carotene supplements on cancer incidence in the alpha-tocopherol beta-carotene cancer prevention study. Am J Clin Nutr. 1995;62(6 Suppl):1427S-1430S.

71. Rautalahti M, Virtamo J, Haukka J, et al. The effect of alpha-tocopherol and beta-carotene supplementation on COPD symptoms. Am J Respir Crit Care Med. 1997;156(5):1447-1452.

72. Hole DJ, Watt GC, Davey-Smith G et al. Impaired lung function and mortality risk in men and women: Findings from the Renfrew and Paisley prospective population study. BMJ. 1996;313(7059):711-5; discussion 715-6.

73. Thomason MJ, Strachan DP. Which spirometric indices best predict subsequent death from chronic obstructive pulmonary disease? Thorax. 2000;55(9):785-788.