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Effect of Smoking on Serum Vitamin D Levels and the Influence on COPD Symptoms Using NHANES 2007 – 2014 Datasets Among Women of Different Ethnic Backgrounds

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Abstract: Smoking is one of the major causes of chronic obstructive pulmonary disease (COPD). The number of women who smoke is increasing and they are at enhanced risk of developing fatal COPD. Symptoms of COPD include frequent coughing, wheezing, whistling, excess phlegm and shortness of breath. According to National Health Interview Survey (NHIS) data during 1999 to 2011, the prevalence of COPD among non-Hispanic whites was generally higher contrasted with non-Hispanic blacks and Hispanics. Among women the annual age-adjusted prevalence was higher than in men, especially among young women. It is likely that nutritional status of COPD patients may impact the outcome of the disease. Deficient levels of vitamin D is common among COPD patients. The goal of this study is to further investigate a potential correlation between smoking and serum vitamin D levels as it influences COPD. The National Health and Nutrition Examination Survey (NHANES) 2007-2014 datasets were utilized for this study. A 10 ng/mL decrease in vitamin D_3 level was associated with an increased number of wheezing and whistling attacks (relative risk (RR) 1.33 (95% CI (1.03, 1.07), P=0.02). There was no evidence of an association between vitamin D_2 with the number of wheezing and whistling attacks. Although there was no evidence of an association between vitamin D_3 (either lower or higher levels) with bringing up phlegm and coughing, there was marginal evidence P<0.1 of an association between vitamin D_2 with bringing up phlegm and coughing. Conclusion: This data confirmed the previous conclusion from an NHANES 2001-2006 investigation that smoking may still influence vitamin D_3 levels among women. Further research is necessary to better understand whether vitamin D_3 has a positive impact on respiratory health in women because they are more likely to be burdened by the symptoms of COPD.

Keywords: NHANES, Cotinine, Vitamin D, Women, Smoking, Chronic Obstructive Pulmonary Disease (COPD)

1. Introduction

The tobacco epidemic continues to be a global threat to public health. The 23rd, 31st and 33rd World Health Assemblies of World Health Organization (WHO) focused on the effect of tobacco smoking as a growing problem of public health in most of the industrialized as well as the developing countries. Among the risk groups affected by the negative health impacts of smoking are pregnant women, lactating mothers, children, and youth. Over time, increasing evidence has shown that smoking is a primary cause of many health concerns, such as chronic obstructive pulmonary disease (COPD) including chronic bronchitis and emphysema, lung cancer, and several other serious health problems [1-3]. Nicotine in tobacco, is a major component in commercial

products and is extremely addictive. Nicotine is recognized as a general risk factor in many devastating health conditions causing death to millions globally each year. Tobacco smoking also has harmful effects on non-smokers who are exposed to tobacco smoke involuntarily through Second-Hand Smoke (SHS) exposure. Tobacco has also been implicated in adverse health outcomes to those non-smokers causing 1.2 million deaths annually. The National Health Interview Survey 1996-2010 and the Medical Expenditure Panel Survey 1998-2011 data show considerably higher cost of health care associated with obesity and smoking among non-Hispanic white women compared to men, ethnic minorities, and younger counterparts associated with smoking, and it was non-discriminatory across gender, ethnicity, and age [4-6].

After nicotine enters the body, it transforms into its major by-product metabolite called cotinine. Measuring cotinine concentration is the most reliable way of determining the level of exposure to nicotine in both Environmental Tobacco Smoke (ETS) exposed smokers and SHS-exposed non-smokers. It is also the preferred method of measuring nicotine exposure because of its longer half-life, specifically in blood serum (~16 hours) compared to urine, saliva, and hair [7, 8].

Of the one billion smokers of the world, approximately 200 million are women, who are more targeted by the tobacco industry, and they tend to smoke "light" cigarettes more than men. In some countries more investigation is needed to better understand the potential trend toward the increasing use of tobacco among women [9]. The National Center for Health Statistics (NCHS) is part of the Centers of Disease Control and Prevention (CDC) organization. NCHS is the principal health statistics agency in the United States and runs the National Health and Nutrition Examination Surveys (NHANES) program. NHANES is a program that evaluates and reviews the health and nutritional status of populations in the United States. The NHANES survey is unique because its information provides vital and health statistics for the nation by combining the attributes of both interviews and physical examinations. A previous analysis from NHANES data showed that women had higher nicotine metabolite ratios (NMR) in the serum, and lower in the urine compared to their male counterparts among both smokers and non-smokers. The NMR was lower both in urine and serum among both male and female smokers than in non-smokers. Non-Hispanic white women had higher ratios in their serum compared to Hispanics and non-Hispanic blacks. The data also showed that the ratio in urine was higher in non-Hispanic blacks than non-Hispanic whites and Hispanics. These data can be further used to study differences in how nicotine is metabolized by women who are smokers or non-smokers [10]. The 2010 "World No Tobacco Day" sponsored by WHO emphasized the marketing of tobacco in relation to gender. The objective of this event was to further discuss the harmful effects of tobacco marketing toward women [11]. Active smokers and second-hand smokers are more susceptible to COPD incidents than non-smokers, and the number of women smokers developing COPD is rapidly increasing. Therefore, women who smoke are at enhanced risk of potentially developing fatal COPD. In industrialized countries, the prevalence of COPD is now almost as high in women as it is in men [12]. This lung disease is a serious public health problem that makes breathing difficult for 16 million Americans. COPD causes airflow blockage and breathing-related illness, of which millions are undiagnosed and therefore, untreated. In 2008, chronic lower respiratory diseases such as COPD became one of the third major causes of mortality [13, 14]. From 2000-2004, 80% of COPD deaths were attributed to smoking. However, much of this disease is potentially preventable by quitting smoking. But the CDC reports from 2017 showed that the occurrence of COPD was greater among women and older adults than men [15, 16]. Symptoms of COPD include frequent coughing, wheezing, whistling, excess phlegm or mucus, shortness of breath and difficulty taking a deep breath [17]. According to one study that analyzed the National Health Interview Survey (NHIS) data during 1999 to 2011, the prevalence of COPD among non-Hispanic whites contrasted with non-Hispanic blacks and Hispanics was generally increased. In women the annual age-adjusted prevalence was higher than in men, especially among young women [18]. A few other studies showed that a high proportion of smoking-related pulmonary patients with COPD falsely declared themselves to be non-smokers, and those who claimed to be non-smokers continued to smoke. The accurate classification of smoking status is vital to the treatment of lung diseases [19, 20]. However, not only cigarette smoking but SHS exposure could also affect the progression of the disease as well. Yet, the importance of the impact of exposure on COPD health outcomes remain unclear [21].

It is likely that nutritional status of COPD patients impacts the outcome of the disease. One element of the nutritional status is the amount of saturated fat intake. The correlation between lung function and fat intake is unclear and remains to be determined. As vitamin D is a known fat-soluble substrate, the results from a NHANES 2007-2012 study previously demonstrated benefits of increased demand for macronutrient substrates with potential anti-inflammatory activity. Overall intakes of fat in COPD patients could theoretically benefit the individuals. It was observed that an increase in lung function was associated with saturated fatty acids (SFA) intake in individuals with COPD. However, specific associated mechanisms are not yet identified [22]. Previously it was shown that vitamin D intake was associated with lower lung cancer risk in non-smoking, post-menopausal women [23]. It also has been observed that low levels of vitamin D and its deficiency are common among COPD patients, and women who currently smoke have lower dietary intakes of vitamin D than non-smokers [24, 25].

Vitamin D can be either acquired from dietary sources or produced in the body (Figure 1).

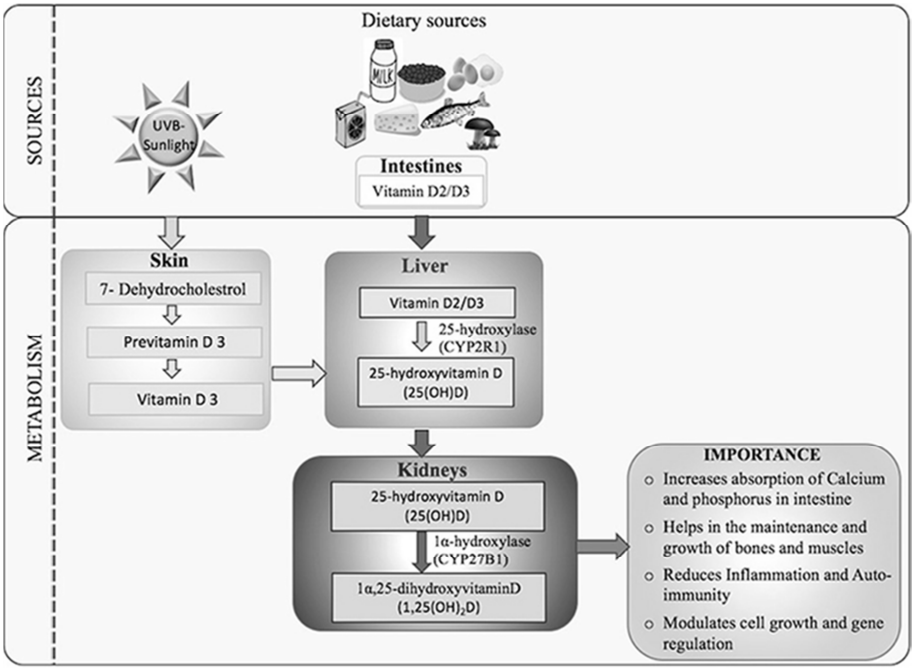

Figure 1. *Sources of Vitamin D and its activation pathway. (Reference: Singh, P., Kumar, M., & Al Khodor, S. (2019). Vitamin D Deficiency in the Gulf Cooperation Council: Exploring the Triad of Genetic Predisposition, the Gut Microbiome and the Immune System. Frontiers in immunology, 10, 1042. https://doi.org/10.3389/fimmu.2019.01042).*

Dietary vitamin D is available in two forms, vitamin D_2 (ergocalciferol) from botanical sources and vitamin D_3 (cholecalciferol) from animal sources, both of which are collectively labeled as vitamin D. However, foods containing natural vitamin D and dietary sources generally account for a small amount of the total vitamin in the body. The primary source of vitamin D in the form of vitamin D_3 can be produced from its non-enzymatic dermal synthesis from 7-dehydrocholestrol in the skin by exposure to ultraviolet (UV) light, mainly from light in UV-B range (280-315 nm) [26, 27]. All the modern-day medical innovations notwithstanding, the deficiency of vitamin D continues to be a global pandemic affecting citizens in both developed and developing countries. The deficiency of the vitamin not only causes bone disorders and fractures in adults, but it also has been associated with other diseases such as cancers, autoimmune diseases, hypertension, and infectious disease as well as respiratory diseases [28, 29].

Brot et al., (1999) performed a cross-sectional study in Denmark on 45 to 58-year-old women (n=510) with different smoking lifestyles that showed the effect of smoking on serum vitamin D. The smokers had significantly decreased levels of the serum vitamin D metabolite, $25(OH)D_3$, suggesting that smoking holds a considerable influence on the vitamin metabolism [30]. Cutillas-Marco et al., (2012), in a cross-sectional investigation, revealed, that smoking was associated with the risk of vitamin D deficiency among southern European Caucasian men and women participants (n=177). The mechanism that explains the effects of cigarette smoking on vitamin D metabolism remains unclear, but it eventually exacerbates bone loss in smokers [31]. The result of NHANES-III from 1988 to 1994 data analyses conducted by Black et al., (2005) regarding the association of vitamin D levels and pulmonary function in people over the age of 20-years old (*n*=14,091). The study showed a strong relationship between serum levels of vitamin D and the volume of air one can exhale (pulmonary function) [32]. There have been other studies that demonstrated the beneficial effects of vitamin D on respiratory health. One study that analyzed NHANES 2001-2006 data showed a significant association of lower vitamin D levels with respiratory symptoms and with COPD [33]. In Belgium, studies done by Janssens et al.,. (2009) (n=414) and Mekove et al., (2016) (n=152) revealed that vitamin D deficiency occurs frequently in COPD patients and correlated with the disease severity as well. Patients admitted to hospital with COPD for exacerbation are a risk group for vitamin D deficiency and insufficiency with stronger prevalence in women than in men [34, 35]. Many epidemiological and experimental investigations have shown possible effects of smoking on vitamin D; however, none of these studies used the serum levels of cotinine as a marker for smoking. Because the concept that vitamin D is associated with chronic disease not involving calcium metabolism is comparatively new, little is understood about the potential mechanisms relating the vitamin to the disease.

According to Mercy Medical Center's report in 2018, approximately 42% of the US residents are vitamin D deficient, with some people such as pre-menopausal female smokers, those with poor nutrition habits, people at the age of 65 and older, non-Hispanic whites who avoid even minimal sun exposure, and those who take prescription medication long term for heartburn, acid reflux, and constipation having even higher levels of deficiency [36]. A NHANES study in 2015 indicated that there was a potential correlation between

the levels of cotinine and vitamin D among women with different ethnic backgrounds. There was a statistically significant and negative relationship between these metabolites. The correlation is higher for non-Hispanic white and non-Hispanic black women with a higher correlation compared to Hispanic and other ethnicities (Table 1).

Table 1. *Correlation between Cotinine and Vitamin D among Women.*

Sample	n	Correlation Coefficient (Rho)	P	Significance
Total	4,272	-0.047	<.01	**
Non-Hispanic White	1,877	-0.119	<.0001	***
Non-Hispanic Black	956	-0.123	<.01	**
Hispanic	1,241	-0.001	0.967	
Other	198	0.046	0.519	
Note: *P<.05, **P<.01, ***P<.001				

Note: *P<.05, **P<.01, ***P<.001.

The study demonstrated that non-Hispanic black women had lower vitamin D (13.3 ng/mL) levels than Hispanic (19.2 ng/mL) or white (24.9 ng/mL) women and the low vitamin D correlated to higher levels of cotinine. Therefore, increased blood serum levels of cotinine, or a higher rate of smoking, may have contributed to decreased vitamin D levels in addition to other known factors such as diet, amount of sun exposure, gender, and ethnicity [37].

The purpose of this investigation is to analyze the effect of smoking on vitamin D status and COPD symptoms by using serum cotinine as the biomarker of tobacco use. In addition to the strong evidence presented in previous epidemiological studies, this investigation will propose a potential answer to this question: *How are cotinine and vitamin D levels influencing symptoms of COPD?*

2. Materials & Methods

The continuous NHANES surveys from 2007 to 2014 collected data in two-year cycles (NHANES 2007-2008, NHANES 2009-2010, NHANES 2011-2012, and NHANES 2013-2014). The continuity allocates annual statistical estimates for broad groups and specific ethnicity groups as well as flexibility in the content of the questionnaires and exam components. Each cycle has data sets from participants, which includes demographic data (Gender, Age, Ethnicity), laboratory data (vitamin D "vitamin D_2, D_3, total vitamin D" and cotinine) and other data such as diet behavior/nutrition, and respiratory health questionnaire data (Table 2).

Table 2. *NHANES Datasets and Variables of interest.*

Years	Laboratory Data	Questionnaire Data	Demographics Data
2007-2014	Total Vitamin D: (25OHD2)+(25OHD3) (nmol/L) Vitamin D3 (nmol/L)(25OHD3) Vitamin D2 (nmol/L)(25OHD2) Cotinine (ng/mL)	Respiratory Health: #wheezing or whistling attacks past year (RDQ080) Bringing up phlegm (mucus) most days (3 months period) – (RDQ050) Coughing most days (over 3 months period) – (RDQ031)	Gender Age Ethnicity

In the ethnicity category there were both Mexican-Americans and other Hispanics, these two were combined to represent the Hispanics category. The entire NHANES dataset is publicly available and no individuals can be linked to any records.

All the eight years of continuous datasets were merged to create a single file for the purpose of statistical analyses. Each participant is assigned a unique identifier known as Sequence Number (SEQN), which assures that all the observations and results are properly linked for each participant. After merging, the data were reviewed for those participants that had missing data (such as refused to answer, or the response was "don't know"), since the missing data may distort the analysis results.

The values of vitamin D_2 and D_3 were converted from nmol/L to ng/mL following the recommended conversion equation by CDC below for parallel comparison with cotinine units in ng/mL (Table 3).

Table 3. *Conversion of Vitamin D values from nmol/L to ng/mL.*

Conversion (D3 "25OHD3"):	1 nmol/L = 0.40066 ng/mL
Conversion (D2 "25OHD2"):	1 nmol/L = 0.41266 ng/mL

The final sample size for this investigation is n=5729 women (Non-Hispanic White n=2274, Non-Hispanic Black n=1175, Hispanic n=1585, and other races, including multi-racial n=695) (Table 4).

Table 4. *Number and Percentage of Participants by the Ethnicity 2007-2014.*

Ethnicity	Number of Participants (n)	Total Percentage (%)
Non-Hispanic White	2274	39.69
Non-Hispanic Black	1175	20.51
Hispanics	1585	27.67
Others	695	12.13
Total:	5729	100

The participants were further sub categorized based on the total blood serum concentrations of their vitamin D and cotinine. Based on the cotinine levels, they were categorized into three groups of Non-smokers/Minor SHS ([Cotinine] <1 ng/mL), Light-smokers/Some SHS (1 ng/mL<[Cotinine]<70 ng/mL) and Heavy-smokers/Major SHS ([Cotinine]>70 ng/mL) according to the Salimetrics® (Table 5).

Table 5. *Number and Percentage of Participants by the Cotinine (Smoking) Status.*

Smoking & Exposure Status	Number of Participants (n)	Total Percentage (%)
Non-smokers/Minor SHS – [Cotinine] <1 ng/mL	3774	71.36
Light-smokers/Some SHS - 1<[Cotinine] <70 ng/mL	468	8.85
Heavy-smokers/Major SHS - [Cotinine] >70 ng/mL	1047	19.8
Total:	5289	100

Also, based upon the vitamin D status, they were categorized into the three groups of deficient, inadequate, and sufficient based on the National Institute of Health (NIH) Office of Dietary Supplements (Table 6).

Table 6. *Number and Percentage of Participants by the Vitamin D Status.*

Vitamin D Status	Number of Participants (n)	Total Percentage (%)
Deficient (<12 ng/mL)	555	10.85
Inadequate (12<[Vit.D]<20 ng/mL)	1393	27.23
Sufficient (20<[Vit.D]<50 ng/mL)	3041	59.45
(Over Upper-Limit)	(126)	(2.46)
Total:	5115	100

Analysis of Covariance (ANCOVA)[1] model was employed to include the continuous variable in addition to the variables of interest, both the dependent and independent variables, as means for control.

Scheffe's method[2] was used to test all possible contrasts at the same time. It applies to the set of all possible contrasts among the factor level means, not just the pairwise differences considered by Tukey's method[3], which it applies simultaneously to the set of all pairwise comparisons. Binomial regression linear model[4] was used to predict the odds of seeing an event, given a vector of regression variables. The Bonferroni [5] test is a type of multiple comparison method used in statistical analysis. It is a test method used to reduce the instance of a false positive. The family-wise error rate 6 was also used to determine the probability of making at least one false conclusion.

Relative Risk (RR)[7] is often used when the study involves comparing the likelihood, or chance, of an event occurring between two groups. Relative Risk is considered a descriptive statistic, not an inferential statistic as it does not determine statistical significance. Relative Risk utilizes the probability of an event occurring in one group compared to the probability of an event occurring in the other group. It requires the examination of two dichotomous variables, where one variable measures the event (occurred vs. not occurred) and the other variable measures the groups (group 1 vs. group 2).

3. Results

A negative binomial generalized linear model was constructed regressing the self-reported number of wheezing and whistling attacks that participants experienced against vitamin D_3 levels. The model included adjustment for subject age, ethnicity, cotinine level, diet behavior and nutrition. Bonferroni's method was employed to control the family-wise error rate for comparisons among subgroups in the model. The hypothesis of interest was that there is a significant association between COPD symptoms and vitamin D levels, after adjusting for other factors that might alter COPD symptoms. The cotinine concentration is in the model as a proxy variable for smoking status. This variable allows for the assertion of an association between vitamin D levels and COPD symptoms independent of smoking status. The need for adjustment using cotinine is represented by the Directed A-cyclic Graph (DAG) below (Figure 2):

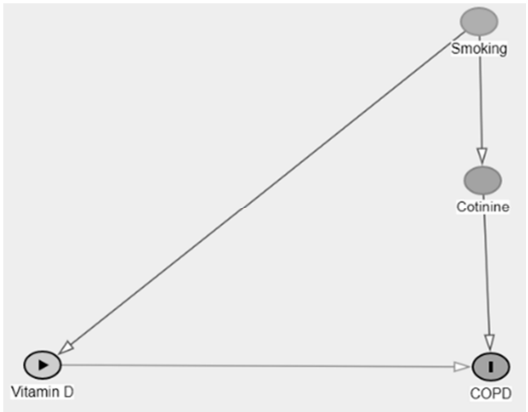

Figure 2. *Directed A-cyclic Graph (DAG).*

1 https://www.statisticssolutions.com/general-uses-of-analysis-of-covariance-ancova/

2 https://www.itl.nist.gov/div898/handbook/prc/section4/prc472.htm

3 https://www.itl.nist.gov/div898/handbook/prc/section4/prc471.htm

4 https://towardsdatascience.com/the-binomial-regression-model-everything-you-need-to-know-5216f1a483d3

5 https://www.investopedia.com/terms/b/bonferroni-test.asp

6 https://www.statisticshowto.com/familywise-error-rate/

7 https://www.statisticssolutions.com/relative-risk/

The arrow connecting vitamin D to COPD is the hypothesis of interest. There is an established relationship between smoking and cotinine concentration, and the ANCOVA model suggests a relationship between smoking and vitamin D. If some measure of smoking status (i.e., cotinine concentration) were not included in the model, it would be unclear whether the change in COPD symptoms were associated with vitamin D levels or due to a latent smoking variable which was also altering vitamin D status. With cotinine concentration in the model it can be said that, even with taking smoking status into account, vitamin D appears to present a relationship with COPD symptoms. Among the total group of the participants 546 individuals had measurements available and were included in the model. Vitamin D_3 showed a protective effect against wheezing and whistling; a 10 ng/mL decrease in vitamin D_3 was associated with an increased risk of wheezing and whistling attack (relative risk (RR) 1.33 (95% CI (1.03, 1.07), P=0.02)). No effect of vitamin D_2 status on RR of wheezing and whistling attacks were identified. A plot of expected wheezing and whistling attacks by vitamin D_3 level is shown in the figure below (Figure 3).

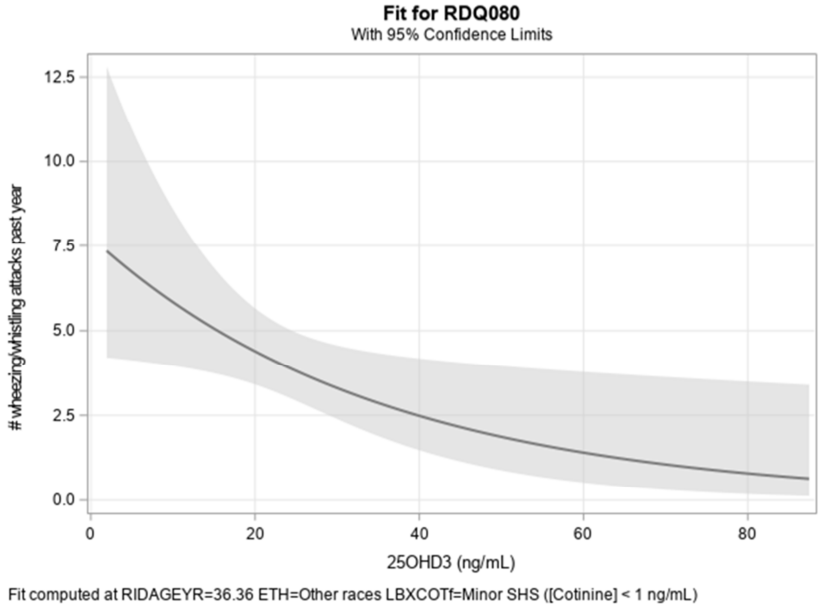

Figure 3. *Number of wheezing & whistling attacks versus serum vitamin D_3 levels.*

Given the SAS application limitation in calculating the CI, instead Relative Risk (or rate ratio "IRR") was used to measure the strength of the association between vitamin D_3 and number of wheezing and whistling attacks in the past year.

$$IRR(D_3) = 0.97197 \geq (1/0.97197)^{10} = 1.328 \text{ IRR for 10 unit decrease in vitamin } D_2$$

$$\text{CI: lower bound} = (1/\exp(-0.0034))^{10} = 1.034, \text{ upper bound} = (1/\exp(-0.0535))^{10} = 1.707$$

Per the outcome, there appears to be association between vitamin D_3 levels and number of wheezing and whistling attacks, which means the participants with lower vitamin D_3 levels experienced higher numbers of wheezing and whistling attacks. There was observational evidence that vitamin D_3 was associated with an increase in number of wheezing and whistling attacks after adjusting for smoking status, age, diet, and ethnicity. Although there was no evidence of association between vitamin D_3 (either lower or higher levels) with bringing up phlegm most days and coughing most days, there was marginal evidence (P<0.1) of an association between vitamin D_2 with bringing up phlegm and coughing. If more datapoints were available, we may have observed a stronger association; but there was no evidence of association between vitamin D_2 with number of wheezing and whistling attacks. A negative binomial model to account for overdispersion of the data and considered some other secondary effect combinations, which improved the fit. It still appears that there is an effect of ethnicity on the protective effect of vitamin D_3 against whistling and wheezing episodes although after controlling for multiple testing, it is not possible to identify exactly the source of this relationship.

As for the heavy-smoker/Major SHS vs non-smoker/Minor SHS vitamin D_3 levels, the mean is indeed lower in the Major SHS group (mean [D_3] = 23.8 ng/mL) vs Minor SHS (mean [D_3] = 23.96 ng/mL). The difference in the means appears to be the result of more non-Hispanic white participants in the heavy-smoker/Major SHS vs non-smoker/Minor SHS. Hence the higher levels of vitamin D_3 observed on average suggest that there are other factors that influence D_3 (ethnicity, age, diet). It is important to account for the differences in these variables among the groups before considering the vitamin D_3 levels. Therefore, we analyzed the effect of smoking groups on vitamin D_3 using a linear model. Data also show, most critically, that the heavy-smoker/Major SHS group is (59.98% non-Hispanic white) vs. non-smoker/Minor SHS

group (35.06% non-Hispanic white), (Table 7).

Table 7. *Ethnicity Distribution Among the Smoking Groups.*

Smoking & Exposure Status	Ethnicity	Ethnicity	Ethnicity	Ethnicity
Non-smokers/Minor SHS	Hispanics (33.41%)	Non-Hispanic White (35.06%)	Non-Hispanic Black (17.62%)	Other Ethnicities (13.91%)
Light-smokers/Some SHS	Hispanics (23.93%)	Non-Hispanic White (37.82%)	Non-Hispanic Black (29.70%)	Other Ethnicities (8.55%)
Heavy-smokers/Major SHS	Hispanics (10.89%)	Non-Hispanic White (59.98%)	Non-Hispanic Black (23.21%)	Other Ethnicities (5.92%)

The conclusion is that the mean [vitamin D_3] among heavy smokers is significantly higher than among non-smokers for the Non-Hispanic White cohort. The difference in the number of people in each ethnic group does impact the statistical analysis but not in a way that we have identified would bias the effect estimates. The manner in which the unequal sample sizes between ethnic groups impacts the analysis is with respect to power: The power to detect a significant difference in mean [vitamin D_3] among heavy smokers vs. non-smokers is reduced as the sample size decreases. Hence, we would expect that the power to detect a difference would be lowest for the Other Ethnicities group and highest for the Non-Hispanic White group. It is possible that, given a larger sample of non-Hispanic White subjects, other interesting relationships might have been identified.

For analysis of a particular ethnic group, the measurements and number of people in other ethnic groups do not impact the statistical estimates used for the difference test. Therefore, it does not appear bias should be present due to imbalanced ethnic groups for these effect estimates. Ethnicity has a considerable influence on total vitamin D_3 values (Table 8).

Table 8. *Mean and Standard Error Vitamin D_3 Comparison based on the Ethnicity.*

Ethnicity	Vitamin D_3 Mean (ng/mL)	Standard Error
Hispanic	21.17	1.6
Non-Hispanic White	29.62	1.6
Non-Hispanic Black	16.42	1.6
Other Ethnicities	21.72	1.7

4. Discussion

Vitamin D can be obtained from different sources in two forms, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Thus, we have a combination of both available to us as part of our lifestyle, either from sunlight exposure, diet and dietary supplement, and fortified foods. Vitamin D in the form of vitamin D_3 can be made from 7-dehydrocholestrol in the skin by exposure to ultraviolet light, mainly from light in UVB spectrum. Biancuzzo et al.. (2013) showed that vitamin D_2 and D_3 both were effective in raising and maintaining the total serum concentration of 25-OH-D. Vitamin D_2 is metabolized in a fashion similar to that of vitamin D_3 to both its 25-hydroxy and 1,25-dihydroxy metabolites [38, 39]. Therefore, as the data showed here, the reason that cotinine affects vitamin D_3 more than vitamin D_2 may be primarily because of the minor chemical structural difference between the two forms. Another reason that it was observed cotinine has more effect on decreasing vitamin D_3 level compared to vitamin D_2, may also be due to the explanation by a previous study which showed that vitamin D_3 is more effective than vitamin D_2 at raising total 25-OH-D levels and most assays of serum vitamin D do not distinguish between the two vitamers [40].

Cigarette-smoking behavior differs substantially by race and gender in the United States among Non-Hispanic blacks and whites as demonstrated by the data analyses from National Youth Tobacco Survey 2004-2013, National Survey on Drug Use and Health 2002-2013, National Health Interview Survey 2004-2013 and NHANES 2001-2012 [41]. As previously reported in one study, higher levels of cotinine in blood per cigarette smoked by blacks compared with whites was explained by both slower clearance of cotinine and higher intake of nicotine per cigarette in blacks. Greater nicotine uptake (30% greater nicotine uptake in blacks than whites), and higher cotinine half-life in blacks than in whites, and therefore greater tobacco effects per cigarette could, in part, explain some of the ethnic differences in smoking-related disease risks [42].

In COPD patients, nutritional status is a well-recognized predictive indicator and poor nutrition was characterized by loss of fat-free body mass or muscle wasting. Many of the COPD patients are in a state of hyper metabolism, which expends more calories per kilogram, potentially caused by the increased work of breathing [43-45]. A previous study showed an association between an increased risk of vitamin D deficiency and COPD, and the characteristics of the disease were significantly related to 25(OH)D levels [46].

Prior investigations have shown that vitamin D has potentially positive effects on respiratory health. One such study that analyzed data from NHANES 2001-2006 showed that lower values of vitamin D were significantly associated with respiratory symptoms and with COPD [47]. Another study that analyzed NHANES 2001-2010 data also showed an association between vitamin D insufficiency and wheezing in adults after stratifying the analysis by ethnicity and current smoking**.** Vitamin D insufficiency was associated with current wheeze in non-Hispanic white and black females [48]. Women, because they are more likely to be significantly burdened by the symptoms of COPD than men, may benefit from more intensive and earlier management of COPD symptoms, specifically wheezing, and this appears most pronounced among young women [49]. Evidence from researchers in China has shown that increased risk of COPD and its severity were inversely associated with vitamin D deficiency, but COPD susceptibility and exacerbation were not associated with low vitamin D serum levels [50, 51]. Study from Sweden revealed that in COPD patients the seasonal

levels of annual vitamin D (late summer to early fall) were significantly lower compared with lung healthy control subjects. Therefore, those authors suggested to monitor vitamin D status more routinely among the patients [52].

The data analyzed in the present study showed that an increase in cotinine concentration is associated with a decrease in total vitamin D among women. There was evidence of an association between cotinine and vitamin D_3, but no evidence was observed as an association with vitamin D_2. However, elevated cotinine levels are associated with a decrease in total vitamin D levels. There is evidence of an association between vitamin D_3 and RDQ080 (lower vitamin D_3 means higher numbers of coughing and wheezing episodes) after adjusting for smoking status, age, diet, and ethnicity. There is no evidence of an association between vitamin D_2 and number of wheezing or whistling attacks. We observed marginal evidence (P<0.1) of an association between vitamin D_2 and bringing up phlegm (mucus) and coughing most days.

5. Conclusion

In addition to cotinine, other factors may affect vitamin D concentrations such as gender, ethnicity, dietary supplement intake and sun exposure. These data calculated from NHANES 2007-2014 confirmed the previous conclusion from NHANES 2001-2006 investigation that smoking affects vitamin D levels among women. Further research is necessary to better understand whether vitamin D exerts beneficial effects on respiratory health in women since they are more likely to be significantly burdened by the symptoms of COPD. Also, further investigations will be needed to examine the effects of vitamin D dietary supplementation on COPD risk, as currently there is limited evidence available. Vitamin D_3 showed a protective effect against wheezing and whistling; a 10 ng/mL decrease in vitamin D_3 was associated with an increased risk of wheezing and whistling attacks (relative risk (RR) 1.33 (95% CI (1.03, 1.07), P=0.02)). No effect of vitamin D_2 status on RR of wheezing and whistling attacks were identified. Per the outcome, there appears to be association between vitamin D_3 levels and number of wheezing and whistling attacks, which means the participants with lower vitamin D_3 levels experienced higher numbers of wheezing and whistling attacks. There was observational evidence that vitamin D_3 was associated with an increase in number of wheezing and whistling attacks after adjusting for smoking status, age, diet, and ethnicity. Although there was no evidence of association between vitamin D_3 (either lower or higher levels) with bringing up phlegm most days and coughing most days**,** there was marginal evidence (P<0.1) of an association between vitamin D_2 and no evidence of an association between vitamin D_2 with number of wheezing and whistling attacks.

Disclosure

The authors declare that they have no competing interests.

IRB Protocol# 23688 - The study was found to be exempt from IRB review by the NCSU IRB office.

References

[1] World Health Organization (WHO) - Tobacco Free Initiative (TFI). (2020, June 17). WHA33.35 WHO's program on smoking and health. Retrieved from https://www.who.int/tobacco/framework/wha_eb/wha33_35/en/

[2] World Health Organization (WHO) - Tobacco Free Initiative (TFI). (2020, June 17). WHA31.56 Health hazards of smoking. Retrieved from https://www.who.int/tobacco/framework/wha_eb/wha31_56/en/

[3] World Health Organization (WHO) - Tobacco Free Initiative (TFI). (2020, June 17). WHA23.32 Health Consequences of Smoking. Retrieved from https://www.who.int/tobacco/framework/wha_eb/wha23_32/en/

[4] World Health Organization (WHO). (2020, June 17). Tobacco. Retrieved from https://www.who.int/health-topics/tobacco#tab=tab_1

[5] World Health Organization (WHO). (2020, May 27). Tobacco. Retrieved from https://www.who.int/news-room/fact-sheets/detail/tobacco

[6] An, R. (2015). Health care expenses in relation to obesity and smoking among U.S. adults by gender, race/ethnicity, and age group: 1998–2011. *Public Health*, *129* (1), 29–36. doi: 10.1016/j.puhe.2014.11.003.

[7] Centers for Disease Control and Prevention (CDC). (2017, April 7). National Biomonitoring Program - Cotinine Factsheet. Retrieved from https://www.cdc.gov/biomonitoring/Cotinine_FactSheet.html

[8] Centers for Disease Control and Prevention (CDC). (2017, April 7). National Biomonitoring Program - Biomonitoring Summary: Cotinine. Retrieved from https://www.cdc.gov/biomonitoring/Cotinine_BiomonitoringSummary.html

[9] World Health Organization (WHO). (2010, May 30). 10 Facts on gender and tobacco - Gender equality is good for health. Retrieved from https://www.who.int/gender/documents/10facts_gender_tobacco_en.pdf

[10] Ram, J. B. (2020). Nicotine metabolite ratios in serum and urine among US adults: variations across smoking status, gender and race/ethnicity. *Biomarkers*, *25* (1), 27–33. doi: 10.1080/1354750X.2019.1688866.

[11] World Health Organization (WHO) - Tobacco Free Initiative (TFI). (2010, May 31). World No Tobacco Day 2010 - Theme: Gender and tobacco with an emphasis on marketing to women. Retrieved from https://www.who.int/tobacco/wntd/2010/announcement/en/

[12] World Health Organization (WHO). (2008). *WHO Report on the Global Tobacco Epidemic - The MPOWER package*. Geneva, Switzerland: WHO.

[13] Centers for Disease Control and Prevention (CDC). (2018, June 6). Chronic Obstructive Pulmonary Disease (COPD) - What is COPD? Retrieved from https://www.cdc.gov/copd/index.html

[14] Kochanek, K. D., Xu, J., Murphy, S. L., Miniño, A. M., & Kung, H.-C. (2011). National Vital Statistics Reports. Deaths: Preliminary Data for 2009. *Division of Vital Statistics*, *59* (4), 1–51. Retrieved from https://www.cdc.gov/nchs/data/nvsr/nvsr59/nvsr59_04.pdf?fbclid=IwAR3SFEndKVF7l-RpvOgSHjudzT4XmLQu8wyQbknhdK1JdIIQ3n62REZcrcc

[15] Centers for Disease Control and Prevention (CDC). Smoking attributable mortality, years of potential life lost, and productivity losses—United States, 2000-2004. *MMWR Morb Mortal Wkly Rep*. 2008; 57 (45): 1226-1228.

[16] Centers for Disease Control and Prevention (CDC). (2019, June 20). Morbidity and Mortality Weekly Report (MMWR) - Chronic Obstructive Pulmonary Disease and Smoking Status — United States, 2017. Retrieved from https://www.cdc.gov/mmwr/volumes/68/wr/mm6824a1.htm

[17] Centers for Disease Control and Prevention (CDC). (2020, June 9). Chronic Obstructive Pulmonary Disease (COPD) - COPD: Symptoms, Diagnosis, and Treatment. Retrieved from https://www.cdc.gov/copd/features/copd-symptoms-diagnosis-treatment.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Ffeatures%2Fcopd-awareness-month%2Findex.html

[18] Ford, E. S., Croft, J. B., Mannino, D. M., Wheaton, A. G., Zhang, X., & Giles, W. H. (2013). COPD Surveillance-United States, 1999-2011. *Chest*, *144* (1), 284–305. doi: 10.1378/chest.13-0809.

[19] Stelmach, R., Arrabal Fernandes, F. L., Carvalho-Pinto, R. M., Athanazio, R. A., Rached, S. Z., Prado, G. F., & Cukier, A. (2015). Comparison between objective measures of smoking and self-reported smoking status in patients with asthma or COPD: are our patients telling us the truth? The Brazilian Journal of Pulmonology and International Databases, 41 (2), 124–132. doi: 10.1590/S1806-37132015000004526.

[20] Centers for Disease Control and Prevention (CDC) (2012). Chronic obstructive pulmonary disease among adults--United States, 2011. *MMWR. Morbidity and mortality weekly report*, *61* (46), 938–943.

[21] Eisner, M. D., Balmes, J., Yelin, E. H., Katz, P. P., Hammond, K., Benowitz, N., & Blanc, P. D. (2006). Directly measured secondhand smoke exposure and COPD health outcomes. BMC Pulmonary Medicine, 6 (12), 1–11. doi: 10.1186/1471-2466.

[22] Cornell, K., Alam, M., Lyden, E., Wood, L., LeVan, T. D., Nordgren, T. M., … Hanson, C. (2019). Saturated Fat Intake Is Associated with Lung Function in Individuals with Airflow Obstruction: Results from NHANES 2007–2012. *Nutrients*, *11* (2), 317–329. doi: 10.3390/nu11020317.

[23] Cheng, T.-Y. D., LaCroix, A. Z., Beresford, S. A., Goodman, G. E., Thornquist, M. D., Zheng, Y., … Neuhouser, M. L. (2013). Vitamin D intake and lung cancer risk in the Women's Health Initiative. *The American Journal of Clinical Nutrition*, *98* (4), 1002–1011. doi: 10.3945/ajcn.112.055905.

[24] Rutten, E., Franssen, F. M., Vanfleteren, L., Spruit, M. A., Hofstra, T., Ubachs, T., … Reynaert, N. (2015). SUN-LB006: Skin Autofluorescence is a Determinant for Vitamin D in Women with COPD. *Clinical Nutrition*, *34* (1), S237. doi: 10.1016/S0261-5614(15)30727-5.

[25] Morabia, A., Bernstein, M. S., & Antonini, S. (2000). Smoking, dietary calcium and vitamin D deficiency in women: A population-based study. *European Journal of Clinical Nutrition, 54* (9), 684-9. doi: 10.1038/sj.ejcn.1601074.

[26] Dixon, K. M., & Mason, R. S. (2009). Molecules in focus Vitamin D. *The International Journal of Biochemistry & Cell Biology*, *41*, 982–985. doi: 10.1016/j.biocel.2008.06.016.

[27] Holick, M. F. (2004). Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *American Journal of Clinical Nutrition*, *79*, 362–371. doi: 10.1093/ajcn/79.3.362.

[28] Holick, M. F., & Chen, T. (2008). Vitamin D deficiency: a worldwide problem with health consequences. *American Journal of Clinical Nutrition*, *87* (4), 1080S–1086S. doi: 10.1093/ajcn/87.4.1080S.

[29] Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N et al.,. Vitamin D and Chronic Diseases. Aging and disease. (2017); 8 (3): 346-353. doi: 10.14336/AD.2016.1021.

[30] Brot, C., Jorgensen, N. R., & Sorensen, O. H. (1999). The influence of smoking on vitamin D status and calcium metabolism. European Journal of Clinical Nutrition, 53, 920–926. doi: 0.1038/sj.ejcn.1600870.

[31] Cutillas-Marco, E., Fuertes-Prosper, Amparo, Grant, W. B., & Morales-Suárez-Varela, M. (2012). Vitamin D deficiency in South Europe: effect of smoking and aging. Photodermatology Photoimmunology and Photomedicine, 28 (3), 159–161. doi: 10.1111/j.1600-0781.2012.00649.x.

[32] Black, P. N., & Scragg, R. (2005). Relationship Between Serum 25-Hydroxyvitamin D and Pulmonary Function in the Third National Health and Nutrition Examination Survey. American College of Chest Physicians, 128 (6), 3792–3798. doi: 10.1378/chest.128.6.3792.

[33] Hendryx, M., & Luo, J. (2015). A test of vitamin D benefits on respiratory health mediated through inflammatory markers. *Chronic Respiratory Disease*, *12* (1), 24–30. doi: 10.1177/1479972314556086.

[34] Janssens, W., Bouillon, R., Claes, B., Carremans, C., Lehouck, A., Buysschaert, I., … Lambrechts, D. (2009). Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax, 65 (3), 205–220. doi: 10.1136/thx.2009.120659.

[35] Mekov, E., Slavova, Y., Tsakova, A., Genova, M., Kostadinov, D., Minchev, D., … Tafradjiiska, M. (2015). Vitamin D Deficiency and Insufficiency in Hospitalized COPD Patients. PLOS One, 10 (6), 1–14. doi: 10.1371/journal.pone.0129080.

[36] Mercy Medical Center - A ministry of the Sisters of Charity Health System. (2018, July 1). 42% Percent of Americans Are Vitamin D Deficient. Are You Among Them? Retrieved from https://www.cantonmercy.org/healthchat/42-percent-of-americans-are-vitaminD-deficient/#:~:text=Unfortunately%2C%20about%2042%25%20of%20the,take%20prescription%20medication%20long%20term

[37] Manavi K, Alston-Mills B, Thompson M, Allen J. Effect of serum cotinine on vitamin D serum concentrations among American females with different ethnic backgrounds. Anticancer Res. 2015; 35 (2): 1211-1218.

[38] Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Ross, A. C., Taylor, C. L., Yaktine, A. L., & Del Valle, H. B. (Eds.). (2011). *Dietary Reference Intakes for Calcium and Vitamin D*. National Academies Press (US).

[39] Biancuzzo, R. M., Clarke, N., Reitz, R. E., Travison, T. G., & Holick, M. F. (2013). Serum Concentrations of 1,25-Dihydroxyvitamin D_2 and 1,25-Dihydroxyvitamin D_3 in Response to Vitamin D_2 and Vitamin D_3 Supplementation. *The Journal of Clinical Endocrinology & Metabolism*, *98* (3), 973–979. doi: 10.1210/jc.2012-2114.

[40] Tripkovic, L., Wilson, L. R., & Lanham-New, S. A. (2017). Vitamin D_2 vs. vitamin D_3: They are not one and the same. *British Nutrition Foundation - Nutrition Bulletin*, *42*, 331–337. doi: 10.1111/nbu.12293.

[41] Caraballo, R. S., Sharapova, S. R., & Asman, K. J. (2016). Does a Race-Gender-Age Crossover Effect Exist in Current Cigarette Smoking Between Non- Hispanic Blacks and Non-Hispanic Whites? United States, 2001–2013. *Nicotine & Tobacco Research*, *18* (1), S41–S48. doi: 10.1093/ntr/ntv150.

[42] Pérez-Stable, E. J., Herrera, B., Jacob III, P., & Benowitz, N. L. (1998). Nicotine Metabolism and Intake in Black and White Smokers. *The Journal of the American Medical Association*, *280* (2), 152–156. doi: 10.1001/jama.280.2.152.

[43] Jones, P., Lucock, M., Veysey, M., & Beckett, E. (2018). The vitamin D-folate hypothesis as an evolutionary model for skin pigmentation: An update and integration of current ideas. *Nutrients, 10* (5), 554. doi: http://dx.doi.org.prox.lib.ncsu.edu/10.3390/nu10050554

[44] Schols AM, Fredrix EW, Soeters PB, Westerterp KR, Wouters EF. Resting energy expenditure in patients with chronic obstructive pulmonary disease. Am J Clin Nutr. 1991; 54: 983–7.

[45] Rawal, G., & Yadav, S. (2015). Nutrition in chronic obstructive pulmonary disease: A review. *Journal of translational internal medicine*, *3* (4), 151–154. https://doi.org/10.1515/jtim-2015-0021

[46] Persson, L. J. P., Aanerud, M., Hiemstra, P. S., Hardie, J. A., Bakke, P. S., & Eagan, T. M. L. (2012). Chronic Obstructive Pulmonary Disease Is Associated with Low Levels of Vitamin D. *PLoS One*, *7* (6), e38934–. doi: 10.1371/journal.pone.0038934.

[47] Hendryx, M., & Luo, J. (2015). A test of vitamin D benefits on respiratory health mediated through inflammatory markers. Chronic Respiratory Disease, 24–30. https://doi.org/10.1177/1479972314556086

[48] Han, Y. Y., Forno, E., & Celedón, J. C. (2017). Vitamin D Insufficiency and Asthma in a US Nationwide Study. *The journal of allergy and clinical immunology. In practice*, *5* (3), 790–796.e1. https://doi.org/10.1016/j.jaip.2016.10.013

[49] DeMeo, D. L., Ramagopalan, S., Kavati, A., Vegesna, A., Han, M. K., Yadao, A., Wilcox, T. K., Make, B. J., & COPD Gene Investigators (2018). Women manifest more severe COPD symptoms across the life course. *International journal of chronic obstructive pulmonary disease*, *13*, 3021–3029. https://doi.org/10.2147/COPD.S160270

[50] Zhu B, Zhu B, Xiao C, Zheng Z. Vitamin D deficiency is associated with the severity of COPD: a systematic review and meta-analysis. *Int J Chron Obstruct Pulmon Dis*. 2015; 10 (1): 1907-1916. https://doi.org/10.2147/COPD.S89763

[51] Zhu M, Wang T, Wang C, Ji Y. The association between vitamin D and COPD risk, severity, and exacerbation: an updated systematic review and meta-analysis. *Int J Chron Obstruct Pulmon Dis*. 2016; 11 (1): 2597-2607. https://doi.org/10.2147/COPD.S101382

[52] Kentson M, Leanderson P, Jacobson P, Persson HL. The influence of disease severity and lifestyle factors on the peak annual 25(OH)D value of COPD patients. *Int J Chron Obstruct Pulmon Dis*. 2018; 13: 1389-1398. https://doi.org/10.2147/COPD.S156121