Effect of Serum Cotinine on Vitamin D Serum Concentrations Among American Females with Different Ethnic Backgrounds

KIANO REZA MANAVI[1], BRENDA P. ALSTON-MILLS[2], MARVIN P. THOMPSON[3] and JONATHAN C. ALLEN[4]

[1]Department of Food, Nutrition and Bioprocessing Sciences; Interdepartmental Nutrition Program, North Carolina State University, Raleigh, NC, U.S.A.;
[2]Department of Animal Science Raleigh, North Carolina State University, Raleigh, NC, U.S.A.;
[3]Department of Food Science and Human Nutrition Michigan State University, East Lansing, MI, U.S.A.;
[4]Department of Food, Nutrition and Bioprocessing Sciences Raleigh, North Carolina State University, Raleigh, NC, U.S.A.

Abstract. *Objective: To investigate the effect of blood serum concentration of cotinine among non-smokers, passive/light smokers and active smoker females in the United States population as it compares to vitamin D blood serum concentrations. Materials and Methods: National Health and Nutrition Examination Survey (NHANES) that is designed to assess the health and nutritional status of adults and children in the United States by Centers for Disease Control and Prevention (n=22,196). Results: The analyses demonstrated that among all three smoking categories, black female active smokers have lower vitamin D (13.374 ng/ml), than hispanic (19.213 ng/ml) or white (24.929 ng/ml) females. It was demonstrated that the active smoker black females have the highest percentage of vitamin D deficiency and inadequacy in the population compared to other ethnic females. Conclusion: The cotinine blood serum concentrations can also affect vitamin D concentrations in addition to other factors such as gender, ethnicity, dietary supplement intake and sun exposure.*

Tobacco smoking in any form, both handmade and manufactured cigarettes, pipes, cigars and other products, has enormous effects on general human health. One of the many effects is damage to lungs. Smoking is related to a number of cancers, most importantly lung cancer in 20% of all adult Americans who smoke (1). The latest statistics from the Centers for Disease Control and Prevention (CDC) has shown that the discontinuation of tobacco use highly reduces causes of death in the United States, which takes the lives of more than 480,000 Americans annually, including more than 41,000 lives from passive smoke exposure (2). Data from Lung Cancer Statistics indicates that more females and males die from lung cancer than any other type of cancer in the United States (3). Human carcinogenicity data has shown that there is an association between lung cancer risk in spouses of smokers and exposure to passive smoking from the spouse who smokes, with 20% risk for female passive smokers and 30% for male passive smokers (4). In the United States, prior to 1920s, smoking among females was much less common than among males because it was not viewed as a respectable social behavior (5). However, as society became more accepting of female cigarette smokers, smoking rates began to rise towards levels nearly as high as those seen in males (6). The closer gap of smoking among females and males has been attributed not only to society tolerance, but also to the tobacco industry's marketing strategies to females as a symbol of freedom (7). The estimated new cases and deaths from both small cell lung cancer and non-small cell lung cancer types combined in 2014 are 224,210 new cases and 159,260 deaths cases, respectively, in the United States (8). Individuals who smoke are 15- to 30-times more likely to get lung cancer or die from it than those who do not smoke. Passive smoking causes lung cancer as well. Two out of five adults who do not smoke are exposed to passive smoke in the United States (9). The primary component in tobacco is nicotine that stimulates on the central nervous system and peripheral nervous system. Cigarette smoking has been the most popular method of nicotine acquisition since the early 20th century, and nicotine still is an ingredient in other tobacco products such as cigars, pipes, tobacco gums and snuff (10). In the

Correspondence to: Dr. Kiano Reza Manavi, Department of Food, Nutrition and Bioprocessing Sciences, Interdepartmental Nutrition Program, North Carolina State University, Raleigh, 1628 Garners Chapel Road. Mt. Olive, NC, 28365 U.S.A. E-mail: kiano_manavi@scientist.com

Key Words: Serum cotinine, serum vitamin D, smoking, lung cancer, ethnicity.

Table I. *Demographic and laboratory variables of study.*

Demographics Data	Age (>18yo)	Gender (M/F)	Race (Hispanic, White, Black)
Laboratory Data	Vitamin D (ng/mL)	Cotinine (ng/mL)	

20th century over a hundred million lives have been lost to the tobacco epidemic globally, with estimated one billion deaths of females and males in twenty-first century alone and nicotine remains as one of the most heavily used chemical in the United States (11). The key metabolite of nicotine is *cotinine*, with a longer plasma half-life (~17 h) than nicotine (~2-3 h), and is used as a biomarker of nicotine exposure (12). Cotinine can be detected in hair, saliva, urine and blood serum. Together with nicotine it is oxidized primarily *via* CYP-2A6 in human liver microsomes (13). Cotinine affects enzymes involved in the synthesis of steroid hormones, estrogen and testosterone *via* releasing neurotransmitters (14, 15).

Although vitamin D is a fat-soluble steroid hormone, some of its biological functions may result from binding of the active form, 1α, 25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$) to the membrane Vitamin D Receptor (mVDR) on cell membranes. Vitamin D deficiency is recognized as a worldwide epidemic. Over the past two decades, different studies have reported and established that this active form of the vitamin reveals potent cell differentiation properties in cancer cells, such as breast, colon, leukemia, and prostate (16). The anti-proliferative and differentiation-inducing effects can be of clinical significance in prevention or treatment of cancer at several target organs breast, colon and prostate, and target tissues such as bone and intestine (17). One study comparing smoker and non-smoker females in Denmark revealed that smoking has a significant effect on vitamin D metabolism (18), and another similar study showed the association of lower serum vitamin D concentration with higher risk of tobacco-related cancers, but not others (19). Furthermore, one study from the Czech Republic showed that among lung cancer patients, very low vitamin D serum concentrations had a higher effect on other cancers such as colorectal, breast and prostate (20). The relationship between receptor binding and cell membrane fluidity in human females has been discussed in one study (21), which may be considered as a factor in vitamin D deficiency among adults, especially among minority groups, black and hispanic females (22).

The objective of our study was to investigate the effect of smoking (assessed by serum cotinine values) on vitamin D serum levels of individuals based on the information available on NHANES.

Materials and Methods

The National Health and Nutrition Examination Survey (NHANES) is a program of studies designed by National Center for Health Statistics (NCHS) of CDC to assess the health and nutritional status of adults and children in the United States. It is a unique database with accurate combinations of interviews and physical examinations. The survey examines samples of approximately 5,000 people each year from all regions to represent a national sample, in which each person represents approximately 50,000 other US residents. All the data from surveys are available in SAS code Version 9.1 statistical software (SAS Institute Inc. Cary, NC). Findings from NHANES data has been used previously in epidemiological studies and health sciences research to determine the prevalence in major diseases, and risk factors for diseases. The data are also used to assess nutritional status and the association with health promotion and disease prevention in the United States. The focus is to help in developing sound public health policy and expand the health knowledge for the nation (23). For the basis of this study, 6 years of data from the Continuous NHANES (two-year cycle) datasets of NHANES 2001-2002, NHANES 2003-2004 and NHANES 2005-2006, were used (24). From the demographic datasets, gender, ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican-American and other Hispanic groups), and age (18-70 years-old) variables were selected. Mexican-American and other Hispanic groups were combined into one group to represent the Hispanic population nationally. The laboratory variables used were, serum Vitamin D (ng/mL) and serum cotinine (ng/mL). All variables are shown in Table I. After locating all the necessary variable datasets provided through the CDC website, the next step was to prepare one unified analytic dataset. In order to perform this task, all the located variables were downloaded as separate data files, appended and merged, cleaned and recoded (such as removing missing data and outliers), formatted and labeled, and saved (25). All the participants in NHANES were assigned a unique identifier known as the "sequence number (SEQN)" that was used to identify each person sampled and used when data files were merged to ensure that all observations were ordered in the same way for each file.

The smoking habits were categorized based on the serum cotinine level (Explanatory Variable) measurements provided by "Guidelines for Interpreting Cotinine Levels "United States" (26): - Adult Non-smokers: 0.1 ng/mL< [Serum Cotinine] <1 ng/mL; - Adult Exposure (Light/Passive-smokers): 1 ng/mL <[Serum Cotinine] <30 ng/mL; - Adult Smokers: [Serum Cotinine] >100 ng/mL;

The Vitamin D (Response Variable) status of participants was divided also in three classes based on the National Institute of Health Office of Dietary Supplements (27): - Vitamin D-deficient: [Serum $25(OH)D_3$] <12 ng/mL; - Vitamin D-inadequate: 12 ng/mL <[Serum $25(OH)D_3$] <20 ng/mL; - Vitamin D-sufficient: [Serum $25(OH)D_3$] > 20 ng/mL;

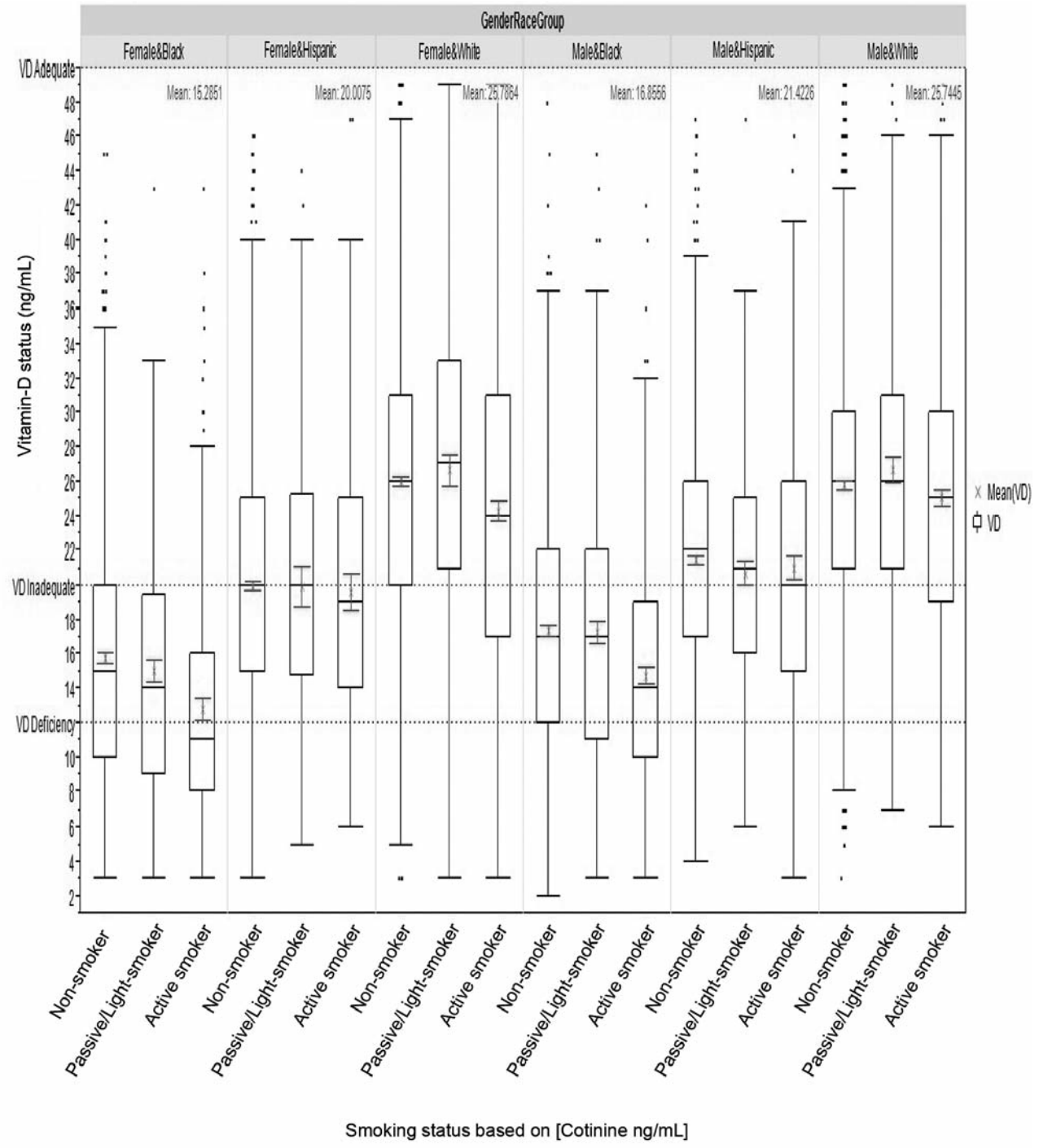

Figure 1. *Vitamin D and mean vitamin D concentrations vs. smoking status (cotinine concentrations).*

Because of the size of the sample, missing data were not considered in this investigation as they were less than 10% of the total sample size suggested by the NHANES documentation. All data analyses were performed using SAS 9.3 using Multiple Logistic Regression methods and the conclusions were interpreted with a significant threshold at 5%.

Results

The first finding of our study was the outcome of comparing vitamin D and mean vitamin D concentrations, *versus* smoking habits based on the cotinine

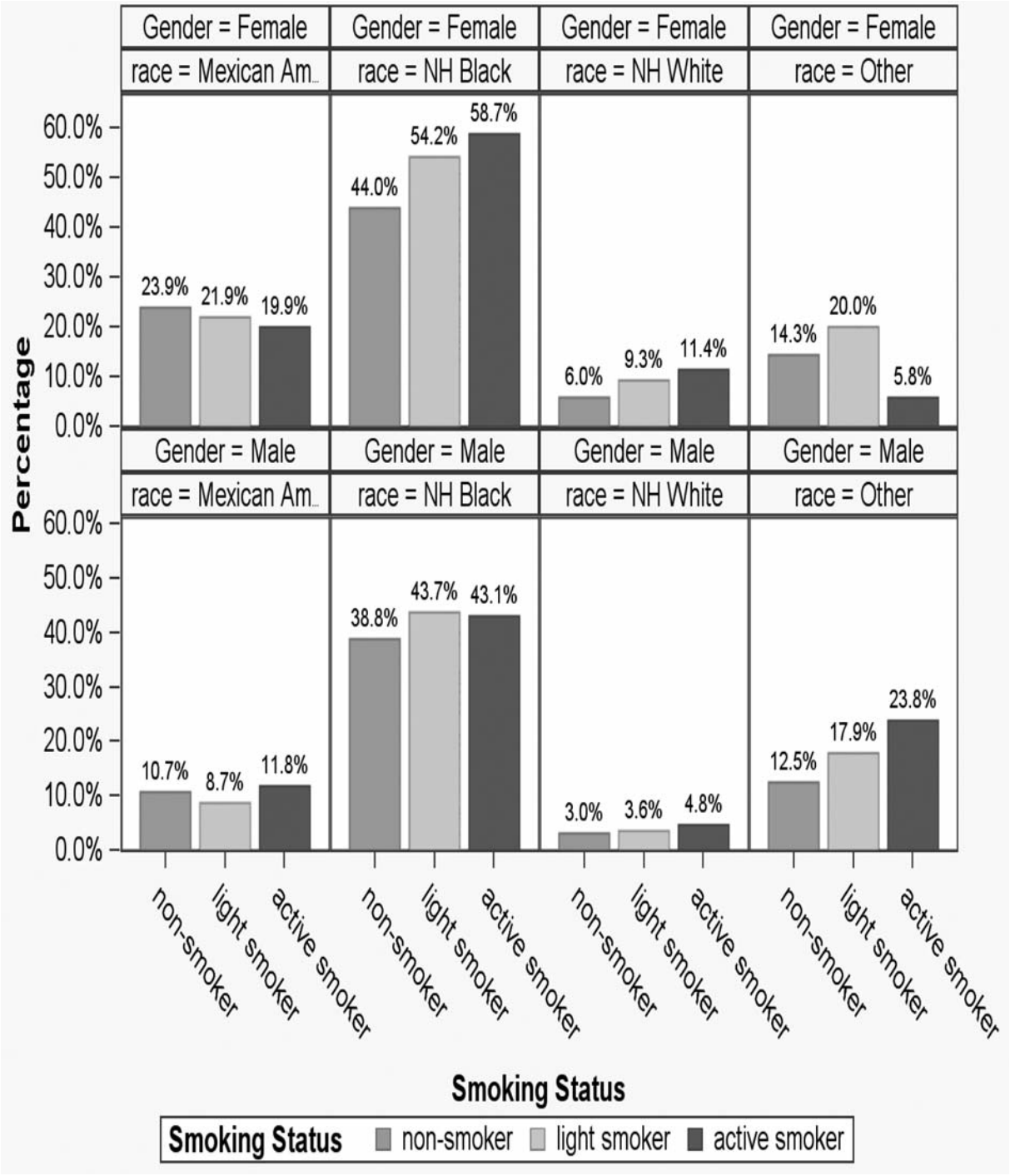

Figure 2. *Descriptive statistics/graphs, adjusted for survey structure bar chart of percentage of the population with vitamin-D deficiency (<12 ng/mL) concentration by smoking status, race and gender.*

concentrations, VD & mean concentrations of (VD) *vs*. smoking habits (Figure 1).

Figure 1 illustrates that the active-smokers among black females have significantly lower mean vitamin D concentrations (13.374 ng/mL), compared to the mean vitamin D concentrations of black females who are passive/light smokers (15.670 ng/mL), and passive/light smokers slightly lower mean vitamin D concentrations than non-smokers black

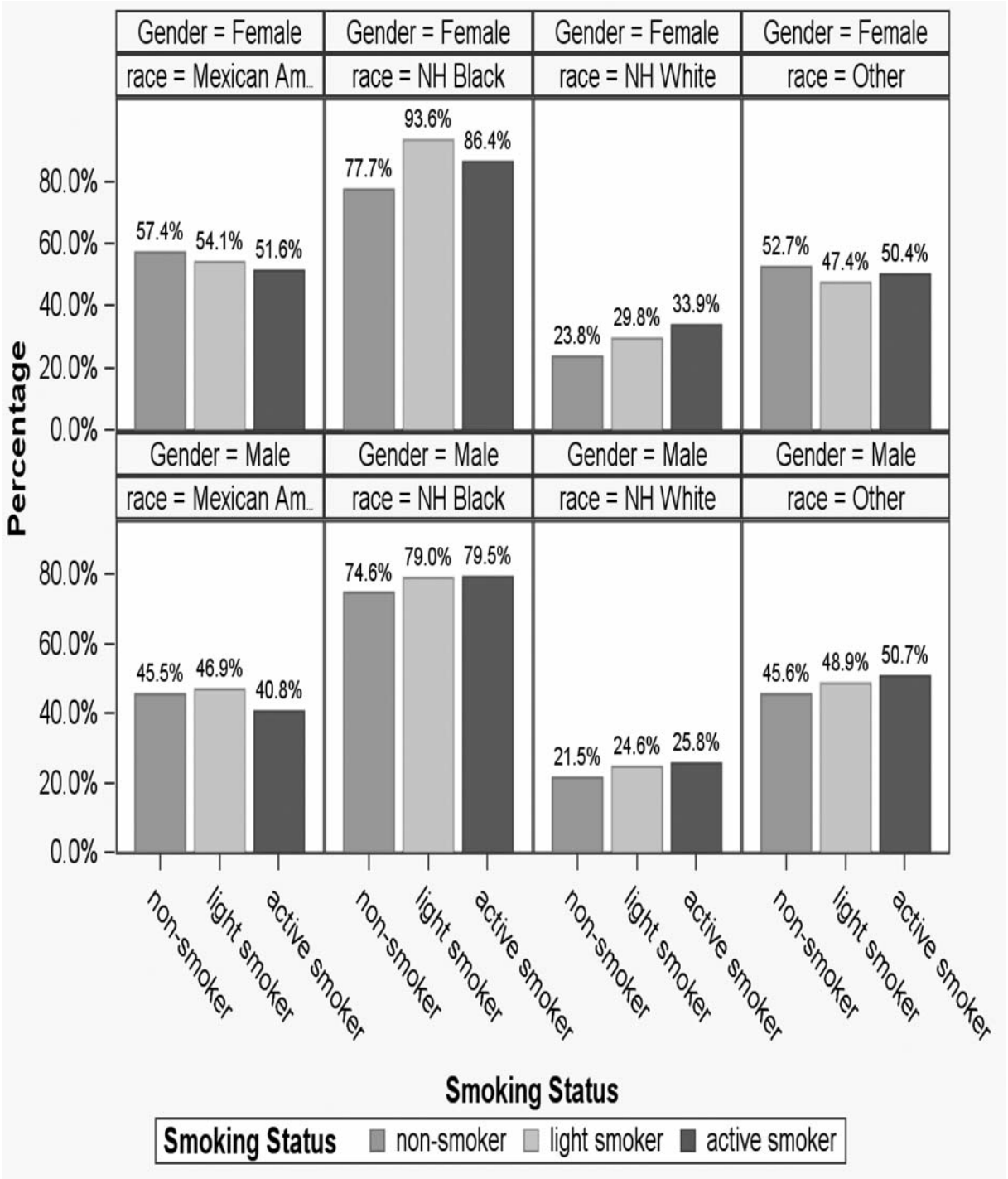

Figure 3. *Descriptive Statistics/Graphs, adjusted for survey structure bar chart of percentage of the population with vitamin-D inadequacy (<20 ng/mL) concentration by smoking status, race and gender.*

females (15.854 ng/mL). Also, the result indicated that the mean serum vitamin D concentration among all three groups is below the inadequacy level (<20 ng/mL), with the active-smokers vitamin D concentration trending to deficiency status in black females. Comparing the vitamin D among all three categories in black females, it showed that the overall vitamin D concentration in active smokers was below deficiency concentration (<12 ng/mL).

Hispanic females who are active-smokers have the lowest mean concentrations of vitamin D (19.213 ng/mL) compared to their passive/light smokers (19.821 ng/mL) and non-smokers (20.231 ng/mL) counterparts. However, within the hispanic females groups in all the three smoking categories, the mean serum concentration of Vitamin D is trending to the inadequate, rather than to the deficiency level (Table II).

White females active-smokers have the lowest mean concentration of vitamin D (24.929 ng/mL) compared to the passive/light smokers (26.562 ng/mL) and non-smokers (26.383 ng/mL) white females. But within the white females groups, the mean concentration of vitamin D of passive/light smokers was slightly higher than non-smoker females. Among all the three smoking categories the mean serum vitamin D concentration was above the inadequate level, yet it was below adequate level (>20 ng/mL) (Table II).

Thus, the active-smoker females overall had the lowest mean vitamin D status. The same pattern was observed among males (data shown). Black males active-smokers (14.181 ng/mL), black males passive/light smokers (14.762 ng/mL), black males non-smokers (14.775 ng/mL); Hispanic males active-smokers (20.569 ng/mL), hispanic males passive/light smokers (21.074 ng/mL), hispanic males non-smokers (22.873 ng/mL); and white males active-smokers (25.353 ng/mL), white males passive/light smokers (25.502 ng/mL), and white males non-smokers (25.491 ng/mL). Further elaboration is not the scope of this paper, since the focus is on females (Table II).

To summarize the results, among all three ethnic groups of females revealed that black females have the lowest mean vitamin D (15.2851 ng/mL) than the hispanic females (20.0075 ng/mL) and white females have the highest mean vitamin D (25.7864 ng/mL). In general, regardless of their smoking lifestyle, a similar pattern is observed in males (16.8556 ng/mL, 21.4226 ng/mL and 25.7445 ng/mL) respectively (Table III).

Based on this finding among all ethnic female groups, black active-smokers females have the highest percentage of vitamin D deficiency concentration (58.7%) compared to hispanic active-smoker females (19.9%) and white active-smoker females (11.4%). The same pattern was observed among male active-smokers respectively (43.1%, 11.8% and 4.8%) (Figure 2).

Based on this finding among all ethnic females groups, black active-smokers females have the highest percentage of vitamin D inadequacy concentration (86.4%) compared to hispanic active-smoker females (51.6%) and white active-smoker females (33.9%). The same pattern was observed among male active-smokers respectively (79.5%, 40.8% and 25.8%). One interesting observation is that among all female ethnic groups of passive/light smokers, black females have the highest prevalence of vitamin D inadequacy (93.6% of the population) compared to hispanic females (54.1%) and white females (29.8%) (Figure 3).

Table II. *Vitamin D concentrations vs. smoking status in ethnic females and males.*

Smoking by Categories	Females (ng/mL)	Males (ng/mL)
Blacks active-smokers	13.374	14.181
Blacks non-smokers	15.854	14.762
Blacks passive/light smokers	15.670	14.775
Hispanics active-smokers	19.213	22.873
Hispanics non-smokers	20.231	21.074
Hispanics passive/light smokers	19.821	20.569
Whites active-smokers	24.929	25.353
Whites non-smokers	26.383	25.491
Whites passive/light smokers	26.562	25.502

Table III. *Overall mean vitamin D concentration in ethnic females and males.*

Overall mean [Vitamin-D] – ng/mL	Blacks population	Hispanic population	White population
Females	15.2851	20.0075	25.7864
Males	16.8556	21.4226	25.7445

Discussion

Approximately 70-80% of nicotine is metabolized to cotinine from each cigarette smoked. Cotinine is the primary metabolite of nicotine, and is currently regarded as the best biomarker of tobacco smoke exposure and can be measured in blood serum, urine, saliva and hair (28). There is a dose-response relationship of plasma cotinine to daily cigarette consumption (29). Additionally, results from one study have shown a strong linear dose-response relationship between serum cotinine concentrations and lung cancer risk in smokers (30, 31), making the serum cotinine concentrations a better method of quantifying risks from cigarette use in epidemiological studies than self-reporting cigarettes questionnaires and surveys (32).

Vitamin D deficiency, the largest pandemic in the world, is defined by most experts as a $25(OH)D_3$ level of less than 20 ng/mL, which could be the result of inadequate irradiation of the skin, insufficient intake from diet and dietary supplement intake, geographic effects such as high altitudes, Body Mass Index (BMI), cholesterol metabolism, cultural impact such as clothing, gender and ethnic backgrounds. Furthermore, the relationship of skin pigment and vitamin D production from sunlight, the darker the skin the more difficult it is to produce vitamin D, which also accounts for low vitamin D values observed in Black and Hispanic females. This study has showen that black females sampled by NHANES had lower vitamin D concentrations (Black: 15.2851 ng/mL, Hispanic:

20.0075 ng/mL and White: 25.7864 ng/mL) compared to other females, and the ethnic background plays an important role in vitamin D status, confirming previous findings that race and gender have effects on vitamin D concentration. To our knowledge, this is the first vitamin D research finding that smoking habits among females may also affect vitamin D metabolism and its concentration in serum. Thus female active-smokers have the lowest mean vitamin D concentration and higher prevalence of vitamin D deficiency and inadequacy within the overall population in the United States. Further research is needed to determine whether, cotinine or other factors associated with smoking interfere with vitamin D metabolism.

The present study illustrated that among all the other factors mentioned and well-understood to affect vitamin D status, the cotinine concentration in blood serum may also be a new factor that should be quantified in vitamin D deficiency and inadequacy research. Therefore, smoking compounds the problem of inadequate vitamin D intake and synthesis. One note to consider is the fact that the high number of observations (n=22,196) will have a great deal of between-subject variation. Since cotinine may have effects on steroid hormone metabolism, it is likely to affect vitamin D metabolism because the vitamin has a secosteroid structure. Given that both these molecules have steroid structures, there may share a further relationship at the molecular level such as binding to receptors. Further research and investigation are needed to better-understand this correlation at the molecular level. This report is the first epidemiological investigation to demonstrate the correlation of cotinine and vitamin D among females of different ethnicities in the United States. Such findings may open new doors in vitamin D research and its anti-cancer therapeutic effects on lung cancer.

Acknowledgements

The Authors would like to express their appreciation and gratitude to: Dr. William H. Swallow, Department of Statistics (Professor Emeritus) at North Carolina State University for his support and guiding us through this study; Miss Lan Dong, graduate student in Statistics at North Carolina State University for assisting me with NHANES statistical data programming and analyses; Dr. Christopher Blanchette for his mentorship.

References

1 JAMA Patient Page: Smoking Cessation. The Journal of the American Medical Association *308(15)*: 1599, 2012.

2 Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention (CDC). [2014]. Current cigarette smoking among U.S. adults aged 18 years and older, [online]. Centers for Disease Control. Available: http://www.cdc.gov/tobacco/campaign/tips/resources/data/cigarette-smoking-in-united-states.html [2014, August 14].

3 Division of Cancer Prevention and Control, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention (CDC). [2014]. Lung Cancer Statistics, [online]. Center for Disease Control. Available: http://www.cdc.gov/cancer/lung/statistics/ [2014, September 2].

4 World Health Organization (WHO): Tobacco Smoke and Involuntary Smoking IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (Summary of Data Reported and Evaluation). International Agency for Research on Cancer *83*: 1-12, 2002.

5 Hitchman SC and Fong GT: Gender empowerment and female-to-male smoking prevalence ratios. Bulletin of the World Health Organization *89*: 195-202, 2011.

6 Waldron I: Patterns and causes of gender differences in smoking. Social Science & Medicine *32(9)*: 989-1005, 1991.

7 O'Keffe A and Pollay RW: Deadly targeting of women in promoting cigarettes. Journal of the American Medical Women's Association *51(1-2)*: 67-69, 1996.

8 National Cancer Institute at the National Institute of Health. [2014]. Lung Cancer, [online]. http://www.cancer.gov/cancertopics/types/lung, [2014, August 17].

9 Division of Cancer Prevention and Control, National Center for Chronic Disease Prevention abd Health promotion, Centers for Disease Control and Prevention (CDC). [2013]. What are the risk factors?, [online]. http://www.cdc.gov/cancer/lung/basic_info/risk_factors.htm [2013, November 21].

10 Litvin EB, Ditre JW, Heckman BW and Brandon TH: Chapter 10/Nicotine. In Drug abuse and addiction in medical illness: 137-150, 2012.

11 World Health Organization (WHO): WHO Report on the Global Tobacco Epidemic. In The MPOWER package: 1-342, 2008.

12 Benowitz NL: Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiologic Reviews *18(2)*: 188-204, 1996.

13 Nakajima M, Yamamoto T, Nunoya K, Yokoi T, Nagashima K, Inoue K. and Kuroiwa Y: Characterization of CYP2A6 involved in 3'-hydroxylation of cotinine in human liver microsomes. The Journal of Pharmacology and Experimental Therapeutics *277(2)*: 1010-1015, 1996.

14 Patterson TR, Stringham JD and Meikle AW: Nicotine and cotinine inhibit steroidogenesis in mouse leydig cells. Life Sciences *46*: 265-272, 1990.

15 Yeh J, Barbieri RL and Friedman AJ: Nicotine and cotinine inhibit rat testis androgen biosynthesis in vitro. Journal of Steroid Biochemistry 33: 627-630, 1989.

16 Mehta RG and Mehta RR: Vitamin D and cancer. Journal of Nutritional Biochemistry *13*: 252-264, 2002.

17 Holick MF: Vitamin D: Its role in cancer prevention and treatment. Progress in Biophysics and Molecular Biology *92*: 49-54, 2006.

18 Brot C, Jùrgensen N and Sùrensen O: The influence of smoking on vitamin D status and calcium metabolism. European Journal of Clinical Nutrition *53*: 920-926, 1999.

19 Afzal S, Bojesen SE and Nordestgaard BG: Low plasma 25-hydroxyvitamin d and risk of tobacco-related cancer. Clinical Chemistry *59(5)*: 1-10, 2013.

20 Pazdiora P, Svobodova S, Fuchsova R, Kucera R, Prazakova M, Vrzalova J, and Topolcan O: Vitamin D in colorectal, breast, prostate and lung cancer: A pilot study. Anticancer Res *31*: 3619-3622, 2011.

21 Berlin E, Bhathena SJ and Judd JT: Dietary fat and hormonal effects on erythrocyte membrane fluidity and lipid composition in adult women. Metabolism *38(8)*: 790-796, 1989.
22 Forrest KYZ and Stuhldreher WL: Prevalence and correlates of vitamin D deficiency in US adults. Nutrition Research *31(1)*: 48-54, 2011.
23 CDC/National Center for Health Statistics, Centers for Disease Control and Prevention (CDC). [2014]. About the National Health and Nutrition Examination Survey. [online]. http://www.cdc.gov/nchs/nhanes/about_nhanes.htm, [2014, February 3].
24 CDC/National Center for Health Statistics, Centers for Disease Control and Prevention (CDC). [2014]. Questionnaires, Datasets, and Related Documentation. [online]. http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm, [2014, February 3].
25 CDC/National Center for Health Statistics, Centers for Disease Control and Prevention (CDC). [2013]. Preparing an Analytic Dataset Course. [online]. http://www.cdc.gov/nchs/tutorials/NHANES/preparing/intro.htm, [2013, December 31].
26 Salimetrics: Guidelines for Interpreting Cotinine Levels: United States. [online]. www.salimetrics.com/assets/.../Spit_Tips_-_Cotinine_Guidelines.pdf, [July 2011].
27 National Institute of Health (NIH) Office of Dietary Supplements [2011]: Vitamin D - Fact sheet for health professionals. [online]. http://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/, [2014, April 4].
28 Centers for Disease Control and Prevention (CDC). [2013]. Cotinine. [online]. http://www.cdc.gov/biomonitoring/Cotinine_BiomonitoringSummary.html, [2013, December, 4].
29 Hukkanen J, Jacob III, P and Benowitz NL: Metabolism and disposition kinetics of nicotine. Pharmacological Reviews *57(1)*: 79-115, 2005.
30 Benowitz NL, Hukkanen J and Jacob III, P: Nicotine chemistry, metabolism, kinetics and biomarkers. Handbook of Experimental Pharmacology *192*: 29-60, 2009.
31 Boffetta P, Clark S, Shen M, Gislefoss R, Peto R and Andersen A: Serum cotinine level as predictor of lung cancer risk. Cancer Epidemiology, Biomarkers & Prevention *15(6)*: 1184-1188, 2006.
32 Perez-Stable EJ, Benowitz NL and Marin G: Is serum cotinine a better measure of cigarette smoking than self-report? Preventative Medicine *24*: 171-179, 1995.

Received August 27, 2014
Revised October 9, 2014
Accepted October 21, 2014