July 2015

Predicted 25-hydroxyvitamin D Score and Risk of Multiple Sclerosis in U.S. Women

Alexandra Purdue-Smithe
University of Massachusetts Amherst

Predicted 25-hydroxyvitamin D Score and Risk of Multiple Sclerosis in U.S. Women

A Thesis Presented

By

ALEXANDRA C. PURDUE-SMITHE

Submitted to the Graduate School of the

University of Massachusetts Amherst in partial fulfillment

of the requirements for the degree of

MASTER OF SCIENCE

May 2015

Public Health

Epidemiology

Predicted 25-hydroxyvitamin D Score and Risk of Multiple Sclerosis in U.S. Women

A Thesis Presented

By

ALEXANDRA C. PURDUE-SMITHE

Approved as to content and style by:

_____
Elizabeth Bertone-Johnson, Chair

_____
Susan E. Hankinson, Member

_____
Susan E Hankinson, Department Chair
Division of Epidemiology & Biostatistics
School of Public Health & Health Sciences

DEDICATION

To my wonderful mother, whose strength, support, and encouragement inspire me in all that I do.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Elizabeth Bertone-Johnson for her exceptional guidance over the past year and a half. Her patience and insight throughout this process has been invaluable to me and without her, this thesis would not have been possible. I owe her many thanks for her tireless efforts on both this thesis and her contributions to my success as a student. I would also like to thank my committee member, Dr. Susan Hankinson for her helpful feedback during the preparation of this manuscript. Additionally, I would like to thank Dr. Kassandra Munger, whose expertise contributed immensely to this project.

I would like to extend my most sincere gratitude to the faculty and staff of the Division of Epidemiology & Biostatistics, who have contributed so much to my learning throughout my pursuit of this degree.

Finally, I would like to thank my friends, family, and teammates whose support and belief in me throughout the past few years has a played an immeasurable role in my success.

ABSTRACT

PREDICTED 25-HYDROXYVITAMIN D SCORE AND RISK OF MULTIPLE SCLEROSIS IN U.S. WOMEN

MAY 2015

ALEXANDRA C. PURDUE-SMITHE, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST

M.S. UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Elizabeth Bertone-Johnson

Multiple sclerosis (MS) is a progressive, autoimmune neurodegenerative disorder affecting nearly 350,000 people in the United States and resulting in significant disability. As an immunomodulator, vitamin D may play a role in the development of MS. Previous studies have observed an inverse association of 25-hydroxyvitamin D (25(OH)D) levels and MS risk in younger populations; however, whether this relationship persists in older adults remains unclear. We prospectively investigated the association between predicted 25(OH)D level and incident MS in the Nurses' Health Study (NHS) (n=121,701) and NHS II (n=116,430). 25(OH)D levels were predicted using validated regression models that include important determinants of vitamin D status, including race, UV-B flux (based on state of residence), physical activity, body mass index, dietary vitamin D intake, alcohol consumption and post-menopausal hormone use. Data on these factors were self-reported on NHS and NHS II questionnaires starting in 1986 and 1991, respectively, and updated every 2-4 years. MS diagnoses were ascertained by self-report and confirmed by medical records. Cox proportional hazards models adjusted for age, ethnicity, latitude of residence at age 15, and BMI at age 18 were used to estimate hazard ratios (HR)s and 95% confidence intervals (CI)s in each cohort. During up to 18 years of follow-up, we

documented 179 definite/probable cases of MS with first symptoms after baseline. Multivariable HRs comparing highest and lowest quintiles of predicted 25(OH)D were 1.09 (95% CI: 0.40-2.96) in the NHS and 0.52 (95% CI: 0.28-0.95) in the NHS II. Higher predicted plasma 25(OH)D may be modestly associated with lower risk of MS, primarily in younger women.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS……..iv

ABSTRACT…….v

LIST OF TABLES…….....viii

CHAPTER

1. INTRODUCTION……...……1

2. METHODS…….…….....7

3. RESULTS……..……15

4. DISCUSSION…….....17

5. CONCLUSION……..23

BIBLIOGRAPHY……..31

LIST OF TABLES

Table **Page**

1. Exclusion criteria: Nurses' Health Study & Nurses' Health Study II……..….24

2. Predictors of serum 25-hydroxyvitamin D from multivariable linear regression models in the Nurses' Health Study & Nurses' Health Study II…….……..25

3. Distribution of predicted 25-hydroxyvitamin D scores at baseline: Nurses' Health Study & Nurses' Health Study II…….....27

4. Distribution of covariates according to age-adjusted mean 25-hydroxyvitamin D score at baseline: Nurses' Health Study (1989) & Nurses' Health Study II (1991)……..28

5. Age-adjusted and multivariate HR and 95% CI of multiple sclerosis by quintile of predicted 25-hydroxyvitamin D score: Nurses' Health Study & Nurses' Health Study II……..…….....29

6. Age-adjusted and multivariate HR and 95% CI of multiple sclerosis by predicted 25-hydroxyvitamin D score restricted to definite cases: Nurses' Health Study & Nurses' Health Study II…….....30

CHAPTER 1

INTRODUCTION

Multiple sclerosis (MS) is a neurodegenerative disorder currently affecting an estimated 350,000 people in the United States.[1] The incidence of MS is relatively low among adolescents (0.43 per 1,000) and individuals older than 60 (2.88 per 1,000). Highest incidence occurs in individuals 35 to 39 years of age.[2] The risk of developing MS is 1.5 to 2.5[3] times higher in women compared to men; the sex-specific incidence rate for women is estimated to be 5.3 per 1,000 compared to 2.3 per 1,000 in men.[2] Additionally, risk of MS is also higher among white individuals compared to black individuals.[4]

MS is an autoimmune disorder in which inflammation of the central nervous system causes progressive degradation of the myelin sheath.[5] Two clinical courses of the disease have been identified. Approximately 85% of MS cases are considered to be relapsing-remitting MS (RRMS), characterized by an evolution of neurologic dysfunction over the course of days to weeks, which subsequently plateaus and resolves.[5] These attacks are considered to be the inflammatory phase of the disease, which is followed by the secondary progressive stage (SPMS) 10-15 years after initial onset in about 50-60%[5] of patients. In the relapsing-remitting phase of MS, patients often experience ataxia, optic neuritis, muscle weakness and numbness as a result of inflammatory lesions affecting brain, optic nerve and spinal cord.[5] As lesions accumulate over a series of relapses, degradation continues as the patient enters the secondary progressive stage, resulting in worsening and non-remitting leg weakness, dementia, ataxia and spasticity, along with other neurological symptoms.[1,5] Roughly 10% of cases have primary progressive multiple sclerosis (PPMS), experiencing the degenerative phase from outset without an initial

relapsing-remitting inflammatory phase.[5] The progression of MS results in substantial disability.[6]

Established risk factors for MS include infection by Epstein-Barr virus, cigarette smoking, high body mass index at age 18 and genetic predisposition.[3,7] There are several genes associated with increased risk of MS. The most prominent genetic risk factor for MS is the HLA-DRB1*1501 risk allele; in individuals of northern European descent, this haplotype confers a 3-fold risk in MS.[8] Prior studies have found no evidence to suggest that micronutrients other than vitamin D are associated with MS.[9]

Vitamin D Hypothesis

Vitamin D plays in an important physiologic role in calcium and blood pressure regulation, neurodevelopment and modulation of immune response.[4] The primary form of vitamin D (colecalciferol) comes from both sun exposure and dietary sources including, but not limited to fatty fish, fortified foods such as milk, and supplements.[4] Sunlight exposure provides significantly more vitamin D than dietary sources. For example, 20 minutes of sun exposure during the summer months provides 10,000 IU of vitamin D compared to 400 IU from 1 serving of fatty fish.[4]

Ecologic studies have observed a latitude gradient of MS risk, with higher incidence rates occurring with increasing latitude.[10] Data from such studies have given rise to the hypothesis that the gradient in incidence rates may be explained by differential sun exposure and therefore, vitamin D exposure.[4]

Vitamin D Metabolism

Cutaneous synthesis of vitamin D occurs as UVB radiation from sunlight reacts with 7-dehydrocholesterol in the skin, forming pre-vitamin D and then cholecalciferol.[4] Cutaneous and dietary cholecalciferol are hydroxylated in the liver to 25-hydroxyvitamin D and later converted to 1,25-dihydroxyvitamin D by the kidney and target tissues.[11]

The action of circulating 1,25-dihydroxyvitamin D is mediated by the vitamin D receptor (VDR), which regulates the expression of vitamin D responsive genes.[12] Because VDR expression has been observed in central nervous system tissue, it has been hypothesized that this may be a site of vitamin D activity.[12]

Increasing circulating levels of 1,25-dihydroxyvitamin D may activate VDR in the central nervous system.[11] Activation of VDR in immune cells and neurons causes a decrease in the inflammatory response of T-helper-1 cells and an increase in the anti-inflammatory response of T-helper-2 cells.[11] Considering that MS is an inflammatory immune reaction of the central nervous system, increased VDR activation due to increased circulating 1,25-dihydroxyvitamin D may modulate the regulatory function of T cells, thereby protecting an individual from inflammation of central nervous tissue. In clinical studies of MS patients, significant correlations between serum 25-hydroxyvitamin D and activity of T cells have been reported.[13,14]

Epidemiology of Vitamin D and Multiple Sclerosis

Six prior epidemiological studies have directly investigated the association of vitamin D (dietary[9] or serum 25(OH)D[15-19]) and MS. Three of these studies were prospective[9,15,16] (two nested case-control[15,16]) and three were case-control studies.[17-19] Of

the retrospective case-control studies, two reported an inverse association[17,18] of serum 25(OH)D and MS, and one reported no association.[19] While the results of two case-control studies support an association of vitamin D and MS, the greatest limitation of the case-control study design is the collection of serum measurements after MS diagnosis. It is possible that lower vitamin D levels among cases are a consequence of MS, rather than an etiologic factor. Therefore, the primary way in which reverse causation can be eliminated in epidemiologic investigations is through prospective analyses.

In the best-designed study, Munger et al used a prospective, nested case-control study design to evaluate serum 25(OH)D level and risk of MS among 7 million U.S. service-men and -women whose blood samples and medical records make up the Department of Defense Serum Repository (DoDSR).[15] The study used at least two serum 25(OH)D measurements from blood samples drawn after entry into the military and prior to a probable/definite diagnosis of MS. In the analyses, 257 incident cases of MS were matched to 514 healthy controls according to age, sex, race/ethnicity, date of sample collection and branch of military service. The race-stratified results indicated a statistically significant inverse association for white men and women whose serum 25(OH)D level fell into the highest quintile (OR=0.38; 95% CI: 0.19-0.75). Among blacks, serum 25 (OH)D in the highest tertile was not associated with MS (OR=1.05; 95% CI: 0.51-2.17). Among Hispanics, the OR of serum 25(OH)D >100 nmol/L compared to serum 25(OH)D <75 nmol/L was not statistically significant (OR=0.61; 95% CI: 0.13-2.93). This was the first study to use repeated 25(OH)D measurements to assess vitamin D status prior to MS diagnosis.

In another prospective study conducted by Munger et al, dietary vitamin D intake was also found to be inversely associated with MS risk within the Nurses' Health Study (NHS) (n=92,253) and Nurses' Health Study II (NHS II) (n=95,310).[9] Vitamin D was assessed using food frequency questionnaires (FFQ) administered every two years, from which total vitamin D intake and supplemental vitamin D was estimated. Multiple sclerosis cases (n=173) through the year 1998 were ascertained by self-report on biennial questionnaires and confirmed as a probable/definite diagnosis by a physician. In the NHS, total vitamin D intake in the highest quintile was inversely associated with risk of multiple sclerosis (RR=0.41; 95% CI: 0.18-0.94) as compared to the lowest quintile. In the NHS II, total vitamin D intake in the highest quintile was non-significantly inversely associated with risk of MS (RR=0.83; 95% CI:0.41-1.67). However, in both the NHS and NHS II, the trend across quintiles was not statistically significant (p-trend= 0.16 and p-trend=0.13, respectively). One limitation of this study was that dietary intake of vitamin D alone may not the most reliable estimate of circulating, bioavailable vitamin D due to the substantial effect of sunlight on serum 25(OH)D levels.

In order to assess the association of vitamin D and MS in the NHS and NHS II, we were interested in using a more comprehensive exposure assessment of vitamin D that incorporated other contributing factors of vitamin D status. Using the NHS and NHS II cohorts provided an excellent opportunity to investigate the association of vitamin D and MS in older women, which the Munger et al DoDSR study was not able to do. Our study was conducted in the same population as the dietary vitamin D study conducted by Munger et al; however, in our analyses, by using predicted 25(OH)D scores, we were

able to use a more comprehensive exposure assessment than dietary vitamin D intake alone.

CHAPTER 2

METHODS

Study Design

We prospectively assessed the association between predicted 25(OH)D score and risk of MS within the Nurses' Health Study and Nurses' Health Study II.

Study Population

The NHS was established in the United States in 1976, when 121,701 married, registered nurses ages 30-55 from 11 states responded to a mailed questionnaire regarding lifestyle habits, health behaviors and medical history. The NHS II includes 116,430 female registered nurses ages 25-42 in the United States who responded to a similar initial questionnaire mailed in 1989. Participants of both cohorts are mailed biennial follow-up questionnaires that assess disease risk factors, new disease diagnoses and significant medical events.

Participants of the NHS were recruited from the registry of the American Nurses' Association according to the age and marital status eligibility criteria. Participants of the NHS were selected from the following 11 states: New York, California, Pennsylvania, Ohio, Massachusetts, New Jersey, Michigan, Texas, Florida, Connecticut, and Maryland, representing the states with the largest number of registrants. Participants of the NHS II were recruited from 14 state nursing boards and were eligible if they met the age criteria and were excluded if they did not provide a social security number or alternative contact person. Participants of the NHS II were selected from the following states: California, Connecticut, Indiana, Iowa, Kentucky, Massachusetts, Michigan, Missouri, New York, North Carolina, Ohio, Pennsylvania, South Carolina, and Texas.

For the present analysis, we excluded women who died prior to baseline or who were missing baseline exposure information. Additionally, we excluded women developing MS who were missing date of first symptoms or who experienced symptom onset prior to baseline. (Table 1) After exclusions, 74,914 individuals in the NHS and 95,106 individuals in the NHS II were included in the main analyses and contributed 26,223,875 and 22,658,634 person-months of follow-up, respectively. In this analysis, 39 women in the NHS and 140 women in the NHS II became incident probable/definite MS cases during follow-up.

Predicted 25-hydroxyvitamin D Scores

Serum 25(OH)D measurement is considered the "gold standard" approach to assessing vitamin D status. However, it is highly variable by season of blood draw and recent sun exposure.[20] Reproducibility of serum measurements is relatively high across 2-3 year intervals, but decreases over longer time periods. In the NHS, the intra-class correlation coefficient (ICC) for plasma 25(OH)D measured 2-3 years apart is 0.72. However, over 10-11 years, the correlation coefficient drops to 0.5, reflecting decreasing reliability with increasing time.[20]

Long-term vitamin D status may be a better predictor of chronic disease risk; however, the expense and difficulty of obtaining repeated measures of serum 25(OH)D in a large prospective cohort study often limits feasibility. Ideally, in a prospective study, we would collect samples and measure serum 25(OH)D for all participants over each follow-up cycle; however, this is unrealistic given budgetary and logistic constraints. While plasma 25(OH)D measurements are available for a small subset of participants in

the NHS and NHS II cohorts, only 57 cases provided a blood sample prior to MS diagnosis.

Because of the limitations of using directly measured 25(OH)D levels to assess the association of vitamin D and MS risk, we have instead used predicted 25(OH)D level as the exposure assessment. The purpose of using predicted scores in lieu of serum measurements was two-fold. First, it enabled us to capture changes in predictors of vitamin D status over longer time periods prior to MS onset. Using predictors of serum 25(OH)D as a proxy for actual serum measurements allowed us to look at a longer potentially relevant etiologic time period. Second, it allowed for increased sample size of eligible cases and greater power to assess a potential difference in the association of vitamin D and MS in older versus younger women.

Development of Predicted 25-hydroxyvitamin D Scores

For both the NHS and NHS II, Bertrand et al created predicted 25(OH)D scores based on correlates of vitamin D status measured from biennial follow-up questionnaires.[20] The scores were derived using the following covariates: race (white, black, other), energy-adjusted vitamin D intake from food sources (≥ 400, 300-399, 200-299, 100-199, <100 IU/d), vitamin D from supplements (≥ 400, 200-399, 1-199, 0 IU/d), UVB-flux based on latitude of state of residence (<113°, 113°, >113°), BMI (<22, 22-24.9, 25-29.9, 30-34.9, >35 kg/m^2), physical activity (quintiles), alcohol intake (0, 1-4.9, 5-9.9, 10 g/day), and hormone use (pre-menopausal, post-menopausal/unknown PMH, post-menopausal/never PMH, post-menopausal/past PMH, post-menopausal/current PMH). Race was included as a predictor as a proxy for skin tone. Alcohol intake and

energy-adjusted vitamin D intake from food sources and supplements was estimated from food frequency questionnaires that asked participants to estimate their average frequency of consumption of specific foods over the preceding 12 months. UVB flux for state of residence was an estimated composite of average UVB radiation based on latitude, altitude and cloud cover. Physical activity in MET-hours/week was estimated from nurses' responses to questions regarding activity frequency and intensity on follow-up questionnaires and was used as a proxy for time spent outdoors.

For each cohort, these covariates, along with age, season of blood draw and laboratory batch, were regressed as a linear function to predict serum 25(OH)D in a "training sample" of nurses (NHS n=2,079; NHS II n=1,497) who had available blood and had served as controls for all previous and ongoing nested case-control studies of vitamin D within the cohorts. Covariate information from the questionnaire cycle closest to the date of blood draw was used in the prediction model building. The regression coefficients for each covariate in this model were used to quantify predicted scores for participants of each cohort. (Table 2) Season of blood draw was not included in the derived scores because it does not affect long-term within-person variation of serum 25(OH)D.

For the NHS, predicted scores for each cycle of follow-up were derived for all living participants beginning in 1986 (the first year of comprehensive physical activity measurement and estimate of UVB-flux). Dietary and supplemental vitamin D estimates from FFQs (assessed every four years) were carried forward from the previous FFQ for non-FFQ years. In the event that a participant had incomplete data on a predictor from a follow-up questionnaire, values from the previous cycle were carried forward one cycle

(with the exception of menopausal status/PMH use). UVB flux was not assessed in 2004; therefore, the value from 2002 was used. For participants who were missing data on a specific predictor for more than one follow-up cycle and for those who did not respond at all to the follow-up questionnaire during a cycle, a prediction score was not created and participants did not contribute to person-time in this cycle.

For the NHS II, prediction scores were created similarly, using the same set of predictors assessed from biennial follow-up questionnaires. The first year of derivation was 1991; scores were derived for all women who responded to the 1991 questionnaire and FFQ and were derived for each questionnaire year thereafter through 2003. For questionnaire years 2005 and 2007, predicted scores from 2003 were carried forward. Missing data on individual predictors were carried forward, as described for the NHS cohort.

Validity of the Prediction Model

A separate, independent "test sample" of nurses in each cohort with available plasma 25(OH)D measurements from a more recent nested case-control study was used to validate the scores estimated by the "training sample" data set. Bertrand et al compared derived scores for the "test sample" that were estimated from the regression coefficients of the "training sample" model to actual plasma 25(OH)D measurements.

The validity of the predicted 25(OH)D scores in the NHS and NHS II was evaluated by comparison to plasma 25(OH)D levels in the aforementioned "test sample" of participants of each cohort (NHS, n=818; NHS II, n=479).[20] The Spearman correlation coefficients between predicted score and plasma 25-hydroxyvitamin D level adjusted for

season, batch, and age were 0.33 and 0.42 for the NHS and NHS II, respectively.[20] For NHS, the prediction model explained 33% of the variability in plasma 25(OH)D. For NHS II, the prediction model explained 25% of serum 25(OH)D variability.[20]

Assessment of Multiple Sclerosis

Documentation of incident MS cases in the NHS and NHS II was initiated by self-report of new diagnosis on one of the biennial follow-up questionnaires. In the event that a nurse indicated a new diagnosis, she was asked permission by investigators to contact her treating physician or neurologist and review medical records. Upon obtaining permission, the physician or neurologist was sent a questionnaire, which addressed the following aspects of the diagnosis: certainty of diagnosis (definite, probable, possible, or not MS), date of symptom onset, laboratory test results, attack history, type of MS and other relevant information pertaining to the diagnosis. Diagnoses were classified as definite, probable, possible, or not MS according to Poser MS diagnostic criteria.[21] Definite cases were defined as clinically definite or laboratory-supported definite MS. Probable cases were defined as clinically probable or laboratory-supported probable MS. For the purposes of this analysis, we included only incident definite/probable MS cases in the main analyses. Cases were ascertained in two-year follow-up intervals.

MS was first included as physician diagnosed condition on the 1992 (NHS) and 1991 (NHS II) questionnaires. Since it is possible that MS may cause an individual to modify behaviors associated with the variables contributing to the 25(OH)D score, MS cases in the NHS who experienced symptom onset prior to the first available prediction

scores in 1986 and MS case in the NHS II who experienced symptom onset prior to 1991 were excluded.

Validity of Multiple Sclerosis Assessment

The validity of MS case ascertainment within the NHS and NHS II has been previously described by Hernan et al.[21] Briefly, the medical records of a small sample of MS cases (n=39) were reviewed by study neurologists blinded to the diagnosis classification assigned by the nurses' treating neurologist. Among probable/definite diagnoses from treating neurologists, classification with study neurologists was concordant 93% of the time. Therefore, diagnoses from the nurses' treating neurologists were used in determining diagnosis classification for all MS cases. In the event that a nurse's diagnosis from her treating neurologist was not available, the study neurologist made the diagnosis after a review of medical records.

Covariate Assessment

Information regarding BMI, latitude of residence, and race was obtained from baseline questionnaires and were updated every two years from returned follow-up questionnaires. Because the aforementioned covariates were included in the prediction models for this exposure, to avoid over-adjustment, they were not included as covariates in the analyses. Instead, we adjusted for age[9,15], cigarette smoking (pack-years)[9], latitude of residence at age 15 (North: ≥41° latitude, Middle: 37-<41° latitude, South: <37° latitude)[9], BMI at age 18 (<18.5, 18.5-24.9, 25-29.9, ≥30 kg/m^2)[3], and ethnicity (Southern European, Scandinavian, other Caucasian and other), based on inclusion in prior studies.

BMI at age 18, latitude of residence at age 15, and ethnicity was assessed at baseline (1976, NHS; 1989, NHS II). Smoking was updated for each follow-up cycle.

Statistical Analysis

First, we calculated general descriptive statistics of the distribution of predicted 25(OH)D score in the NHS and NHS II. (Table 3) To evaluate confounders, we cross-tabulated covariates by age-adjusted mean predicted 25(OH)D score at baseline (1986 and 1991, respectively). (Table 4) Generalized linear models were used to assess age-adjusted mean scores by covariates. Person-months were calculated from month of return of baseline questionnaire to month of MS symptom onset, death from any cause, or end of follow-up (2004 for NHS and 2009 for NHS II), whichever occurred first.

Analyses were conducted separately for each cohort (NHS and NHS II). Cox proportional hazards models were used to evaluate the association between predicted 25(OH)D score and MS, adjusting for covariates. To assess predicted 25(OH)D score and risk of MS, predicted scores were analyzed as a categorical variable (quintiles). Hazard ratios and 95% confidence intervals are reported. (Table 5) Mantel extension tests were used to assess trend across quintiles.

A sensitivity analysis was conducted restricting to only definite MS cases. Age-adjusted and multivariable Cox proportional hazards models were repeated excluding probable cases. Hazard ratios and associated 95% confidence intervals are reported. (Table 6) All analyses were conducted using SAS v9.3 (SAS Institute Inc, Cary, NC).

CHAPTER 3

RESULTS

Table 3 shows the distribution of predicted scores in each cohort at baseline. Mean predicted 25(OH)D score was lower in the NHS than in the NHS II. In the NHS, scores in the lowest quintile ranged from 2.49 to 24.44, which was a greater range than any other quintile. The same was true in the NHS II; scores in the lowest quintile ranged from 14.48 to 28.55. The average age of symptom onset of cases was 54 years in the NHS and 45 years in the NHS II.

Because of high statistical power in this analysis, even small differences in the distribution of all covariates according to age-adjusted mean predicted 25(OH)D scores at baseline were statistically significant. (Table 4) In the NHS, women who were 55-59 years old at baseline had the lowest mean predicted 25(OH)D score and women who were 45-49 years old at baseline had the highest mean predicted 25(OH)D score. Baseline mean 25(OH)D score was highest amongst women younger than 30, and lowest amongst women older than 45 in the NHS II. In both the NHS and NHS II, women of Scandinavian ethnicity had highest mean age-adjusted predicted score and women of "other" ethnicity had the lowest. Women who reported BMI at age 18 greater than 30 kg/m^2 had the lowest predicted age-adjusted mean score in both cohorts. In the NHS, women who had a middle latitude of residence at age 15 had the lowest age-adjusted mean predicted 25(OH)D score at baseline, while those who had a southern latitude of residence at age 15 had the highest. In the NHS II, age-adjusted mean 25(OH)D score was lowest for southern latitude of residence at age 15 and highest for northern latitude.

In both cohorts, age-adjusted mean 25(OH)D score was lowest for women who smoked greater than 25 packs per year.

Table 5 shows age-adjusted and multivariable-adjusted hazard ratios of MS by quintile of predicted 25(OH)D score. In the NHS, comparing Q5 vs. Q1 of predicted 25(OH)D score was not associated with risk of MS (HR=1.15, 95% CI=0.42-3.12) (p-trend=0.97). However, in the NHS II, women in the highest quintile (median=34.76) of predicted 25(OH)D had a significant lower risk of developing MS (HR=0.52, 95% CI=0.28-0.95), with some evidence of a linear trend (p-trend=0.07).

Table 6 shows age-adjusted and multivariable-adjusted hazard ratios of MS by predicted 25(OH)D score in analyses restricted to definite cases. In the NHS, results were somewhat stronger in analyses limited to definite cases, though our power for this comparison was low and the trend was not significant (p-trend=0.47). Women in the highest quintile of predicted score had a non-significant lower risk of MS (HR=0.58, 95% CI=0.13-2.57). In the NHS II, the inverse association was stronger when restricted to definite cases. For example, women in the highest quintile of predicted score experienced a 59% lower risk of developing MS as compared to those in the lowest quintile (HR=0.41, 95% CI=0.20-0.85). The linear trend was also significant (p-trend=0.04).

CHAPTER 4

DISCUSSION

We observed an inverse association of predicted 25(OH)D score and risk of MS in the NHS II with some evidence of a dose-response relationship, although this association was not observed in the NHS. This difference may be due to variation in the association across age groups.

The findings of our study in the NHS II cohort are consistent with prior literature in younger adults. Although no prior studies have used a predicted 25(OH)D score as a proxy for vitamin D status, one prospective study of serum 25(OH)D and one prospective study of dietary vitamin D intake found a similar association. In a nested case-control study of U.S. service-men and –women, Munger et al reported a 62% lower risk of MS in white individuals in the highest quintile of serum 25(OH)D compared to those in the lowest quintile. In this population, the average age of MS onset was 28.5 years, which is closer to the age during follow-up of NHS II members than NHS members. For the NHS, the youngest possible age at start of follow-up in our study was later (40 years) compared to the NHS II (27 years). Earlier cases in the NHS were excluded because of the timing of the start of the prediction modeling in 1986, making this an older cohort during the exposure window than the NHS II. In the NHS, the average age of onset was 54, which may have contributed to the lack of association for this cohort.

In a prospective study of dietary vitamin D intake in the NHS and NHS II, Munger et al found that total dietary vitamin D intake in the highest quintile was associated with a 59% decreased risk of MS in the NHS (RR=0.41; 95% CI: 0.18-0.94). In the NHS II, total dietary vitamin D intake in the highest quintile was associated with a

17% decreased risk of MS (RR=0.83; 95% CI: 0.41-1.67). In the NHS, among the four cases with supplemental vitamin D ≥ 400 IU/day, there was a 65% decreased risk of MS compared to non-supplement users with a non-significant trend (p-trend=0.10). In the NHS II, among the 14 cases with supplemental vitamin D ≥ 400 IU/day, there was 30% decreased risk of MS compared to non-supplement users and the trend was not significant (p-trend=0.18).

One reason that Munger et al may have found a strong inverse association of dietary vitamin D intake and MS in the NHS cohort while our study did not may be due to the younger cases included in their analysis. By design, our study excluded all cases of MS that experienced symptom onset prior to 1986 (the first year of available predicted scores), meaning that the youngest possible age of MS cases included in our analysis was 40. Their study included cases collected from 1980 to 1998, which means that the youngest possible cases included in their analysis was 34. This discrepancy in the age of cases may partially explain the strong inverse association of dietary vitamin D intake in the NHS that Munger et al found while we observed no association. The lack of association that we observed in the NHS (older women) and the strong inverse association that we observed in the NHS II (younger women) is consistent with the highest incidence rates occurring in younger women and lower incidence rates occurring with increasing age.

In the NHS II, results from this study are consistent with ours; however, we observed an even stronger association than Munger et al and our results were statistically significant. The non-significant findings in the NHS II that Munger et al observed for dietary vitamin D intake may have been affected by non-differential misclassification of

the exposure. Because vitamin D status is influenced by a variety of different factors (particularly sun exposure), dietary vitamin D intake may not be the most reliable of vitamin D status. In our study, by using predicted 25(OH)D scores as a proxy for vitamin D status rather than dietary vitamin D intake alone, other predictors of vitamin D status were incorporated into the exposure, reducing non-differential misclassification. This may explain the stronger results we observed in the NHS II compared the dietary vitamin intake study.

Our study has several strengths. First, in prospective cohorts of 121,701 and 116,430 women, there were 26,223,875 and 22,658,634 person-months of follow-up contributed in the NHS and NHS II, respectively. Follow-up for each questionnaire cycle is at least 89% and is even higher for prospective analyses and thus, selection bias due to differential follow-up in this study is unlikely. Another strength of our study is that we were able to censor MS cases at date of first symptoms. MS cases often experience first symptoms long before physician diagnosis. Symptoms of MS, which are exacerbated by heat, may have induced behavior change related to vitamin D exposure before date of diagnosis and thus, censoring at date of symptom onset rather than date of diagnosis reduces the potential for reverse causation. In our study, because predicted 25(OH)D scores are updated biennially, we were able to capture changes in exposure status throughout the duration of follow-up. By using the NHS and NHS II cohorts, we were able to assess the association of vitamin D status and risk of MS in older adult women using a proxy of vitamin D status, an association that has not previously been elucidated.

Because repeated 25(OH)D measurements are not available for most cohorts, the use of predicted 25(OH)D scores as a proxy for blood measurements is a practical and

cost-effective way to assess the association between vitamin D and MS. Using predicted 25(OH)D scores in lieu of serum measurements may best be viewed as a trade-off of strengths. By using updated predicted scores in prospective analyses as a proxy for serum measurements, we sacrificed direct measurements but gained the ability to prospectively assess vitamin D over a longer time period prior to MS onset.

There are also some limitations to our study. Predicted 25(OH)D scores were estimated from self-reported covariates including race, energy-adjusted vitamin D from food, vitamin D from supplements, BMI, physical activity, alcohol intake and hormone use.[20] It is possible that women may have inaccurately reported some of these covariates, which may have caused participants to fall into inappropriate quintiles of predicted 25(OH)D score. Several predictors of vitamin D status including skin tone, time spent outdoors, sunscreen use and genetic polymorphisms (i.e., VDR and VDBP expression) were not directly measured and incorporated in the derivation of predicted scores. However, race and physical activity were used as proxy measurements for skin tone and time spent outdoors, respectively, in an effort to include these factors.

It is important to note that the Spearman correlation coefficients between predicted scores and serum measurements adjusted for age, batch, and season of blood draw for the NHS and NHS II are 0.33 and 0.40, respectively. Serum 25(OH)D has moderate within-person variability, and thus is not a true "gold standard" of vitamin D status. In the NHS, the intra-class correlation coefficient (ICC) for serum 25(OH)D over 2-3 years is 0.72[20], which indicates fairly high reproducibility. However, over 10-11 years, the ICC is 0.50[20], indicating decreasing reproducibility with increasing time. Fitting the predictors to a single serum 25-hydroxyvitamin D measurement likely

underestimated the correlation coefficients between serum values and predicted scores. According to Bertrand et al, in a comparison of quintiles of serum 25(OH)D and predicted 25(OH)D scores, 24.8% of NHS participants and 29.9% NHSII participants fell into identical quintiles. 59.8% of NHS participants and 66.5% of NHSII participants fell into the same or adjacent quintiles and only 5% or less of the participants fell into extreme opposite quintiles.[20]

Misclassification of the exposure is likely and if present, caused a bias towards the null. The middle quintiles may have been particularly affected; however, comparison between extreme quintiles (i.e., low versus high score) is likely a more robust comparison because of the low rate of misclassification into extreme opposite quintiles. While the predicted scores do not directly measure vitamin D deficiency/sufficiency per se, their relative ranking of women into quintiles is likely to be generally accurate for extreme opposite ends of the spectrum. Women who fell into the lowest quintile of predicted 25(OH)D score likely correspond to women who are truly vitamin D deficient and vice versa. Importantly, if misclassification of the exposure occurred, we would expect that the true association would be even stronger than we observed.

It could be argued that null results in the NHS are the result of non-differential misclassification of exposure rather than a true lack of an association for older women. However, the difference in Spearman correlation coefficients of serum 25(OH)D and predicted score between the NHS (r=0.33) and NHS II (r=0.40) is relatively small, and is unlikely to explain a null association in the NHS considering such a strong inverse association in the NHS II. It is unlikely that the NHS experienced a much higher degree

of non-differential misclassification of exposure than the NHS II, and thus, we attribute the difference in results to a potential variation in the association across age groups.

For the NHS and NHS II, the covariates used to create the prediction scores accounted for 33% and 25% of the variability in serum 25(OH)D.[20] The remaining unexplained variability can likely be attributed to measurement error of predictor variables and missing information regarding other determinants of vitamin D status, such as genetic factors.[20] We do not have available information regarding infection of Epstein-Barr virus (a known risk factor for MS) for the entire NHS and NHS II cohorts; however, this is unlikely to confound the association between predicted 25(OH)D score and MS. While EBV infection is associated with risk of MS, it is not associated with serum 25(OH)D.[7] Additionally, genes associated with increased risk of MS such as *HLA-DRB1*1501*, have not been associated with serum 25(OH)D levels.[8] Essentially, some of the remaining variability of serum 25(OH)D unexplained by the covariates of the prediction scores is likely to be related to genetic factors affecting plasma 25(OH)D, but these same genetic factors have not been consistently associated with risk of MS.[4] Because of this, we expect the effect of confounding by genetic factors to be relatively small. Other dietary components may interact with vitamin D; however, other vitamins and minerals have not been consistently associated with risk of MS.[9] Therefore, we also expect the effect of confounding by other dietary factors to be relatively small.

The results of this study are generalizable to other women of similar age in the United States. The association of predicted 25(OH)D score and MS may differ in adolescents and thus, findings may not be generalizable to this group.

CHAPTER 5

CONCLUSION

In conclusion, the results of our study expand upon the existing literature that supports a protective role of vitamin D on risk of MS. Our analyses suggest that predicted 25(OH)D score, as a proxy for vitamin D status, is associated with lower risk of MS in younger women; however, this association was not observed in older women.

TABLES

Table 1. Exclusion criteria: Nurses' Health Study & Nurses' Health Study II

	NHS	**NHSII**
	N	N
Original study sample	121,701	116,430
Missing date of first symptoms	14	45
MS onset prior to first score derivation	127	111
Death prior to baseline	2293	5
Missing baseline prediction scores	43193	21163
Final study sample	75,914	95,106

Table 2. Predictors of serum 25-hydroxyvitamin D from multivariable linear regression models in the Nurses' Health Study & Nurses' Health Study II

Predictor	**NHS**	***P***	**NHSII**	***P***
	n=2079		n=1497	
	Difference in 25(OH)D (ng/ml; β)		Difference in 25(OH)D (ng/ml; β)	
Intercept	22.69		35.78	
Age (years)	0.07	0.07	−0.23	<0.0001
Race		<0.001		<0.001
White	0		0	
Black	−11.3		−6.42	
Asian	–		−5.55	
Hispanic	–		−6.83	
Other	−1.63		1.98	
UV-B flux category		<0.0001		0.67
1 (highest)	0		0	
2	−2.69		−0.16	
3	−1.29		−0.66	
4	–		−0.6	
5 (lowest)	–		–	
Dietary vitamin D (μg/d)		<0.0001		0.003
<2.5	0		0	
2.5- <5	0.92		1.56	
5- <7.5	2.19		1.87	
7.5- <10	3.43		3.55	
≥10	3.33		2.49	
Supplementary vitamin D (μg/d)		<0.0001		<0.001
0	0		0	
0.025- <5	2.85		0.76	
5- <10	1.57		2.05	
≥10	3.15		2.7	
BMI (kg/m^2)		<0.0001		<0.0001
< 19	–		2.22	
< 22 (19-21.9 in NHS II)	0		0	
22-24.9	−0.57		−0.38	
25-29.9	−1.95		−2.35	
30-34.9	−3.32		−5.09	
≥ 35	−8.16		−6.17	

Predictor	NHS	*P*	NHSII	*P*
Quintile of physical activity		<0.0001		<0.0001
1 (lowest)	0		0	
2	1.77		0.99	
3	1.15		1.2	
4	2.13		3.07	
5 (highest)	3.66		3.79	
Post-menopausal hormone use		<0.001		0.12
1	0		0	
2	−1.66		0.17	
3	−2.11		1.94	
4	−1.17		1.53	
5	−0.66		0.71	
Alcohol intake (g/d)		<0.0001		<0.001
0	0		0	
> 0- <5	0.24		1.34	
5- <10	1.33		2.38	
≥10	2.62		2.69	
Season of blood draw		<0.0001		<0.0001
Autumn	0		0	
Summer	1.18		1.33	
Spring	−2.68		−5.55	
Winter	−3.35		−5.61	

*Table adapted from Bertrand et al[20]

Table 3. Distribution of predicted 25-hydroxyvitamin D scores at baseline: Nurses' Health Study & Nurses' Health Study II

	N	M (SE)	Range	Median
NHS				
Predicted 25-hydroxyvitamin D score	75914	27.30 (0.01)	37.12	27.49
Predicted 25-hydroxyvitamin D score				
Q1	15172	21.60 (0.02)	21.95	22.60
Q2	15106	25.57 (0.01)	2.14	25.59
Q3	15240	27.50 (0.004)	1.84	27.49
Q4	15174	29.39 (0.005)	2.02	29.36
Q5	15222	32.40 (0.01)	9.13	32.00
NHS II				
Predicted 25-hydroxyvitamin D score	95106	31.20 (0.01)	26	31.64
Predicted 25-hydroxyvitamin D score				
Q1	19013	25.92 (0.02)	14.07	26.45
Q2	18997	29.80 (0.005)	2.26	29.88
Q3	19029	31.64 (0.003)	1.61	31.64
Q4	19045	33.23 (0.004)	1.68	33.22
Q5	19022	35.42 (0.007)	6.38	35.19

Table 4. Distribution of covariates according to age-adjusted mean 25-hydroxyvitamin D score at baseline: Nurses' Health Study (1989) & Nurses' Health Study II (1991)

Covariate	**NHS**	**NHS II**
	Mean 25(OH)D Score (SE)	Mean 25(OH)D Score (SE)
Ethnicity		
S. European	27.17 (0.04)	31.37 (0.03)
Scandinavian	28.42 (0.07)	31.81 (0.05)
Other caucasian	27.68 (0.02)	31.47 (0.01)
Other	26.18 (0.03)	27.57 (0.04)
BMI at age 18		
<18.5	27.98 (0.04)	32.24 (0.03)
18.5-24.9	27.52 (0.02)	31.42 (0.01)
25-29.9	25.26 (0.05)	28.61 (0.04)
>=30	23.69 (0.07)	26.99 (0.07)
Latitude of residence at age 15		
North	27.79 (0.02)	31.32 (0.02)
Middle	26.80 (0.02)	31.29 (0.02)
South	28.46 (0.05)	31.18 (0.03)
Smoking (pack-years)		
Never	27.26 (0.12)	31.09 (0.01)
<10	27.67 (0.03)	31.76 (0.03)
10-24	27.61 (0.04)	31.24 (0.03)
25+	27.13 (0.02)	30.52 (0.06)

Table 5. Age-adjusted and multivariate HR and 95% CI of multiple sclerosis by quintile of predicted 25-hydroxyvitamin D score: Nurses' Health Study & Nurses' Health Study II

	Person-months	**Age-adjusted**	**Multivariate***	***P*-trend**
	Cases (N)	HR (95% CI)	HR (95% CI)	
NHS	26,223,875 N=39			*P*=0.97
Q1		1.0 (Referent)	1.0 (Referent)	
Q2		1.24 (0.49-3.13)	1.27 (0.49-3.27)	
Q3		0.49 (0.15-1.63)	0.49 (0.14-1.65)	
Q4		0.99 (0.37-2.64)	0.97 (0.35-2.67)	
Q5		1.13 (0.45-3.01)	1.15 (0.42-3.12)	
NHS II	22,658,634 N=140			
Q1		1.0 (Referent)	1.0 (Referent)	*P*=0.07
Q2		0.79 (0.48-1.28)	0.81 (0.49-1.35)	
Q3		0.71(0.43-1.17)	0.74 (0.43-1.26)	
Q4		0.82 (0.51-1.33)	0.87 (0.51-1.46)	
Q5		0.49 (0.28-0.87)	0.52 (0.28-0.95)	

*Adjusted for age, ethnicity, latitude of residence at age 15, BMI at age 18 and smoking

Table 6. Age-adjusted and multivariate HR and 95% CI of multiple sclerosis by predicted 25-hydroxyvitamin D score restricted to definite cases: Nurses' Health Study & Nurses' Health Study II

	Person-months	**Age-adjusted**	**Multivariate***	***P*-trend**
	Cases (N)	HR (95% CI)	HR (95% CI)	
NHS	26,223,875 N=20			
Predicted 25(OH)D				*P*=0.47
Q1		1.0 (Referent)	1.0 (Referent)	
Q2		0.98 (0.28-3.38)	1.00 (0.28-3.55)	
Q3		0.39 (0.08-2.00)	0.37 (0.07-2.00)	
Q4		0.98 (0.28-3.40)	0.92 (0.25-3.36)	
Q5		0.63 (0.15-2.63)	0.58 (0.13-2.57)	
NHS II	22,658,634 N=102			
Predicted 25(OH)D				*P*=0.04
Q1		1.0 (Referent)	1.0 (Referent)	
Q2		0.69 (0.39-1.22)	0.71 (0.39-1.29)	
Q3		0.73 (0.41-1.27)	0.76 (0.42-1.39)	
Q4		0.73 (0.42-1.28)	0.77 (0.42-1.42)	
Q5		0.38 (0.19-0.77)	0.41 (0.20-0.85)	

*Adjusted for age, ethnicity, latitude of residence at age 15, BMI at age 18 and smoking

BIBLIOGRAPHY

1. Anderson DW. Revised estimate of the prevalence of multiple sclerosis in the united states. Ann Neurol. 1992;31(3):333-6.

2. Alonso A. Incidence of multiple sclerosis in the United Kingdom. J Neurol. 2007;254(12):1736-41.

3. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. part II: Noninfectious factors. Ann Neurol. 2007;61(6):504-13.

4. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. The Lancet Neurology. 2010 6;9(6):599-612.

5. Manji H. Oxford handbook of neurology. Oxford; New York : Oxford University Press; 2007.

6. Ytterberg C. Variations in functioning and disability in multiple sclerosis. J Neurol. 2008;255(7):967-73.

7. Ascherio A. Environmental risk factors for multiple sclerosis. part I: The role of infection. Ann Neurol. 2007;61(4):288-99.

8. Ferrari S. Gene variants for osteoporosis and their pleiotropic effects in aging. Mol Aspects Med. 2005;26(3):145.

9. Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004 January 13;62(1):60-5.

10. Goldberg P. Multiple sclerosis: Vitamin D and calcium as environmental determinants of prevalence. The International journal of environmental studies. 1974;6(2-3):121-9.

11. Smolders J, Damoiseaux J, Menheere P, Hupperts R. Vitamin D as an immune modulator in multiple sclerosis, a review. J Neuroimmunol. 2008 2;194(1–2):7-17.

12. Kampman MT, Steffensen LH. The role of vitamin D in multiple sclerosis. Journal of Photochemistry and Photobiology B: Biology. 2010 11/3;101(2):137-41.

13. Royal W. Peripheral blood regulatory T cell measurements correlate with serum vitamin D levels in patients with multiple sclerosis. J Neuroimmunol. 2009;213(1-2):135-41.

14. Smolders J. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. PLoS ONE. 2009;4(8):1.

15. Munger KL. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA: the Journal of the American Medical Association. 2006;296(23):2832.

16. Salzer J, Hallmans G, Nyström M, Stenlund H, Wadell G, Sundström P. Vitamin D as a protective factor in multiple sclerosis. Neurology. 2012;79(21):2140-5.

17. Kragt J. Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women. Multiple Sclerosis Journal. 2009;15(1):9-15.

18. Barnes MS. Assessment of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 concentrations in male and female multiple sclerosis patients and control volunteers. Multiple Sclerosis Journal. 2007;13(5):670-2.

19. Soilu-Hanninen M. 25-hydroxyvitamin D levels in serum at the onset of multiple sclerosis. Multiple Sclerosis Journal. 2005;11(3):266-71.

20. Bertrand KA, Giovannucci E, Liu Y, Malspeis S, Eliassen AH, Wu K, et al. Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three US cohorts. Br J Nutr. 2012;108(10):1889-96.

21. Hernan MA. Geographic variation of MS incidence in two prospective studies of US women. Neurology. 1999;53(8):1711.

22. Giovannucci E. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst. 2006;98(7):451-9.

23. Munger K. Body size and risk of MS in two cohorts of US women. Neurology. 2009;73(19):1543-50.