ABSTRACT

Title of Thesis: ASSOCIATION OF SERUM VITAMIN D AND KEY CO-NUTRIENTS IN RELATION TO HYPERTENSION: A CROSS-SECTIONAL STUDY BASED ON NHANES DATA

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Observational studies demonstrate strong associations between deficient serum vitamin D (25(OH)D) levels and cardiovascular disease. To further examine the association between vitamin D and hypertension (HTN), data from the 2003-2006 National Health and Nutrition Examination Survey were analyzed to assess whether the association between vitamin D and HTN varies by sufficiency of key co-nutrients necessary for metabolic vitamin D reactions to occur. Logistic regression results demonstrate independent effect modification by calcium, magnesium, and vitamin A on the association between vitamin D and HTN. Among non-pregnant adults with adequate renal function, those with low levels of calcium, magnesium, and vitamin D levels had 1.75 times the odds of HTN compared to those with sufficient vitamin D levels (p = <0.0001). Additionally, participants with low levels of calcium,

magnesium, vitamin A, and vitamin D had 5.43 times the odds of HTN compared to those with vitamin D sufficiency ($p = 0.0103$).

ASSOCIATION OF SERUM VITAMIN D AND KEY CO-NUTRIENTS IN RELATION TO HYPERTENSION: A CROSS-SECTIONAL STUDY BASED ON NHANES DATA

by

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Dedication

I dedicate this work to my husband and family who have provided encouragement, support, and the gift of time to commit to my graduate studies and this thesis, in particular.

I also give thanks to God for the persistence, strength and courage to move into this field of study to supplement my knowledge of nutrition, and hopefully, to contribute to public health through nutrition and epidemiology.

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List of Abbreviations

AMPM – Automated Multiple-Pass Method

BMI – Body Mass Index

CI – Confidence interval

CRP – C reactive protein

CVD – Cardiovascular disease

DASH – Dietary Approaches to Stop Hypertension

EAR - Estimated Average Requirement

eGFR - Estimated Glomerular Filtration Rate

HgA1c - Glycated hemoglobin

HTN – Hypertension

IOM – Institute of Medicine

IRB – Institutional Review Board

MEC – Mobile Examination Center

MPH – Masters of Public Health

NHANES – National Health and Nutrition Examination Survey

NIST - National Institute of Standards and Technology

OR - Odds Ratio

SAS - Statistical Analysis Systems

U.S. - United States

VDR –Vitamin D Receptor

WHI - Women's Health Initiative trial

25(OH)D - Serum 25-hydroxyvitamin D

Chapter 1: **INTRODUCTION**

An estimated one in three Americans, approximately 77.9 million individuals, has high blood pressure or hypertension (HTN) (1). HTN is a major risk factor for heart disease and stroke, the first and fourth leading causes of death in the United States, respectively (2).

Observational studies demonstrate strong associations between deficient vitamin D levels and cardiovascular mortality, stroke, coronary heart disease, and fatal heart failure (3–5). In contrast, the Institute of Medicine's (IOM) 2011 report concluded that the evidence regarding the impact of vitamin D to improve cardiovascular outcomes was "inconsistent, inconclusive as to causality, and insufficient to inform nutritional requirements" (6).

The last decade has witnessed an explosion of scientific research into vitamin D, along with a steady stream of sometimes conflicting vitamin D study reports (7,8). New information clarifies the mechanisms of actions of vitamin D, the heart and cardiovascular system (9). While the public hears about the purported benefits of achieving 'sufficient' levels of the nutrient, the medical community debates the efficacy of vitamin D therapy in light of contradictory clinical trial results, and seeks an appropriate cutoff value or range for determining adequacy (10–16). Vitamin D researchers recommend utilizing different study designs to test nutrients in order to avoid the cost in money and time of large trials that yield disappointing results (17,18).

This analysis investigated a novel biochemical 'systems' approach that considered whether the association between vitamin D levels and HTN varied by the sufficiency status of co-nutrients in the vitamin D biochemical pathway (19–21). The system of interest included serum 25-hydroxyvitamin D (25(OH)D), the co-nutrients that support the chemical transformation change from the inactive to the active form of vitamin D, and assessment of cases of HTN. Previous studies have been conducted to examine whether a lack of vitamin D is associated with an increased risk of having HTN (8,22–26).

This study examined an additional question: Does an insufficiency of the co-nutrients that support vitamin D metabolism modify the risk of having HTN? If co-nutrients levels are inadequate, necessary biochemical reactions may be limited or may not occur. Such negative or mixed results may help to explain the IOM finding of insufficient and inconclusive evidence to demonstrate the efficacy of vitamin D regarding cardiovascular outcomes.

Chapter 2: RESEARCH QUESTIONS AND SPECIFIC AIMS

Long Term Objectives

The long term objective was to demonstrate the impact of vitamin D on the critical health outcome of hypertension (HTN), and explore the potential public health benefits that may be achieved with provision of sufficient levels of the nutrient. Inadequate vitamin D status is associated epidemiologically with multiple disparate health outcomes such as osteoporosis, cardiovascular disease (CVD), cancer, diabetes, and autoimmune diseases (8,27,28). HTN was selected for this research study because of the public health burden related to this disease, and to explore the reasons for the mixed outcomes of previous clinical trials.

Importance of Study

Many clinical trials of nutrients have yielded disappointing results, leading to confusion and skepticism on the part of the public and healthcare providers of the value and role of nutrition for health outcomes (29-31). Attempting to study isolated nutrients in relation to a disease outcome may be a key study design omission, responsible, in part, for the null or inconclusive results observed in many nutrient trials, and those regarding CVD in particular (31). A 'systems' model may provide some insight into improved methodology for conducting future nutrient trials (20). This study involved analysis of calcium, iron, magnesium, niacin, vitamin A, and zinc as key co-nutrients that are required physiologically for the vitamin D pathway to function to its full capacity (9,32,33).

Specific Aims

The specific aims of this thesis were twofold: 1) to evaluate the association between vitamin D and HTN; and 2) to evaluate the association between vitamin D and HTN, analyzed with and without adequate levels of vitamin D co-nutrients. Adequate level of vitamin D co-nutrients was defined as meeting the Estimated Average Requirement (EAR) cutoff for the respective co-nutrients investigated in this study (34). The EAR is the national standard defined by the Institute of Medicine, used in population nutrition studies as a measure of the prevalence of inadequacy for each nutrient within a group, defined as meeting fifty percent of the recommended intake of a healthy person in the group on the occasion of the nutrient assessment.

Vitamin D is measured by serum 25(OH)D, a continuous variable that is reported both in nanograms per milliliter or in millimoles per liter. In this thesis, serum 25(OH)D was categorized as a binary variable using less than 29 ng/mL or greater than or equal to 30 ng/mL as the cutoff values.

The study definition of HTN include the following criteria:

- High blood pressure of >140 millimeters of mercury (mmHg) systolic and/or
- High blood pressure of >90 mmHg diastolic or
- Currently taking medication to lower blood pressure

Study Hypotheses:

1. H_O: The association between vitamin D status and hypertension is not significant after adjustment for potential confounders.

   H_A: The association between vitamin D status and hypertension is significant after adjustment for potential confounders.

2. H_O: The association between vitamin D status and hypertension does not vary by vitamin D co-nutrient sufficiency status.

   H_A: The association between vitamin D status and hypertension does vary by vitamin D co-nutrient sufficiency status.

Study Overview

The study population included civilian, non-institutionalized, adult males and non-pregnant females greater than or equal to 20 years, from the 50 U.S. States and Washington, D.C. Two cycles of the biennial 2003-2004 and 2005-2006 NHANES were selected for analysis. The independent variable was serum 25(OH)D; the dependent variable was HTN. The statistical software utilized was Statistical Analysis Systems (SAS) (SAS version 9.4, SAS Institute, Cary NC).

Chapter 3: BACKGROUND

The role of vitamin D in maintaining calcium homeostasis within the classical vitamin D endocrine system that impacts skeletal health has been well established since the mid-1970s (35). Vitamin D is synthesized in the body by the interaction of sunlight with 7-dehydrocholesterol in the skin with heat to form vitamin D_3 (36). In the bloodstream, D binding-protein carries D_3 from the skin, or from absorbed D_2 or D_3 from food or dietary supplement sources to the liver or to fat cells for storage. In the liver, the 25α-hydroxylase enzyme adds an OH group to form the prohormone 25(OH)D. This is the form that is measured to assess adequacy of vitamin D stores (12,37,38). Bound to D binding-protein, 25(OH)D is carried to the kidneys where it undergoes second enzyme catalyzed addition of an OH group to form the active steroid hormone: $1,25(OH)_2D$ (35). From the kidney, bound again by D binding-protein in the blood, the active hormone vitamin D attaches to vitamin D receptors (VDR) to perform many different functions.

A major shift in vitamin D research took place once investigators discovered that the VDR was part of a nuclear family of receptors that directly or indirectly controls DNA synthesis (39). In order for a cell to respond to vitamin D, VDR must be located on the cell wall (40). VDR have now been found on cell membranes throughout the body.

Ample, compelling epidemiological evidence links the active hormone to many cardiovascular mechanisms in the human body (21). Low serum vitamin D levels are inversely related to high blood pressure through three main processes: VDR directly suppresses renin promotion in renal juxtaglomerular cells as part of the Renin

Angiotensin Aldosterone System (RAAS), which controls blood pressure; effects on large and small blood vessels to enable contraction and relaxation; and interaction with heart muscle cells to decrease cardiac contractility (41,42).

Vitamin D plays an active role in the cardiovascular system (43). VDR are located in all large and small vessels of the cardiovascular system, in the lining of the innermost or intima single cell endothelial layer, the middle smooth muscle layer, and the outer walls of the vessels (44). Vitamin D deficiency is inversely related to abnormal lipid levels, atherosclerosis, and all-cause mortality (45,46). Adequate vitamin D decreases foam cell formation, vascular inflammation and macrophage activation (47). Overall, 1,25(OH)D, the active hormone form of vitamin D, down-regulates several inflammatory and cell adhesion molecules that impact vascular tone, vascular angiogenesis, and HTN directly or indirectly (9).

Existing Knowledge

The leading cause of death in the United States (U.S.) is heart disease. Roughly one in three Americans will die of CVD, and 60% will experience a major vascular event before dying (1). The age-standardized mortality rate attributable to CVD in 2010 was 236.6 per 100,000. The adjusted fraction attributable to HTN was 40.6% (95% Confidence Interval (CI): 24.5%-54.6%). The prevalence of HTN in the 2010 population among adults at least 20 years of age was 33% (33.6% in males, 32.2% in females).

The prevalence of HTN is highest in Non-Hispanic Black females (47.0%), followed by Non-Hispanic Black males (42.6%), Non-Hispanic White males (33.4%), Non-Hispanic White females (30.7%), Mexican American males (31.1%), Mexican

American females (28.8%), American Indian/Alaskan Native (28.8%), Hispanic or Latino (22.2%) and Asian (18.7%), respectively (48).

Gaps in Literature

Adequacy of co-nutrients is rarely considered in human trials. In nutrient testing of animals, the provision of known co-nutrients are carefully monitored and provided to ensure the adequacy of the 'feed' so that co-nutrients do not limit the action of the independent variable (49). Nutrients perform roles in a biochemical system.

The sheer number of reactions that have been discovered to date is indicative of why vitamin D is reported to impact so many organ systems and disease states (50). The active hormone ($1,25(OH)_2D$) is directly and indirectly involved in approximately 800 chemical reactions in the human body and is a seco-steroid hormone. A seco-steroid can change shape, and can perform more functions because it can assume different forms. (37,51). Steroid hormones have a high affinity for their corresponding nuclear receptors as they dock with DNA to enable nuclear synthesis reactions. A major finding was that VDR forms a heterodimer or unit of two different molecules, with the vitamin A nuclear receptor, described by the acronym RXR. The VDR-RXR unit docks with deoxyribonucleic acid (DNA) to induce and regulate nuclear synthesis reactions. Vitamin A is essential for many genomic vitamin D reactions, including those that influence HTN. In the synthesis of DNA, the RXR-VDR complex requires zinc finger enzymes, iron and niacin (40,52). Zinc is also required for the synthesis of retinol binding protein, the transport molecule for

vitamin A in the bloodstream (53). Lack of zinc compromises the ability of the body to access vitamin A liver stores to distribute the nutrient to target tissues (54).

Magnesium is a key vitamin D co-nutrient, needed by the parathyroid glands for parathyroid hormone to be secreted to induce the synthesis of the active form of vitamin D. Magnesium is a stabilizing co-nutrient in almost every vitamin D enzymatic reaction (32,55).

Sufficient calcium is needed to optimize vitamin D functions in cellular signaling pathways; calcium is also one of the regulators of the quantity of VDR found on cell walls in a process termed 'heterologous regulation' (56,57).

Relation of Aims to Long Term Relevance

National guidelines and public health messages regarding HTN do not specifically address the need for adequate vitamin D. Cardiovascular health is multifactorial, and sufficient vitamin D impacts well-known risk factors such as blood pressure, diabetes, atherosclerosis, BMI, heart health, muscle strength, and more.

The efficacy of the Dietary Approaches to Stop Hypertension (DASH) diet has been demonstrated clinically to decrease high blood pressure (58). The DASH diet includes food that contains vitamin D, but vitamin D is not directly addressed. The American Heart Association addresses guidelines for dietary fats, sodium, potassium, and alcohol intake in their dietary recommendations, yet the need for adequate vitamin D intake or supplementation is absent (1,59).

Clinical trials of vitamin D dosing have yielded mixed results, but have provided insight into the range of effective vitamin D dosage (60). Investigators have learned that dosages less than 700 International Units were ineffective in vitamin D

deficient patients to decrease the risk of fractures and falls; extremely high dosages increased negative clinical symptoms, though rarely the classical symptom that is most feared, namely hypercalcemia (61,62). Meta-analyses have combined analyses of heterogeneous amounts of vitamin D ranging from inadequate doses, to more physiologic dosages, to extremely high doses. The conclusion reached from these combined trials is that the results cannot be used to provide public health guidelines on outcomes other than bone health (29). While the IOM based their recommendations for vitamin D on the serum 25(OH)D level that will prevent overt bone diseases, other researchers recommend that vitamin D serum goals should be at least 30 ng/mL (75 nmol/L) to decrease risk of CVD and HTN (16,63–65). A new model of analysis is needed for nutrition studies (11,19,24).

Chapter 4: RESEARCH DESIGN AND METHODS

Overall Study Design

Cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) were analyzed, utilizing the comprehensive demographic, physical, laboratory, and dietary components of the survey. The 2003-2004 and 2005-2006 survey cycles were selected for analysis (66).

The NHANES is a cross-sectional study, designed to monitor the health and nutritional status of the nation, and conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (66). The NHANES is the most complex of the national surveys and is unique in that it combines home interviews, physical examination, dietary intake and supplement data, and comprehensive laboratory testing of participants for both known and unknown diseases.

The NHANES employs a complex, multistage, probability sampling design (67). The participant sampling process is designed to survey the U.S. civilian, non-institutionalized population, excluding Indian reservations. Certain subgroups are over-sampled in order to obtain a sufficient group sample to generate more precise and reliable samples of public health estimates for these subgroups. Each person is assigned a sample weight proportional to the number of people in the population represented by that sample person to control for the non-randomized study selection process. Roughly 5,000 participants are surveyed each year from approximately 15 counties. Survey cycles are released for a two year period and contain approximately 10,000 participants per biennial cycle (66).

The multi-stage study design is based on information from the 2000 U.S. Census. Individuals are selected for a full home interview to obtain the demographic information and dietary supplement information of the household member(s). A subsample of the above are selected for examination at the mobile examination center (MEC) based on the demographic characteristics needed to obtain representative information on the diverse U.S. population.

Study Population Inclusion and Exclusion Criteria

The inclusion criteria for this study encompassed all of the following:

1) Adults at least 20 years of age, male and non-pregnant females
2) Laboratory value for 25(OH)D, vitamin A, and creatinine

The exclusion criteria included any of the following:

1) Less than 20 years of age
2) Pregnancy
3) Compromised renal function which alters vitamin D metabolism, defined as participants on dialysis or estimated glomerular filtration rate (eGFR) <50 mL/min/1.73m^2 or stage 3B renal disease (9,68,69)
4) Lack of data to compute eGFR: participants without creatinine blood draw or weight measurement
5) Incomplete 24 hour dietary recall record

Data Source for Analysis

The 2003-2004 and 2005-2006 survey cycles were used because these cycles contained the most recently released data containing serum 25(OH)D data available at the time of analysis. The total sample size included in the original merged and

appended files was 20,470; the subpopulation number for logistic regression was 6,583.

Dependent Variable

Hypertension was the dependent or outcome variable for this study. Hypertension or abnormally high blood pressure was defined as:

1) Greater than 140 mm Hg systolic blood pressure (SBP) and/or greater than 90 mm Hg diastolic blood pressure (DBP) or
2) Currently taking medication to lower blood pressure

Blood pressure was measured by physicians with certified training in blood pressure measurement following strict protocol during the medical examination at the Mobile Examination Center (MEC). Specifics regarding the equipment are available on the NHANES website (70). Systolic and diastolic blood pressure were measured a maximum of five times to attempt to obtain three reliable measurements. If a measurement was interrupted, another attempt was made to obtain a good reading. A maximum of four readings per patient were recorded, and the readings were averaged during the coding process.

HTN was coded as 1 if the participant averaged SBP greater than 140 mmHg or DBP greater than 90 mmHg, or answered positively to the question regarding whether he/she was taking a prescription for HTN. If none of the above conditions were met, HTN was coded 0.

Independent Variable

Serum vitamin D was the independent or exposure variable in this thesis, and the recommended nutritional biomarker to measure for total exogenous or

endogenous sources of vitamin D from diet, dietary supplements containing vitamin D, and/or exposure to UVB light, respectively (71,72). Sufficient 25(OH)D was defined in this study as greater than or equal to 30 ng/mL; low vitamin D levels as less than 29 ng/mL. The serum 25(OH)D lab specimens measured in the MEC were tested using a DiaSorin RIA kit from Stillwater, MN (72).

Widespread variation in vitamin D lab results spurred the creation of the Vitamin D Standardization Program in November, 2010 led by the Office of Dietary Supplements to standardize 25(OH)D concentration measurements in national health surveys based on the National Institute of Standards and Technology (NIST) standard reference materials (73,74). In October of 2015, the CDC announced a strong recommendation that researchers utilize NHANES website 25(OH)D conversion equations to standardize the vitamin D survey values to the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method used in the most recent NHANES survey cycles, and compatible with the NIST reference materials. This study incorporated the regression equations for all analyses.

Vitamin D lab results are known to differ by season because of participant sun exposure and seasonal changes in the angle of the sun (75). The laboratory data were collected in the south during the winter and in the north in the summer (70). In the analysis phase, the season of vitamin D collection was tested as a potential confounder.

Data Analysis

SAS version 9.4 software was used for the analysis of the 2003-2006 NHANES data. The variables relevant to the study purpose were selected from the NHANES participant questionnaire, laboratory, demographic, and dietary data.

As a result of the survey multi-stage sampling design, some ethnic groups were over-sampled in order to gain adequate numbers to make valid inferences about the group, while for other measurements, subsampling is used (67). For example, not every subject that was interviewed in the home took part in the MEC examination. Special variance procedures for this type of sampling design were available for use in SAS utilizing programs and were utilized in this analysis, including Proc Surveyfreq, Surveymeans, and Surveylogistic. Weight codes were applied for the specific survey years being analyzed, for dietary intake, and data analysis for measurements that take place in the MEC. Dietary day 1 weights were utilized for this study to preserve the weighting system of the complex survey design. Table 1 (See Appendix C) displays the sample size and response rates for the screening, interview and examination components of the NHANES 2003-2006 cycles.

The Poverty Index Ratio was excluded from analysis, whereas the education variable was retained as a potential confounder of the association between vitamin D and HTN. The rationale was that while educational attainment and income were strongly associated, participants are generally less willing to report income, but more likely to report level of education. Missing data were evaluated for all variables used. Undetectable lab values were re-coded as missing based on NHANES codebook information (76). Participants with missing values for vitamin A, vitamin D, MEC

examination, and creatinine were omitted from analysis. Data imputation was avoided in general, but was employed in the following circumstances: pregnancy data were missing for many participants. Therefore, males and women older than 60 years were imputed as not pregnant.

Categorical variables were created for continuous variables such as vitamin D, HgA1c, creatinine, age, and the six vitamin D co-nutrients, including serum vitamin A, zinc, iron, niacin, magnesium, and calcium. Binary categories were created for co-nutrients, waist circumference, and creatinine as being above or below the cutoff point. Binary categories were also coded for nominal data such as gender, moderate physical exercise, and season of vitamin D collection. Variables coded for multiple categories included race, education, body mass index, and smoking. The vitamin D values were re-coded again at the end of the analysis phase in order to utilize the regression equations provided in the recent recommendations by the CDC to link the results with the NIST reference materials (73,77).

A correction factor was recommended for the creatinine lab value by the CDC for the 2005-2006 data cycle. This correction equation was applied to the creatinine values, and incorporated into the equations for men and women, respectively, to calculate estimate glomerular filtration rates (eGFR). A binary variable was then created to indicate eGFR greater than or equal to 50 mL/min/m^2 as 0, indicating sufficient renal function, and less than 50 mL/min/m^2 as 1, indicting renal insufficiency. Participants with an eGFR less than 50 mL/min/m^2 were omitted from further analysis since poor renal function alters the metabolism of vitamin D which could bias the study results.

Once the online NHANES data were downloaded, merged, appended, and cleaned, univariate logistic analyses of the socio-demographic and health variables were conducted to better understand the distribution and determinants of the dataset. Table 2 (See Appendix C) displays preliminary descriptive Rao-Scott Chi-Square tests. The weighted column percentiles and *p* values were displayed as a frequency distribution of socio-demographic and health variables by vitamin D status. Two-tailed *p* values of less than 0.05 were considered significant for all analysis results. The lower category of 25(OH)D levels includes serum measurements in the range of 1-29 ng/mL; participants with serum 25(OH)D laboratory values in this category were defined as having low vitamin D status. Those in the upper category with vitamin D levels of greater than or equal to 30 ng/mL were defined as having sufficient vitamin D status.

Table 3 (See Appendix C) provides univariate logistic analyses of the same descriptive statistics of socio-demographic and health variables as Table 2 but by HTN status--as having or not having HTN. The socio-demographic and health variables were determinants or potential determinants of vitamin D or HTN or both, having been pre-selected based on preparatory literature research described earlier for this study.

The resulting *p* values indicated whether the variable met the criteria for consideration as a confounder; that is, the variable must be associated significantly with the exposure and with the outcome, but not be part of the causal pathway between the exposure and outcome variables. The socio-demographic and health variables measured included age, gender, race/ethnicity, education level, poverty-

income ratio, smoking, moderate physical activity, season of serum vitamin D measurement, body mass index (BMI), waist circumference, C-reactive protein (CRP) and HgA1c.

Appropriate survey cycle weights were used in each analysis to preserve the weighting scheme necessary to generate reliable estimates that are applicable to the U.S. population. During Logistic regression analyses, preservation of the weighting scheme entailed not restricting the subpopulation until the SurveyLogistic call by incorporating a domain statement. Through the use of the domain statement, the subpopulation was restricted to non-pregnant adults 20 years and older with adequate renal function, with creatinine, vitamin A, and vitamin D lab values, and with reliable food intake records.

Analysis I

Three main analyses were conducted utilizing logistic regression to test the study hypotheses. For the first analysis, descriptive statistics were generated as the association was determined of each covariate to vitamin D as the exposure variable, then to HTN as the outcome variable. The variable was examined for effect modification and then stratified to obtain an Odds Ratio (OR), 95% confidence intervals (CI) and *p* values using a Wald test (See Tables 4_a and 4_b in the appendices).

Analysis II

The second analysis addressed the association of vitamin D status and HTN utilizing multivariate logistic regression. The first model of this analysis was performed without adjustment for potential confounders to provide the crude OR. The second model was adjusted for potential confounders, observing for changes in

the strength of the association between vitamin D and HTN that were greater than 10 percent. (See Table 5 in the appendices).

Analysis III

The third analysis calculated the total nutrient intake of each co-nutrient from the sum of the day 1 dietary re-call and the total mean daily intake of dietary supplements. If the participant did not consume dietary supplements, then only the nutrients consumed from the day 1 dietary intake were used. The EAR is the intake level that is estimated to meet the requirements of approximately half of the healthy participants in a group; this dietary reference was used in preference to the Recommended Dietary Allowance which is designed to meet the nutrient requirement of 97 to 99 percent of healthy individuals in a group (78). The EAR was the more reasonable cutoff to utilize since dietary intake was assessed from one 24-h dietary recall record.

The nutrient totals for each co-nutrient were regressed in a bivariate analysis--as meeting or not meeting the respective EAR levels for each of the co-nutrients under consideration: calcium, iron, magnesium, niacin, zinc, and serum levels of vitamin A. Each nutrient was assessed using logistic regression to obtain the OR, CI, and *p* value for having HTN by the total nutrient level of adequacy or inadequacy.

Serum retinol was substituted as an objective measurement for vitamin A instead of the EAR because the NHANES dietary supplement data provided insufficient detail for beta carotene, which can covert to vitamin A (54,79). Normal plasma retinol has a broad range of 30-80 ug/dL (80). The range of recommended adequate plasma vitamin A values of greater than or equal 40 ug/dL predict normal

retinol function 95% of the time, whereas less than 30 ug/dL is considered suboptimal (81).

The main aim of the third analysis was to examine whether the association between vitamin D and hypertension varies by vitamin D co-nutrient sufficiency status. Co-nutrients of vitamin D that demonstrated significant effect modification were retained for further analysis. (See Table 6 in the appendices).

Human Subjects

For the present analyses, the University of Maryland College Park Institutional Review Board reviewed the protocol for this thesis and determined the study did not meet the definition for human subject research on January 30, 2015.

Chapter 5: RESULTS

Descriptive Statistics

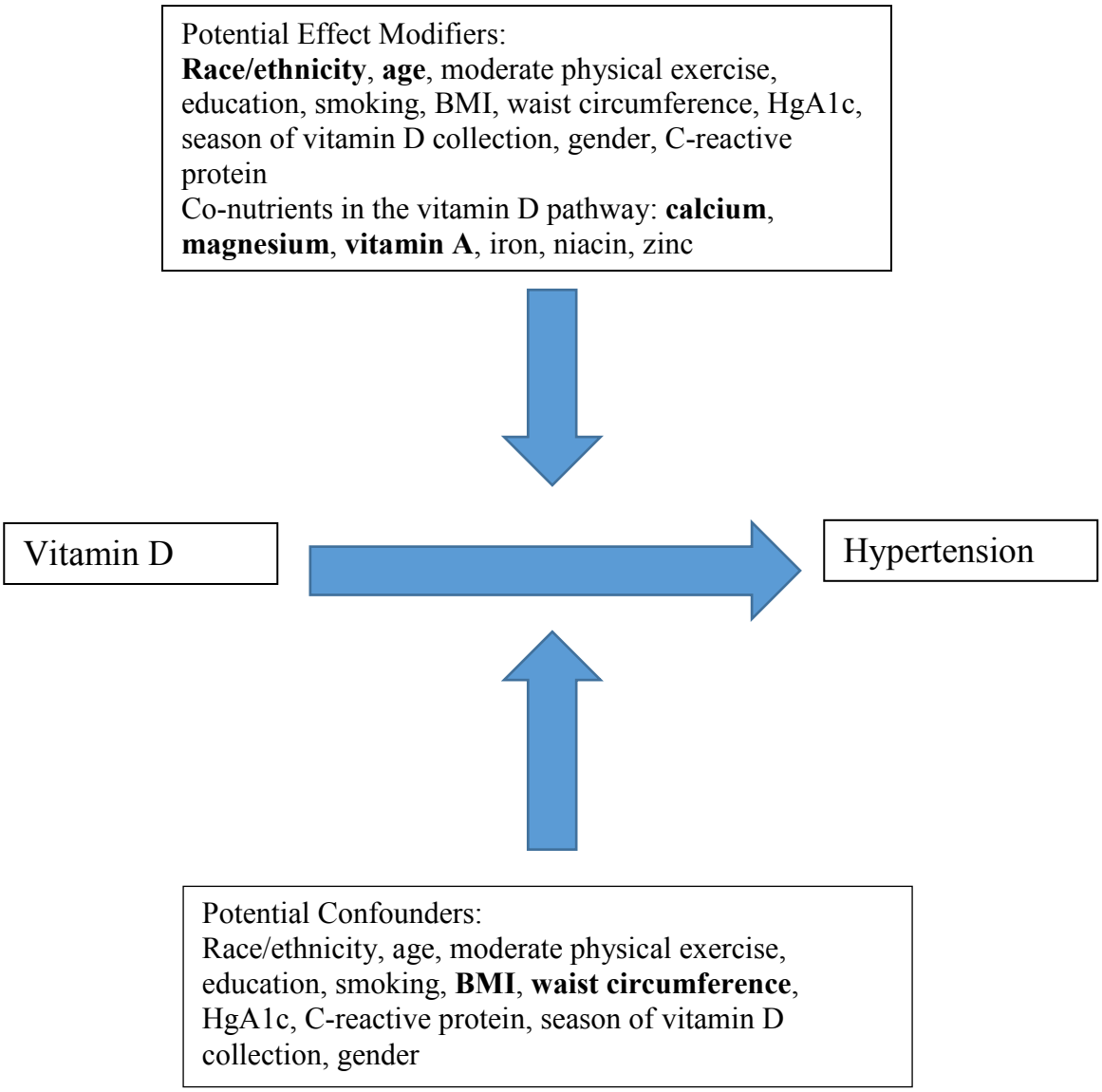
Among non-pregnant adults with adequate kidney function, 61.1% of those with low vitamin D status (n = 1538) had HTN compared to 38.9% of those with adequate vitamin D levels (n = 671), $p = 0.0006$. Adults with low vitamin D status had 1.28 the odds of having HTN as compared to those with sufficient vitamin D levels (95% CI: 1.12-1.45, $p = 0.0002$).

Age and race/ethnicity demonstrated effect modification with p values of 0.0010 and 0.0047 respectively, so were excluded as confounders in the final multivariate logistic regression model. Gender, season of vitamin D collection, and CRP did not meet the criteria for confounding: the p values were not statistically significant in association to both the exposure and outcome variables; they were omitted from further analysis. (See Tables 4_a, and 4_b in Appendix C).

The following socio-demographic and health characteristic variables met the criteria described previously for potential confounders of the association between serum 25(OH)D status and HTN: moderate physical activity, BMI, waist circumference, education, HgA1c, and smoking. No multicollinearity was demonstrated in the testing of the covariates for collinearity and variance inflation factors. During multivariate logistic regression, the independent effects of the covariates on HTN was tested: those with less than ten percent change in the OR for HTN were eliminated from the model. Only BMI and waist circumference remained as confounders to adjust for in the final model.

Figure 1

Diagram of the Association between Vitamin D and Hypertension: Effect Modifiers and Confounders*



***Bolded** variables demonstrated significant *p* values at the $\alpha = 0.05$ level in the final analysis

Multivariate Logistic Regression

Multivariate Logistic regression was conducted to test whether the association between vitamin D status and hypertension was significant after adjustment for potential confounders. The crude analysis of the association between vitamin D and HTN without adjustment for confounders demonstrated an OR of 1.28, (95% CI: 1.12-1.45, $p = 0.0002$).

Adjustment for Confounding

The two covariates of were then added to the Logistic regression analysis model. Table 5 illustrates the results of the analysis. After adjustment for waist circumference and BMI, among non-pregnant adults with adequate renal function, those with low vitamin D status demonstrated 1.05 times the odds of having HTN when compared to those with sufficient vitamin D levels (95% CI: 0.92-1.21, $p = 0.467$).

Co-Nutrient Analysis

Niacin, zinc, and iron did not demonstrate significant effect modification in the association of vitamin D to HTN. Effect modification was demonstrated by magnesium in relation to the association between vitamin D and HTN ($p = 0.0447$). Effect modification signifies that a potentially biologic phenomenon of interest is being described. Additionally, among non-pregnant adults with kidney sufficiency, in those with an insufficient EAR level of total magnesium (less than 350 mg/day), the odds of having HTN if they also had low vitamin D status was 1.42 times the odds for adults with vitamin D sufficiency (95% CI: 1.19-1.70, $p = 0.0001$).

Calcium was found to be an effect modifier of the association between vitamin D and HTN ($p = 0.0157$). The odds of having HTN if participants had both low calcium and vitamin D status was 1.48 times the odds for adults with vitamin D sufficiency (95% CI: 1.22-1.78, $p = <.0001$).

Utilizing logistic regression, calcium and magnesium together were found to modify the association between vitamin D and HTN. Among non-pregnant adults with adequate renal function, those with low levels of calcium, magnesium and vitamin D had 1.75 times the odds of also having HTN when compared to those with sufficient vitamin D levels (95% CI: 1.42-2.16, $p = <.0001$).

Vitamin A serves as a further effect modifier of the association between vitamin D and HTN. Among adults with insufficient vitamin A levels (less than 39.9 ug/dL), the odds of having HTN if they also had low vitamin D status was 3.09 times the odds for adults with vitamin D sufficiency (95% CI: 1.66-5.76, $p = 0.0004$). In contrast, among adults with sufficient vitamin A levels (greater than or equal to 39.9 ug/dL), the odds of having HTN if they also had low vitamin D status was 1.29 times the odds for adults with sufficient vitamin D levels (95% CI: 1.14-1.47, $p = <.0001$). Additionally, among adults with low calcium, magnesium and vitamin A levels, those who also exhibited low vitamin D status had 5.43 times the odds of also having HTN when compared to those with sufficient vitamin D levels (95% CI: 1.49-19.77, $p = 0.0103$). In contrast, among non-pregnant adults with adequate renal function, those with low calcium, low magnesium and adequate vitamin A, those who also had low vitamin D status had 1.66 times the odds of having HTN as those with sufficient vitamin D (95% CI: 1.33-2.06, $p = <.0001$).

All of the co-nutrients were associated with vitamin D at the *p* level of <.0001. However, only vitamin A and calcium were significantly associated with HTN (*p* = <.0001 and 0.0025, respectively). Since they are both part of the causal pathway between vitamin D and HTN, they did not meet the criteria for confounding.

Chapter 6: DISCUSSION

Association between Vitamin D and HTN

The observed crude association between vitamin D status and HTN (OR=1.28, 95% CI: 1.12-1.45, $p = 0.0002$) is supported by prior research (24,42,82–87). Wang et al. researched the association between vitamin D levels and risk of CVD using the Framingham Heart Study Offspring Cohort prospective study (25). The hazard ratio of CVD in participants who exhibited also HTN and had low vitamin D status was 2.13 (95% CI: 1.3-3.48) compared to a hazard ratio of 1.04 (95% CI: 0.55-1.96) in CVD subjects without HTN.

Using NHANES III 1988-1992 data, Judd et al. studied the association of vitamin D with HTN and age. The authors included only participants who had never been told that they had HTN to minimize the potential for lifestyle changes that may occur in persons with a HTN diagnosis (82). Key findings were that the systolic blood pressure in the white population with vitamin D levels >80 nmol/L (32 ng/mL) was 20% lower than whites with insufficient or deficient status, and that only 8% of the NH Black population had vitamin D levels greater than 80 nmol/L. As reported in the background section, the NH Black population has the highest rates of HTN by race/ethnicity in the U.S (48).

In this study, effect modification by race was demonstrated. This confirmed the findings by Judd et al who found significantly lower 25(OH)D status at all levels of HTN compared to Whites. (82). Darker skin tone is associated with lower 25(OH)D levels in the U.S., and this raises questions about whether this finding is a biological or socio-economic health disparity issue (88–90). Durazo-Arvizu et al

demonstrated that serum vitamin D level does not differ by black race physiologically, but rather by geographic location in relation to the equator (a proxy for adequate sun exposure) (91). A double blind, randomized controlled vitamin D dose response trial by Gallagher et al examined the change in serum vitamin D levels after oral vitamin D supplementation in young, vitamin D deficient NH Black and White women (92). The Black women had lower baseline values at a mean of 12.4 ng/mL, but both races had similar serum responses at the 6 month and 12 month measurement intervals. Gallagher's group also ruled out differences by race of intestinal absorptive response in a randomized controlled double blind vitamin D dose response trial in older African American women (93). Forman et al demonstrated a significant decrease in blood pressure measurements in a randomized, placebo-controlled, double blind, 3 month trial in a population of African Americans with administration of 3 different doses of vitamin D supplements in the experimental arm (94).

Effect modification was demonstrated by age in this analysis, also a confirmation of the Judd study (82). Judd et al found that participants with low vitamin D status had higher increases in blood pressure with age than those who had 25(OH)D levels greater than 32 ng/mL. Adequate levels of vitamin D are associated with suppression of the RAAS, with changes in calcium metabolism, and with vasculoprotective effects that may all influence HTN (22,84).

There is much discussion in the literature surrounding the optimal level of 25(OH)D for human health beyond the level set by the IOM of 20 ng/mL for decreased risk of bone disease (4,11,12,15,63,71,90,95–103). For this study, low

25(OH)D status was defined as less than 29 ng/mL; sufficient vitamin D as greater than or equal to 30 ng/mL. The sufficient level approximates the physiological 25(OH)D value where adequate levels of vitamin D hormone inhibit secretion of parathyroid hormone (PTH) (7,84,90,104,105).

To examine the outcome of HTN, this study investigated a novel nutrient 'systems' approach to consider whether the association of vitamin D status with hypertension varied by the level of key co-nutrients that are necessary for important reactions to occur in vitamin D systems or pathways. The alternate hypothesis was not rejected for three co-nutrients, namely, calcium, magnesium and vitamin A.

Each of the three co-nutrients demonstrated significant effect modification in the association between vitamin D and HTN. This study found that non-pregnant adults with normal renal function had 42% greater odds of having HTN if they had low magnesium and vitamin D levels compared to those with sufficient vitamin D status; participants with low calcium and low vitamin D status had 48% greater odds of HTN compared to those with adequate vitamin D levels; and those with low vitamin A and D levels more than a threefold increase in odds of HTN compared to those with sufficient vitamin D status. This association was greater than fivefold when all three co-nutrients were examined together (See Table 7 in the appendices). If these findings are confirmed in cohort or clinical trials, application of this knowledge may change the treatment of HTN and other vitamin D related diseases.

Total calcium and magnesium, derived from diet and/or average dietary supplement intake per day, demonstrated significant effect on the association between vitamin D and HTN when the EAR level of each nutrient was used as the cutoff

criteria for adequacy. Niacin, iron and zinc were not significant at the EAR cutoff level. In examining the chemical reactions that take place as 25(OH)D is metabolized from the inactive to the active steroid form, these co-nutrients are clearly necessary. While the EAR was a reasonable test cutoff value to use in a nutritional population study to explore the level of each co-nutrient that may be required for the metabolic reactions involving vitamin D, future studies could explore alternate levels for the co-nutrients that did not demonstrate significance in this study. In the case of total iron intake, 5.2% of the study population had low iron levels (n = 353). This small number may have contributed to the inability to detect a significant difference if it did exist.

Deng et al added support to the thesis findings of magnesium effect modification (55). Deng investigated the role of magnesium on the association between serum 25(OH)D status and mortality, and reported that total magnesium intake above the EAR was associated with decreased levels of deficient and insufficient levels of 25(OH)D. Sufficient levels of vitamin D were associated with decreased risk of mortality from all-cause CVD, and from colorectal cancer. In stratified analysis, magnesium was found to interact with 25(OH)D ($P_{\text{interaction,}}$ <0.001) for deficient/insufficient levels of vitamin D.

Magnesium is critical for blood pressure regulation, for synthesis and secretion of parathyroid hormone to regulate the conversion of vitamin D to its active form for endocrine functions, and for the 25-hydroxylase, 1-α hydroxylase and the 24-hydroxylase enzymes required to activate and deactivate vitamin D (33,106–108). Zitterman noted the association of low magnesium to low vitamin D levels, and the

relationship of low magnesium levels to CVD (32). The author called for more attention to magnesium levels when assessing vitamin D status, and for additional study regarding the dose-response relationship of magnesium to vitamin D, which this thesis studied.

Vitamin D and Calcium

The Women's Health Initiative (WHI) study included a multi-center, randomized clinical trial with a calcium and vitamin D arm (30,109–111). This study is frequently cited as rationale for the lack of effect of vitamin D on cardiovascular outcomes, and was one of the most influential studies used in the IOM 2009 calcium and vitamin D review (25,112). Although there are some important strengths of this study, such as large sample size, there are critical limitations that may explain why it did not find an association between vitamin D and HTN. These limitations included: High levels of independent calcium usage decreased the ability to detect a difference between groups; insufficient dosage of vitamin D provided to achieve an effect; poor adherence to study medications; interaction of the hormonal study arms of the WHI with the calcium vitamin D component; and cardiovascular disease was a secondary outcome--the trial was primarily designed to describe fracture outcomes.

However, Berridge described the vitamin D dependent calcium pathways involved in HTN, with emphasis on regulation of the Renin-Angiotensin-Aldosterone System (RAAS), the system responsible for increases in blood pressure (57). While sufficient vitamin D dampens the expression of the RAAS, other metabolic processes, such as the inflammatory state induced with obesity, can enhance the RAAS. This

may explain why including BMI and waist circumference in the final logistic regression model nullified the results demonstrated in the crude OR.

Vaidya et al indirectly described calcium and vitamin D effect modification in the relationship between the RAAS system and calcium regulatory systems (113–115). The classical vitamin D endocrine system has been well described for decades, the major components being the parathyroid glands, calcium and vitamin D. Less is known about the regulation of RAAS by vitamin D. An additional piece of the puzzle involves the regulation of aldosterone with PTH. Vaidya provides an excellent review of the mechanisms responsible for the above physiological interactions. Vaidya et al questioned whether other factors required in vitamin D metabolism may account for the mixed results of previous studies, and stated that further research was needed to answer the question. This thesis system study aids in addressing the question.

Vitamin D, Vitamin A, Calcium, and Magnesium

Effect modification among the four nutrients has not been reported previously to this author's knowledge. Previous studies report mixed results regarding the association between vitamin D, calcium, and HTN. McCarron noted that dairy products were found to be strongly associated with decreased HTN in observational studies (116). This important observation regarding the potential impact of dairy products illustrates the benefit of providing multiple nutrients together to support the vitamin D co-nutrient system. Dairy products provide a mixture of nutrients of importance to HTN—calcium, magnesium, B vitamins, vitamin A, potassium and vitamin D.

Additional research demonstrated that many nutrients work together to decrease HTN and support the premise of a systems approach to nutrition study (117–119). The DASH Diet is a major research accomplishment in this area, with proven results in lowering blood pressure (58). The DASH diet requires adequate provision of lowfat dairy products, fiber, fruits and vegetables, while providing the vitamin D co-nutrient mix.

STRENGTHS & LIMITATIONS

Study Strengths

This study has several strengths. A systems approach for testing whether the association between vitamin D and hypertension varies by vitamin D co-nutrient sufficiency status had not been completed previously to the author's knowledge.

The NHANES data are particularly suited to this type of preliminary testing because of the comprehensive array of data collected annually in demographic, physical, laboratory, and dietary arms of the survey. Secondary data analysis is much less expensive than a clinical trial or a cohort study design.

The NHANES is a thoroughly planned, reliably conducted, national study that publishes data on a biennial basis for use in the public domain (120). The data can be generalized for the U.S population with the appropriate use of study weights to compensate for the non-random study design. A strength of the NHANES is the large sample size. The two survey cycles were merged and appended to increase the sample size and to increase the reliability of the statistical estimates as recommended in the NHANES tutorials and analytic guidelines (121).

The United States Department of Agriculture's database has provided food and nutrient information for over 115 years, and undergoes planned and continuous updating (122). Dietary intake data were gathered in a tested, systematic manner utilizing the Automated Multiple Pass Method (AMPM) to assist participants to recall the foods and quantities of food consumed in the last 24 hours (123). The 5–step AMPM process was conducted by trained interviewers using standardized text to elicit responses from participants to decrease re-call bias and increase reliability (124–126). The United States Department of Agriculture validated the AMPM process using the doubly labeled water method.

Mineral Validation of the 24-h Dietary Recall

Although some have raised concerns about the validity of NHANES dietary data, validation studies have demonstrated that the data are very accurate. For example, Rhodes et al compared the accuracy of self-reported dietary intake of sodium intake compared to objective results from a 24–h urinary excretion tests. The overall accuracy of the self-reported data demonstrated a mean of 0.93 for men (CI: 0.89-0.97) and 0.9. (CI: 0.87-0.94) for women. Subjects with a normal BMI classification demonstrated the highest degree of accuracy (125). Validation studies indicated that estimates of vitamins and mineral intake from the AMPM data were within 90% of the true mean and that under-reporting would shift the data results toward the null. Subar et al stated that dietary intake errors will generally cause underestimations of statistical estimates, that spurious associations caused by residual confounding are rare, and that strong results are likely to be true, especially if consistent with other studies (127).

This thesis utilized information from the 24-h dietary intake data for calcium, magnesium, zinc, iron, and niacin. Vitamin D and vitamin A were measured objectively utilizing serum lab draws with standardized procedures and preferred laboratories for analysis of the blood. The serum vitamin D data were recently re-validated as described in the dependent variable section, and the recommended regression equations that link the serum vitamin D lab values to the NIST reference material were incorporated into the current analysis. Blood pressure was measured using standardized protocols with calibrated instruments. Height, body weight, and waist circumference were measured, not estimated.

The inclusion and exclusion criteria for this study assisted in avoiding confounding because of the link between renal disease, vitamin D metabolism and HTN. Vitamin D metabolism is also altered in pregnancy, which is why pregnant women were excluded from the population.

Study Limitations

In recent years great debate and controversy has arisen over the known measurement error inherent in the process of the 24 hour dietary recall in regards to total energy intake. Critics state that the measurement error is systematic and renders the energy information in the 24-h recall useless (128). Federal researchers maintain that the limitations are known and can be corrected with statistical methods when caloric intake is analyzed (124,129). This thesis utilized the 24-h dietary intake data, but did not use energy intake.

Only Day 1 dietary intake was used for the purpose of this study. The recall process introduces an element of bias as the information gathered is reliant on the

memory of the participant (130). Additional challenges include the difficulty in estimating the amount of food consumed and incomplete knowledge of the food components of a mixed food product. A potential bias is for the individual to report a healthy dietary intake because of the desire to be perceived as socially correct. Studies find that participants under-report their intake, particularly in the obese population. A 24-h intake does not assume to take into consideration the intra-personal intake variation that takes place from day to day. Further research could incorporate two days of 24-h intake.

Dietary supplement data are also reliant largely on participant recall regarding the quantity and duration of supplements taken. The investigators who conduct the in-home interviews ask to see the dietary supplement containers, if available, to obtain the exact label information to decrease the risk of systematic measurement bias (131).

The EAR for vitamin A was not tested because of the lack of availability of complete beta carotene dietary supplement data. Therefore, the serum level of vitamin A was chosen as an objective measurement. There are challenges associated with the appropriate cutoff of serum vitamin A as described in the methods section. Examination of both deficient and excessive serum vitamin A levels would enhance future studies.

Potassium and sodium are nutrients that play important roles in HTN (58). Since these minerals are not part of the metabolic pathway of vitamin D, the effects of potassium and sodium were not considered in this analysis, but could be incorporated in future research.

While the NHANES is useful for the generation of hypotheses that can be further explored in cohort studies or clinical trials, cross-sectional study designs cannot be used to prove cause and effect. The problem is the risk of reverse causation because of lack of temporal information—not knowing which came first, the exposure or the outcome.

The 2003-2006 survey cycles had been analyzed in many published studies, though not with the same study design. Although the most current serum vitamin D test results available to the public were utilized when this thesis analysis was undertaken, more recent 2007-2008 and 2009-2010 NHANES cycles containing serum vitamin D data were released in October of 2015 (72).

The population restrictions utilized for the study analysis limit the generalizability of the study to non-institutionalized U.S. residents of the same age group, with non-pregnant status and adequate renal function. While covariates that were significantly associated independently with vitamin D and HTN were controlled for in the multivariate logistic regression, the role of residual confounding cannot be ruled out.

PUBLIC HEALTH SIGNIFICANCE

A recommended goal for future nutritional studies is to avoid studying nutrients in isolation, as this is not a valid representation of the chemical environment of the human body. Many nutritional studies have demonstrated disappointing results when testing isolated nutrients in clinical trials, leading to skepticism regarding the value of nutritional recommendation to decrease the risk of disease. Instead, the system of nutrients that are required for the metabolism of the nutrient of interest

requires control during the study in order for research to yield results that can truly mitigate disease risk and benefit public health. This thesis suggests that a change in study design may be needed to explore the impact of nutrition on disease outcomes such as HTN.

This systems approach could form the basis for future cohort studies or clinical trials to test the relationship of vitamin D to an outcome of interest such as HTN, while ensuring adequate provision of the vitamin D co-nutrients. Since iron, zinc and niacin did not demonstrate significance in this study as vitamin D co-nutrients, other cutoff points could be explored to better understand the level at which a deficiency or excess of each co-nutrient may alter the ability of vitamin D to be metabolized from the inactive to active forms to impact a disease outcome.

If significance of the association between vitamin D and HTN are confirmed in future trials that provide sufficient levels of vitamin D co-nutrients, then public health guidelines would need to change to reflect the evidence. Clinicians and the public who incorporate this knowledge into clinical practice may decrease the incidence of HTN for the future and assist patients with prevalent HTN to better manage their health (132,133).

Appendices

APPENDIX A: MPH COMPETENCIES ADDRESSED IN THESIS

The following table illustrates the complete list of MPH competencies addressed in this thesis.

Competencies for MPH in Epidemiology	Thesis	Addressed in this Thesis
1) Identify vital statistics and key sources of data for epidemiological purposes.	✓	Vital statistics and the NHANES dataset as available in the public domain were utilized for the analysis.
2) Describe a public health problem in terms of magnitude, person, time and place.	✓	The magnitude of the association between different levels of 25(OH)D and HTN in a population that is representative of the U.S. using 2 cycles of the biennial NHANES data was examined.
3) Discuss the principles and limitations of public health screening programs.		This competency was met during the MPH internship.
4) Comprehend basic ethical and legal principles pertaining to the collection, maintenance, use and dissemination of epidemiologic data.	✓	Ethical considerations for participants of the NHANES were incorporated in the analysis of the data. A human subject research determination form was submitted to the University of Maryland IRB and approved on 1/30/15.
5) Explain the importance of epidemiology for informing scientific, ethical, economic, and political discussion of health issues.	✓	The importance of clear analysis models in studying nutrient topics such as vitamin D for informing scientific, ethical, economic, and political discussion of health issues, especially as related to hypertension was demonstrated in this thesis.
6) Apply the basic terminology and definitions of epidemiology.	✓	This study addressed the distribution and determinants of 25(OH)D as associated with HTN.
7) Calculate basic epidemiology measures.	✓	Descriptive statistics of proportions, and *p* values for the demographic characteristics in the adult, non-pregnant, civilian population $\geq$20 years, relative to vitamin D status were provided in Tables 2 and 3.
8) Communicate epidemiologic information to lay and	✓	This objective was met in the written thesis proposal and defense reports, and in the oral

professional audiences.		thesis proposal and defense presentations to the Thesis Committee.
9) Differentiate among the criteria for causality.	✓	Causality was addressed in the strengths and limitations section in regard to cross-sectional studies.
10) Draw appropriate inferences from epidemiologic data.	✓	Inferences were drawn from the study and presented in the results and discussion sections of the document.
11) Describe epidemiologic study designs and assess their strengths and limitations.	✓	This competency was met in the strengths and limitations section of the study.
12) Evaluate strengths and limitations of epidemiologic reports.	✓	Strengths and limitations were addressed in the Public Health Significance chapter.
13) Calculate advanced epidemiologic measures.	✓	Analyses included analyses such as Chi-Square tests, univariate and multivariate logistic regression to calculate odds ratios, *p* values, and confidence intervals.
14) Design, analyze, and evaluate an epidemiologic study.	✓	This competency was demonstrated by the thesis process in designing a study, conducting the analysis and interpreting the study results in the thesis.
15) Demonstrate skills in public health data collection and management.	✓	Multiple components of the NHANES data including past medical history, physical examination, laboratory, and dietary intake and supplementation were merged, appended and managed appropriately in the current analyses.
16) Design interventions to reduce prevalence of major public health problems.	✓	This thesis analyzed a model for testing the relation of co-nutrients of an independent nutrient variable in relation to an outcome, in this case, HTN. Results from this model could be replicated in newer release of NHANES survey cycles and could be tested in cohort or clinical trials for HTN. The co-nutrient model could be utilized in a similar manner for analysis of other nutrients related to public health outcomes.
17) Demonstrate program administration and organizational leadership		This objective was met in the thesis proposal, analysis, and formation of the Thesis Committee in accordance with the University of Maryland MPH process.

APPENDIX B: NHANES Variable Tables

Participant Variable, Survey, and Sample Weights

#	Variable	Type	Label	Note
1	SEQN	N/A	Respondent Sequence Number	
2	SDDSRVYR	N/A	Data Release Number	
3	SDMVPSU	Interval	Masked Variance Pseudo-PSU	
4	SDMVSTRA	Interval	Masked Variance Pseudo-Stratum	
5	WTMEC2YR	Continuous	Full Sample 2 Year Mobile Exam Center (MEC) Exam Weight	
6	RIDEXMON	Categorical	Six month time period	
7	RIDSTATR	Categorical	Interview Examination Status	1 = Interview, 2 = Interview and MEC
8	WTDRD1	Continuous	Dietary day one sample weight	

Demographic

#	Variable	Type	Label	Note
9	RIAGENDR	Categorical	Gender	
10	RIDAGEYR	Interval	Age at Screening Adjudicated – Recode	
11	RIDRETH1	Categorical	Race/Ethnicity – Recode	
12	DMDEDUC2	Categorical	Education Level – Adults 20+	
13	INDFMPIR	Continuous	Ratio of Family Income to poverty	
14	RIDEXPRG	Categorical	Pregnancy Status at Exam – Recode	

Laboratory Measurements

15	LBDVID	Continuous	Vitamin D	ng/mL
16	LBDVIASI	Continuous	Vitamin A	umol/L
17	LBXCRP	Continuous	C-reactive protein	mg/dL
18	LBXGH	Continuous	Glycohemoglobin	%
19	LBXCR	Continuous	C-reactive protein	mg/dL

Dietary

20	DR1DRSTZ	Interval	Dietary recall day 1	
21	DR1INIAC	Interval	Niacin	mg
22	DR1ICALC	Interval	Calcium	mg
23	DR1IMAGN	Interval	Magnesium	mg
24	DR1IZINC	Interval	Zinc	mg
25	DR1TIRON	Interval	Iron	mg
Dietary Supplement Variables				
26	DSD090	Interval	How long supplement taken?	days
27	DSD103	Interval	Days Supplement Taken	Past 30 Days
28	DSD122Q	Interval	Quantity of supplement taken daily	Number
29	DSDSUPID	Character	Ingredient ID Number	
30	DSDSUPP	Character	Supplement Name	
31	DSDTYPE	Character	Formulation Type	
32	DSDSERVQ	Continuous	Serving Size Quantity	
33	DSDSERVU	Character	Serving Size Unit	
34	DSDINGID	Character	Ingredient ID Number	
35	DSDINGR	Character	Ingredient Name	
36	DSDQTY	Continuous	Ingredient Quantity	
37	DSDUNIT	Character	Ingredient Unit	
38	DSDCAT	Character	Ingredient Category	
39	DSD010	Character	Any Supplements Taken?	
40	DSD010A	Character	Any Antacids Taken?	
41	DSD103	Continuous	Days supplement taken Past 30 Days?	
42	DSD122Q	Continuous	Quantity of Supplement Taken Daily	
43	DSD090	Continuous	How Long Supplements Taken?	

Hypertension Health Indicators

44	BMXBMI	Continuous	Body Mass Index	kg/m^2
45	BMXWAIST	Continuous	Waist Circumference	cm
46	BPQ050A	Categorical	Now taking prescribed medicine for HBP	
47	BPQ040A	Categorical	Taking prescription for hypertension	
48	BPQ030	Categorical	Told had high blood pressure 2+ times	
49	BMXWT	Continuous	Weight	kg
50	BPXSY1	Interval	Systolic: Blood pressure (1^{st}) mm Hg	Variables 50-53 averaged
51	BPXSY2	Interval	Systolic Blood pressure (2^{nd}) mm Hg	
52	BPXSY3	Interval	Systolic Blood pressure (3^{rd}) mm Hg	
53	BPXSY4	Interval	Systolic Blood pressure (4^{th}) mm Hg	
54	BPXDI1	Interval	Diastolic Blood pressure (1^{st}) mm Hg	Variables 54-57 averaged
55	BPXDI2	Interval	Diastolic Blood pressure (2^{nd}) mm Hg	
56	BPXD13	Interval	Diastolic Blood pressure (3^{rd}) mm Hg	
57	BPXDI4	Interval	Diastolic Blood pressure (4^{th}) mm Hg	
58	SMQ020	Categorical	Smoked at least 100 cigarettes in life	
59	SMQ040	Categorical	Do you now smoke cigarettes?	
60	PAD320	Categorical	Moderate activity over past 30 days	
61	PAQ180	Categorical	Average level of physical activity each day	

APPENDIX C: TABLES

Table 1: NHANES 2003-2006 Sample Size and Response Rates

NHANES Survey Years	Screened	Interviewed	Unweighted Interview Response Rate (percent)	MEC examined	Unweighted MEC Response Rate (percent)
2003-2004	12,761	10,122	79	9,643	76
2005-2006	12,862	10,384	80	9,950	77

Table 2: Socio-Demographic and Health Characteristics of NHANES 2003-2006 Adult Participants by 25(OH)D Status

	Serum 25 (OH)D		
	1-29 ng/mL	≥30 ng/mL	
Categorical Variables	Weighted Column %	Weighted Column %	***p* value[a]**
Age in Years			0.1081
20-39	41.19	37.94	
40-64	45.72	47.32	
≥65	13.08	14.74	
Gender			0.0001
Men	42.53	51.49	
Women	57.47	48.51	
Race/Ethnicity			<.0001
Non-Hispanic White	42.53	88.38	
Non-Hispanic Black	32.77	2.31	
Other Hispanic	3.81	2.11	
Mexican-American	12.06	4.28	
Other	8.83	2.92	
Education Level			<.0001
Less than High School Graduate	23.61	12.63	
High School Graduate	25.33	26.94	
Some College or AA degree	32.73	31.67	
College Graduate or Above	18.34	28.77	
Poverty-Income Ratio			<.0001
Less than 1.0	18.19	8.31	
1.0 – 5.0	65.09	62.32	
> 5.0	16.72	29.38	
Smoking			<.0001
Never	49.94	47.69	
Former	19.36	27.28	
Current	30.70	25.03	
Moderate Physical Activity Last 30 Days			<.0001
Yes	46.76	67.64	
No	53.24	32.36	
Season of Serum 25(OH)D Collection			<.0001
May 1 to Oct 31	43.62	67.82	
Nov 1 to April 30	56.38	32.18	
Hypertension			<.0001
No	66.18	72.74	
Yes	33.82	27.26	
BMI, kg/m^2			<.0001
<18.5	1.71	1.74	
18.5-24.9	21.49	36.07	
25-29.9	27.41	36.60	
30-39.9	36.64	22.90	
≥40	12.75	2.69	
Waist Circumference			<.0001
Normal Waist Circumference	35.50	53.35	
Elevated Waist Circumference	64.50	46.65	
CRP, mg/dL			0.0209
<3.00	97.31	98.82	
≥3.00	2.69	1.18	
HgA1c, %			<.0001
<6.4	90.29	96.25	
≥6.5	9.71	3.75	

a = Rao-Scott Chi-Square

Table 3: Socio-Demographic and Health Characteristics of NHANES 2003-2006 Adult Participants by Hypertension Status

Categorical Variables	Non-Hypertensive Weighted Column %	Hypertensive Weighted Column %	*p* value[a]
Age in Years			<.0001
20-39	50.92	9.01	
40-64	42.63	57.48	
≥65	6.44	33.51	
Gender			0.9855
Men	49.27	49.23	
Women	50.73	50.77	
Race/Ethnicity			<.0001
Non-Hispanic White	75.98	76.72	
Non-Hispanic Black	9.35	13.29	
Other Hispanic	2.87	1.70	
Mexican-American	7.49	3.59	
Other	4.32	4.71	
Education Level			0.0002
Less than High School Graduate	14.56	17.87	
High School Graduate	24.40	31.08	
Some College or AA degree	33.83	28.23	
College Graduate or Above	27.21	21.82	
Poverty-Income Ratio			0.0012
Less than 1.0	11.48	9.21	
1.0 – 5.0	61.22	67.94	
> 5.0	27.30	22.85	
Smoking Status			<.0001
Never	49.07	46.06	
Former	21.34	34.66	
Current	29.58	19.29	
Moderate Physical Activity Last 30 Days			<.0001
Yes	64.44	55.83	
No	35.56	44.17	
Season of Serum 25(OH)D Collection			0.5033
May 1 to Oct 31	61.81	60.01	
Nov 1 to April 30	38.19	39.99	
Vitamin D			<.0001
<29 ng/dL	25.35	31.65	
≥30 ng/dL	74.65	68.65	
BMI, kg/m^2			<.0001
<18.5	2.23	0.68	
18.5-24.9	37.36	19.48	
25-29.9	33.93	35.29	
30-39.9	23.28	35.69	
≥40	3.20	8.87	
Waist Circumference			<.0001
Normal	57.29	27.96	
Elevated	42.71	72.04	
CRP, mg/dL			0.115
<3.00	98.28	98.86	
≥3.00	1.72	1.14	
HgA1c, %			<.0001
<6.4	97.44	88.27	
≥6.5	2.56	11.73	

a = Rao-Scott Chi-Square Test

Table 4_a: Assessment of Effect Modification for the Association Between Vitamin D and HTN: NHANES 2003-2006 Adult Participants				
	OR of HTN	95% Confidence Interval		
Categorical Variables (n= 6721)	Low vs Sufficient VitD	**Lower**	**Upper**	***p* value**[a]
Age in Years				
vitD*Age				0.0010
20-39 (n = 2410)	1.66	1.06	2.6	0.0263
40-64 (n = 2870)	1.38	1.15	1.669	0.0007
≥65 (n = 1441)	1.07	0.79	1.435	0.6745
Gender				
vitD*Gender				0.6335
Men (n = 3513)	1.23	1.02	1.479	0.0337
Women (n = 3208)	1.32	1.07	1.615	0.0082
Race/Ethnicity				
vitD*Race				0.0047
NH White (n = 3399)	1.34	1.16	1.56	<.0001
NH Black (n =1448)	0.85	0.63	1.139	0.2754
Other Hispanic (n = 210)	1.41	0.59	3.401	0.4420
Mexican-American (n = 1400)	1.43	0.94	2.181	0.0937
Other (n = 264)	0.75	0.41	1.357	0.3386
Education Level				
vitD*Education				0.1273
Less than High School Graduate (n = 1807)	1.10	0.85	1.429	0.4763
High School Graduate (n = 1657)	1.08	0.86	1.362	0.5026
Some College or AA degree (n = 1923)	1.49	1.15	1.947	0.0029
College Graduate or Above (n = 1332)	1.25	1.02	1.524	0.0342
Smoking				
vitD*smoking_status				0.3475
Never Smoked (n = 3381)	1.24	1.02	1.512	0.0299
Current Smoker (n = 1629)	1.58	1.15	2.181	0.0053
Former Smoker (n = 1709)	1.25	1.04	1.516	0.0197
Moderate Physical Activity				
vitD*mod_exer				0.8778
YES (n = 3595)	1.23	1.04	1.456	0.0179
NO (n = 3125)	1.21	1.04	1.405	0.0134
				a = Wald Test

Table 4_b: Assessment of Effect Modification and Confounding for the Association Between Vitamin D and HTN: NHANES 2003-2006 Adult Participants				
	OR of HTN	95% Confidence Interval		
Categorical Variables (n= 6721)	**Low vs Sufficient VitD**	**Lower**	**Upper**	***p* value[a]**
Season of Vitamin D Collection				
vitD*Season				0.6363
Nov 1 to Apr 30 (n = 3120)	1.21	1.00	1.462	0.0511
May 1 to Oct 31 (n = 3601)	1.29	1.11	1.493	0.0008
BMI, kg/m^2				
vitD*BMI				0.1185
<18.5 (n = 1859)	0.88	0.07	1.1	0.2672
18.5-24.9 (n = 84)	0.45	0.07	2.73	0.3843
25-29.9 (n = 2359)	1.25	0.99	1.586	0.0619
30-39.9 (n = 2010)	1.02	0.84	1.244	0.8479
≥40 (n = 393)	0.94	0.43	2.06	0.8760
Waist Circumference				
vitD*bmxwaist_MF				0.6948
Normal Waist Circumference (n = 2973)	1.04	0.84	1.294	0.7129
Elevated Waist Circumference (n = 3644)	1.10	0.94	1.28	0.2398
HgA1c				
vitD*HgA1c				0.3305
Normal: <6.4% (n = 6114)	1.21	1.07	1.368	0.0026
High: ≥6.5% (n = 591)	0.90	0.476	1.683	0.7304
				a = Wald Test

Table 5: Adjusted Logistic Regression Model of the Assocation Between Vitamin D and HTN: NHANES 2003-2006 Adult Participants

		AOR	95% CI	*p* value
		1.05	0.92-1.21	0.467
Body Mass Index				
	<18.5	1.38	0.61-3.12	0.4423
	18.5-24.9	Referent		
	25-29.9	1.73	0.69-4.31	0.2401
	30-39.9	1.66	0.71-3.89	0.2429
	≥40	2.72	1.21-6.13	0.016
Waist Circumference				
	Elevated vs Normal	3.1	2.67-3.6	<0.001

CI = Confidence Interval
OR = Odds Ratio
p value from Wald Test
Adjusted for 2 confounders, including BMI and Waist Circumference

Table 6: Vitamin D Co-Nutrient Levels of NHANES 2003-2006 Adult Participants by Vitamin D Status and HTN

	Hypertension			
	OR of HTN	95% CI		*p* value[a]
Vitamin D Co-Nutrient Variables	Low vs Sufficient VitD Status	Lower	Upper	
Calcium				
<EAR (n = 3076)	1.48	1.22	1.78	<.0001
≥EAR (n = 3507)	1.12	0.96	1.32	0.1449
Assoc to HBP	1.18	1.06	1.32	0.0025
Assoc to vitD	1.83	1.65	2.02	<.0001
vitD*Calcium				0.0157
Iron				
<EAR of 6 mg/day (n = 353)	1.19	0.70	2.00	0.5246
≥EAR of 6 mg/day (n = 6394)	1.26	1.10	1.45	0.0012
Assoc to HBP	1.19	0.93	1.51	0.1705
Assoc to vitD	1.74	1.39	2.17	<.0001
vitD*Iron				0.8049
Magnesium				
<EAR (n =4228)	1.42	1.19	1.70	0.0001
≥EAR (n = 2355)	1.12	0.93	1.33	0.2337
Assoc to HBP	0.99	0.87	1.12	0.813
Assoc to vitD	1.69	1.45	1.98	<.0001
vitD*Magnesium				0.0447
Niacin				
<EAR of 12 mg/day (n = 727)	1.18	0.72	1.94	0.5203
≥EAR of 12 mg/day (n = 5855)	1.28	1.11	1.48	0.0007
Assoc to HBP	1.08	0.85	1.37	0.5444
Assoc to vitD	1.64	1.31	2.07	<.0001
vitD*Niacin				0.7683
Vitamin A				
<39.9 ug/dL (n = 723)	3.09	1.66	5.76	0.0004
≥39.9 ug/dL (n = 5858)	1.29	1.14	1.47	<.0001
Assoc to HBP	0.57	0.45	0.74	<.0001
Assoc to vitD	3.13	2.42	4.03	<.0001
vitD*Vitamin A				0.006
Zinc				
<EAR of 9.4 mg/day (n = 2056)	1.87	1.37	2.56	<.0001
≥EAR of 9.4 mg/day (n = 4665)	1.18	1.01	1.38	0.0349
Assoc to HBP	1.13	0.99	1.29	0.0642
Assoc to vitD	1.63	1.40	1.91	<.0001
vitD*Zinc				0.1176

a **= Wald Test**

Table 7: Effect Modification of Calcium and Magnesium on the Assocation Between Vitamin D and HTN: NHANES 2003-2006 Adult Participants		
	Low Magnesium	**Adequate Magnesium**
Low Calcium	OR: 1.75 (95% CI: 1.42-2.16, p = <0.0001)	OR: 1.18 (95% CI: 0.09-1.54, p = 0.2280)
Adequate Calcium	OR: 0.90 (95% CI: 0.56-1.47, p = 0.6799)	OR: 1.18 (95% CI: 0.98-1.42, p = 0.0866)
CI = Confidence Interval OR = Odds Ratio		

Table 8: Effect Modification of Calcium, Magnesium and Vitamin A on the Assocation Between Vitamin D and HTN: NHANES 2003-2006 Adult Participants		
	Low Magnesium	Adequate Magnesium
Low Calcium, Low VitA	OR: 5.43 (95% CI 1.49, 19.77, p = 0.0103)	OR: 2.08 (95% CI 0.79, 5.49, p = 0.1406)
Low Calcium, Adequate VitA	OR: 1.66 (95% CI 1.33, 2.06, p = <0.0001)	OR: 0.87 (95% CI 0.53, 1.43, p = 0.5765)
Adequate Calcium, Low VitA	OR: 1.66 (95% CI 0.56, 4.91, p = 0.3640)	OR: 2.88 (95% CI 1.13, 7.30, p = 0.0261)
Adequate Calcium, Adequate VitA	OR: 2.08 (95% CI 0.79, 5.49, p = 0.1406)	OR: 1.16 (95% CI 0.97, 1.37, p = 0.1055)
CI = Confidence Interval OR = Odds Ratio		

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