ABSTRACT

Title of thesis: ASSOCIATION BETWEEN C-REACITVE PROTEIN AND SERUM 25-HYDROXYVITAMIN D: A NEGATIVE ACUTE PHASE REACTANT

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Objective: The aims of this study were to determine whether serum 25-hydroxyvitamin-D (25-OH-vitamin D) is a negative acute phase reactant and whether class of diagnosis modifies the association between C-Reactive Protein (CRP) and 25-OH-vitamin D.
Methods: Multiple linear regression analysis was utilized to assess the association between CRP and 25-OH-vitamin D in 1,043 patients with acute and chronic diseases and normal volunteers.
Results: After adjusting for confounding factors, the association between CRP and 25-OH-vitamin D was statistically significant. Class of diagnosis did not modify this association.
Conclusions: 25-OH-vitamin D is demonstrated to be a negative acute phase reactant in this group of patients; Therefore, it is not an accurate marker of vitamin D status in the setting of inflammation. These findings support that 25-OH-vitamin D should be interpreted cautiously when CRP is elevated and that evaluating 25-OH-vitamin D in the context of CRP will improve accuracy of 25-OH-vitamin D interpretation.

ASSOCIATION BETWEEN C-REACITVE PROTEIN AND SERUM 25-HYDROXYVITAMIN D: A NEGATIVE ACUTE PHASE REACTANT

By

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Chapter 1: Introduction

Over recent years, several studies have been published which link hypovitaminosis D to increased risk for an array of acute and chronic inflammatory diseases, including cancer, diabetes, rheumatoid arthritis, cardiovascular disease, and mortality.[1-7] In response to the conclusions of these studies, many experts have argued in favor of an increase in the threshold at which serum vitamin D levels are considered adequate.[1, 2, 8] Additionally, many researchers have suggested that a large majority of the worldwide population is vitamin D insufficient and could benefit from vitamin D supplementation.[1, 2] Concerns surrounding this have grown and the term pandemic is now being utilized to describe the current hypovitaminosis D status of the world. [1, 9] Prescriptions and sales of vitamin D supplements have escalated at a rapid rate[10], yet a causal relationship between vitamin D status and disease still has not been demonstrated due to an absence of adequate randomized controlled trials investigating this association.[4]

On the other hand, recent data have suggested that 25-hydroxyvitamin D (25-OH vitamin D), the circulating form of the vitamin, believed to be the most accurate indicator of an individual's vitamin D status[1, 4], is a negative acute phase reactant.[11] This is momentous, as it reveals that 25-OH vitamin D is decreased in response to inflammation, ruling it an inaccurate indicator of vitamin D status in the setting of inflammation. Moreover, this suggests that low 25-OH vitamin D levels are the consequence of inflammatory diseases, rather than a cause for these diseases, potentially debunking the current widespread interpretation of the existing literature.[11] It is imperative that researchers work to discern whether the trajectory being followed with widespread prescription of vitamin D is based on a fallacious interpretation of laboratory results.

Chapter 2: Research Question and Specific Aims

The primary aim of this study is to shed further light on the question of whether serum 25-OH vitamin D responds as a negative acute phase reactant. The following aims and hypotheses were used to guide this study:

1. Is 25-OH vitamin D significantly lower in patients with higher C-Reactive Protein (CRP) levels compared to those with lower CRP levels in a group of hospitalized patients with a variety of sources of acute and chronic inflammation?

CRP is a laboratory marker of general inflammation.[12] Other studies have looked at longitudinal data, in which the change of CRP and 25-OH vitamin D levels were assessed over time by comparing results before and after a particular inflammation-driving event, such as a surgical procedure or an acute myocardial infarction. To the best of my knowledge, this will be the first study that examines this association across hospitalized patients with a variety of diagnoses.

2. Does class of diagnosis function as an effect modifier in the examination of the association between CRP and vitamin D within this hospitalized population?

Diagnosis impacts the source, degree, and duration of inflammation present. Previous studies that have examined this relationship have found conflicting results, which could be due to different sources of inflammation studied in each one. Therefore, it may be necessary to stratify the results based on this variable.

The research hypothesis was that vitamin D levels would be significantly lower in patients with higher CRP levels compared to those with lower CRP levels and that the magnitude of this association would vary significantly based on class of diagnosis.

If the conclusion of this study were consistent with that hypothesized, this would indicate that 25-OH vitamin D is an inaccurate marker of vitamin D status when inflammation is present and would suggest that vitamin D supplementation might currently be prescribed in excess. While vitamin D toxicity is rare, other potential unintended consequences of high-dose vitamin D supplementation are understudied, yet sales for these products have grown at an astronomical rate over recent years.[10] Further, the Vitamin D Council is recommending that adults with infrequent exposure to the sun take 5,000 international units of vitamin D daily, which exceeds the tolerable upper limit set by the Institute of Medicine (IOM) of 1000 international units. [10]

Of note, Mursu et al. concluded that there may be increased total mortality risk associated with use of vitamin supplements in older women[13], and in a sample of 15,167 subjects from the National Health and Nutrition Examination Survey, Amer et al. concluded that 25-OH vitamin D at levels greater than 21ng/mL is associated with higher CRP levels.[14] Both of these studies suggest the potential for some negative effects of micronutrient supplementation, as do studies that have demonstrated associations between high serum 25-OH vitamin D levels and increased all-cause mortality, cancers at some sites like the pancreas, and greater risk of cardiovascular events.[15] Also noteworthy, the hospitalized population is one in which the addition of more pills to a medication regimen could contribute unnecessarily to discomfort, nausea, and cost. Finally, elevated levels of calcium and vitamin D, can contribute to calcification and damage to organs, such as the kidneys and blood vessels. [15]

Chapter 3: Background

3.1 Existing knowledge

3.1.1 Vitamin D

The primary role of vitamin D is to regulate concentrations of calcium, phosphorous, and magnesium in the bloodstream and contribute to bone growth.[1] Over the past decade, vitamin D has recognized to function as a hormone and has been linked to a variety of additional body functions, such as immune system regulation and associated conditions, including autoimmune disorders, cardiovascular disease, and cancer.[1-7] 25-OH vitamin D is the major circulating form of vitamin D and circulates bound to a specific protein known as vitamin D binding protein (VDBP), which is found widespread throughout the body in a variety of different tissues.[15, 16] Both endogenous and exogenous sources of vitamin D exist. Endogenous vitamin D is produced when the skin is exposed to sunlight, whereas exogenous vitamin D is obtained from ingestion of foods and dietary supplements containing vitamin D. [17, 18]

The IOM has identified the acceptable level of 25-OH vitamin D to be 20ng/mL for bone health and reported that serum concentrations above 30ng/mL do not consistently provide additional health benefits.[15] Many vitamin D experts argue that a higher cutoff should be utilized with some citing 50ng/mL as a more appropriate target for optimal health of other body systems.[8, 15] However, the IOM cites concern associated with the frequent use of this figure, given the inconsistent representation in the literature and lack of a unified body tasked with the responsibility of establishing guidance on this subject.[15]

Using the cutoff recommended by the IOM for diagnosing vitamin D deficiency of serum concentration </=20ng/mL, National Health and Nutrition Examination Survey (NHANES) data suggest that the prevalence of vitamin D deficiency in the United States is 41.5%.[2] However, experts believing that a higher cutoff is more appropriate would argue that these figures should be much higher.[1] Risk factors for vitamin D deficiency include older age, darker skin tone (due to requirement for longer duration of sun exposure for synthesis vitamin D compared with fair skin counterparts), obesity (due to decreased bioavailability secondary to sequestration within adipose tissue), nutrient malabsorption, kidney dysfunction and other comorbidities, and lack of sunlight exposure, possibly due to season or hospitalization.[1, 8, 17, 18, 19, 20] Some medications also influence vitamin D metabolism.[21]

3.1.2 C-Reactive Protein and Inflammation

CRP is one key protein component of the immune system made by the body in response to infection, trauma, or stress.[22] CRP is recognized as an indicator of inflammation from an array of sources.[12] The influence inflammation has on a variety of nutrition markers has been recognized over recent years.[22] For example, serum or plasma levels of iron, zinc, vitamin A, albumin and prealbumin decrease in the setting of inflammation and are therefore recognized as negative acute phase reactants.[19, 23, 24] It is recommended that CRP levels be checked simultaneously, in order to interpret these results in the context of a given CRP level.[25] When CRP is elevated, it is advised to rely on alternative methods for assessment of nutrition status, such as reported dietary intake, physical assessment, and weight changes.[26] Current practice for clinical interpretation of

these lab values, based on the presence or absence of inflammation, is a very similar relationship to that being hypothesized for this study related to vitamin D.

3.2 Analyses performed by others

Waldron et al. conducted a longitudinal cohort study among 30 patients undergoing elective orthopedic surgery.[11] Serum CRP, 25-OH vitamin D, and VDBP were measured before and 48 hours after knee or hip arthroplasty.[11] Results of this study demonstrated a statistically significant increase in CRP and a statistically significant decrease in 25-OH vitamin D and VDBP when pre- and post-operative mean values were compared, thereby suggesting that both 25-OH vitamin D and VDBP decrease in response to the inflammation incurred by surgery.[11] Similarly, Reid et al. conducted a cohort study of 33 patients undergoing elective knee anthroplasty and demonstrated that 25-OH vitamin D levels significantly declined after surgery and remained significantly below baseline measurement at three months post-surgery.[3] The results of this study support those of the previous study by demonstrating that vitamin D levels decrease in response to the inflammation incurred by surgery.[3] Third, Louw et al. conducted a similar analysis in a prospective cohort study involving 26 patients undergoing orthopedic surgery and found that vitamin levels were decreased in response to the inflammatory response.[27] As a result, they urged for the use of caution when interpreting serum vitamin levels during times of inflammation.[27] Finally, in a cross-sectional study, Amer et al. demonstrated a significant negative correlation between CRP and 25-OH vitamin D when 25-OH vitamin D was $\leq$ 21 ng/ml in a group of asymptomatic adults, based on a sample of 15,167 subjects from NHANES data.[14]

Contrastingly, Barth et al. conducted a cohort study among 32 patients following acute myocardial infarctions and concluded the opposite.[28] In this study, it was determined that 25-OH vitamin D levels remained unchanged after this event and is likely an accurate marker of vitamin D status in patients surrounding a myocardial infarction.[28] Likewise, Newens et al. concluded that 25-OH vitamin D levels do not change from vitamin D levels during malarial infection after recovery from malarial infection in 14 subjects followed for two to six weeks after malarial infection.[29]

Of the few randomized controlled trials that have been conducted, results have been contradictory with some revealing a decrease in inflammatory biomarkers and others concluding no effect, upon supplementation with vitamin D.[7, 30 31, 32] Schleithoff et al. conducted a double-blind, randomized, placebo-controlled trial in which patients with congestive heart failure (n = 93 completed the study) were randomized to receive either a vitamin D and calcium supplement or a placebo for 90 days.[30] Results indicated a statistically significant decrease in parathyroid hormone and anti-inflammatory cytokine interleukin 10 after 9 months of supplementation, compared with baseline levels, while there was no significant difference in survival rate between the two groups.[30] Van Den Berghe et al. randomized 22 critically ill patients in an intensive care unit to receive either 200 international units or 500 international units vitamin D supplementation. Results indicated that CRP decreased over time and that this decrease was more pronounced in the higher dose supplementation group.[31] Witham et al. conducted a randomized, parallel group, double-blind, placebo-controlled trial in a group of 105 patients with systolic heart failure, in which patients were randomized to receive either 100,000 international units vitamin D or a placebo at baseline and at 10 weeks.[32]

Multiple measurements were utilized to assess physical function and quality of life at various timepoints and there was no improvement observed upon any measure for those in the treatment group.[32]

3.3 Knowledge gaps

The conflicting results of these studies demonstrate a lack of consensus related to the influence of inflammation on the accuracy of 25-OH vitamin D measurements. Secondly, these differing findings indicate that there may be differences in this association dependent on the source of the inflammation. To my knowledge, there have been no studies to date that investigate this relationship in patients with chronic disease-driven inflammation.[3] For these reasons, it is imperative that studies are conducted to assess this association in a broader range of patients and to specifically investigate the possibility of effect modification on the basis of class of diagnosis, a gap that is evident in the existing literature.

3.4 Importance of the study

If 25-OH vitamin D were a negative acute phase reactant, this would hold substantial significance to human health. This would indicate that cases of vitamin D deficiency are currently being over-diagnosed worldwide and prescription of vitamin D supplementation is being done in excess. This also may explain one remaining mystery related to vitamin D, which is that while 25-OH vitamin D levels increase in response to increased intake of vitamin D, the relationship is nonlinear.[17] Perhaps the reason is that the degree of inflammation present for each individual mediates this relationship.

The results of this study also pinpoint a new factor that should be considered in past and future vitamin D studies by including CRP as a confounding factor in their

analysis. For example, a study that was recently published in May 2014 by Hansson et al. concluded that vitamin D levels affect outcomes in pediatric hematopoietic stem cell transplantation.[5] However, it is possible that the true factor responsible for the difference in outcomes between those with high versus low levels of vitamin D could be that vitamin D levels actually served as a surrogate indicator of inflammation status for those having lower levels of inflammation associated with their disease. If inflammation, measured as CRP, had been included in the multivariate analysis model, it would be interested to see if the same results were reached.

The 2010 Dietary Guidelines for Americans state that the nutrients should preferentially come from foods.[33] Therefore, some health professionals have questioned the accuracy of the notion that supplemental vitamin D in pill form is necessary to prevent vitamin D deficiency. There are limited natural dietary sources of vitamin D and exposure to sunlight is generally inadequate to allow for synthesis of vitamin D in the quantities researchers are now suggesting are adequate for optimal health.[2, 8] It is important to keep in mind that supplementation of nutrients in pill form is not always beneficial and can even elicit undesired side effects or outcomes. Therefore, it will be important to discern whether the trajectory being taken is based on a fallacious interpretation of those laboratory results.

Chapter 4: Research Design and Methods

4.1 Study design

A retrospective study design was conducted utilizing electronic health record data from a clinical research data repository, the National Institutes of Health (NIH) Clinical Center Biomedical Translational Research Information System (BTRIS). BTRIS is a data repository available to NIH intramural researchers, which was developed to consolidate clinical research data into one single database.[34] One intended use of this database is for the reuse of clinical research data captured in the electronic health record to answer new research questions.[34] The database currently captures more than half a million subjects (~515,000) and over 9 billion rows of data.[35] Each row of data yields a variety of data types for a given subject, ranging from a given vital sign or medication administered to a laboratory test result or medical problem.[34] Subjects include an international population with a variety of diseases and conditions, including some with very rare diagnoses and normal volunteers. For the purposes of this study, data were pulled from a variety of completed and active clinical research protocols that have been conducted across several NIH institutes.

4.2 Subjects and data pairing

There were 1,043 subjects included in this analysis. **Figure 1** outlines the steps taken throughout the data acquisition and cleaning process. First, a limited dataset query was conducted to capture deidentified data on all subjects admitted to the NIH Clinical Center since the year 1976 that have had CRP and 25-OH vitamin D levels measured (n = 2,100). Second, all subjects under the age of 18 years were deleted (485 subjects eliminated), leaving a total of 1,615 subjects in the sample. Third, vitamin D levels were

paired with the highest CRP value measured over the previous 3 months for each subject. This 3 month cutoff value was selected based on a previous study that demonstrated a lasting impact of inflammation, or CRP, on vitamin D levels for 3 months after the elevated level.[3] Subjects without a CRP level checked within 3 months of a vitamin D level were eliminated (316 subjects were eliminated), bringing the sample size to 1,299 subjects. For any subjects with multiple possible data pairs, the first pair only was selected, in order to ensure that all data pairs remain independent of one another for compliance with assumptions of statistical tests. This decision was made based on the idea that earlier pairs would be closer to baseline and may be less likely to be influenced by any treatments or other factors occurring through study participation at the NIH Clinical Center.

Next, all subjects taking vitamin D supplementation that exceeded 400 international units at the time the lab was checked were excluded (266 eliminated), leaving a total of 1,033 subjects in the sample. The cut off of 400 international units was selected based on the fact that this is the amount of vitamin D contained in most general multivitamins, including those prescribed at NIH. Multivitamin use or use of a vitamin supplement containing 400 international units or less of vitamin D was assessed as a potential confounder in the analysis.

High-sensitivity CRP data was obtained from one Principal Investigator, Dr. Jack Yanovski. Approximately 25% of the sample was composed of patients that had been admitted under one of his protocols. In order to increase the proportion of CRP values that were generated from high-sensitivity CRP testing, these were substituted for the original subjects from his protocol that had initially been gathered through BTRIS with

regular CRP values. In order to ensure there was no duplication of these subjects, all subjects from the original dataset enrolled on Dr. Yanovski's protocol were eliminated. The high-sensitivity CRP data provided by Dr. Yanovski was then added. A greater number of subjects was available in the newly added sample than were initially removed from the dataset, hence the sample size of the CRP-HS subset increased following this step. Finally, 49 subjects were eliminated due to missing BMI data. This brought the total final analytic sample size to 1,043 subjects.

4.3 Description of variables

4.3.1 Dependent variable

The dependent variable of interest was serum vitamin D levels, measured as total 25-OH vitamin D. This variable was treated as a continuous variable. Of the different forms of vitamin D that can be measured in the blood, this form has been identified among vitamin D experts and clinicians as the most reliable marker of vitamin D status.[1,6]

4.3.2 Independent variable

The independent variable of interest was inflammation, measured as CRP, and also treated as a continuous variable. CRP is described as a non-specific test, meaning that it is an indicator of inflammation without identifying any specific source or type of inflammation.[12] Infections, inflammatory diseases such as lupus or inflammatory bowel disease, cancer, and post-operative status are examples of potential sources of such inflammation.[12] Obesity has also been cited as a condition in which CRP may be elevated.[2, 20] CRP levels can also be used to assess risk for heart disease.[12] To give an idea of significance of CRP levels, results are generally interpreted as follows:[12]

- <1.0mg/L → Low risk of heart disease

- 1.0 – 3.0mg/L → Average risk of heart disease
- 3.0 – 8.0mg/L → High risk for heart disease
- >8mg/L → Serious inflammation or infection

Contrary to some other markers of inflammation, CRP responds quickly to changes in levels of inflammation and therefore is an accurate marker of acute changes in inflammation.[12] Both high-sensitivity and conventional CRP data were included in this dataset. The high-sensitivity CRP test, which was recently developed, is able to detect lower levels of inflammation and is therefore more useful than standard CRP values for assessing risk of cardiovascular disease and associated complications.[36] Both measures are still recognized as acceptable markers of inflammation.[35]

4.3.3 Potential confounders

Several additional variables were examined as potential confounders. Age and BMI were analyzed as continuous variables. Race/ethnicity were analyzed as a categorical variable with categories designated as White, non-Hispanic; Black, non-Hispanic; Hispanic or Latino; or other. Sex was analyzed as a binary variable with all subjects categorized as male or female. Comorbidities and medications known to potentially influence vitamin D levels were both considered as possible confounders. They were categorized as binary variables, as presence or absence of a comorbidity of interest and taking or not taking a medication of interest. Comorbidities accounted for in this assessment included kidney dysfunction, malabsorbtive syndrome, and end-stage liver disease.[12, 19] Medications accounted for in this assessment included estrogen, Isoniazid, Thiazide, Antacids, Anti-seizure medications, bile acid sequestrants, Rifampin, mineral oil, Orlistat or Alli, and steroids.[21, 37] Season was analyzed as a categorical

variable based on date upon which 25-OH vitamin D levels were measured and was classified as Winter (December through February), Spring (March through May), Summer (June through August), or Fall (September through November). These cutoffs were selected in order to be consistent with other analyses that have been conducted in the literature. Finally, class of diagnosis was categorized into groups with other similar diagnoses. Ten categories were utilized, including lipodystrophy, auto-immune diseases, normal volunteers, Turner syndrome, immune defect disorders, ovarian dysfunction, alcohol dependence, lipid processing and storage disorders, chronic graft versus host disease, disturbances of amino-acid transport, and other diseases. For more information about the specific diagnoses included under each of these categories, please refer to **Appendix A**. These variables were each selected based on evidence in the literature that they influence vitamin D levels or metabolism.

4.4 Statistical analysis

Secondary data analysis was conducted in this study. A descriptive analysis was performed first, in order to assess the distribution of each of the variables of interest across the study sample and to identify missing values. Sharpiro-Wilk tests were used to check whether the normality assumption was violated for any continuous variable and to determine whether non-parametric tests were needed. Next, bivariate analysis was implemented to evaluate the unadjusted relationship between each covariate and the dependent variable, 25-OH vitamin D. Non-parametric tests were used to compare the medians of the continuous outcome variable (25-OH vitamin D) between groups of categorical variables. Wilcoxon-Mann-Whitney test was used for two-group comparison and Kruskal-Wallis test was used for multiple-group comparison. Two-sided tests were

applied, given that there is no reason to assume a single direction of interest. Since all continuous variables violated the normal assumption, Spearman's rank correlation coefficients were calculated to assess the association between CRP and 25-OH vitamin D levels, as well as to examine the unadjusted relationship between each continuous covariate and the dependent variable, 25-OH vitamin D.

From there, multiple linear regression was utilized to further assess the relationship between CRP and 25-OH vitamin D. Potential confounding effects (including age, sex, BMI, season, race/ethnicity, and medications and comorbidities known to potentially influence vitamin D levels) were assessed in the model. Decisions about which variables to include in the final model were made based on the statistical analysis, as well as based on the literature. Any variables deemed to be confounders evidenced by producing a change in the regression coefficient estimate for the association between CRP and 25-OH vitamin D of greater than 10%, were included in the final linear regression model. Variables identified for inclusion in the model based on a 10% change included race/ethnicity and multivitamin supplementation. BMI was also included in the final model based on evidence in the literature that BMI has an association with both CRP and vitamin D. [1, 2, 18, 19, 20, 21, 38] Individuals with higher BMI are known to have higher CRP levels and lower 25-OH vitamin D levels.[1, 2, 18, 19, 20, 21, 38] One noteworthy point is that the mechanism for the association between obesity and vitamin D exceeds that which could be attributed to the inflammation associated with obesity. Based on existing literature, vitamin D is sequestered within the excess adipose stores, thereby reducing its bioavailability. [20]

The final model was selected as follows:

$$E(\text{Vitamin D}) = \text{Intercept} + \beta_1 \text{CRP} + \beta_2 \text{Other races} + \beta_3 \text{Hispanic or Latino} + \beta_4 \text{Black} + \beta_3 \text{MVI} + \beta_4 \text{BMI}$$

Class of diagnosis was temporarily added to the model, along with the associated interaction term, in order to test for significance as an effect modifier. However, it was determined not to contribute significantly to the model based on the p-value associated with the interaction term, and thus was removed.

Variance inflation factors (VIFs) were calculated to test for collinearity, and the standard threshold value of 10 was used when conducting such tests. No evidence of multicollinearity was observed ($VIF \leq 4.59$ for all variable categories). Confidence intervals were reported, and a type I error rate α of 0.05 was utilized to determine which results were statistically significant. Statistical analysis was conducted using SAS version 9.3 software.

4.5 Human subjects and IRB

A human subjects research determination form was submitted to the University of Maryland Institutional Review Board (IRB) and was determined to be exempt for full board review. This process has also been confirmed through the NIH IRB, as well. Official certificates are available from both institutions and are included in **Appendices B and C** of this report.

Chapter 5: Results

5.1 Descriptive statistics

The study analytic sample included 1,043 patients with a majority being female (71%) and white (72%). The mean age of the patients was 39 years (ranging from 18 to 79 years), and the mean BMI was 28 kg/m2 (ranging from an underweight status of 13.0 kg/m2 to an obesity class III status of 58.2 kg/m2). The sample was approximately evenly distributed among the four seasons and between type of CRP measured (56% with non-high-sensitivity CRP vs. 44% with high-sensitivity CRP). The majority of patients did not have any of the comorbidities of interest and were not taking any of the medications of interest, including multivitamin or other vitamin supplement containing at most 400 international units of vitamin D. Diagnoses were classified into 10 different disease types, with the majority of patients classified as normal volunteers (28%) or as having ovarian dysfunction (16%). For the independent variable, the mean CRP value was 0.87 mg/dL (ranging from a normal value of 0.01 to a value indicating severe inflammation of 33.6 mg/dL). For the dependent variable, the mean 25-OH vitamin D level was 24.67 ng/mL (ranging from a severely deficient value of 1 to an optimal value of 88 ng/mL). **Table 1** illustrates details of the descriptive statistics.

5.2 Bivariate analysis

The bivariate analysis revealed significant differences in median 25-OH vitamin D levels between categories for multivitamin ($p = 0.0008$), race ($p < 0.0001$), season ($p = 0.03$) and class of diagnosis ($p = 0.002$), and significant correlation between 25-OH vitamin D with CRP ($p < 0.0001$), age ($p = 0.04$), and BMI ($p < 0.0001$). A marginally significant association was observed for 25-OH vitamin D with comorbidities ($p = 0.09$).

There were not significant differences observed for the other variables, including sex and medications. These results show the existence of significant relationships between the dependent variable and independent variable, as well as between the dependent variable and some of the confounding factors.

5.3 Model selection

As described in the methods section, the final model was identified based on 10% change in estimate method and based on the literature. After substituting in the β estimates from the analytical output, the equation reads as follows:

E(Vitamin D) = 28.63 + (-0.39)*CRP + (7.16)*White + (1.89)*Hispanic or Latino + (-1.63)*Black + (-2.59)*MVI + (-0.23)*BMI

5.4 Results

The β estimate for the association between CRP and vitamin D was attenuated after multi-variable adjusted analysis but a statistically significant, negative association remained. Based on the change in estimate analysis, race was identified as the variable with the greatest degree of confounding effects (yielded a 21% change in estimate). Multivitamin supplementation was determined to be the second greatest contributor of confounding effects (yielded a 14% change in estimate).

Additional noteworthy results are evident based on the data displayed in **Table 2**. Among race groups, after adjusting for CRP, BMI and multivitamin, Blacks have a decrease of 25-OH vitamin D of -8.79 ng/mL ($p < 0.0001$), Hispanic or Latino have a decrease of 25-OH vitamin D of 5.27 ng/mL ($p = 0.0002$), and other races have a decrease of -7.16 ng/mL ($p < 0.0001$), compared with Whites. Also those taking a multivitamin or low-dose vitamin D supplement would have an increase of 25-OH

vitamin D of 2.59 ng/mL ($p = 0.01$) compared with those that are taking a supplement after adjusting for other factors. Finally, for each one unit increase in BMI, 25-OH vitamin D would be expected to decrease by 0.23 ng/mL ($p < 0.0001$) after adjusting for other factors.

Lastly, the hypothesis that class of diagnosis serves as an effect modifier of this association is rejected ($p = 0.19$).

Chapter 6: Discussion

6.1 Conclusions

A greater understanding of the relationship between inflammation and vitamin D is vital to help clinicians accurately interpret laboratory results of patients. As was discussed throughout the introduction, there has been considerable discrepancy among studies previously conducted to examine the association between inflammation and vitamin D. It is suspected that this is due to small sample size, limited representation of different degrees and sources of inflammation, and inability to account for key confounding variables, such as race and vitamin supplementation. This study improves upon many of these prior limitations and fills many remaining research gaps, as it analyzes data from a large sample of subjects with a wide variety of health conditions. It also is one of the first studies to investigate the association between CRP and 25-OH vitamin D in lower levels of inflammation and was able to account for both acute and chronic sources of inflammation. Finally, this study also included assessment for a wider range of confounding factors.

The findings of this study support that there is an association between CRP and 25-OH vitamin D, and that a change in clinical and research practice may be needed. These results, in conjunction with the conclusions of supporting studies cited, suggest that the accuracy of 25-OH vitamin D levels declines when inflammation is present. From a clinical application standpoint, this is a highly important finding, as it provides evidence to support a need to concurrently check a CRP level when checking a 25-OH vitamin D level. When CRP is elevated, 25-OH vitamin D levels should be interpreted cautiously and checking levels when CRP is lower would be beneficial. Lastly, use of

alternative tools for assessment of vitamin D status is recommended, especially when inflammation is present. Examples of such factors may include diet recall, assessment of risk for malabsorption, questions about lifestyle and sunshine exposure, and DEXA scans to assess bone mineral density.

Specifically, the results of this study demonstrate that for every 1 mg/dL increase in CRP, 25-OH vitamin D decreases by 0.39 ng/mL. Clinically, this would suggest that the association between CRP and 25-OH vitamin D is most important at higher levels of inflammation. For example, a patient with a CRP of 1 mg/dL would not be expected to have a clinically relevant change in 25-OH vitamin D. However, a patient with a CRP of 20 mg/dL may have a reduction in 25-OH vitamin D of 8 ng/mL, which could certainly alter the results of the test and decisions surrounding vitamin D supplementation. Further, it should be recognized that the data pairing approach used in this study would be expected to result in a conservative estimate of this association, since the association would be strongest when levels are measured closest to one another. In other words, Reid et al. found that the greatest changes occurred at the point of the systemic inflammatory response and then diminished but remained statistically significant at the 3 month post-operative mark.[3] Therefore, one could expect that if our data pairs all reflected the peak CRP level paired with a 25-OH vitamin D level measured close to that value, an even stronger association may have been observed, which may demonstrate greater clinical significance at a wider variety of CRP levels.

From a research perspective, one key recommendation is that investigators should assess CRP, or other markers of inflammation, as potential confounding factors in all future studies examining 25-OH vitamin D as a predictor of other diseases and outcomes.

The findings of this study combined with the existing literature support the conclusion that inflammation could be confounding the results of such studies. One example of a study that could have benefited from assessing inflammation as a confounding factor was described in the introduction. Hansson et al. concluded in a paper published in May 2014 that vitamin D levels affect outcomes in pediatric hematopoietic stem cell transplantation.[5] However, it is possible that the true factor responsible for the difference in outcomes between those with high versus low levels of vitamin D could be that those with seemingly higher vitamin D levels actually served as a surrogate indicator for those having lower levels of inflammation associated with their disease. It would be interesting to see if the same results held true if inflammation had been included in the multivariate analysis model.

The statistically significant findings related to race and multivitamin use as confounding factors described in the results section are consistent with existing literature. Given what is known about skin pigmentation and ability to synthesize the active form of vitamin D from sunlight, it was expected that whites would have higher levels of vitamin D and Blacks would have lower levels of vitamin D, independent of other factors. [1, 8, 17, 18, 19, 20] A majority of this study population was White, which explains why adjustment for race had such a significant contribution to the attenuation in the β estimate for CRP. The unadjusted estimate was confounded by the fact that Whites generally have lower inflammation levels and higher 25-OH vitamin D levels compared with other races, thereby magnifying the association. [17, 18, 20, 39] One would also expect that supplementation of vitamin D, even at low levels, would result in an increase in vitamin D level after controlling for other variables. A smaller proportion of the sample was

taking a multivitamin, explaining why a smaller contribution to the attenuation occurred for this variable, compared with race. Nonetheless, vitamin supplementation is associated with a decrease in inflammation and an increase in 25-OH vitamin D, thereby falsely elevating the β estimate for CRP in the unadjusted analysis.[40] Finally, several prior studies have demonstrated that 25-OH vitamin D levels are lower and CRP is higher in individuals with higher BMI, which again would falsely magnify the unadjusted association. [20, 38]

As for the lack of effect modification by class of diagnosis, it could be that this is due to a couple of different factors. First, there may be some inflammation driving events unaccounted for, such as scheduled procedures or fevers, which would not be reflected in the class of diagnosis variable but which would also alter the source and degree of inflammation. For example, if a patient had a class of diagnosis generally associated with low amounts of inflammation but that person had recently undergone a procedure that caused an acute sudden increase in inflammation, this would be unaccounted for in the class of diagnosis variable. Second, a lack of adequate sample size within each class of diagnosis could have limited the power to detect significance. Or a third possibility is that it is not actually the source of the inflammation that modifies the association between CRP and 25-OH vitamin D after all. This is supported by the results of this study, which indicate that across a wide variety of diagnoses, in patients with both high and low levels of inflammation, and in the setting of both chronic and acute sources of inflammation, a significant association holds true.

6.2 Comparison with existing studies

A majority of studies that have demonstrated a significant decline in 25-OH vitamin D in response to inflammation have been studied in small samples of patients with limited representation of a variety of conditions, given that most of these studies looked at patients specifically undergoing orthopedic surgery. This lends to the question of whether or not there is something specifically related to this type of surgery that is causing the reduced 25-OH vitamin D levels observed in these cases, rather than the inflammation itself as was concluded by the authors. In other words, it is unclear whether these results could be generalized to a wider variety of patients, given the limited study population. However, the fact that the present study sample contained a wide variety of diagnoses for which orthopedic surgeries was not indicated and still found positive results, rules out this as the sole contributor to the findings in these studies, thereby supporting the findings that 25-OH vitamin D is in fact a negative acute phase reactant.

As noted previously, the a priori hypothesis was that class of diagnosis would modify this association. This hypothesis was made based on the idea that the type and degree of inflammation, such as that generated by a surgical procedure versus that generated by a chronic illness for instance, may contribute differences to the association between CRP and 25-hydroxyvitamin D. It is important to note that this present study included a wide variety of types and sources of inflammation. The fact that a positive and significant association was observed in a sample with such a varied set of health conditions and status supports that this association may be applicable to many other populations.

This also brings up the point of degree of inflammation present in the study subjects. In the present study, the mean CRP value was 0.87 mg/dL, which is significantly lower than the post-operative mean CRP values of the studies that demonstrated statistically significant, positive findings (Waldron et al. mean post-operative CRP: 8.12 mg/dL; Reid et al. median day 3 post-operative CRP: 18.9 mg/dL). Unfortunately, CRP values were not reported for either of the two studies described with null findings. Regardless, there is now evidence demonstrating a significant association between CRP and 25-OH vitamin D in a population that contains both high and low levels of inflammation.

Similarly, one can also speculate about the influence of the duration of the inflammation on vitamin D levels. Many of the prior studies with positive findings were investigating the association between CRP and 25-OH vitamin D in the setting of an acute inflammatory response related to surgery, whereas many of the subjects in the present study had chronic diseases or long-standing illnesses that are associated with chronic inflammation at varying levels. Again, there is now evidence demonstrating a significant association between CRP and 25-OH vitamin D in a population with both acute and chronic drivers of inflammation.

Upon closer review of the studies presented previously which reported null findings in contrast to the results of this present study, some notable limitations were evident. First, in the study conducted by Newens et al., which looked at changes in 25-OH vitamin D levels during malarial infection, the small sample size is a limitation. There were only 25 subjects included in the analysis and some of these were lost to follow-up. Furthermore, attrition occurred disproportionately between groups in that

subjects with higher baseline 25-OH vitamin D levels were less likely to return for follow-up, and this may have introduced bias. Additionally, the baseline levels were checked upon admission, which occurred after the infection and associated symptoms were already present, which may mean that even the baseline levels were affected by inflammation. So, when 25-OH D levels were compared to baseline, the difference would have been muted or less than actual. Finally, the authors did not collect information on race, which was found to be the most prominent confounding factor in this study and thereby may have significantly confounded their results.

Second, in the study by Barth et al. looking at 25-OH vitamin D levels before and after acute myocardial infarction, limitations were also noted. Again the sample size was small (n = 32). Also the labs were again checked after initial presentation with symptoms, suggesting that the 25-OH vitamin D levels may have already been affected by the existing inflammation, thereby reducing the difference. Additionally, no data were collected related to vitamin D supplementation, which was another major confounding factor identified in the present study, even after exclusion of any subjects taking >400 international units of vitamin D per day. The fact that even low levels of vitamin D supplementation significantly confounded the unadjusted association in the present study suggests that this could be a very significant limitation of this study by Barth et al.

There are multiple other noteworthy differences between the present study and prior studies. One such difference is the decision to pair 25-OH vitamin D levels with the highest CRP value recorded within 3 months prior. This decision was made based on the study by Reid et al. which found that 25-OH vitamin D levels remained significantly below baseline levels up to 3 months after surgery and the associated rise in CRP. [3] Of

note, this does exceed the half-life of vitamin D (15 days)[17], but it is possible that the mechanisms by which 25-OH vitamin D levels drop create a lasting effect on vitamin D metabolism. For example, a possible explanation could be if vitamin D binding proteins or receptors were affected.

From the standpoint of considerations for future studies, another noteworthy factor is that only CRP was analyzed as a marker of inflammation. This is recognized as a non-specific marker that increases in response to a variety of sources of inflammation, making it the ideal biomarker for inflammation. However, there are still some diseases or drivers of inflammation that are not necessarily reflected in CRP values. For example, per communication with Dr. Alexandra Freeman, a physician working at the NIH Clinical Center for the National Institutes of Allergy and Infectious Disease, some patients in this study have underlying immune defects that may result in inflammation that is not reflected in CRP values. There are multiple other measures of inflammation, such as β-2-microglobulin and erythrocyte sedimentation rate that could be considered in future studies.

6.3 Strengths and limitations

Several strengths and limitations are evident in this study. Beginning with the strengths, one key component is that the data are pulled directly from an electronic medical record, thereby avoiding any potential recall bias that can be introduced based on retrospective self-reported data. Another strength is the large analytic sample size (n = 1,043) and wide age range (ages 18 – 79 years), which helps to increase the power and validity of the results. Third, this study design allows for a quick and inexpensive means by which to examine this association in a population that has not already been tested.

Fourth, capturing 25-OH vitamin D values that occur at the time CRP is measured or up to three months after demonstrates temporality, as each vitamin D reflects the period in which inflammation, or lack thereof, would be expected to influence the results. Further, use of this particular dataset allows for a unique capture of a large group of individuals with a variety of diagnoses, which allowed for assessment of the theory of whether discrepancy observed in previous studies may be related to the source of inflammation, a gap that is evident in the existing literature. And finally, there is a fairly wide range of characteristics and lab values captured in this sample, including age range, CRP range, 25-OH vitamin D levels, and BMI.

There are also some limitations that come along with the reuse of electronic health record data, such as the potential introduction of bias based on the inclusion and exclusion criteria for each individual protocol on which the patients were enrolled. Every patient admitted at the NIH Clinical Center must be enrolled on a given study protocol. Each of the many study protocols on which the patients included in this study population were initially enrolled had different inclusion and exclusion criteria, which may have influenced the characteristics of the sample. This makes this population unique relative to other hospitalized populations in ways that are difficult to predict.

Similarly, there may be some additional unknown potential confounders, such as unknown comorbidities, medications, procedures, or other factors that could influence CRP and/or vitamin D levels. Also, the inability to dictate the time points at which both of the labs of interest were ordered is a limitation, as this results in pairing of values that were measured at varying amounts of time from one another, up to three months difference. Additionally, this may restrict representation of this relationship during

situations in which CRP is very high within this dataset. In other words, while CRP levels >100mg/L are often observed in the patient population being studied, it is not as common for 25-OH vitamin D levels to be tested alongside the tracking of CRP. Therefore, there is a small number of patients captured in this dataset with very high CRP levels relative to that observed in the patient population at the NIH Clinical Center, due to a lack of corresponding 25-OH vitamin D levels for those values. Regardless, being able to explain this association at lower levels of CRP is a significant and meaningful contribution to the literature. Additionally, this helped to fill another gap in the existing literature, as most studies described in this paper were conducted in patients with acute, high level inflammation, such as that driven by surgery. The values represented in this study are more representative of a greater proportion of people. For instance, secondary to obesity or other underlying chronic conditions, many people function daily with a chronic low level of inflammation. Of note, given that this study is conducted in hospitalized patients, the results may not be generalizable to the greater population, although the fact that 25% of the sample is composed of normal volunteers may suggest some generalizability.

Final potential limitations can be identified as the fact that sun exposure using home location was not controlled for, despite recognition of proximity to the equator as having a potential impact on 25-OH vitamin D levels. This decision was made based on the fact that some patients had been in the Clinical Center in Bethesda, MD for several months, thereby making their home address an inaccurate marker of proximity to the equator. Plus, Mansoor et al. demonstrated that the shielding of skin from the sun makes vitamin D deficiency common even in highly sunny locations, such as Pakistan.[1]

Lastly, data related to dietary intake of vitamin D are not available for this study sample. Given that we found that taking a multivitamin or very low level vitamin D supplement (no more than 400 international units of vitamin D) had a statistically significant influence on the results, this may be contributing confounding effects in this analysis. Further, a study sample with a majority of inpatient, hospitalized patients would be expected to potentially have a high degree of variability of vitamin D intake, as some may have abnormally high or low appetites for a variety of reasons. That said, no other studies have been able to account for this variable either. Finally, vitamin D supplementation could only be accounted for in those patients taking the supplement during the admission when the CRP and 25-OH vitamin D measurements were taken. There may have been some instances in which patients were taking vitamin D supplementation and stopped upon admission that were missed. Therefore, it is possible that some of the vitamin D levels in the study were inflated by previous vitamin D supplementation that was unable to be accounted for in the model.

Chapter 7: Public Health Significance

Current widespread belief among researchers and clinicians alike is that vitamin D deficiency is a cause of a growing number of inflammatory diseases. However, some newer evidence suggests that vitamin D may actually respond as a negative acute phase reactant, thereby decreasing in the setting of acute inflammation independent of true changes in vitamin D status. This could indicate that 25-OH vitamin D levels are not an accurate measure of vitamin D status in the setting of inflammation, a hypothesis that the results of this study strongly support.

This gain of information related to the relationship between CRP and vitamin D is highly relevant to clinical practice. Recognizing that this negative acute phase response does occur in this population with a variety of sources of inflammation, such as that driven by cancer, infection, or general chronic lower levels of inflammation, is imperative in order to ensure that clinicians are accurately identifying vitamin D deficient patients. At this point, it appears that more frequent checking of 25-OH vitamin D levels and trending with CRP levels is necessary to accurately diagnose a vitamin D deficiency. Also, considering additional assessment factors to determine deficiency risk is highly important, particularly in the setting of inflammation.

Given that discrepancy remains within the literature and that this study demonstrated a significant association between CRP and vitamin D, further studies of this relationship will be highly beneficial. For future studies, it would be recommended to impart alternative data pairing methods, such as utilizing the closest chronological CRP value to each 25-OH vitamin D level. More longitudinal studies with larger and more varied study populations would be ideal for demonstrating temporality, as well.

Incorporating additional inflammatory markers into the analysis could be useful. And finally, it is recommended that all future studies investigating disease outcomes associated with 25-OH vitamin D level assess for the confounding effects of inflammation.

Chapter 8: MPH Competencies Addressed in Thesis

In completing this study, several MPH competencies were addressed. Key sources of epidemiological data and vital statistics have been utilized during the literature review. Vitamin D deficiency has been described in terms of magnitude, person, time and place when citing prevalence statistics and other associated parameters. In justifying the public health significance and long term relevance of this study, the importance of epidemiology for informing scientific, ethical, economic, and political discussion of health issues has been explained. Epidemiological terminology and definitions were utilized throughout the verbal and written reporting of this study, as well as epidemiology measures. Written and oral presentation of the proposal and findings of this study to various groups will achieve the communication of epidemiologic information to lay and professional audiences. When commenting on strengths and limitations of this study, as well as other studies reviewed as references, Hill's causal criteria, strengths and limitations of different epidemiology study designs, and strengths and limitations of epidemiologic reports were all addressed. Advanced epidemiologic measures were calculated during the data analysis and results reporting phases. The entire process of completing this thesis pertains to designing, analyzing, and evaluating an epidemiological study. Finally, ability to collect and manage public health data was demonstrated through the data acquisition and cleaning phases of this thesis.

In summary, the following competencies are those addressed in this thesis:

Competencies for MPH in Epidemiology	Internship	Project/Thesis
1. Identify vital statistics and other key sources of data for epidemiological purposes	☑	☑
2. Describe a public health problem in terms of magnitude, person, time and place.	☐	☑
3. Discuss the principles and limitations of public health screening programs.	☑	☐
4. Comprehend basic ethical and legal principles pertaining to the collection, maintenance, use and dissemination of epidemiologic data.	☑	☐
5. Explain the importance of epidemiology for informing scientific, ethical, economic and political discussion of health issues.	☑	☑
6. Apply the basic terminology and definitions of epidemiology.	☐	☑
7. Calculate basic epidemiology measures.	☐	☑
8. Communicate epidemiologic information to lay and professional audiences.	☑	☑
9. Differentiate among the criteria for causality.	☐	☑
10. Draw appropriate inferences from epidemiologic data.	☐	☑
11. Describe epidemiologic study designs and assess their strengths and limitations.	☐	☑
12. Evaluate the strengths and limitations of epidemiologic reports.	☐	☑
13. Calculate advanced epidemiology measures.	☐	☑
14. Design, analyze, and evaluate an epidemiologic study.	☐	☑
15. Demonstrate skills in public health data collection and management.	☑	☑
16. Design interventions to reduce prevalence of major public health problems.	☑	☐
17. Demonstrate program administration and organizational leadership.	☑	☐

Figure 1. Data Acquisition and Cleaning

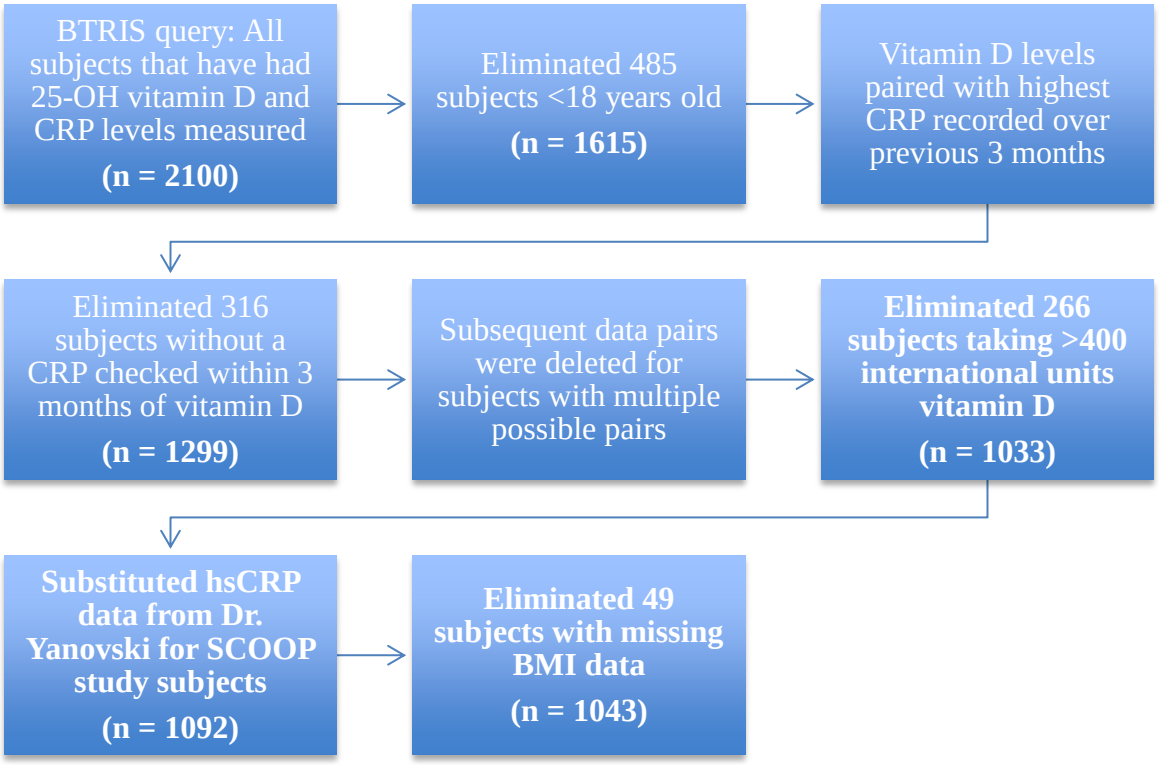

Table 1. Descriptive Characteristics (n = 1,043)

Categorical Variables			
Characteristic	**Category**	**Number**	**Percent**
Sex	Female	739	70.85
	Male	304	29.15
Race	White	750	71.91
	Black	179	17.16
	Hispanic or Latino	65	6.23
	Other	49	4.70
Season	Spring	289	27.71
	Winter	261	25.02
	Fall	248	23.78
	Summer	245	23.49
Medications	No	818	78.43
	Yes	225	21.57
Multivitamin intake	No	858	82.26
	Yes	185	17.74
Class of diagnosis	Normal volunteer	293	28.09
	Ovarian dysfunction	165	15.82
	Other	128	12.27
	Turner syndrome	117	11.22
	Auto-immune disease	76	7.29
	Alcohol dependence	65	6.23
	Disturbances of amino acid transport	52	4.99
	Immune defect disorder	44	4.22
	Lipid processing and storage disorders	37	3.55
	Lipodystrophy	33	3.16
	Chronic Graft Versus Host Disease	33	3.16
Comorbidities	None	921	88.30
	Present	122	11.70

Continuous Variables				
Characteristic	**Mean**	**Standard deviation**	**Minimum**	**Maximum**
Age (years)	38.95	12.43	18.00	79.00
25-OH Vitamin D (ng/mL)	24.61	11.84	1.00	88.00
CRP (mg/dL)	0.87	1.82	0.01	33.60
BMI (kg/m^2)	28.23	7.10	13.04	58.19

Table 2. Results of Unadjusted and Adjusted Analysis

	Unadjusted analysis			**Adjusted analysis***		
	β	**p-value**	**95% CI**	**β**	**p-value**	**95% CI**
CRP	-0.47	0.02	(-0.86, -0.07)	-0.39	0.04	(-0.77, -0.02)
Race						
Black	-9.68	<0.0001	(-6.54, 0.55)	-8.79	<0.0001	(-10.62, -6.96)
Hispanic or Latino	-5.05	0.0005	(-2.53, 5.80)	-5.27	0.0002	(-8.09, -2.46)
Other	-6.69	<0.0001	(3.44, 9.93)	-7.16	<0.0001	(-10.36, -3.96)
White	-	-	-	-	-	-
Multivitamin						
Taking	3.24	0.0007	(1.37, 5.12)	2.59	0.01	(0.81, 4.37)
Not taking	-	-	-	-	-	-
BMI	-0.32	<0.0001	(-0.42, -0.22)	-0.23	<0.0001	(-0.33, -0.13)

***Adjusted for race, multivitamin supplementation and BMI.**

Appendix A. Table Illustrating Diagnoses Included in Each Category

	Category	**Class of diagnosis Included**
0	Lipodystrophy	
1	Auto-immune diseases	Systemic lupus erythematosus; rheumatoid arthritis; polyarthritis; osteoarthritis; synovitis; Sarcoidosis; arthropathy; pyoderma gangrenosum and acne syndrome (PAPA); crohn's disease
2	Normal volunteer	
3	Turner syndrome	
4	Immune defect disorder	Chronic granulomatous disease; Job's syndrome; disseminated mycobacterium avium intracellulare infection
5	Ovarian dysfunction	Premature ovarian failure; polycystic ovary syndrome
6	Alcohol dependence	
7	Other	Gland or hormone disorders [thyroid dysfunction; hyperparathyroidism; adrenal gland neoplasm; pheochromoctoma; insulin resistance]; non-autoimmune-associated pain disorders [dermamyositis; polymyositis; fibromyalgia; myositis; unspecified disorder of autonomic nervous system; erythromelalgia; carpal tunnel]; Acute infection or recurrent febrile disease [familial Mediterranean fever]; disorders of the bone marrow [diamond-blackfan anemia; sickle cell; dyskeratosis congenita]; cancer [T-cell lymphoma; ewing's sarcoma; primary brain tumor-glioblastoma; chronic myelogenous leukemia; malignant neoplasm of kidney; hodgkins lymphoma; acute promyelocitic leukemia; acute myelogenous leukemia; large B-cell lymphoma; multiple myeloma; breast cancer; prostate cancer; peripheral T-cell lymphoma; chronic lymphocytic leukemia; recurrent refractory mantel cell lymphoma; chronic lymphocytic leukemia, chronic lymphocytic; non-hodgkin's lymphoma; myelodysplasia]; chronic lung disease [lymphangioleiomyomatosis; cystic fibrosis]; hemochromatosis; hepatic fibrosis; kidney disease; osler-weber-rendu syndrome; erdheim-chester disease
8	Lipid processing and storage disorders	Fabry-anderson disease; gaucher disease; homozygous familial hypercholesterolemia; lipoprotein deficiencies-abetalipoproteinemia; lecithin cholesterol acyltransferase deficiency; homozygous hypobetalipoproteinemia; homozygous hypobetalipoproteinemia; sitosterolemia
9	cGVHD	
10	Disturbances of amino acid metabolism	Cystinosis; hermansky-pudlak syndrome; methylmalonic acidemia; alkaptonuria

Appendix B. UMD IRB Exemption Certificate

UNIVERSITY OF MARYLAND
INSTITUTIONAL REVIEW BOARD

1204 Marie Mount Hall
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TEL 301.405.4212
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DATE:	October 20, 2014
TO:	Kelly Verdin
FROM:	University of Maryland College Park (UMCP) IRB
PROJECT TITLE:	[672898-1] MPH Thesis: Does Inflammation (measured at CRP) Predict Vitamin D Levels (measured as 25-OH vitamin D)?
SUBMISSION TYPE:	New Project
ACTION:	DETERMINATION OF EXEMPT STATUS
DECISION DATE:	October 20, 2014
REVIEW CATEGORY:	Exemption category *#4*

Thank you for your submission of New Project materials for this project. The University of Maryland College Park (UMCP) IRB has determined this project is EXEMPT FROM IRB REVIEW according to federal regulations.

We will retain a copy of this correspondence within our records.

If you have any questions, please contact the IRB Office at 301-405-4212 or irb@umd.edu. Please include your project title and reference number in all correspondence with this committee.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within University of Maryland College Park (UMCP) IRB's records.

Appendix C. NIH IRB Exemption Certificate

National Institutes of Health

Agreement for BTRIS Limited Data Set Use and Exclusion from IRB Review

Kelly VERDIN
10 Center Dr Bethesda, MD 20814
Building B2-2426, Tel: 301 594 8128
CC, CC OD OCOO ND CNS

BTRIS

OFFICE OF HUMAN SUBJECT RESEARCH PROTECTIONS

The NIH Office of Human Subjects Research Protections has determined that the following research with Limited Data Set(s) from BTRIS does not meet the definition of human subjects research pursuant to 45 CFR 46 and OHRP guidance:

Title of Proposed Research Study:
Association Between Vitamin D and CRP

Description of Proposed Research Study:
This is a follow-up to a prior query in order to obtain a greater number of subjects with values in the desired ranges. The aim of this study is to assess whether vitamin D levels are suppressed in the setting of inflammation.

The NIH researchers will comply with all NIH policies for data security, confidentiality and privacy. This document serves as a record of the BTRIS Data Use Agreement between the user and BTRIS.

Agreement#: BTRIS_2014_812_VERDIN_K_CC

Date: Friday, October 03, 2014

Query performed on behalf of: Myself

Other researchers having access to data: None

Note: Some NIH conducted or supported research involving coded private information or specimens may be subject to Food and Drug Administration (FDA) regulations. The FDA regulatory definitions of human subject (21 CFR 50.3(g), 21 CFR 56.102(e)) and subject (21 CFR 312.3(b), 21 CFR 812.3(p)) differ from the definition of human subject under HHS regulations at 45 CFR 46.102(f). Anyone needing guidance on such FDA-regulated research should contact the FDA.

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