VITAMIN D DEFICIENCY AND REPLACEMENT: RELATIONSHIPS TO CARDIOVASCULAR HEALTH

By

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RELATIONSHIPS TO CARDIOVASCULAR HEALTH

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Abstract

Background: Cardiovascular disease is the most common cause of mortality and morbidity in the United States as well as many other nations. Recent evidence supports an association of vitamin D deficiency with hypertension, peripheral vascular disease, diabetes mellitus, metabolic syndrome, coronary artery disease, and heart failure. We wished to study the association of vitamin D deficiency in a cohort of patients followed by a large cardiovascular practice at an academic medical institution, as well as the association of vitamin D replacement with improvement in cardiovascular outcomes.

Methods: Serum vitamin D measurements for 5 years and 8 months (1/1/2004 to 10/8/2009) from The University of Kansas Hospital were obtained. These values were matched to patient demographic, physiologic and disease state variables from the cardiovascular database. Serum vitamin D levels were analyzed as a continuous variable and as normal (≥30ng/ml) or deficient. Descriptive statistics, univariate analysis, multivariate analysis, survival analysis, and Cox proportional hazard modeling were performed.

Results: 10,899 patients were available for analysis. Mean age was 58.3 +/- 14.9 years. There were 7758 (71%) women and 3141 (29%) men. Mean weight was 185.7 +/-52.0 lbs and BMI was 29.9 +/- 7.7 Ejection fraction was 57.2 +/- 10.4%. Mean vitamin D was 24.1 +/- 13.6 ng/ml.. 3294 (29.7%) subjects were in normal range (≥30ng/ml) and 7665 (70.3%) were deficient (<30ng/ml). Vitamin D deficiency was

found to be associated with several cardiovascular disease states including hypertension, coronary artery disease and cardiomyopathy; as well as diabetes and death (all P's < .05). Logistic regression analysis found vitamin D deficiency to be a strong predictor of death (OR 2.64, CI 1.901-3.662, P < .0001). This association persisted with other clinical variables such as, BMI, gender, and ejection fraction added to the model and was confirmed by survival as well as hazard function analysis. Vitamin D replacement conferred substantial survival benefit (OR for death 0.39, CI 0.277-0.534, P < .0001) and was particularly beneficial in vitamin D deficient patients. The interaction of vitamin D deficiency and supplementation was analyzed as well as the association of vitamin D deficiency and certain coronary artery disease risk factors.

Conclusions: Vitamin D deficiency is a significant risk factor for several cardiovascular disease states and is a significant independent predictor of reduced survival. Vitamin D supplementation improves survival with greater benefit in deficient patients. Prospective randomized trials of vitamin D supplementation in patients with cardiovascular diseases are warranted, as well as consideration for increased supplementation in the general public.

Keywords: Vitamin D, Cardiovascular Diseases, Risk Factors

Introduction

Background

Cardiovascular disease is the most common cause of mortality and morbidity in the United States as well as many other nations. Recent evidence supports an association of Vitamin D deficiency with hypertension, peripheral vascular disease, diabetes mellitus, metabolic syndrome, coronary artery disease, and heart failure. It is emerging as a major and widespread cardiovascular risk factor.

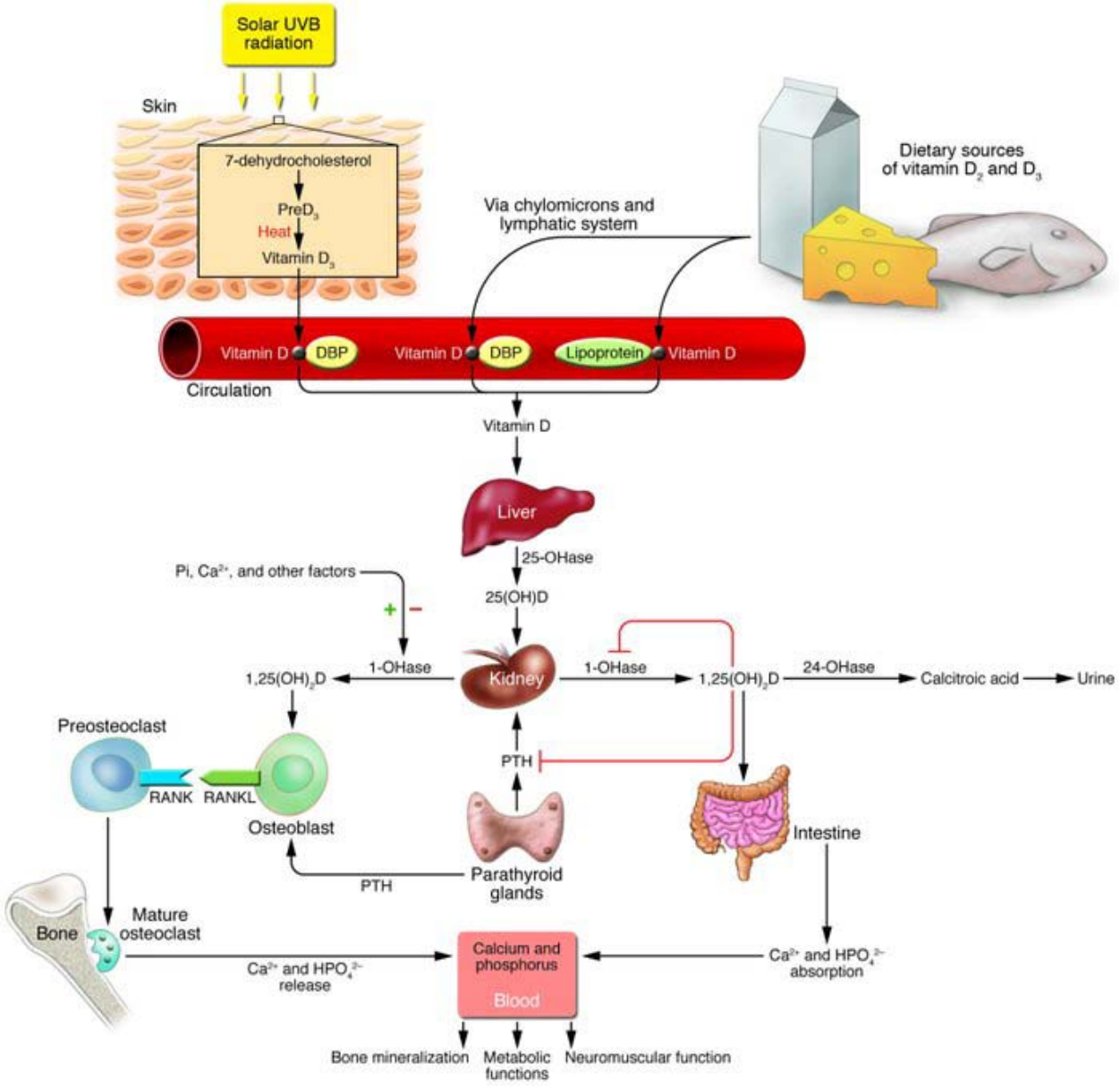
Vitamin D Metabolism

Vitamin D belongs to a group of secosteroid molecules that are traditionally associated with bone and calcium metabolism. Although 5 forms of vitamin D (designated D1 to D5) are known, vitamin D2 and D3 are the most studied forms (Table 1). Ergocalciferol or vitamin D2 is principally synthesized in plants and

Table 1 Forms of Vitamin D

Class	Chemical Composition	Source
Vitamin D_1	Combination of Ergocalciferol and Lumisterol	
Vitamin D_2	Ergocalciferol-made from Ergosterol or pre-Vitamin D_2	Made by invertebrates, fungus and plants in response to UV irradiation; Not made by vertebrates
Vitamin D_3	Cholecalciferol-made from 7-Dehydrocholesterol or pre-Vitamin D_3	Made in the skin as a response to UVB reacting with 7-Dehydrocholesterol.
Vitamin D_4	Dihydroergocalciferol-Vitamin D_2 without 22,23 double bond	Ineffective form of Vitamin D
Vitamin D_5	Sitocalciferol-made from 7-Dehydrositosterol	May have anti-tumor properties

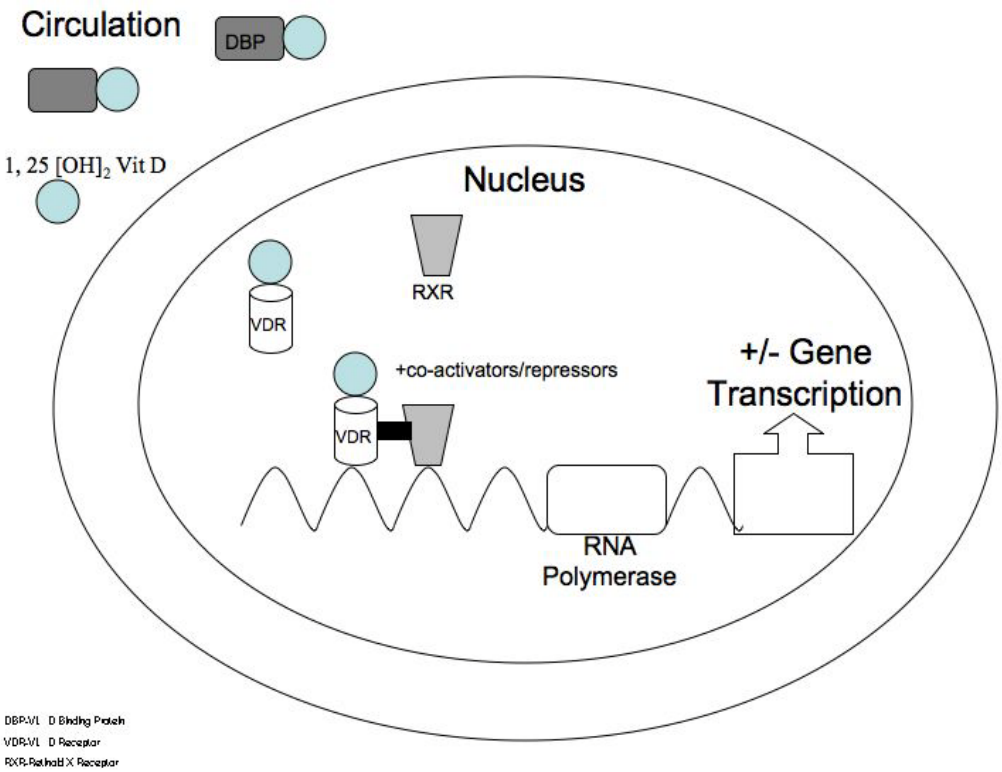
Figure 1 Metabolic pathways of Vitamin D

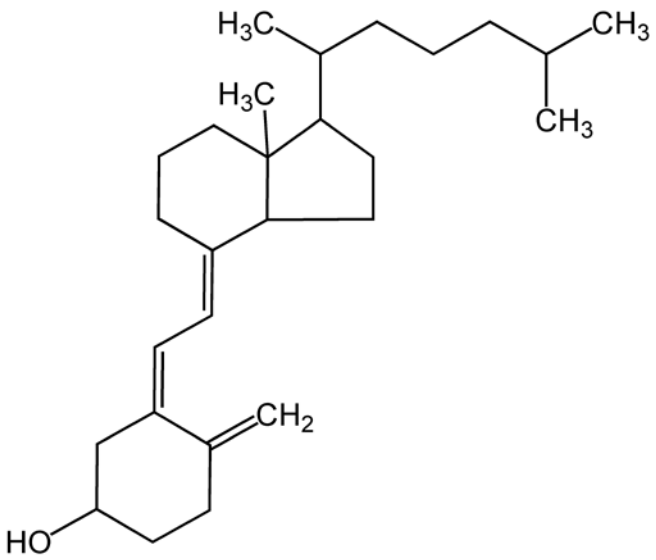


invertebrates and is consumed in the human diet and as supplements or fortified products. Cholecalciferol or vitamin D3 is mainly of vertebrate animal origin and commonly consumed from oily fish. Cholecalciferol is also synthesized in the skin after exposure of 7-dehydrocholesterol to solar ultraviolet radiation (Figure 1).

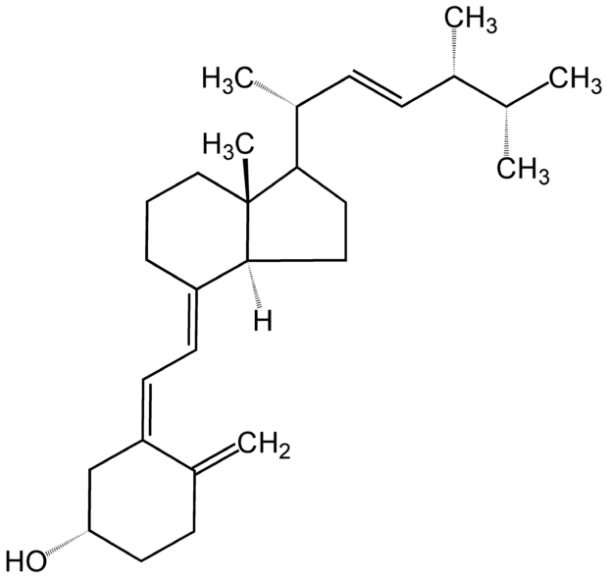
Approximately 80-90% of vitamin D comes from cutaneous conversion, with only 10-20% from dietary sources. Both endogenous and consumed Vitamin D are stored in fat tissues and released into the circulation. Vitamin D is bound to a circulating glycoprotein called vitamin D binding protein (DBP). The liver converts Vitamin D to 25 [OH] Vitamin D which is largely inert. In a rate limiting step, the kidneys convert 25 [OH] Vitamin D to its active form 1, 25 [OH]2 vitamin D, which is bound to specific receptors (VDR) at several sites in the body (Figure 2). Serum levels of 1,25dihydroxyvitamin D are primarily determined by renal production, which is closely associated with calcium homeostasis and is up regulated by parathyroid hormone, which increases in response to low serum calcium levels.

Figure 2 Vitamin D Structure, Transport and Receptor Mechanics





Cholecalciferol (D3)

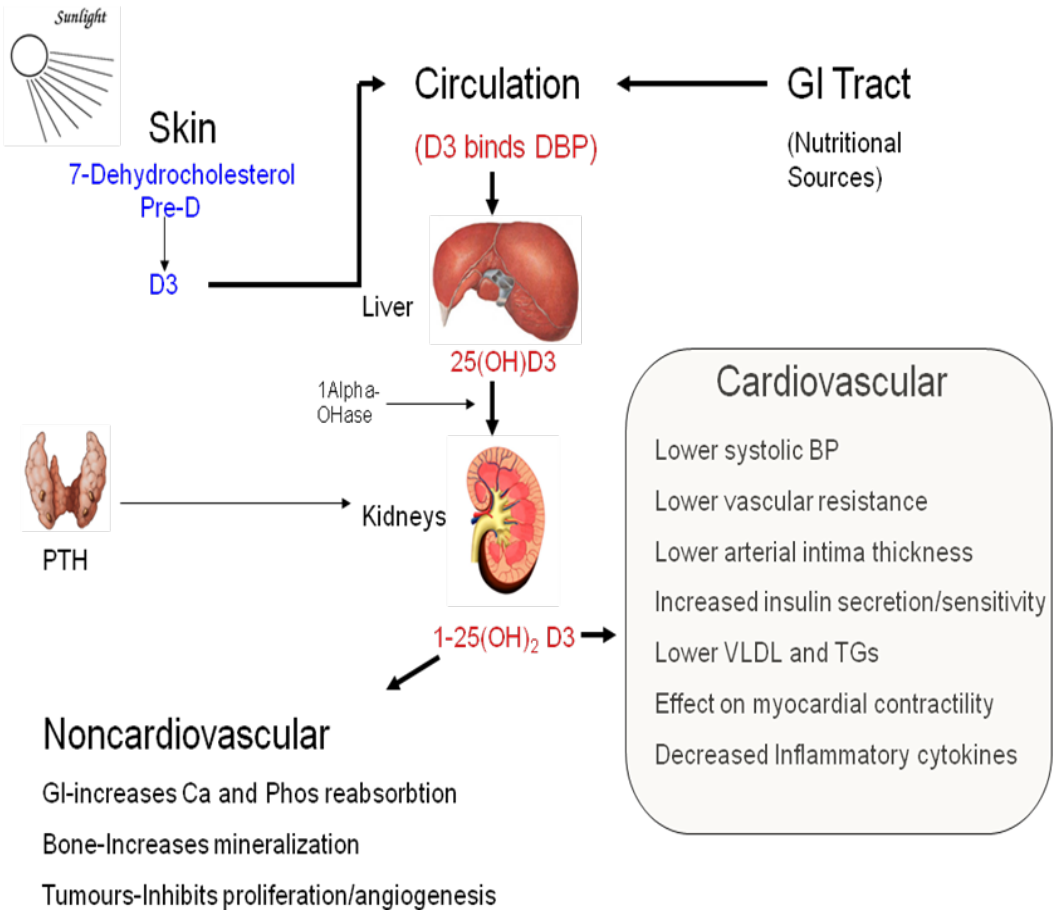


Ergocalciferol (D2)

In humans, VDR is an intranuclear class II steroid hormone receptor. The conjugated Vitamin D with its receptor forms a heterodimer complex with retinoid X receptor.

Together with several other factors and an activator, this complex attaches to Vitamin D responsive elements on DNA and alters gene expression[1]. VDR are found in enterocytes, osteoblasts, parathyroid cells, and the distal renal tubule cells. Vitamin D increases calcium absorption from gut and renal tubular cells and suppresses parathyroid hormone. It acts on osteoblasts and increases bone mineral density. Recent studies have revealed that VDRs also have a significant presence in the liver, immune system, skeletal system, and cardiac muscle[2]. Proposed cardiovascular and other physiologic effects are shown in Figure 3.

Figure 3 Vitamin D absorption and effects



Laboratory Measures of Vitamin D

Serum 25[OH] Vitamin D is the major circulating metabolite of vitamin D and reflects vitamin D input from cutaneous synthesis and dietary intake. Although 1,25[OH]2 vitamin D is the biologically active form of vitamin D, serum 25[OH] vitamin D is regarded as the best indicator of vitamin D status in individuals without kidney disease. In contrast, 1, 25[OH]2 vitamin D levels can be normal or even elevated in patients with vitamin D deficiency.

Multiple assay methods are available for measurement of serum levels of vitamin D, detailed discussion of which is beyond the scope of this paper. Variation in assay sensitivity makes comparison of vitamin D levels from different studies problematic.

Normal ranges for serum 25[OH] vitamin D concentrations are not well established. In a large meta-analysis, serum 25[OH] vitamin D concentrations and multiple end points were compared to establish optimal serum levels. The most beneficial serum concentrations of 25[OH] vitamin D were observed at levels >30 ng/mL (>75 nmol/L) with optimal levels between 36 – 40 ng/mL (90 - 100 nmol/L). Many experts and clinical laboratories define vitamin D insufficiency as 25[OH]D levels between 21 to 29 ng/ml while levels less than 20 ng/ml (50 nmol/l) indicate vitamin D deficiency [3]. In many patients, current daily recommended doses of 200 to 600 IU have not been shown to reach beneficial serum concentrations [4].

Vitamin D Epidemiology

Worldwide, most humans typically expose 5% or less of their skin to infrequent periods of unshielded sunlight, a behavior which commonly leads to vitamin D deficiency. This is far less solar exposure than that experienced in most historical human cultures and free living primates. It is estimated that 30% to 50% of the general population suffer from vitamin D deficiency. In one study, 36% of young healthy free-living adults in the USA aged 18 to 29 years had vitamin D deficiency at the end of winter [5]. In elderly and institutionalized patients, the prevalence of vitamin D deficiency is higher [6]. The Third National Health and Nutrition Examination Survey (NHANES III) reported the prevalence of vitamin D deficiency in the U.S. to be between 25% and 57% of adults [7]. The risk factors for vitamin D deficiency include advanced age, dark skin color, institutionalized or homebound status, increased distance from the equator (Figure 4), winter season, clothing, sunscreen, air pollution, smoking, obesity, malabsorption, renal disease, liver disease, and medications [3].

Recommended Daily Intake and Toxicity:

The present recommended daily allowance is 400 IU daily. However as noted above, intake in this range may not be adequate to avoid deficiency in many people.[4] The Food and Nutrition Board has established the tolerable upper intake level for vitamin D at 2000 IU/day but this has been questioned [8]. It is estimated that African Americans with low sun-exposure need an intake of 2100-3100 IU/d of vitamin D

Figure 4 Area of the United States above the 37th parallel at risk of reduced sunlight exposure.

orally throughout the year to achieve a serum 25-[OH] vitamin D concentration of ≥30mg/mL (75 nmol/L) [9]. Total-body sun exposure easily provides the equivalent of 250 µg (10,000 IU) vitamin D/day, suggesting that this is a physiologic limit [10].

Hypervitaminosis D is described as rare and typically results from massive doses of oral supplementation over prolonged time and presents with symptoms related to hypercalcemia which includes anorexia, nausea, and vomiting followed by polyuria, polydipsia, weakness, nervousness, pruritus, and eventually even renal failure. Recent evidence suggests that vitamin D is well tolerated over a large intake range [8]. A healthy female tolerated an oral dose of 150,000 IU daily for 28 years without any toxicity [11]. Other case reports describe 2 patients who had self-prescribed supra-

therapeutic doses of vitamin D and had serum concentrations of 260 nmol/L and 1126 nmol/L for months to years without significant hypercalcemia or other side effects [12].

Present Study Outline

The goals of this study were to study the association of vitamin D levels with coronary artery disease, hypertension, diabetes and other disease processes, to the assess the relationship of vitamin D levels to survival, and to evaluate the association of vitamin D supplementation with outcomes. The study population was a retrospective sampling from a cohort of patients followed by a large cardiology practice at an academic medical institution, the University of Kansas Hospital and Medical Center in Kansas City, Kansas. We defined the optimal concentration of 25[OH] vitamin D as at least 30 ng/ml and vitamin D deficiency as a 25[OH]D level of <30 ng/ml. For this study serum vitamin D levels were analyzed both as a continuous variable and as normal ($\geq$30ng/ml) or deficient.

The 1st set of hypotheses were: null: vitamin D levels are not associated with cardiovascular outcomes and survival; and alternative: vitamin D levels are associated with cardiovascular outcomes and survival at the .05 level of significance.

The 2nd set of hypotheses were: null: vitamin D supplementation is not associated with improved cardiovascular outcomes and survival; and alternative: vitamin D supplementation is associated with improved cardiovascular outcomes and survival.

Methods

Serum vitamin D measurements for 5 years and 8 months (1/1/2004 to 10/8/2009) from the University of Kansas Hospital were obtained. Our laboratory uses the DiaSorin® (Stillwater, MN) chemiluminescence immunoassay method to measure total serum vitamin D (both 25 hydroxy D2 and D3 forms of vitamin D.) The laboratory assay did not change over this period. These values were matched to patient demographic, physiologic and disease state variables from the cardiovascular database at the medical center and provided in de-identified format. Diagnoses were derived from the patient problem list in the patients' electronic medical record based on International Classification of Diseases, 9th Revision (ICD-9) codes. Death was determined from the Social Security Death Index.

A total of 24,895 samples were tested in the University of Kansas Hospital laboratory. There were 14,261 unique patients. The lowest recorded value for patients with multiple measurements was used for analysis. Database query yielded information on 11,017 matching patients. After excluding patients who were less than 18 years of age, 10,899 patients were available for analysis. This data was then "cleaned" and formatted to allow statistical analysis in Microsoft Excel 2007. The data was then imported into SAS 9.1.3 for statistical analysis and modeling. Descriptive statistics, univariate analysis [(unpaired t tests for continuous variables, chi square analysis for categorical variables, logistic regression for odds ratios (OR) and confidence intervals (CI)], multivariate analysis using logistic regression for OR

and CI, survival analysis, and Cox proportional hazard modeling were performed. A .05 level of significance was used throughout.

The SAS syntax developed for the analysis is provided in Appendix 1. Sample routines for each type of analysis are provided. Multiple variations of these routines were performed to assess various models and variable combinations.

Results

Demographics and Univariate Analyses

General descriptive statistics include mean age 58.3 +/- 14.9 years with normal distribution (Figure 5). There were 7758 (71%) women and 3141 (29%) men. Mean weight was 185.7 +/-52.0 lbs and body mass index (BMI) was 29.9 +/- 7.7 (Figure 6). Both weight and BMI distributions were near normal with right skew. Ejection fraction (by echocardiogram) was 57 +/- 10% with left skew.

Descriptive statistics for vitamin D values included mean 24.1 +/- 13.6 ng/ml and median 22.5 with right skew (Figure 7). 3234 (29.7%) subjects were in normal range (≥30ng/ml) and 7665 (70.3%) were deficient (<30ng/ml).

Baseline characteristics for the subjects with and without vitamin D deficiency are shown in Table 2.

Univariate analysis was then performed with the odds ratios of an event if the subject was vitamin D deficient vs. not (as a dichotomous predictor variable) presented in Table 3. Vitamin D deficiency was significantly associated with several

Figure 5 Age distribution of subjects

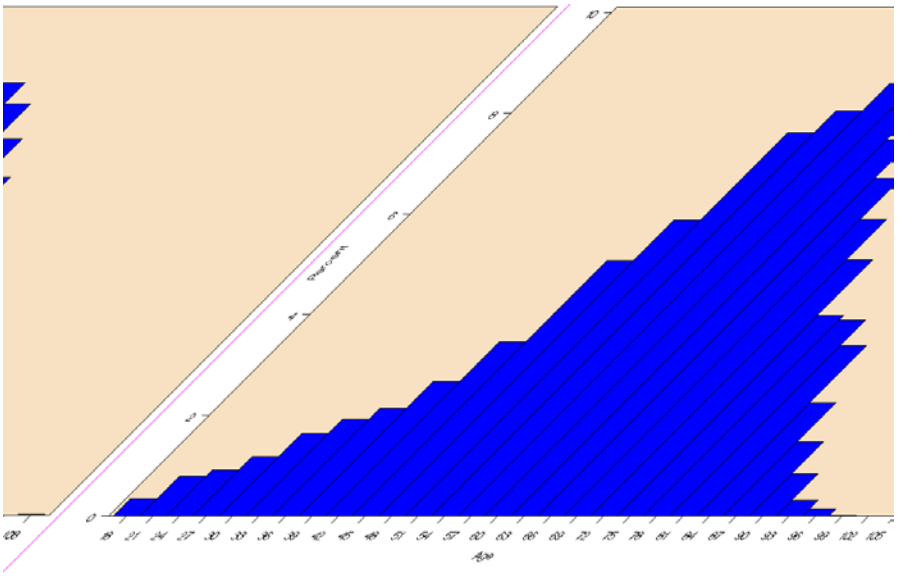
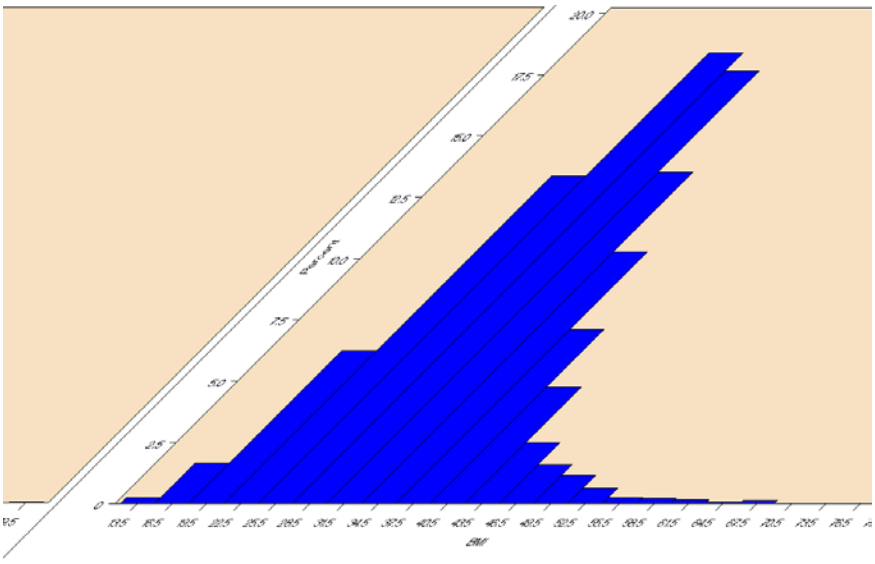


Figure 6 BMI distribution of subjects



cardiovascular disease states including coronary artery disease, atrial fibrillation, diabetes, cardiomyopathy, and hypertension, as well as death. All are positive associations other than that for atrial fibrillation.

Figure 7. Vitamin D distribution of subjects

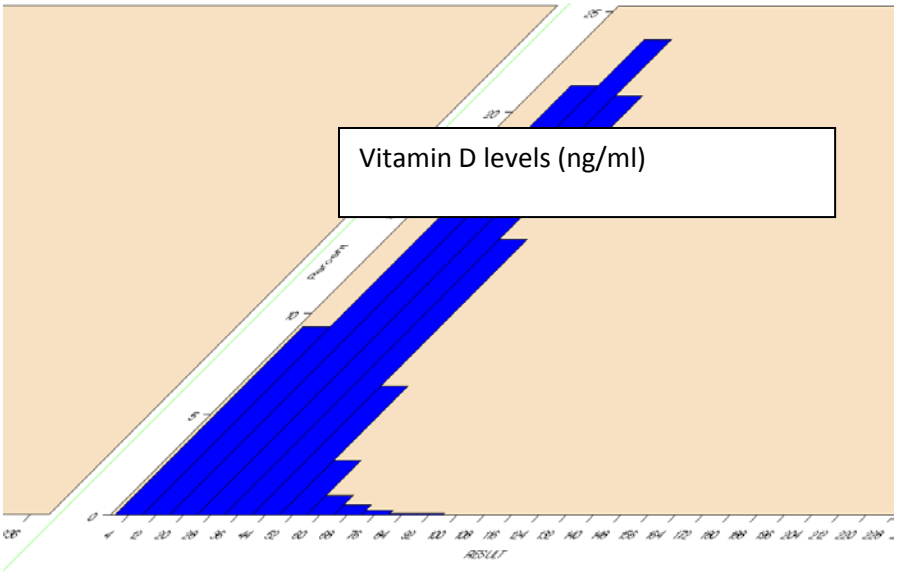


Table 2 Baseline Characteristics ( ) = %

Variable	Vitamin D Deficient n =7665	Not Vitamin D Deficient n=3234	p
Atrial fibrillation	201 (6)	398 (5)	.03
Aspirin use	997 (31)	2254 (29)	NS
Angiotension converting enzyme inhibitor use	725 (22)	2089 (27)	<.0001
Age	60 +/- 15	58 +/- 15	<.0001
BMI	28 +/- 7	31 +/- 8	<.0001
Coronary artery disease	306 (9)	830 (11)	.03
Cardiomyopathy	95 (3)	288 (4)	.03
Cholesterol level	166 +/- 43	171 +/- 54	.02
Creatinine level	1.38 +/- 6.29	1.58 +/- 2.98	NS
Ejection fraction	58 +/- 10	57 +/- 10	NS
Diabetes	294 (9)	1436 (19)	<.0001
Gender (female)	2503 (77)	5255 (69)	<.0001
Death	43 (1)	293 (4)	<.0001
HDL cholesterol level	52 +/- 18	47 +/- 16	<.0001
Hypertension	938 (29)	2795 (36)	<.0001
LDL level	92 +/- 33	97 +/- 39	.002
Statin use	1098 (34)	2611 (34)	NS
Triglyceride level	115 +/- 80	140 +/- 126	<.0001
Valvular heart disease	181 (6)	487 (6)	NS
Vitamin D level	40 +/- 11	17 +/- 7	<.0001
Vitamin D supplement use	689 (21)	2423 (32)	<.0001
Vitamin supplement (any)	1097 (34)	2992 (39)	<.0001

NS = not significant at 0.05 level

Table 3 Univariate Analysis: Odds Ratio of Event if Vitamin D Deficient

Event	**OR**	**CI**	**P**
Death	2.95	2.135-4.073	<.001
CAD	1.16	1.012-1.334	.03
Atrial Fibrillation	0.83	0.693-0.984	.03
Diabetes	2.31	2.018-2.633	<.001
Cardiomyopathy	1.29	1.019-1.633	.03
Hypertension	1.40	1.285-1.536	<.0001

Vitamin D Deficiency: Logistic Regression

Logistic regression using death as the dependent variable and the variables above as well as vitamin D deficiency yielded an overall model $P < .0001$ with results in Table 4. Vitamin D deficiency was a strong predictor of death along with coronary artery disease, diabetes, cardiomyopathy, and hypertension.

Table 4 Logistic regression for death as dependent variable

Predictor	**OR**	**CI**	**P**
CAD	2.71	2.062-3.573	<.0001
Vitamin D deficiency	2.64	1.901-3.662	<.0001
Diabetes	1.45	1.114-1.891	.006
Cardiomyopathy	3.29	2.359-4.596	<.0001
Hypertension	1.53	1.183-1.969	.001

Stepwise selection was performed with all 5 variables included in model, with entry criteria = .10, and removal criteria = .15. All variables were retained in the model with c statistic = .734, suggesting acceptable goodness of fit for the model. Logistic regression for death as the outcome variable with vitamin D level as a continuous predictor variable resulted in an odds ratio of .95 (CI .944-964), P < .0001 for the model. Higher vitamin D levels are protective. In a multivariate model as above, the vitamin D level as a continuous variable OR was .96 with the OR for the other variables and overall model significance similar as for the model with Vitamin D as a categorical variable. Stepwise selection with all 5 variables included in the model and similar entry and removal criteria resulted in all variables being retained in the model and a c statistic = .758.

Logistic regression for death was performed with additional clinical variables added (Table 5). The overall model was predictive with P < .0001 and with vitamin D remaining a very strong predictor variable. By correlation analysis age and coronary artery disease appeared to be significantly correlated. When age was removed from the model, coronary artery disease became a significant predictor of death (OR 1.45, CI 1.056-1.986, P = .02).

Stepwise selection of the expanded model resulted in hypertension, coronary artery disease and creatinine dropping out. Vitamin D deficiency was the strongest predictor with OR 2.36 (CI 1.583-3.511), P < .0001. c = .709.

Table 5 Logistic regression for death with additional clinical variables added

Event	**OR**	**CI**	**P**
Vitamin D deficiency	2.42	1.622-3.612	<.0001
CAD	1.28	0.926-1.769	NS
Diabetes	1.45	1.051-1.994	.02
Cardiomyopathy	1.52	1.018-2.283	.04
Hypertension	0.81	0.585-1.110	NS
Age	1.02	1.008-1.033	.001
BMI	0.96	0.935-0.980	.0003
Ejection Fraction	0.98	0.970-0.994	.003
Creatinine	1.01	0.986-1.039	NS
Gender (female)	.0.63	0.464-0.844	.002

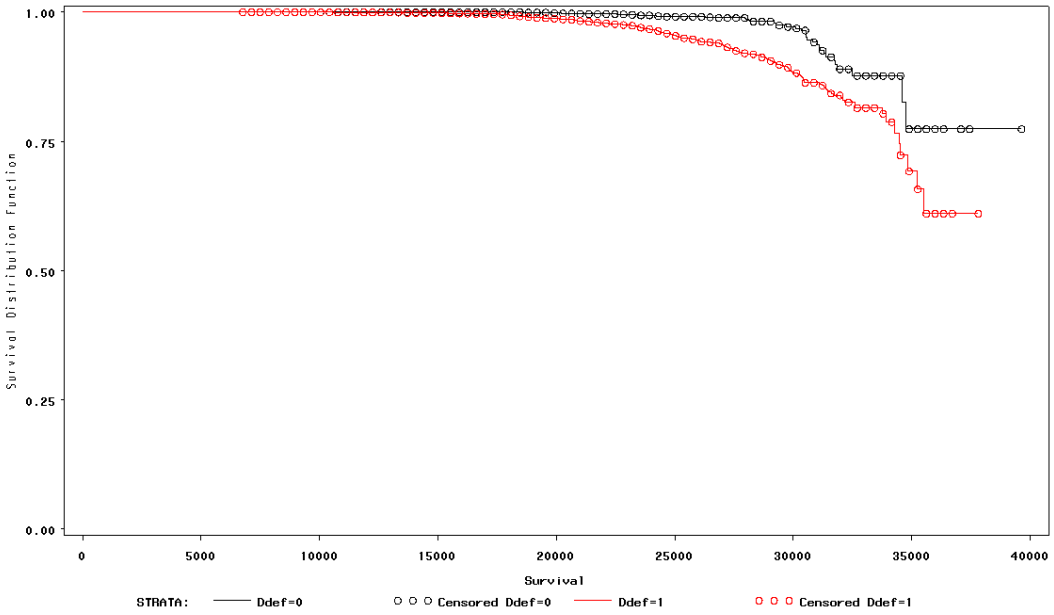
Vitamin D Deficiency: Survival Analysis and Cox Proportional Hazards Modeling

Survival was then studied. Survival method 1 examined lifelong survival calculated as survival time = date of death – date of birth if the patient died, otherwise survival = end of study (October 9, 2009) – date of birth. This survival estimate may be biased because it extends survival observation retrospectively before the period of data analysis. However it was felt reasonable to analyze this lifelong survival interval in relation to a contemporary vitamin D measurement. The correlation of the results

with those obtained with the 2nd survival method below may support the importance of vitamin D in long term outcomes.

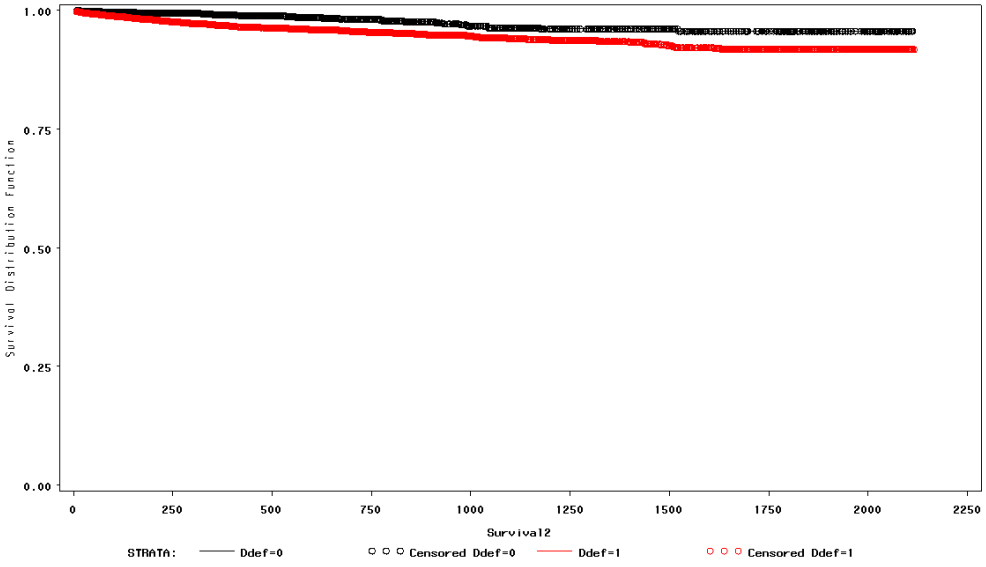
Survival curves are presented in Figure 8 with consistently better survival for those patients not vitamin D deficient (P < .0001 for homogeneity of strata).

Figure 8 Survival Curve Method 1

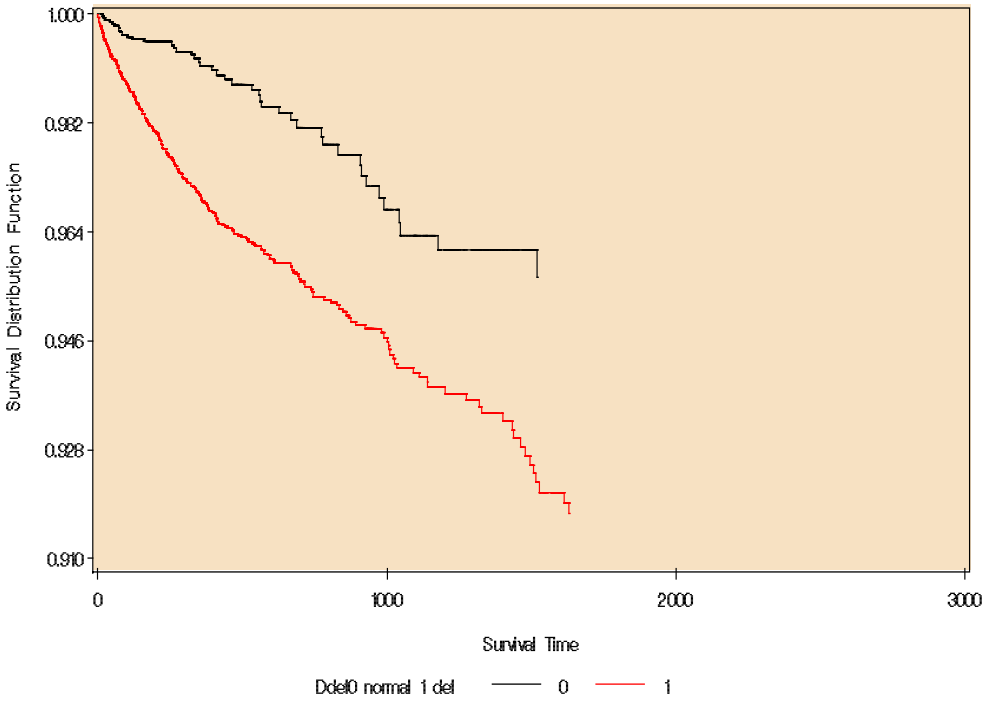


Survival method 2 calculated survival as survival2 = Date of death – date of collection, otherwise Survival 2 = End of study – date of collection. This may be a more appropriate means of assessing survival relative to the measurement of vitamin D level and clinical parameters. The survival curve for method 2 is given in Figure 9 (P < .0001 for homogeneity of strata).

Figure 9 Survival Curve Method 2



Expanded Scale shown below:



Hazard ratios using the Cox proportional hazards model were then calculated with vitamin D deficiency as a dichotomous variable with results shown in Table 6 for the disease state variables, using survival method 1 ($P < .0001$ for both vitamin D deficiency and overall model with vitamin D deficiency being the strongest individual predictor). Hazard function analysis with additional predictive variables (age, BMI, EF, gender) was then performed with overall $P < .0001$ and vitamin D remaining a significant independent predictor. Vitamin D deficiency remained the variable with the highest OR (2.52). Hazard function analysis was also performed using survival method 2 and vitamin D deficiency as a dichotomous variable (Table 7) with the overall model being highly predictive as well as vitamin D as an independent predictive variable ($P < .0001$ for both). Similar results were found in the expanded clinical model when hazard ratios were generated using survival method 2. Therefore, survival whether assessed by a traditional method from study onset (method 2) or by extension to birth (method 1) shows that vitamin D deficiency is associated with decreased survival by the proportional hazards method.

Table 7 Hazard Ratios for Death with Vitamin D deficiency (dichotomous)-Survival Method 2

Predictor	**Hazard Ratio**	**CI**	**P**
CAD	2.617	2.006-3.414	<.0001
Diabetes	1.497	1.162-1.929	.0018
Cardiomyopathy	2.766	2.033-3.764	<.0001
Hypertension	1.586	1.236-2.036	.0003
Vitamin D deficiency	2.174	1.574-3.003	<.0001

Vitamin D Supplementation: Relationship and Interaction with Vitamin D Deficiency

For the next portion of the study, the impact of vitamin D supplementation was studied. Data was coded as either 1 for supplement use recorded in the electronic medical record or 0 if no use was documented. Use was more common in vitamin D deficient patients, with 31.6% of deficient patients receiving supplements vs. 21.3% of patients with normal vitamin D values receiving supplements (OR 1.71, P<.0001).

Death vs. vitamin D deficiency was stratified by vitamin D supplementation. With supplementation, the OR for death = 1.46 (CI 0.760-2.799) (P=NS)). Without supplementation the OR for death = 3.72 (CI 2.563-5.396) (P<.0001). Controlling for vitamin D replacement, the common OR = 3.07 (CI 2.222-4.228) (P<.0001) (by Cochran-Mantel-Haensel analysis). The Breslow-Day test for homogeneity of OR = 0.01.

To further study this interaction, the Cox proportional hazards model was then run with only the variables for vitamin D deficiency and supplementation included. The hazard ratio using survival method 1 for deficiency was 3.70 (CI 2.680-5.097) and for supplementation was 0.51 (CI 0.389-0.665)(both P's < .0001). When the model was run stratifying vitamin D deficiency by supplementation, the hazard ratio was 3.69 (CI 2.674-5.085), P < .0001. Using survival method 2 the hazard ratio for deficiency was 2.60 (CI 1.864-3.540)(P < .0001). When stratified by supplementation the hazard ratio for deficiency as 2.60 (CI 1.884-3.578) using survival method 2.

Next the possible interaction between vitamin D deficiency (as a dichotomous variable) and supplementation was studied by creating an interaction variable for vitamin D deficiency*supplementation (interactionA). When this interaction term was added to a hazard model with vitamin D deficiency and supplementation, the overall model P remained significant as did that for vitamin D deficiency (both P's < .0001, hazard ratio for deficiency 4.515). The interaction term was significant at P = .0036 with a hazard ratio of 0.330, while vitamin D supplementation was no longer a significant predictor. The model was significant by both Wald statistic and by likelihood ratio test (P < .0001). Proportionality testing was highly significant (P = .0036), suggesting an important interaction. A second non-time dependent interaction variable (interactionB) was developed as the product of the continuous value of the vitamin D measurement and the dichotomous value of vitamin D replacement as described below in the final model section. This interaction variable yielded a highly significant outcome (P<.0001) by proportionality testing, suggesting that the vitamin D deficiency-supplementation interaction is indeed important.

Vitamin D Supplementation: Logistic Regression and Cox Proportional Hazards Modeling

The analyses for death by logistic regression and by Cox proportional hazard models were then repeated both with the limited, disease related variable sets as well as with the expanded variable models, either adding either the interaction term A or B and retaining vitamin D supplementation as a variable, or with the interaction term

included and vitamin D supplementation removed. This was done using both survival methods 1and 2 with similar results. In all cases, both with full model testing and by stepwise selection (using inclusion criteria of .10 and removal criteria of .15), vitamin D supplementation alone became a nonsignificant predictor while the interaction term was highly significant. In general the level of significance and association hazard ratios for the interaction term were similar whether the interaction term was included alone or with vitamin D supplementation also included. These levels of significance and hazard ratios were also similar for models containing vitamin D supplementation alone with no interaction term. Table 8 gives an example of these relationships, with basic models of vitamin D deficiency, supplementation, and the interaction term.

Table 8 Example of Relative Contribution of Interaction Term of Vitamin D Deficiency*Supplementation in Hazards Model

Model	Variables	Hazard Ratio	P
1	Vitamin D Deficiency	3.37 (2.680-5.097)	<.0001
	Vitamin D Supplement	0.51(0.389-0.665)	<.0001
2	Vitamin D Deficiency	4.52 (3.121-6.534)	<.0001
	Vitamin D Supplement	1.35(0.682-2.684)	.3877
	Interaction Term	0.33 (0.157-0.696)	.0036
3	Vitamin D Deficiency	4.22 (3.045-5.834)	<.0001
	Interaction Term	0.45 (0.334-0.598)	<.0001

I interpret these results as indicating that vitamin D deficiency and replacement are highly associated. It may be reasonable on a practical basis to consider models with vitamin D supplement as a predictor variable rather than the interaction term, for

simplicity or for generation of a risk scoring system, as this is more intuitive. However for this study, the interaction term will be included in final model development below along with main effects.

Therefore vitamin D supplementation improved survival overall, and to a greater degree in deficient patients. The univariate overall risk of death was reduced for subjects on supplements with OR 0.62 (CI 0.469- 0.806), P = .0004. When both vitamin D deficiency and replacement were entered as predictor variables in a regression model, the OR for vitamin D deficiency was 3.12 (CI 2.250- 4.297) and the OR for vitamin supplements was 0.56 (CI 0.428- 0.737), both P's < .0001.

When added to the expanded regression model for death the vitamin D supplement OR was 0.43, P < .0001 (Table 9). Removal of age in the model did not significantly change the outcomes. When added to the disease state only regression model for death the vitamin D supplement OR was 0.44, P < .0001 (Table 10). Stepwise selection results are given in Table 11.

Table 9 Logistic Regression for death with Vitamin D replacement added to expanded model

Predictor	**OR**	**CI**	**P**
CAD	1.32	0.970-1.799	.0769
Diabetes	1.71	1.254-2.326	.0007
Cardiomyopathy	2.35	1.643-3.326	<.0001
HTN	0.86	0.629-1.170	NS
Vitamin D Supplement	0.43	0.313-0.587	<.0001
Atrial Fibrillation	1.25	0.883-1.761	NS
Age	1.02	1.008-1.033	.0012
Gender (female)	0.64	0.480-0.861	.003
BMI	0.96	0.936-0.981	.0003
Creatinine	1.01	0.985-1.030	NS

Table 10 Logistic Regression with Vitamin D replacement added to disease state model:

Predictor	**OR**	**CI**	**P**
CAD	2.45	1.852-3.245	<.0001
Diabetes	1.67	1.281-2.172	.0001
Cardiomyopathy	3.09	2.189-4.355	<.0001
HTN	1.62	1.249-2.091	.0003
Vitamin D Supplement	0.44	0.335-0.589	<.0001
Atrial Fibrillation	2.13	1.543-2.929	<.0001

Vitamin D replacement was associated with a significantly lower occurrence of death in the models above, suggesting a protective role. When added to the hazard model using survival method 1the vitamin D supplementation hazard ratio for death was 0.40 (CI 0.335-0.576) for subjects on replacement with P < .0001. Using survival method 2 the vitamin D supplementation odds ratio for death was .46 (CI 0.342-0.589) with P <.0001.

Survival by vitamin D supplementation using method 1 is shown in Figure 10, which demonstrates that survival was improved by supplementation. Survival by vitamin D supplementation and deficiency was also studied, with overlap of the survival curves, but suggestion of important interaction between vitamin D deficiency and replacement (Figure 11). Significant differences in survival were seen only for the vitamin D deficient patients.

Figure 10 Survival by Vitamin D supplementation

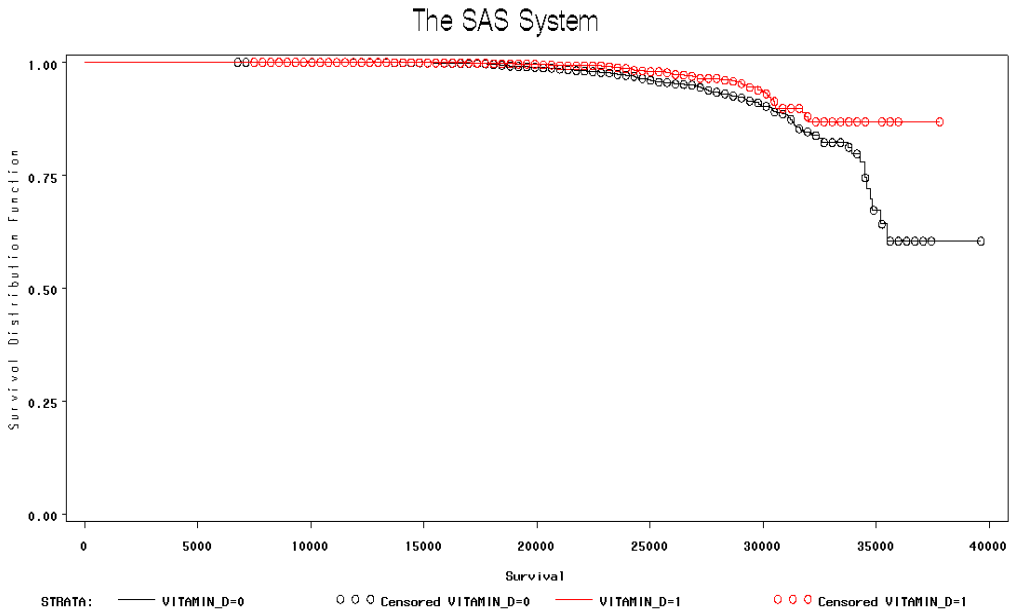
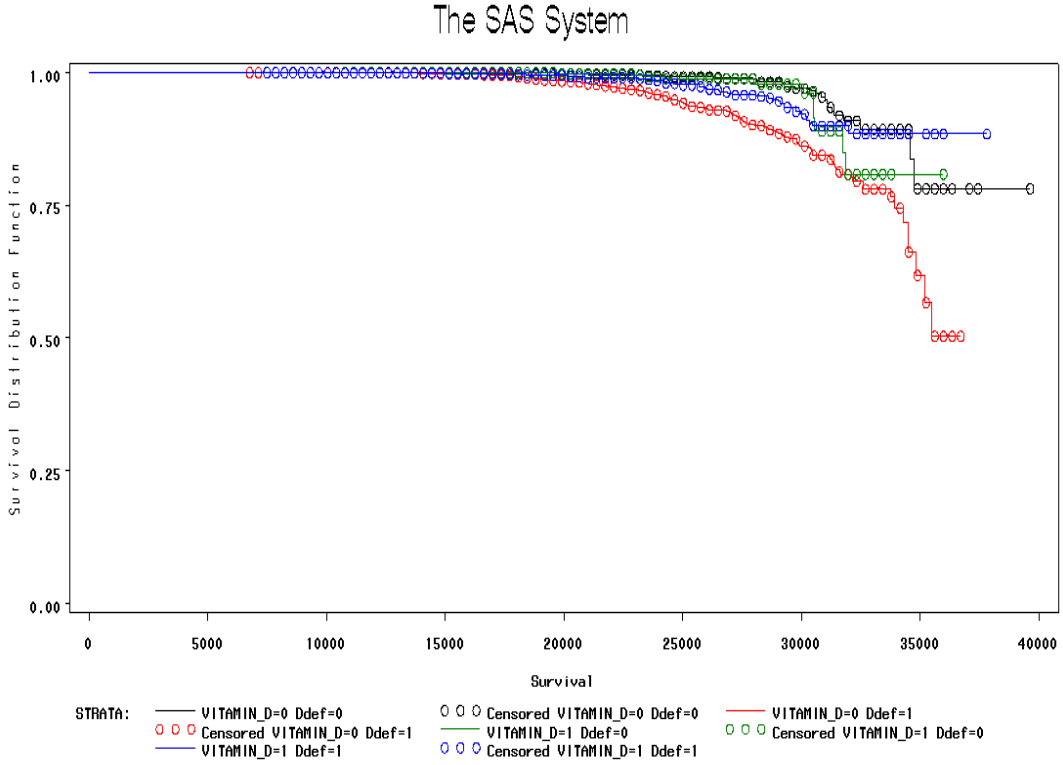
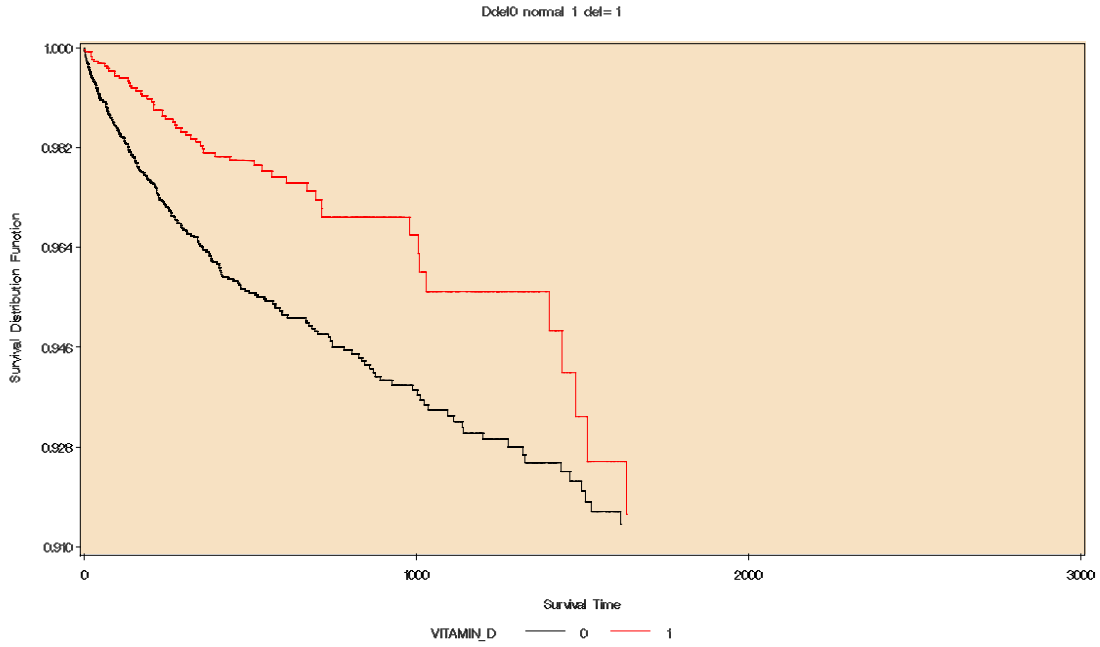


Figure 11 Survival Curves by Vitamin D deficiency and replacement. Combined curves below.



Separate Surival Curves for Vitamin D deficient patients (top) and non deficient patients (bottom) by Vitamin D replacment with expanded scale. Patients on supplements represented by the red lines, those not on supplements represented by the black lines. Significant differences in survival are seen only for the deficient patients.



Vitamin D Supplementation: Interactions with Other Medications

To assess whether use of any vitamin reduced the positive association of vitamin D replacement (or the negative association of vitamin D deficiency) with death, an expanded analysis including these factors was added to the model with significant clinical predictors as well as vitamin D deficiency as a dichotomous variable. The results are given in Table 12 and indicate that any use of vitamin supplement does not reduce the associations of vitamin D replacement or deficiency on mortality. The overall P was <.0001 with c = .772.

Table 12 Logistic regression using stepwise selection with vitamin D replacement and any vitamin use added to model

Predictor	**OR**	**CI**	**P**
CAD	2.37	1.784-3.140	<.0001
Diabetes	1.44	1.106-1.881	.0069
Cardiomyopathy	3.12	2.219-4.395	<.0001
HTN	1.63	1.263-2.114	.0002
Vitamin D Supplement	0.31	0.214-0.447	<.0001
Gender (female)	0.49	0.392-0.621	<.0001
Vitamin D Deficiency	2.87	2.061-4.005	<.0001
Any Vitamin	1.54	1.123-2.101	.0072

These relationships continued to be significant when statin and aspirin use were added to the regression model. This argues against the benefit of vitamin D

supplements being due primarily to associated use of other medications which have shown survival benefit in prior studies (Table 13).

Table 13 Logistic regression using stepwise selection model with statin and aspirin use added to model.

Predictor	**OR**	**CI**	**P**
CAD	3.06	2.215-4.226	<.0001
Diabetes	1.61	1.222-2.113	.0007
Cardiomyopathy	3.51	2.478-4.985	<.0001
HTN	1.91	1.450-2.506	<.0001
Vitamin D Supplement	0.45	0.339-0.602	<.0001
Statin Use	0.41	0.296-0.556	<.0001
Vitamin D Deficiency	2.68	1.923-3.735	<.0001
Aspirin	1.43	1.063-1.924	.0182
Gender (female)	0.49	0.390-0.620	<.0001

Vitamin D Deficiency and Supplementation: Final Model

Extensive modeling was performed testing different combinations of parameters and using Hosmer and Lemeshow goodness-of-fit testing to develop a model which included important parameters without excess variables. A model which fulfilled these criteria and produced a goodness-of-fit statistic indicating adequacy of the model included vitamin D deficiency, gender, coronary artery disease, cardiomyopathy, vitamin D supplementation, and the Vitamin D deficiency*supplementation term. Because of the significant interaction between

vitamin D deficiency and replacement separate analyses were done for the deficient and non deficient subjects. The details of the logistic regression model for death for the vitamin D deficient subjects are given in Table 14. Vitamin D supplementation is a significant negative predictor variable for death. Table 15 provides the model for the subjects not vitamin D deficient. Note that vitamin D supplements are not a significant predictor of death for these subjects.

Table 14 Final Logistic Regression Model for Death (Vitamin D as dichotomous variable) for vitamin D deficient subjects.

Variable	OR	P
Diabetes	1.63 (1.229-2.162)	0.0007
Coronary Artery Disease	2.37 (1.749-3.219)	<.0001
Cardiomyopathy	3.22 (2.218-4.662)	<.0001
Gender (female)	0.52 (0.402-0.658)	<.0001
Hypertension	1.57 (1.185-2.072)	0.0016
Vitamin D Supplement	0.38 (0.280-0.519)	<.0001

Model:

Logit p(death) = -3.20 + 0.86(Coronary Artery Disease) + 1.17(Cardiomyopathy) – 0.66(Gender/female) + 0.49(Diabetes) + 0.45(Hypertension) – 0.97(Vitamin D Supplement)

Odds Ratio for Death = $e^{-3.20 + 0.86(\text{Coronary Artery Disease}) + 1.17(\text{Cardiomyopathy}) - 0.66(\text{Gender/female}) + 0.49(\text{Diabetes}) + 0.45(\text{Hypertension}) - 0.97(\text{Vitamin D Supplement})}$

P < .0001

c = 0.745

Table 15 Final Logistic Regression Model for Death (Vitamin D as dichotomous variable) for subjects not Vitamin D deficient. Vitamin D supplementation was not a significant predictor variable.

Variable	OR	P
Coronary Artery Disease	3.00 (1.447-6.201)	0.0031
Cardiomyopathy	2.88 (1.209-6.841)	0.0169
Gender (female)	0.40 (0.202-0.770)	0.0064
Hypertension	2.23 (1.147-4.337)	0.0181

Model:

Logit p(death) = -4.41 + 1.10(Coronary Artery Disease) + 1.06(Cardiomyopathy) – 0.93(Gender/female) + 0.80(Hypertension)

Odds Ratio for Death = $e^{-4.41 + 1.10(\text{Coronary Artery Disease}) + 1.06(\text{Cardiomyopathy}) - 0.93(\text{Gender/female}) + 0.80(\text{Hypertension})}$

$P < .0001$

$c = 0.778$

Table 16 presents a final logistic regression model for death in the subjects not on vitamin D supplementation who are vitamin D deficient (as a dichotomous variable. For patients on vitamin D supplements vitamin D deficiency was not a significant predictor of death.

Table 16 Final Logistic regression model for death for subjects not on vitamin D supplements (for patients on supplements vitamin D deficiency was not a significant predictor of death).

Variable	OR	P
Vitamin D Deficiency (dichotomous)	3.51 (2.400-5.125)	<.0001
Coronary Artery Disease	2.99 (2.180-4.106)	<.0001
Cardiomyopathy	3.34 (2.234-4.994)	<.0001
Gender (female)	0.45 (0.344-0.579)	<.0001
Hypertension	1.87 (1.419-2.458)	<.0001

Model:

Logit p(death) = - 4.40 + 1.25(Vitamin D Deficiency) + 1.10(Coronary Artery Disease) + 1.21(Cardiomyopathy) - 0.81(Gender/female) + 0.62(Hypertension)

Odds Ratio for Death = $e^{-4.40 + 1.25(\text{Vitamin D Deficiency}) + 1.10(\text{Coronary Artery Disease}) + 1.21(\text{Cardiomyopathy}) - 0.81(\text{Gender/female}) + 0.62(\text{Hypertension})}$

P < .0001

c = 0.776

Table 17 repeats the analysis presented in Table 17 including the continuous vitamin D level as a predictor variable for patients not on vitamin D supplements. Note the c value of .799. These models appear to have predictive value and are readily available clinical parameters which could be utilized in a risk assessment scale.

Table 17 Final Logistic regression model for death for subjects not on vitamin D supplements when vitamin D level as a continuous variable was included in the model (for patients on supplements vitamin D level was not a significant predictor of death).

Variable	OR	P
Vitamin D Level (continuous)	0.95 (0.934-0.958)	<.0001
Coronary Artery Disease	3.15 (2.289-4.331)	<.0001
Cardiomyopathy	3.37 (2.246-5.063)	<.0001
Gender (female)	0.46 (0.358-0.602)	<.0001
Hypertension	1.80 (1.365-2.373)	<.0001

Model:

Logit p(death) = - 2.29 – 0.06(Vitamin D Level) + 1.15(Coronary Artery Disease) + 1.22(Cardiomyopathy) - 0.77(Gender/female) + 0.58 (Hypertension)

Odds Ratio for Death = $e^{-2.29 – 0.06(\text{Vitamin D Level}) + 1.15(\text{Coronary Artery Disease}) + 1.22(\text{Cardiomyopathy}) – 0.77(\text{Gender/female}) + 0.58(\text{Hypertension})}$

$P < .0001$

Vitamin D Deficiency and Coronary Artery Disease Risk Factors

To examine the relationship between BMI and vitamin D deficiency, linear regression with BMI as the predictor variable and vitamin D level (continuous) as the dependent variable showed a highly significant negative association (beta -0.3134, $P < .0001$). Therefore higher BMI was associated with lower vitamin D levels. Logistic regression with vitamin D deficiency as a dichotomous variable showed a similar association, with OR for BMI 1.057 (CI 1.046-1.068) ($P < .0001$), again confirming

increased risk of vitamin D deficiency with increasing BMI. These findings may explain one mechanism for the association between obesity and cardiovascular disease processes.

The association between vitamin D level as a continuous predictor variable and LDL cholesterol measurement as the dependent variable was also examined. There was a significant negative association between vitamin D level and LDL value, with a beta of -0.1956, P = .0005). A similar analysis with HDL cholesterol as the dependent variable found a significant positive association between vitamin D level and HDL measurement (beta 0.1734, P < .0001). Likewise vitamin D levels were associated with triglyceride measurements. The beta was -1.126 with P < .0001). Thus for every increase of 1 unit of vitamin D, triglyceride levels dropped by over 1 unit.

These analyses demonstrate significant associations between vitamin D levels and known risk factors for cardiac disease.

Discussion:

A number of cardiovascular disease states have been associated with vitamin D deficiency. A summary follows, arranged by disease process.

Vitamin D and Hypertensive Vascular Disease

Essential hypertension is a major risk factor for cardiovascular disease. Vitamin D appears to be related to blood pressure control via multiple pathways. Calcitriol levels are inversely related to serum renin activity [13]. Similarly a drop in blood pressure was seen in subjects who were exposed to ultraviolet B radiation which converts vitamin

D to 25 [OH] vitamin D [14]. The effects of calcitriol on suppression of renin activity are probably secondary to increased intracellular calcium levels [15,16]. Vitamin D replacement in deficient subjects significantly improved flow mediated dilatation of the brachial artery suggesting the role of vitamin D in the sensitivity of vascular smooth muscle cells [17].

Initial small retrospective observational studies have studied correlations between vitamin D levels and systolic blood pressure.[18-20] In a small study from Belgium of 25 patients with hypertension, 25 [OH] vitamin D levels were significantly inversely correlated with systolic blood pressure, diastolic blood pressure, and calf vascular resistance [18]. In a subsequent study involving normotensive men, a similar inverse correlation between 1, 25 $[OH]_2$ vitamin D and systolic blood pressure was observed [19].

A large cross-sectional national study involving a non-institutionalized population aged >20 years, the third National Health and Nutrition Examination Survey (NHANES III), was carried out in the United States between 1988–1994. This study population was used to evaluate the cross-sectional relationship between serum 25-hydroxyvitamin D concentrations and blood pressure [21]. After excluding those who were on antihypertensive medications, a total of 12,644 patients were included in the analysis. The mean blood pressure varied inversely with serum 25 [OH] vitamin D levels, with the association remaining significant after adjustment for age, sex, race-

ethnicity, and physical activity. The impact of vitamin D deficiency in the elderly (age > 50 years) was highly significant (p=0.021) [21].

Interventions with vitamin D replacement were attempted to support the hypothesis that changes in vitamin D status affect blood pressure. In a randomized study, women older than 70 years who had 25 [OH] D levels less than 20 ng/ml were randomly assigned to receive supplementation with calcium 1200 mg/day only or calcium 1200 mg/day plus vitamin D (cholecalciferol) 800 IU/day. Within 8 weeks of treatment, the systolic blood pressure in the group treated with vitamin D dropped on an average of 13mm of Hg (p=0.02) [22] . In a similar randomized study involving patients with diabetes mellitus and serum 25[OH] D levels less than 20 ng/ml, patients were randomly assigned to receive a one-time dose of ergocalciferol 100,000 IU or a placebo. Vitamin D supplementation produced a significant decrease in systolic blood pressure [23]. Similar benefits of vitamin D on blood pressure were noticed in other small studies [14,20].

Vitamin D and Peripheral Vascular Disease

25[OH]D levels were inversely correlated to calf vascular resistance and positively correlated with calf blood flow [18]. Similar associations were identified in the NHANES study. After multivariable adjustment for demographics, co-morbidities, physical activity level, and laboratory measures, low 25[OH] vitamin D levels were associated with a higher prevalence of peripheral arterial disease [24]. Vitamin D deficiency is also strongly associated with increased thickness of intima-media in

carotid arteries [25]. One-third of the excess risk of PAD in an African American population was attributed to racial differences in vitamin D status [26]. Similarly, a high prevalence of vitamin D deficiency with secondary hyperparathyroidism was observed in a non-diabetic population with peripheral vascular disease [27]. No interventional studies have been reported to identify the specific effect of vitamin D replacement on peripheral vascular disease to the present.

Vitamin D and Diabetes Mellitus

Diabetes mellitus is a major risk factor for coronary artery disease and a sign of a profound metabolic derangement. Pancreatic beta cell dysfunction, peripheral tissue resistance to insulin, and chronic inflammation appear to be the possible mechanisms for the role of vitamin D in the expression of diabetes mellitus [28]. Vitamin D receptors have been found in pancreatic islets indicating the possible role for vitamin D in insulin secretion [29]. Basal insulin secretion rate was not altered in VDR knockout mice[30] but insulin secretion rate after a challenge with glucose diet was impaired in vitamin D deficiency [31]. Vitamin D might affect intracellular calcium levels in pancreatic cells which is an important stimulus for insulin secretion. In the peripheral tissues, VDR was found in skeletal muscles and adipose tissue. Vitamin D also has been shown to control insulin receptor expression and insulin responsiveness for glucose transport [32], establishing its role in both insulin secretion and sensitivity.

Observational human studies showed the seasonal variation [33] and geographical variation [34] of type 1 diabetes mellitus and its relationship to vitamin D deficiency.

The European Community sponsored Concerted Action on the Epidemiology and Prevention of Diabetes study found a 33% reduction in the risk of developing childhood-onset type-1 DM in children who received vitamin D supplementation [35]. Seasonal variations of glycemic control attributed to vitamin D level fluctuations were reported [36]. Replacing vitamin D improves insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients [37,38] as well as HbA1C levels [39]. Metabolic syndrome is also prevalent in patients with vitamin D deficiency [40].

Although the data from observational studies is strong, expected benefit from replacement of vitamin D on fasting blood glucose, glucose tolerance, or insulin sensitivity has not been observed in all studies [41,42]. The variance may be due to ethnic differences, VDR gene polymorphisms [43] or inadequate dosing regimens. Nevertheless, two meta-analyses pooling large amounts of data did show benefit with vitamin D replacement [29,44].

Vitamin D and Lipid Metabolism

Serum levels of 1, 25-$(OH)_2$-vitamin D are inversely correlated to VLDL and triglyceride levels [45]. Vitamin D deficiency may cause an abnormal lipid profile by increasing peripheral insulin resistance and by contributing to metabolic syndrome. Studies have suggested that statin therapy may increase vitamin D levels, a finding that may account for some of the non-lipid pleiotropic actions of statins [44-49]. It is postulated that inhibition of HMG Co-A (3-hydroxy-3 methylglutaryl coenzyme A) reducatse enzyme by statins result in increased 7-dehydrocholesterol, a substrate for

the enzyme. This excess 7-dehydrocholesterol is then converted to 25-hydroxycholecalciferol by sunlight or the CYP11A1 enzyme, thereby increasing vitamin D levels [47,49,50]. In addition, a recent study looked at reduction in vitamin D receptor signaling in diabetics and found increased foam cell formation in macrophages, an early sign of atherosclerosis [51].

Vitamin D and Coronary Artery Disease

As discussed previously, hypertension, diabetes mellitus, and lipid levels are effected by vitamin D. Vitamin D has also been shown to effect endothelial function [17,22], and decrease vascular calcification [52]. Calcification of coronary arteries was inversely correlated with vitamin D levels [53]. Earlier observations in the 1980s and 1990s found geographic and seasonal differences in mortality from ischemic heart disease (IHD) [45,54]. The initial suggestion of vitamin D as a protective factor came from a study which showed mortality from IHD was inversely proportional to the hours of sunlight in the United Kingdom [45].

Larger cross-sectional observations came from The National Health and Nutritional Examination Surveys (NHANES) conducted between 1988–1994 and 2000–2004. In the initial survey, a total of 16,603 men and women aged 18 years or older were studied. Participants with IHD and stroke had a greater frequency of 25[OH] D deficiency ($p<0.0001$) [55]. This was confirmed by the recent study involving 8,351 adults. The prevalence of vitamin D deficiency was 74% in patients with coronary artery disease and heart failure [56]. The lowest quartile of 25[OH] vitamin D level

(<17.8 ng/mL) was independently associated with all-cause mortality in the general population [57]. Together, the findings of these epidemiologic studies suggest that suboptimal vitamin D status is associated with poor cardiovascular outcomes.

Multiple cohort studies evaluated the role of vitamin D prospectively in long-term cardiovascular outcomes in subjects with no history of cardiovascular disease. In renal dialysis patients, untreated vitamin D deficient subjects were at significantly increased risk for early mortality [58]. Similarly, healthy male health professionals aged 40 to 75 years with no history of coronary artery disease, vitamin D deficiency (25[OH]D <15 ng/mL) exhibited a 2-fold increased rate of myocardial infarction over 10 year period [59]. In the Framingham Offspring Study, subjects with no history of cardiovascular disease and severe vitamin D deficiency (25(OH)D <10 ng/mL) experienced a hazard ratio of 1.80 (95% CI, 1.05–3.08) for developing a first cardiovascular event after 5 years of follow-up compared with subjects with of 25(OH)D levels of >15 ng/mL [60]. In more than 3000 subjects undergoing coronary angiography, those with severe vitamin D deficiency (25(OH)D <10 ng/mL) had 3 to 5 times the risk of death from sudden cardiac death, heart failure or fatal stroke during a 7-year follow-up period compared with those who had optimal levels of vitamin D (25(OH)D >30 ng/mL) [61,62].

Although the significance of vitamin D deficiency in coronary artery disease is well established in observational studies, few studies have been conducted to evaluate the impact of vitamin D supplementation on the risk of cardiovascular mortality. In the

Women's Health Initiative CAD trial, postmenopausal women were randomized to vitamin D 400 IU daily and 1000mg of calcium supplementation and were followed for 7 years. These supplements had no significant effect on mortality rates [63]. Elderly individuals living in the community, aged between 65 to 85 years were given 100,000 IU oral vitamin D3 (cholecalciferol) supplementation or matching placebo every four months over five years. After 5 years, the relative risk for total mortality did not change (Odds ratio 0.88, CI 0.74 to 1.06, P=0.18) [64]. However neither of these studies provided what may be optimal daily doses of supplemental vitamin D.

Vitamin D and Heart Failure

Cardiomyocytes express vitamin D receptors [65]. Histological examination of ventricular muscle from vitamin D deficient rats revealed a significant decrease in myofibrillar area and increase in extracellular space with collagen; and restoring normal calcium levels did not prevent the increase in myocardial collagen [66]. Increased extracelluar space with collagen in the myocardium is associated with reduced ejection fraction and CHF. In mice, VDRs are closely associated with t-tubule proteins and are ideally positioned to exert an immediate effect on signal transduction mediators and ion channels. VDR knockout mice developed an altered rate of relaxation and increased cardiomyocyte hypertrophy [65].

The major potential mechanisms which may explain the direct protective effects of vitamin D against heart failure include: effects on myocardial contractile function, regulation of natriuretic hormone secretion, effects on extracellular matrix

remodeling, reduced left ventricular hypertrophy, and the regulation of inflammatory cytokines [67]. Indirectly vitamin D can also affect heart function by altering parathyroid hormone and serum calcium levels. The initial evidence in humans came from dialysis patients. In uremic cardiomyopathy patients, treatment with 1 microgram of 1, alpha-hydroxycholecalciferol daily for 6 weeks produced a decrease in plasma PTH concentration and an increase in fractional fiber shortening on M-Mode echocardiogram ($p < 0.025$) [68].

Observational studies showed that osteoporosis, osteopenia and low serum 25 [OH] vitamin D levels are common in congestive heart failure (CHF) patients [69]. This may explain in part the ethnic variance in the incidence of CHF and serum vitamin D levels [70]. A case-control study demonstrated that CHF patients and controls differed in several vitamin D associated lifestyle factors such as urban dwelling, sports club membership and number of summer holidays during earlier periods of lives [52]. In a study involving African American patients with left ventricular ejection fraction less than 35%, vitamin D deficiency (levels $\leq$ 30ng/mL) was associated with decompensated CHF and prolonged hospital stays [71]. Low vitamin D levels were associated with poor outcomes in patients with end stage heart failure awaiting heart transplantation [72]. A study on VDR gene polymorphisms in patients with end stage renal disease (ESRD) when compared to normal subjects suggested that vitamin D signaling is implicated in the regulation of left ventricular mass and hypertrophy in ESRD patients ($p<0.001$) [73].

Hemodialysis patients with secondary hyperparathyroidism when treated with IV calcitriol showed pronounced reductions in interventricular wall thickness ($P = 0.01$), left ventricular posterior wall thickness ($P < 0.05$), and left ventricle mass index ($P < 0.01$) [74]. Similarly vitamin D supplementation reduced the inflammatory markers in CHF patients and improved serum parathyroid hormone levels. However there was no significant direct survival benefit with vitamin D supplementation demonstrated in this study [75].

Vitamin D and Arrhythmia

Correction of vitamin D deficiency and hypocalcemia resulted in control of incessant ventricular tachycardia and cardiomyopathy in a recent report [76]. A rare case of fetal atrial flutter was reported in vitamin D resistant rickets [77]. In an animal study, rats fed a vitamin D deficient diet for 12 weeks developed significant shortening of QT interval despite normal serum calcium levels when compared to normal rats [78]. Vitamin D deficiency can result in hypocalcemia and prolongation of QTc interval, but current evidence on vitamin D deficiency as an etiological factor for arrhythmia has not been established.

Vitamin D and Mortality

Several references already cited as well as additional studies and meta-analysis suggest that vitamin deficiency has a negative association with survival while supplementation decreases overall mortality.[53,79-81]

Limitations of the present study:

This was a retrospective, observational trial with a selected population, introducing possible bias. The study population derived from patients who had their vitamin D levels measured in a hospital laboratory and who were patients in a cardiovascular practice and included in its electronic medical records. Extrapolation to other populations may not be appropriate. Isolated vitamin D measurement may not reflect long-term levels. We made an arbitrary decision to use the lowest vitamin D measurement for analysis as this value was felt to most likely represent the subjects' baseline non supplemented level. We were unable to accurately associate the timing of vitamin D measurement and supplement initiation. Dose and duration of supplementation were not analyzed. Inclusion of vitamin D in multivitamin supplements (typically 400 IU per tablet) was not considered. Projection of recommendations for large scale supplementation awaits prospective, randomized trials.

Summary

In this retrospective cohort study of patients followed in a large cardiovascular practice at an academic medical center, with data obtained from electronic medical records; vitamin D deficiency was shown to be significantly positively associated with several cardiovascular outcomes and poorer survival, consistent with prior studies. Our data also shows an association of vitamin D supplementation with better survival. This association is stronger in vitamin D deficient subjects than in those who

are not deficient, suggesting an incremental benefit of vitamin D supplementation for patients who are deficient. This benefit is independent of use of other medications such as aspirin or statins.

Conclusions

Observational studies strongly associate vitamin D deficiency with a variety of cardiovascular diseases beyond defects in bone and calcium metabolism. Vitamin D has multiple associations and mechanisms which potentially may impact cardiovascular health (Figures 1 and 3)[1-4,53,79-85]. Our study shows a strong association of vitamin D deficiency with coronary artery disease, diabetes, cardiomyopathy, and death. When included in survival and hazard models with several disease states, vitamin D deficiency is a strong independent predictor of death. Several studies have reported on the association between obesity and low vitamin D levels[82-85], which we also observed in our study population. As the prevalence of overweight status is increasing in the United States as well as many other developed and emerging nations, vitamin D deficiency may be increasingly common in the future. In addition our study showed an association between vitamin D deficiency and unfavorable serum lipid values.

Since vitamin D deficiency is widespread, strategies directed at population based supplemental programs may prove beneficial.[80] To date however, prospective studies evaluating vitamin D supplementations are few and have not consistently shown benefit. It is possible that the lack of benefit in these studies may have resulted from

suboptimal levels of vitamin D supplementation or other unknown factors. Many prior studies of vitamin D supplementation have employed doses of 400-800 IU, which may not be adequate to assure adequate serum levels, with more appropriate daily supplement doses suggested as 1000-2000 IU.[80,86] Nevertheless, the growing body of observational data and consistent findings of relatively high rates of low vitamin D serum levels warrant further well designed studies to investigate the relationship between vitamin D and cardiovascular health. Our study shows a significant association of vitamin D supplement use and improved survival, supporting the potential benefit of active vitamin D replacement.

Further investigation of these topics is warranted. Long-term prospective studies of various vitamin D dosage supplements in both healthy and diseased populations are indicated, as well as consideration of recommendations for supplementation in the general population while these studies are in progress. A recently announced prospective randomized study planning to enroll 20,000 elderly people will examine the potential cardiovascular benefits of vitamin D and omega-3 supplementation [87]. It is anticipated that this study will answer many of the questions raised from the observational and smaller interventional studies on vitamin D and cardiovascular disease processes described in this paper, as well as our own analysis.

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Appendix 1
SAS Program: (sample code, multiple iterations were used for different aspects of the analysis)

```
dm 'log;clear;output;clear;';
options ls=76 ps=88 ;
data vitd;
set storage.vitd;


endofstudy2 = Today()- 30;
endofstudy = 18187;

if death = 1 then Survival =  DOD - BD;
else Survival = endofstudy - BD;

if death = 1 then Survival2 = DOD - COL_DATE;
else Survival2 =  endofstudy - COL_DATE;

proc univariate normal plot; var result; run;

proc reg; model TRIGLYCER = RESULT;    run;


/*proc lifetest plot = (S);
time survival2*death(0);
strata Ddef;
run;

proc lifetest plot = (S);
time survival*death(0);
strata Ddef;
run;

proc phreg;
model survival*death(0) = cad diabetes ddef htn age bmi
gender_recode/rl ties = exact;
run;

proc phreg;
ddeftm=ddef*log(survival);
CADtm=CAD*log(survival);
model survival*death(0) = cad cadtm ddef ddeftm /rl ties = exact;
proportionality_test: test ddeftm, cadtm;
run;

proc phreg;
ddeftm=ddef*log(survival);
CADtm=CAD*log(survival);
model survival*death(0) = result vitamin_d interactd /rl ties =
exact;
proportionality_test: test interactd;
```

```
run;

proc logistic descending; model death = ddef CAD HTN cardiomyo
diabetes age BMI Cardia_EF creatinine gender_recode; run;

proc logistic descending; model death = ddef CAD HTN cardiomyo
diabetes age BMI Cardia_EF creatinine gender_recode /
        selection=stepwise sle=0.1 sls=0.15 scale=none aggregate;
run;

proc freq; tables vitamin_d*ddef*death/CMH; run;

proc freq; tables ddef*afib/CMH; run;

proc logistic descending; model death = ddef vitamin_D CAD HTN
cardiomyo diabetes age BMI Cardia_EF creatinine gender_recode; run;

proc logistic descending; model death = ddef vitamin_D CAD HTN
cardiomyo diabetes AFIB; run;

proc phreg;
model survival*death(0) = cad vitamin_d diabetes ddef htn age bmi
gender_recode/rl ties = exact;
run;

proc phreg;
model survival*death(0) = cad vitamin_d diabetes ddef htn cardiomyo
afib/rl ties = exact;
run;

proc freq; tables vitamin_D*death/CMH; run;

proc freq;
tables DDEF*DEATH VITAMIN_D*DDEF*DEATH / MEASURES CHISQ AGREE CMH
      NOPERCENT;
run;


proc lifetest plot = (S);
time survival*death(0);
strata vitamin_d ddef;
run; */

proc logistic descending;
model DEATH = CAD DIABETES CARDIOMYO HTN VITAMIN_D / corrb covb;
run;

proc logistic descending;
model DEATH = CAD DIABETES CARDIOMYO HTN VITAMIN_D AFIB AGE
GENDER_RECODE
BMI CREATININE / selection=stepwise sle=0.1 sls=0.15 corrb covb;
run;
```

```
proc reg; model result = BMI; run;


/* dm 'log;clear;output;clear;';
options ls=76 ps=88 ;


data vitd;
set storage.vitd;


endofstudy2 = Today()- 30;
endofstudy = 18187;

if death = 1 then Survival =  DOD - BD;
else Survival = endofstudy - BD;

if death = 1 then Survival2 = DOD - COL_DATE;
else Survival2 =  endofstudy - COL_DATE;

proc lifetest plot = (S);
time survival2*death(0);
strata Ddef;
run;

proc lifetest plot = (S);
time survival*death(0);
strata Ddef;
run;

proc contents data = vitd;  run;

proc univariate plot normal;
var age result; run;

proc freq;
tables G; run;

proc univariate plot normal;
var weight BMI cardia_ef; run;

proc freq;
tables ddef; run;

proc freq; tables vitamin_d*ddef*osteopenia/CMH; run;

proc logistic descending; model death = result; run;

proc logistic descending; model death = ddef CAD HTN cardiomyo
diabetes; run;

proc logistic descending; model death = result CAD HTN cardiomyo
diabetes; run;
```

```
proc logistic descending; model death = ddef CAD HTN cardiomyo
diabetes age BMI Cardia_EF creatinine gender_recode; run;

proc logistic descending; model DEATH = CAD DIABETES CARDIOMYO AGE
BMI CARDIA_EF DDEF /
        selection=stepwise sle=0.1 sls=0.15 scale=none aggregate;
run;

proc phreg;
model survival2*death(0) = cad diabetes cardiomyo ddef age bmi
cardia_EF/rl ties = exact;
run;

proc phreg;
model survival2*death(0) = cad diabetes ddef htn age bmi/rl ties =
exact;
run; */



  *** Proportional Hazards Models *** ;
  options pageno=1;
  proc phreg data=STORAGE.vitd;
     model SURVIVAL * DEATH (0) = DDEF AFIB CAD DIABETES CARDIOMYO
HTN VHD
     VITAMINANY VITAMIN_D A_INHIBITOR STATINS ASA GENDER_RECODE
INTERACTB /RL ties = exact
        selection= n;  strata vitamin_d;
  run;

  proc lifetest data=storage.vitd plot =(S);
  time survival*death(0);
  strata interactB ddef;
  run;


  proc lifetest data=storage.vitd plot =(S);
  time survival*death(0);
  strata ddef;
  run;

 *** Logistic Regression Analysis ***;
  options pageno=1;
  proc logistic data=STORAGE.vitd DESCEND;
     model DEATH =   DDEF diabetes cardiomyo gender_recode
       /ctable lackfit;
  run;

*** Logistic Regression Analysis ***;
  options pageno=1;
  proc logistic data=STORAGE.vitd DESCEND;
     model DEATH =   ddef interactB diabetes cardiomyo gender_recode
cad
```

```
      /ctable lackfit;
  run;
```

```
final model goodness of fit

dm 'log;clear;output;clear;';
options ls=76 ps=88 ;


data vitd;
set storage.vitd;


endofstudy2 = Today()- 30;
endofstudy = 18187;

if death = 1 then Survival =  DOD - BD;
else Survival = endofstudy - BD;


options pageno=1;
  proc logistic data=STORAGE.vitd DESCEND;
     model DEATH =   DDEF CAD cardiomyo gender_recode vitamin_D
       /ctable lackfit;
  run;
```

Appendix 2. Final model SAS output

Final Model with Vitamin D as a dichotomous variable

11:48 Thursday, March 18, 2010 1

The LOGISTIC Procedure

Model Information

Data Set	STORAGE.VITD	
Response Variable	Death	Death
Number of Response Levels	2	
Model	binary logit	
Optimization Technique	Fisher's scoring	

Number of Observations Read	10899
Number of Observations Used	10899

Response Profile

Ordered Value	Death	Total Frequency
1	1	336
2	0	10563

Probability modeled is Death='1'.

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	3001.633	2693.854
SC	3008.930	2737.633
-2 Log L	2999.633	2681.854

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	317.7791	5	<.0001
Score	439.9622	5	<.0001
Wald	333.4625	5	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-4.0427	0.1764	525.1706	<.0001
Ddef	1	1.0915	0.1677	42.3572	<.0001
CAD	1	1.2081	0.1370	77.7529	<.0001

CARDIOMYO	1	1.2325	0.1760	49.0589	<.0001
Gender_recode	1	-0.7283	0.1175	38.4141	<.0001
VITAMIN_D	1	-0.7584	0.1448	27.4458	<.0001

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
Ddef	2.979	2.144	4.138
CAD	3.347	2.559	4.378
CARDIOMYO	3.430	2.429	4.842
Gender_recode	0.483	0.383	0.608
VITAMIN_D	0.468	0.353	0.622

Association of Predicted Probabilities and Observed Responses

Percent Concordant	68.9	Somers' D	0.492
Percent Discordant	19.7	Gamma	0.556
Percent Tied	11.5	Tau-a	0.029
Pairs	3549168	c	0.746

The LOGISTIC Procedure

Partition for the Hosmer and Lemeshow Test

		Death = 1		Death = 0	
Group	Total	Observed	Expected	Observed	Expected
1	596	5	2.62	591	593.38
2	1802	8	15.14	1794	1786.86
3	1561	22	18.24	1539	1542.76
4	943	23	18.92	920	924.08
5	3228	71	79.45	3157	3148.55
6	361	14	12.12	347	348.88
7	1494	76	74.22	1418	1419.78
8	914	117	115.30	797	798.70

Hosmer and Lemeshow Goodness-of-Fit Test

Chi-Square	DF	Pr > ChiSq
8.5599	6	0.1999

Classification Table

	Correct		Incorrect		Percentages				
Prob Level	Event	Non-Event	Event	Non-Event	Correct	Sensi-tivity	Speci-ficity	False POS	False NEG
0.000	336	0	10563	0	3.1	100.0	0.0	96.9	.
0.020	290	4435	6128	46	43.4	86.3	42.0	95.5	1.0
0.040	193	8346	2217	143	78.3	57.4	79.0	92.0	1.7
0.060	111	9865	698	225	91.5	33.0	93.4	86.3	2.2
0.080	81	10204	359	255	94.4	24.1	96.6	81.6	2.4
0.100	77	10226	337	259	94.5	22.9	96.8	81.4	2.5
0.120	73	10259	304	263	94.8	21.7	97.1	80.6	2.5
0.140	73	10259	304	263	94.8	21.7	97.1	80.6	2.5
0.160	34	10454	109	302	96.2	10.1	99.0	76.2	2.8
0.180	31	10474	89	305	96.4	9.2	99.2	74.2	2.8
0.200	31	10474	89	305	96.4	9.2	99.2	74.2	2.8

0.220	22	10474	89	314	96.3	6.5	99.2	80.2	2.9
0.240	22	10527	36	314	96.8	6.5	99.7	62.1	2.9
0.260	22	10527	36	314	96.8	6.5	99.7	62.1	2.9
0.280	22	10527	36	314	96.8	6.5	99.7	62.1	2.9
0.300	22	10527	36	314	96.8	6.5	99.7	62.1	2.9
0.320	22	10527	36	314	96.8	6.5	99.7	62.1	2.9
0.340	22	10527	36	314	96.8	6.5	99.7	62.1	2.9
0.360	22	10527	36	314	96.8	6.5	99.7	62.1	2.9
0.380	0	10563	0	336	96.9	0.0	100.0	.	3.1

Vitamin D in final model as a continuous variable

Model Information

Data Set	STORAGE.VITD	
Response Variable	Death	Death
Number of Response Levels	2	
Model	binary logit	
Optimization Technique	Fisher's scoring	

Number of Observations Read	10899
Number of Observations Used	10899

Response Profile

Ordered Value	Death	Total Frequency
1	1	336
2	0	10563

Probability modeled is Death='1'.

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Deviance and Pearson Goodness-of-Fit Statistics

Criterion	Value	DF	Value/DF	Pr > ChiSq
Deviance	1435.6946	2858	0.5023	1.0000
Pearson	4016.1785	2858	1.4052	<.0001

Number of unique profiles: 2864

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	3001.633	2663.880
SC	3008.930	2707.658
-2 Log L	2999.633	2651.880

The LOGISTIC Procedure

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	347.7538	5	<.0001
Score	461.2933	5	<.0001
Wald	352.1680	5	<.0001

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-2.2358	0.1354	272.7708	<.0001
result	1	-0.0463	0.00552	70.4926	<.0001
CAD	1	1.2353	0.1368	81.4861	<.0001
CARDIOMYO	1	1.2304	0.1764	48.6520	<.0001
VITAMIN_D	1	-0.7465	0.1449	26.5494	<.0001
Gender_recode	1	-0.7058	0.1174	36.1435	<.0001

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
result	0.955	0.944	0.965
CAD	3.439	2.630	4.497
CARDIOMYO	3.423	2.422	4.836
VITAMIN_D	0.474	0.357	0.630
Gender_recode	0.494	0.392	0.621

Association of Predicted Probabilities and Observed Responses

Percent Concordant	75.6	Somers' D	0.533
Percent Discordant	22.3	Gamma	0.544
Percent Tied	2.0	Tau-a	0.032
Pairs	3549168	c	0.766

The LOGISTIC Procedure

Partition for the Hosmer and Lemeshow Test

Group	Total	Death = 1 Observed	Death = 1 Expected	Death = 0 Observed	Death = 0 Expected
1	1092	9	5.02	1083	1086.98
2	1088	7	8.85	1081	1079.15
3	1092	11	11.83	1081	1080.17
4	1090	8	14.91	1082	1075.09
5	1087	19	18.46	1068	1068.54
6	1087	18	23.23	1069	1063.77
7	1092	30	30.03	1062	1061.97
8	1090	47	38.08	1043	1051.92
9	1091	53	53.96	1038	1037.04
10	1090	134	131.64	956	958.36

Hosmer and Lemeshow Goodness-of-Fit Test

Chi-Square	DF	Pr > ChiSq
10.3286	8	0.2427