AN ABSTRACT OF THE THESIS OF

Wyatt Messenger for the degree of Honors Baccalaureate of Science in Health Management and Policy presented on May 1, 2009. Title: Independent Association of Vitamin D with Prevalent and Incident Outcomes of Heart Attack and Stroke in Elderly Men.

Abstract approved: _____
Dr. Jackilen Shannon, PhD

BACKGROUND: The association of vitamin D with heart attack and stroke remains unclear. METHOD: Using data from the Osteoporotic Fractures in Men Study (MrOS), we collected dietary vitamin D data from 5,995 participants and serum (25)OH vitamin D from 1,606 participants. The association of vitamin D with prevalent heart attack and strokes was studied using an unconditional logistic regression model. Then, incidence of heart attack and stroke was prospectively studied using a log binomial model. RESULTS: In the analysis of prevalence, a non-significant inverse relationship between vitamin D and heart attack and stroke was observed. However, participants in the highest quartile of dietary vitamin D reported significantly fewer prevalent strokes than men in the lowest quartile (prevalence ratio, 0.72; p=0.04). In the incidence analysis, the data was largely inconclusive. In the dietary vitamin D analyses, the highest quartile dietary vitamin D was generally associated with a non-significant decreased odds of heart attack (odds ratio, 0.98; p=0.91) and stroke (odds ratio 0.97; p=0.98). Similarly, in the serum analysis, the highest quartile of serum vitamin was associated with a non-significant decreased odds of heart attack (odds ratio, 0.82; p=0.49) and stroke (odds ratio 0.57; p=0.10). CONCLUSION: Vitamin D was not significantly protective against incidents of heart attack and stroke. While the majority of our results showed a minor protective effect, it was too small to make definitive conclusions.

Key words: vitamin D, heart attack, stroke

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Independent Association of Vitamin D with Prevalent and Incident Outcomes of Heart Attack and Stroke in Elderly Men: A Prospective Cohort Study

by

Wyatt Messenger

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Wyatt Messenger, Author

TABLE OF CONTENTS

TABLE OF TABLES

INTRODUCTION

Despite years of research and major advances in understanding, prevention, and treatment, 1 in 3 Americans still die from cardiovascular disease (CVD). As CVD has been correlated with higher latitudes and winter months, many investigators have hypothesized that hypovitaminosis D may be a risk factor for CVD (Spencer et al, Spengos et al, Krause et al). Several studies have shown an association with hypovitaminosis D and risk factors of CVD, such as hypertension (Scragg et al, Forman et al, Martins et al, Lind et al), hyperlipidemia (Carbone, Martins et al), and metabolic syndrome (Reis et al, Chiu et al). Regardless of the connection between hypovitaminosis D and risk factors for CVD, recent epidemiological investigations have been largely inconclusive in showing an association between vitamin D and CVD.

In a community-based case-control study, which matched 179 patients presenting symptoms of myocardial infarction (MI) patients with controls, MI patients were found to have significantly lower mean 25-hydroxyvitamin D3 levels than controls (32.0 versus 35.5 nmol/L; p = 0.017) (Scragg et al). In a large prospective cohort study of 3,258 participants, low serum 25(OH) vitamin D and 1,25(OH) vitamin D were significantly associated with higher cardiovascular mortality (HR, 2.22; 95% CI, 1.57-3.13; and HR, 1.82; 95% CI, 1.29-2.58; respectively) (Dobnig et al).In a cross-sectional case control study, the mean *Z* score of serum 25(OH) vitamin D in 44 acute stroke was –1.4 SD units (95% CI, –1.7, –1.1), with 77% of patients falling in the insufficient range (Poole). In a cohort study following elderly subjects for ten years, researchers found that low dietary vitamin D (p=0.011) and serum 1,25-dihydroxyl-vitamin D (p=0.0053) were significantly associated with outcomes of stroke (Marniemi et al). Similarly, in prospective case-control study using data from the Health Professionals Follow-up Study, low levels of serum 25(OH)D were significantly associated with a higher risk of heart attack (Giovannucci et al).

Conversely, a cohort study of post-menopausal women from Iowa, dietary and supplemental vitamin D was not associated with risk of ischemic heart disease mortality. In fact, a non-significant increased risk of ischemic heart disease was associated with

higher vitamin D (Bostick et al). In a randomized trial with 32,282 post-menopausal women, the participants received either vitamin D (400 IU/day) and calcium (500 mg/day) or a placebo. After 7 years of follow-up, vitamin D and calcium did not have a protective effect on either CHD or heart attacks (hazard ratio 1.04; 95% CI, 0.92-1.18) (Hsia et al). In a case-control study, researchers studied 73 patients with coronary heart disease or having a history of an acute myocardial infarction and matched them with 70 controls. After performing a multivariate logistic regression analysis, participants with higher levels of serum vitamin D were at increased risk of having coronary heart disease (odds ratio 3.18: 95% CI, 1.31-7.73) (Rajasree).

Despite these discrepancies, as the majority of elderly populations are vitamin D deficient (Gloth et al, Goldray et al, McKenna et al) and cardiovascular disease continues to burden our health care systems, there needs to be continued large studies on the association of vitamin D deficiency and/or insufficiency and heart disease and stroke. To our knowledge, this is the first multi-center cohort study, using a representative sample of minorities, to comprehensively study the association of vitamin D with heart attack and stroke. In our study, we hypothesized that vitamin D would be associated with a reduction in risk of heart attack and stroke.

METHODS

POPULATION

The Osteoporotic Fractures in Men (MrOS) has been previously described (Blank et al). Briefly, MrOS is a prospective cohort study designed to elucidate predictors of fractures in older men (aged 65 and older) at 6 separate testing sites (Birmingham, Alabama; Palo Alto, California; San Diego, California; Minneapolis, Minnesota; Portland, Oregon; Pittsburgh, Pennsylvania). Exclusion criteria included: (1) inability to walk without assistance from another person, (2) bilateral hip replacements, (3) inability to provide self reported data, residence not near a study site, (4) were judged by an investigator to have a medical condition that would result in imminent death, (6) or inability to understand and sign informed consent (Orwoll 572). The MrOS cohort study began enrollment in March

of 2000 through April of 2002 and was designed to last 13.5 years with three separate follow-up visits every 4.5 years. Our analysis will only include data through the first follow-up visit. Of the 5,995 men enrolled at baseline, 5,194 completed visit 2 and are eligible for our analysis. We measured serum samples from a sub-cohort of men 1,606 men. This study was approved by Institutional Review Board at Oregon Health and Sciences University. All subjects consented to their participation in the study.

ASSESSMENT OF FACTORS AFFECTING VITAMIN D STATUS

Upon enrollment, participants completed a self-administered questionnaire, including medical history, food frequency questionnaire, and lifestyle summary; a clinical interview questionnaire, which included information about medication use; clinical measurements, including pulse rate, anthropometric measures, and blood pressure; and biological specimen acquisition, including serum. With the exception of the food frequency questionnaire, the same measurements and questionnaires were repeated at visit 2. Data collected from the study was compiled and analyzed using SAS software (SAS Corporation, Cary, North Carolina).

ASSESSMENT OF SELF-REPORTED HEART ATTACK AND STROKE

We studied prevalence and incidence of self-reported heart attacks and strokes. As part of the self-administered questionnaire, participants were asked two separate questions “Has a doctor or other health care provider ever told you that you had or have a stroke, blood clot in the brain, or bleeding in the brain?” and “Has a doctor or health care provider ever told you that you had a heart attack, coronary or myocardial infarction?” Prevalence was defined as a history of heart attack or stroke reported at visit 1. We defined “calculated incidence” of non-fatal heart attack or stroke as any event that was reported at visit 2 that was not reported at baseline. Because date of diagnosis was not available for this analysis the “calculated incidence” is not characterized as person years, but is simply a dichotomous variable. We used information from death certificates to determine incidence of fatal heart attack and stroke between visit 1 and visit 2. Non-fatal and fatal heart attack and strokes were merged to create a variable to express an incident heart

attack, a heart attack occurring between visit 1 and 2. A portion of the general cohort participated in a sleep sub-study, which verified heart attack and stroke incidents. We used data from this study to adjudicate reports of non-fatal heart attack or stroke in the men that participated in this sub-study.

ASSESSEMENT OF VITAMIN D

We measured vitamin D using dietary and serum measures. First, daily dietary intake of vitamin D was measured at baseline using a validated self-administered food frequency questionnaire (Block 98). Every participant reported the type and amount of food and beverage they consumed. The questionnaire was modified specifically to ensure adequate determination of vitamin D and calcium intake from foods and contained 9 categorical responses for frequency of consumption of each food type ranging from never to every day. Nutrient analyses were conducted by Block Nutrition Analysis Systems. This information was combined with information gathered about vitamin supplementation in order to estimate the average daily intake of vitamin D, as well as other dietary factors.

From a sub-cohort of 1606 men, we used serum vitamin D as the second measure of vitamin D levels. Serum for vitamin D analyses was collected at baseline, processed and immediately frozen at -80. Concentrations of 25(OH)D and 1,25(OH)2D were determined at the Mayo Clinic Reference Laboratories (Dr. Ravinder Singh). The 25(OH)D assay is performed by mass spectrometry, thus providing a accurate and precise method free of the artifacts that have affected other radioimmunoassay based methods.(130) Samples were prepared by incubation for 15 minutes with stable isotope 25(OH)D3-d6 followed by precipitation with acetonitrile. 50 μL of the supernatant is injected onto Cohesive Turbo Flow Cyclone extraction columns followed by chromatography on a Supelco LC-18 column and analysis by tandem mass spectrometry. Functional sensitivity is 4 ng/mL for 25OHD2 and 2 ng/mL for 25(OH)D3. The assay is linear to 200 ng/mL for both analyses. Inter assay precision is <10%.

STATISCAL ANALYSIS

Using a log binomial model, we examined the cross-sectional association of prevalent heart attack and stroke with dietary+supplemental vitamin D and serum vitamin D. The referent quartile was set as the lowest measure of vitamin D. We estimated an age adjusted model and a multivariate-adjusted model. The additional potential confounders used for the multivariate mode were race, education, alcohol use, exercise, total calorie intake, height, total cholesterol, low density lipids, high density lipids, triglycerides, glucose, insulin, systolic blood pressure, hypertension, diabetes, statins, diabetes medication, blood pressure reducing medication, and anticoagulation medication. Covariates were entered independently into the main model. Variables were considered confounders and maintained in the multivariate analysis if the regression coefficient of the primary independent variable changed 10% or more after adding the potential confounding variable to the model.

After studying the association of prevalent heart attack and stroke with vitamin D, we created a "calculated incidence" group to study longitudinal association. All subjects who reported a history of heart attack or stroke at visit 1 were excluded from these analyses, such that any participant who reported having had a heart attack of stroke at visit 2 was considered an incidence. These cases were combined with the fatal cases of heart attack and stroke to complete our "calculated incidence" group.

Using the incident heart attack and stroke groups, we analyzed their baseline characteristics with respect to the heart attack-free and stroke-free groups. Like the prevalent analysis, the baseline characteristics we studied included their demographics, lifestyle factors, anthropometric measures, lipids, glucose and insulin levels, systolic blood pressure, medical history, medication use, and body composition. After the men were categorized based on these conditions, a student's t-test was used to evaluate whether a significant difference existed between the cases and men not reporting an incidence. Both heart attack and stroke were analyzed separately.

In our final analysis, we used an unconditional logistic regression model to evaluate the risk of a heart attack and stroke at different quartiles of vitamin D. Before beginning, participants were removed if they had a previous history of a heart attack or stroke for the heart attack and stroke analysis, respectively, or if they refused to complete the food frequency questionnaire. We began the analysis by categorizing participants into quartiles based of dietary+supplemental vitamin D and serum vitamin D. Then, a log transform was used to remove the skew in the distribution of dietary+supplemental vitamin D. We used an age-adjusted and multivariate-adjusted model. The same potential confounders that were used in the prevalent analysis were used for the incident analysis. Again, the variables were maintained in the multivariate model if the regression coefficient of the primary independent variable changed 10% or more after adding the potential confounding variable to the model.

RESULTS

PREVALENT RESULTS

Of the 5,995 men in the cohort, 828 men reported prevalent heart attack and 344 reported prevalent stroke. From the sub-cohort of men giving serum samples, 251 men reported a previous heart attack and 98 men reported a previous stroke. The median dietary+supplemental vitamin D was 430 IU/day. The median serum vitamin D was 25.1 for the sub-cohort, which gave serum samples. The exact quartile distributions for dietary vitamin D and serum vitamin D are provided (**Table 1**).Selected baseline characteristics are given for men reporting a heart attack or a stroke (**Table 2**). We performed a student's t-test to determine a whether a significance existed between the participants reporting a prevalent heart attack and the participants not reporting a heart attack. The same process was repeated for participants reporting a stroke. Alcohol consumption was significantly lower for men reporting a history of a heart attack ($p<0.0001$) and stroke ($p=0.03$). Participants that reported a history of a heart attack had significantly higher triglyceride levels ($p=0.02$). Men reporting a heart attack ($p<0.0001$) or stroke ($p<0.0001$) were significantly more likely to report a history of hypertension.

The prevalence ratios that were produced by the log-binomial model are presented (**Table 3**). Comparing highest and lowest quartiles, higher dietary vitamin D was strongly inversely associated with a decreased risk of heart attack (prevalence ratio, 0.86; p=0.02). After adjustment for possible confounders, the prevalence ratio no longer remain significant; however, the protective effect of dietary vitamin D remained strong (prevalence ratio, 0.86; p=0.13). Higher serum vitamin D was only weakly associated with fewer prevalent heart attacks (prevalence ratio 0.95, p=0.75). Men in the highest quartile of dietary vitamin D was reported significantly fewer prevalent strokes after adjustment for possible confounders (prevalence ratio, 0.72; p=0.04). There was no significant changes in reports of stroke between men with high and low serum vitamin D. (p=0.73).

Table 1. Quartile distributions for dietary vitamin D and serum vitamin D in the general MrOS cohort and serum-reporting sub-cohort.

	Dietary vitamin D, (IU/day)	***Serum 25(OH)D, (ng/mL)***
Quartile 1	158.65	<19.90
Quartile 2	158.65 to <430.54	19.90 to < 25.09
Quartile 3	430.54 to <568.09	25.09 to < 29.80
Quartile 4	≥ 568.09	≥ 29.80

Table 2. Baseline characteristics of participants reporting a history of a heart attack and participants reporting a history of a stroke.

	Prevalent Heart Attack			**Prevalent Stroke**		
	No (n=5160)	**Yes (n=835)**	***p-value***	**No (n=5651)**	**Yes (n=344)**	***p-value***
Demographics						
Age (years)	73.4 ± 5.8	75.1 ± 5.8	*<0.0001*	73.5 ± 5.8	76.1 ± 6.0	*<0.0001*
Race						
White, non-Hispanic	89.2	91		89.4	89.8	
African-American	4.1	3.7		4	5.2	
Asian	3.5	1.6		3.2	2.6	
Hispanic	2.1	2.2		2.2	0.6	
Other	1.1	1.6	*0.04*	1.2	1.7	*0.16*
Education						
< High school	35.8	29.7		35.1	31.7	
High school	41.1	42.3		41.2	42.2	

College	16.8	20		17.2	18.6	
Graduate school	6.3	8	*0.002*	6.5	7.6	*0.55*
Study Site						
Birmingham	15.8	18.2		16.2	16	
Minneapolis	16.7	16.9		16.6	18.9	
Palo Alto	16.9	15.1		16.9	11.9	
Pittsburgh	16.1	21		16.5	20.9	
Portland	17	15.3		16.7	17.7	
San Diego	17.4	13.5	*0.0006*	17	14.5	*0.05*
Lifestyle / Diet						
% Current smoking	3.4	3.6	*0.78*	3.5	2.6	*0.39*
% Current alcohol drinking	65.9	56.7	*<0.0001*	64.9	59	*0.03*
Physical activity score	147.8 ± 67.7	138.8 ± 71.2	*0.0004*	148.1 ± 68.2	121.0 ± 64.3	*<0.0001*
Total caloric intake	1632.3 ± 643.5	1557.7 ± 608.1	*0.13*	1625.3 ± 638.3	1566.1 ± 650.6	*0.1*
Anthropometric Measures						
BMI (kg/m^2)	27.4 ± 3.8	27.5 ± 3.9	*0.3*	27.4 ± 3.8	27.1 ± 3.8	*0.13*
Weight (kg)	83.2 ± 13.3	82.7 ± 13.5	*0.32*	83.3 ± 13.2	80.8 ± 14.1	*0.0006*
Height (cm)	174.3 ± 6.8	173.3 ± 6.6	*<0.0001*	174.2 ± 6.8	172.4 ± 7.1	*<0.0001*
Lipids						
Total cholesterol (mg/dl)	195.9 ± 33.6	177.1 ± 33.5	*<0.0001*	193.6 ± 34.3	188.1 ± 33.4	*0.006*
LDL (mg/dl)	116.6 ± 30.5	99.3 ± 29.7	*<0.0001*	114.5 ± 31.1	109.3 ± 28.4	*0.005*
HDL (mg/dl)	49.4 ± 14.7	46.1 ± 14.1	*<0.0001*	48.9 ± 14.7	48.3 ± 13.8	*0.49*
Triglyceride (mg/dl)	149.8 ± 97.5	159.2 ± 103.9	*0.02*	151.0 ± 98.4	152.2 ± 100.4	*0.84*
Glucose level (mg/dl)	99.0 (16.0)	101.0 (21.0)	*0.003*	100.0 (16.0)	100.0 (21.0)	*0.78*
Insulin level (μIU/ml)	7.6 (6.0)	8.2 (6.8)	*<0.0001*	7.7 (6.2)	7.4 (5.6)	*0.57*
Systolic blood pressure (mmHg)	138.5 ± 18.9	136.4 ± 19.8	*0.003*	138.0 ± 18.8	142.0 ± 21.5	*0.001*
Medical History						
History of hypertension	41	56.1	*<0.0001*	42.2	57.6	*<0.0001*
History of diabetes	9.9	16.9	*<0.0001*	10.5	16.9	*0.0003*
Medication Use						
Statins	21.9	59.6	*<0.0001*	26.3	42	*<0.0001*
Diabetes medications	7.5	13.9	*<0.0001*	8.2	11.4	*0.04*
Antihypertensives	33.5	62.7	*<0.0001*	36.7	53.3	*<0.0001*
Anticoagulants	35.7	71.6	*<0.0001*	38.8	74	*<0.0001*
Body Composition Measures						
Total body fat mass (kg)	21.7 ± 7.1	21.9 ± 6.9	*0.4*	21.8 ± 7.1	21.4 ± 7.2	*0.3*

Total body lean mass (kg)	58.1 ± 7.4	57.3 ± 7.7	*0.003*	58.1 ± 7.4	55.9 ± 7.9	*<0.0001*
Truncal fat mass (kg)	12.2 ± 4.3	12.4 ± 4.1	*0.28*	12.3 ± 4.3	12.0 ± 4.3	*0.38*

Table 3. Estimated prevalence ratios of heart attack and stroke for quartiles of dietary+supplemental vitamin D and serum vitamin D

		HEART ATTACK					
		Dietary+Supplemental Vitamin D			**Serum Vitamin D**		
		Cases	Age Adjusted	**Multivariate**†	**Cases**	Age Adjusted	**Multivariate**
Quartile 1 (lowest)		231	1 (referent)	1 (referent)	68	1 (referent)	1 (referent)
Quartile 2		298	0.84	0.93	65	0.95	0.95
Quartile 3		210	0.91	0.93	57	0.86	0.86
Quartile 4 (highest)		190	0.80	0.86	61	0.95	0.95
		STROKE					
		Dietary+Supplemental Vitamin D			**Serum Vitamin D**		
		Cases	Age Adjusted	**Multivariate**†	**Cases**	Age Adjusted	**Multivariate**
Quartile 1 (lowest)		93	1 (referent)	1 (referent)	28	1 (referent)	1 (referent)
Quartile 2		87	0.92	0.90	20	0.73	0.73
Quartile 3		87	0.94	0.91	28	1.06	1.06
Quartile 4 (highest)		77	0.8	0.72	22	0.91	0.91

†Adjusted for age and anti-coagulant medication

INCIDENT RESULTS

During the 4.5 year follow up period, there were 400 participants (306 non-fatal, 95 fatal) that had a heart attack during follow-up and 282 men (233 non-fatal, 49 fatal) had a stroke. There were 4 participants that reported an incident heart attack, but were removed from the analysis because they refused the food frequency questionnaire. From the sub-cohort of men giving serum samples, there were 116 participants (88 non-fatal, 28 fatal) that had a heart attack during follow-up and 76 (61 non-fatal, 15 fatal) men that reported a stroke. Selected baseline characteristics are given for men reporting a heart attack or a stroke (**Table 4**). We performed a student's t-test to determine a whether a significance existed between cases and non-cases for both heart attack and stroke separately. After evaluating possible confounders, we found that glucose levels (p=0.006) and insulin (p=0.05) were significantly higher in men that had a heart attack; yet both glucose

(p=0.91) and insulin (p=0.28) were not significantly different in men with a stroke and men not reporting a stroke. Physical activity was significantly lower outcomes of heart attacks (p=0.002), but was not a significant indicator of stroke outcomes (p=0.69).

The odds ratio output of the unconditional regression models are presented (**Table 5**). Comparing the lowest and highest quartiles of vitamin D, higher dietary vitamin D (odds ratio, 0.98; p=0.91) nor higher serum vitamin D (odds ratio, 0.82; p=0.49) were significantly associated with outcomes of heart attack in our multivariate models. The stroke data produced similar results. The multivariate-adjusted hazards ratio for the highest dietary vitamin D quartile was not significantly lower for stroke (odds ratio 0.97; p=0.98) compared to the lowest quartile of vitamin D; and despite having a stronger inverse relationship, higher serum vitamin D was also not significantly associated with decreased incident stroke (odds ratio 0.57; p=0.10).

Table 4. Baseline characteristics of heart attack cases and stroke cases

	Incident Heart Attack			Incident Stroke		
	No (n=4760)	Yes (n=400)	*p-value*	No (n=5369)	Yes (n=282)	*p-value*
Demographics						
Age (years)	73.3 ± 5.8	75.3 ± 6.3	*<0.0001*	73.4 ± 5.8	75.2 ± 5.9	*<0.0001*
Race						
White, non-Hispanic	89.1	90		89.3	92.6	
African-American	4.1	5		4.1	1.8	
Asian	3.6	1.5		3.2	3.6	
Hispanic	2.1	2		2.3	1.4	
Other	1.1	1.5	*0.19*	1.2	0.7	*0.24*
Education						
< High school	36.3	29.5		35.1	36.5	
High school	41	41.8		41.4	37.6	
College	16.5	20.5		17.1	19.5	
Graduate school	6.2	8.3	*0.01*	6.5	6.4	*0.57*
Study Site						
Birmingham	15.9	14.8		16.2	16.3	
Minneapolis	16.6	18.3		16.5	19.5	
Palo Alto	16.7	18.3		16.8	18.1	

Pittsburgh	15.8	19		16.6	14.2	
Portland	17.1	15.8		16.8	16.3	
San Diego	17.7	14	*0.22*	17.1	15.6	*0.69*
Lifestyle / Diet						
% Current smoking	3.3	4.3	*0.34*	3.5	2.8	*0.54*
% Current alcohol drinking	66.3	60.3	*0.01*	65.1	60.6	*0.12*
Physical activity score	148.6 ± 67.7	137.5 ± 68.0	*0.002*	148.2 ± 67.9	146.3 ± 74.9	*0.69*
Total caloric intake	1626.5 ± 634.4	1701.4 ± 741.3	*0.05*	1619.6 ± 644.4	1608.9 ± 621.6	*0.78*
Anthropometric Measures						
BMI (kg/m^2)	27.3 ± 3.8	27.7 ± 3.9	*0.08*	27.4 ± 3.8	27.4 ± 3.6	*0.92*
Weight (kg)	83.2 ± 13.3	83.5 ± 13.3	*0.62*	83.3 ± 13.3	83.2 ± 12.6	*0.91*
Height (cm)	174.3 ± 6.8	173.6 ± 6.6	*0.04*	174.2 ± 6.8	174.2 ± 7.0	*0.95*
Lipids						
Total cholesterol (mg/dl)	196.2 ± 33.8	193 ± 31.3	*0.09*	193.7 ± 34.3	191.6 ± 33.5	*0.35*
LDL (mg/dl)	116.9 ± 30.5	113.6 ± 30.4	*0.05*	114.6 ± 31.1	112.2 ± 30.8	*0.23*
HDL (mg/dl)	49.6 ± 14.7	46.5 ± 13.3	*0.0001*	49.0 ± 14.6	48.6 ± 15.3	*0.7*
Triglyceride (mg/dl)	148.6 ± 96.7	164.2 ± 105.5	*0.003*	150.9 ± 98.2	154.3 ± 101.4	*0.59*
Glucose level (mg/dl)	99.0 (16.0)	102.0 (22.0)	*0.006*	100.0 (16.0)	98.0 (17.5)	*0.91*
Insulin level (μIU/ml)	7.5 (6.1)	8.1 (6.5)	*0.05*	7.7 (6.1)	8.0 (7.6)	*0.28*
Systolic blood pressure (mmHg)	138.2 ± 18.7	141.7 ± 20.4	*0.001*	137.7 ± 18.8	142.3 ± 20.0	*<0.0001*
Medical History						
History of hypertension	40.5	46.3	*0.02*	41.9	47.2	*0.08*
History of diabetes	9.3	17	*<0.0001*	10.3	14.2	*0.04*
Medication Use						
Statins	21.3	29.3	*0.0003*	26.4	24.8	*0.58*
Diabetes medications	7	13.2	*<0.0001*	8	11.8	*0.03*
Antihypertensives	32.4	46.4	*<0.0001*	36.5	40	*0.25*
Anticoagulants	34.7	47.7	*<0.0001*	38.3	48.1	*0.002*
Body Composition Measures						
Total body fat mass (kg)	21.7 ± 7.1	22.0 ± 7.2	*0.33*	21.7 ± 7.1	22.2 ± 5.8	*0.33*
Total body lean mass (kg)	58.1 ± 7.4	58.0 ± 7.4	*0.84*	58.1 ± 7.4	57.6 ± 7.4	*0.26*
Truncal fat mass (kg)	12.2 ± 4.3	12.5 ± 4.4	*0.17*	12.2 ± 4.3	12.5 ± 4.1	*0.31*

Table 5. Estimated odds ratios of incident heart attack and stroke for quartiles of dietary+supplemental vitamin D and serum vitamin D

	HEART ATTACK					
	Dietary+Supplemental Vitamin D			Serum Vitamin D		
	Cases	Age Adjusted	Multivariate	Cases	Age Adjusted	Multivariate
Quartile 1 (lowest)	89	1 (referent)	1 (referent)	31	1 (referent)	1 (referent)
Quartile 2	117	1.28	1.28	26	0.81	0.81
Quartile 3	96	1.07	1.07	35	1.13	1.13
Quartile 4 (highest)	94	0.98	0.98	24	0.82	0.82
	STROKE					
	Dietary+Supplemental Vitamin D			Serum Vitamin D		
	Cases	Age Adjusted	Multivariate	Cases	Age Adjusted	Multivariate*
Quartile 1 (lowest)	68	1 (referent)	1 (referent)	21	1 (referent)	1 (referent)
Quartile 2	77	1.12	1.12	17	0.85	1
Quartile 3	68	1	1	23	0.72	0.69
Quartile 4 (highest)	69	0.97	0.97	16	0.59	0.57

*Adjusted for age and diabetes medication

DISCUSSION

In both the cross-sectional and longitudinal analyses, our results suggested that vitamin D had a weak, non-significant protective effect against heart attack and stroke. Although the majority of our results were not significant, dietary vitamin D deficiency had a significant inverse association with prevalent stroke (p=0.04), higher dietary vitamin D was borderline significantly protective against prevalent heart attack (p=0.13), and serum vitamin D had a borderline significant (p=0.13) inverse association with longitudinal outcomes of stroke. Dietary vitamin D tended to be a stronger indicator of heart attack and stroke outcomes in the cross-sectional analysis, while serum vitamin D was the stronger indicator in the longitudinal analysis.

Our study contributes to the limited knowledge of the relationship of vitamin D and CVD by adding to the small body of prospective cohort studies. To our knowledge, there have been three large epidemiological studies similar to ours and all three have supported a significant inverse association of vitamin D with cardiovascular disease. These three studies are the Framingham Offspring Study, the Health Professionals Follow-up Study, and the Ludwigshafen Risk and Cardiovascular Health (LURIC) study.

In the Framingham Offspring Study (FOS), researchers prospectively studied the association of serum vitamin D with cardiovascular disease events in 1,739 elderly participants. Cardiovascular disease events were significantly higher (p=0.01) in participants with <15 ng/mL 25(OH) vitamin D (Wang et al), supporting the association of hypovitaminosis D with CVD. Similarly, in the Health Professionals Follow-up Study (HPFS), researchers used a case-control study to evaluate the effects of serum 25(OH) vitamin D on heart attack outcomes. There were 454 cases of heart attack during the 10 year follow-up. Cases and controls were divided by 4 categories of common definitions of "deficient" (≤15 ng/mL), "insufficient" (15.1-29.9 ng/mL), and "sufficient" (≥30 ng/mL). After multivariate adjustment, deficient men had an increased risk of a heart attack compared to men with sufficient vitamin D (relative RR, 2.42; 95% confidence interval, 1.53-3.84; P<.001 trend) (Giovannucci et al). In the LURIC study, 3,258 participants were measured for 25(OH) vitamin D and 1,25(OH) vitamin D at baseline and followed up for cardiovascular mortality. Participants in the lowest quartile of 25(OH) vitamin D and 1,25(OH) vitamin D were higher for cardiovascular mortality when compared to the highest quartile (hazard ratio [HR], 2.08; 95% CI 1.60-2.70; HR, 1.61; 95% CI 1.25-2.07, respectively) (Dobnig et al).

Pieces of our study support the evidence in these studies; however, the majority of our data show only a weak association between vitamin D and cardiovascular disease. Possible explanations for weak findings include the following: (1) differences in demographic make-up of the populations being studied, (2) the use of different thresholds of vitamin D levels for evaluating the effects of vitamin D, and (3) insufficient vitamin D levels to adequately measure a protective effect.

First, in the FOS and LURIC study, the cohort being studied was completely white and therefore may have limited making conclusions about the general population, especially as vitamin D deficiency and CVD rates are higher among many non-white populations (Martins et al, Zadshir et al, Irabarren et al, Wolf et al). Our study used recruitment strategies intended to result in a cohort that was representative of the population.

Second, in the Framingham Offspring Study, the effects of vitamin D were analyzed only at very low levels (<10 ng/mL and <15 ng/mL). The study had inadequate statistical power to address whether the therapeutic effects of vitamin D were also evident at mild insufficiency (15 ng/mL – 30 ng/mL) or sufficiency (>30 ng/mL) (Wang et al). In the HPFS, vitamin D was again divided based on deficiency (< 15 ng/mL), mild insufficiency (≥15 ng/mL – 29.9 ng/mL), and sufficiency (≥30 ng/mL) (Giovannucci et al). Conversely, in our study, vitamin D was divided into quartiles and was not studied at standard cut points as it was beyond the scope of our study. Whether the protective effects are more evident at certain thresholds is debatable. Nevertheless, establishing thresholds for effective dosages for vitamin D may have led to discrepancies between our results and the other studies.

Third, the weak inverse association between vitamin D and heart attack and stroke may also be the result of a general insufficiency in our population. Some researchers have argued that adequate dietary vitamin D consumption may need to be as high as 1000 IU/day; yet, between 400 IU/day to 600 IU/day is regarded as standard recommendation for elderly populations (Utiger, Thomas et al, Bischoff-Ferrari et al). In our study, our median dietary+supplemental vitamin D was 430.54 IU/day. Our distribution of dietary vitamin D was slightly elevated, yet still typical amongst elderly populations (Yetley, Hsia).

Like dietary vitamin D, serum vitamin D deficiency and sufficiency is not well defined. Serum 25(OH) vitamin D insufficiency and sufficiency have been set at <15ng/mL and >30ng/mL, respectively (Bischoff-Ferrari et al). The distributions of 25(OH) vitamin D in our study are similar to the results of the FOS and HPFS, but lower than NHANES III distributions (Wang et al, Giovannucci et al, Martins et al). Some studies have shown that the most advantageous levels of 25(OH) vitamin D begin around >30 ng/mL (75 nmol/L) (Birschoff-Ferrari et al, Zimmermann). Given that less than 25% had serum vitamin D at this level or higher, our results may be too low to reveal the stronger protective effects of

vitamin D realized at higher levels. Analysis of extremely low or extremely high levels of vitamin D was beyond the range of our study.

To our knowledge, the largest clinical control that has studied the effects of vitamin D and calcium supplementation on coronary and cerebrovascular events came from the Women's Health Initiative randomized trial. The results of this study, which found no reduction in risk for woman supplemented with calcium/vitamin D compared to controls, opposed the findings of the FOS, the HFPS and the LURIC study. In this study, women were randomized for either 500 mg calcium carbonate with 400 IU/day of dietary vitamin D or a placebo. After 7 years of follow-up, women assigned to calcium/vitamin D had no significant change in risk of myocardial infarction and coronary heart disease (hazard ratio, 1.04; 95% CI interval, 0.92 to 1.18) or stroke (hazard ratio, 0.95; 95% CI interval, 0.82 to 1.10) (Hsia et al).

Despite having a large sample size and following participants prospectively, the data from the Women's Health Initiative is limited, since it relies completely on dietary vitamin D. While dietary vitamin D and vitamin D supplementation can increase serum vitamin D levels, the amount of vitamin D obtained from UV exposure compared to dietary sources varies tremendously among different populations. Moreover, even current the recommended daily allowance of 400 IU is heavily debated. Some researchers have argued that the positive effects of dietary vitamin D are not significant until it is consumed at 1000 IU per day or higher (Zittermann et al). As less than 50% of the study participants from the Women's Health Initiative consumed more than 400 IU per day, the results from this study are even more limited.

While there are many similarities between the results of the Women's Health Initiative and our own study, our results generally support a weak inverse association of vitamin D and cardiovascular disease. Nevertheless, it is possible that the weak protective effect of vitamin D against heart attack and stroke was the result of confounders that were not included in the models. However, this is unlikely. Due to the large sample size, many of the standard confounders for heart attack and stroke that we used in our models did not

change our results by more than 5%, supporting the protective effects of vitamin D in our results. Another explanation for the discrepancy between the Women's Health Initiative results and our own may also be due to the population demographics. In our study, the population was confined to men 65 and older, while the Women's Health Initiative included only women, who were 50 and older. Between the sex and age differences, the discrepancy in our results may be explained. Probably the largest contributor to the differences in the dietary data from the Women's Health Initiative and our own is due to the inconsistencies of testing dietary vitamin D. However, this will be discussed in further detail later.

BIOLOGICAL EXPLANATION

Despite the lack of a strong association between vitamin D and outcomes of heart attack and stroke in our study, there are three biological explanations for why vitamin D may protect against heart attack and stroke: (1) the effects vitamin D may have on blood coagulation, (2) the role of vitamin D in the renin-angiotensin system (RAS), and (3) the association of vitamin D with inflammatory response elements that lead to atherosclerosis.

First, many researchers studying the link between vitamin D and the prevention of heart attack and stroke point to vitamin D's anti-thrombotic properties as the therapeutic mechanism. Vitamin D acts as a ligand for the vitamin D receptor that binds to genes, which encode proteins that control coagulation in the blood. In *in vitro* studies, vitamin D was found to upregulate thrombomodulin (Koyoma et al, Ohsawa et al). Thrombomodulin is crucial in the activation of protein C and protein S, which provides negative feedback to coagulation. Moreover, thrombomodulin also deactivates thrombin and consequently inhibits the conversion of fibrin to fibrinogen and further coagulation in the blood. Besides vitamin D's role in regulating thrombomudulin, there is also evidence to suggest that vitamin D inhibits transcription factors related to the genes coding for tissue factor, which initiates coagulation in the blood (Ohsawa et al, Chung et al). In the past 5 years, research on vitamin D's anticoagulant properties was expanded to *in vivo* models with vitamin D receptor knockout (VDRKO) mice. Researchers found that

VDRKO mice experienced increased platelet aggregation and tissue factor expression when compared to wild type mice. Additionally, when mice were injected with lipopolysaccharide (LPS), VDRKO mice developed multi-organ thrombus formation (Aihara et al).

Besides vitamin D's role in thrombus reduction, vitamin D may protect against heart attack and stroke by regulating blood pressure through the renin-angiotensin system (RAS). Research on vitamin D and the RAS pathway, shows that vitamin D inhibits the production of renin in the kidneys (Li et al). Since renin initiates the RAS pathway, which leads to vasoconstriction and increased water reabsorption in the kidneys, researchers believe that vitamin D may indirectly regulate blood pressure via the RAS pathway. When researchers tested their *in vitro* findings on vitamin D receptor knockout (VDRKO) mice, they found that VDRKO mice had significantly higher levels of renin expression and incidents of hypertension (Li et al). Similarly, when wild-type were injected with calcitriol, renin transcription was down-regulated (Li et al). In clinical studies, after calcitriol was administered to high-renin hypertensive patients, a reduction in blood pressure was observed (Burgess et al, Resnick et al, Kimura et al).

Vitamin D may also have a regulatory role in the inflammatory processes that lead to atherosclerosis. Experimental data show that calcitriol has been shown to suppress the release of the pro-inflammatory cytokine interleukin 6 (IL-6) (Muller et al). In both *in vivo* studies and epidemiological studies, IL-6 has been linked with artherosclerosis (Huber et al, Jenny et al). In epidemiological studies, IL-6 has been associated with myocardial infarction (Ridker et al), stroke (Cesari et al), and cardiovascular disease mortality (Cesari et al, Harris et al). Besides IL-6, calcitriol may enhance anti-inflamatory IL-10 expression in epidermal cells (Michel et al), while down-regulating IL-10 in the absence of calcitriol (Canning et al). In *in vivo* models, IL-10 deficiency is associated with atherosclerosis (Mallat et al). Epidemiological studies have shown that IL-10 has been found to be strongly inversely correlated with acute coronary syndromes (Heeschen et al) and stroke (Van Exel et al).

LIMITATIONS

There are some limitations that are specific to our study that deserve to be mentioned. Just like any cross-sectional study, our prevalent data may be affected by a cause-and-effect bias. When interpreting cross-sectional outcomes, it cannot be determined if vitamin D status affects disease outcomes or if the disease outcomes affect vitamin D status. This may be especially relevant to our cross-sectional vitamin D analysis, where dietary vitamin D was a strong indicator of heart attack (p=0.13) and stroke (p=0.04). Due to cause-and-effect bias, prevalent cases may have changed their vitamin D consumption and supplementation after the event. However, if vitamin D consumption and supplementation was not altered after the event, we could assume that vitamin D may protect against heart attack and stroke. This assumption cannot be made, though, due to limitations in our data. For this reason, our longitudinal analysis is a more valid method of analysis as it captures the vitamin D profile of the participant before the incident occurs. Nevertheless, with such a large sample size, it still remains a valid method of analysis.

Given that our non-fatal outcomes were self-reported, participants may have been affected by recall bias, which could have altered the results by a patient over-reporting or under-reporting a prior episode. It is possible that a patient with a history of symptoms of a heart attack or stroke, but without being diagnosed by a health professional, as the questionnaire states, may have falsely reported a history. While these may have detrimentally altered the results, we were able to account for false-positives two separate ways. First, it is very possible that the same mechanism that contributed to a heart attack or stroke would also contribute to the symptoms of a false positive, and therefore, one could assume that the effects of vitamin D could still be studied. However, this is beyond the scope of our study. Second, it can be assumed that participants that reported a false positive at the follow-up visit would have done the same at baseline and would have been removed from the longitudinal analysis for having a previous history of an incidence.

Vitamin D is ingested through dietary sources, such as milk or fortified cereals, or may be endogenously produced from the skin by sunlight exposure. However, the exact relation between dietary intake and the effect on serum vitamin D levels remains inconclusive, and certainly varies between geographic regions and even individuals. Therefore, using dietary vitamin D as an indicator of serum vitamin D levels has its limitations. As a result, serum 25(OH)D remains the standard for reporting vitamin D levels. Although calcitriol (1,25(OH) vitamin D) is the metabolically active form, it breaks down too quickly to be a reliable source of measurement. 25-OH Vitamin D is the calcitriol precursor and is much more stable in the body, having a half life of 12-19 days (Zittermannn). Even though 25(OH) vitamin D is currently the best measure for vitamin D, it still may be problematic to extrapolate serum 25-OH vitamin D levels at baseline to represent vitamin D levels during the follow-up period, especially across seasons. Despite these inherent limitations of both dietary and serum vitamin D sampling, our large population may soften the effects of under-reporting and over-reporting dietary vitamin D or the variations of serum vitamin D due to the season or weather at the time the sample was taken.

PERSPECTIVE

As cardiovascular disease continues to be leading cause of death in the world and burden our health care systems, it is essential that we find effective preventative treatments. While there is growing evidence to support a protective effect of vitamin D against CVD in cohort studies, the results have not been supported in clinical trials. In light of these contradictions, more studies and clinical trials are required to make definitive claims about the association of vitamin D with cardiovascular disease.

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