Relation Between Serum 25-Hydroxyvitamin D and C-Reactive Protein in Asymptomatic Adults (From the Continuous National Health and Nutrition Examination Survey 2001 to 2006)

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The inverse relation between vitamin D supplementation and inflammatory biomarkers among asymptomatic adults is not settled. We hypothesized that the inverse relation is present only at lower levels and disappears at higher serum levels of vitamin D. We examined the relation between 25-hydroxyvitamin D [25(OH)D] and C-reactive protein (CRP) using the continuous National Health and Nutrition Examination Survey data from 2001 to 2006. Linear spline [single knot at median serum levels of 25(OH)D] regression models were used. The median serum 25(OH)D and CRP level was 21 ng/ml (interquartile range 15 to 27) and 0.21 mg/dl (interquartile range 0.08 to 0.5), respectively. On univariate linear regression analysis, CRP decreased [geometric mean CRP change 0.285 mg/dl for each 10-ng/ml change in 25(OH)D, 95% confidence interval [CI] −0.33 to −0.23] as 25(OH)D increased ≤21 ng/ml. However, an increase in 25(OH)D to >21 ng/ml was not associated with any significant decrease [geometric mean CRP change 0.05 mg/dl for each 10-ng/ml change in 25(OH)D, 95% CI −0.11 to 0.005) in CRP. The inverse relation between 25(OH)D below its median and CRP remained significant [geometric mean CRP change 0.11 mg/dl for each 10-ng/ml change in 25(OH)D, 95% CI 0.16 to −0.04] on multivariate linear regression analysis. Additionally, we observed a positive relation between 25(OH)D above its median and CRP [geometric mean CRP change 0.06 mg/dl for each 10-ng/ml change in 25(OH)D, 95% CI 0.02 to 0.11) after adjusting for traditional cardiovascular risk factors. In conclusion, from this cohort of asymptomatic adults, independent of traditional cardiovascular risk factors, we observed a statistically significant inverse relation between 25(OH)D at levels <21 ng/ml and CRP. We found that 25(OH)D at a level ≥21 ng/ml is associated with an increase in serum CRP. It is possible that the role of vitamin D supplementation to reduce inflammation is beneficial only among those with a lower serum 25(OH)D. **(Am J Cardiol 2012;109:226–230)**

The cardiovascular protection offered by vitamin D and its analogues is probably mediated by modulation of inflammatory cytokines. Vitamin D receptors are expressed by both monocytes and endothelial cells.[1,2] In various cell lines, including monocytes, vitamin D supplementation decreases the production of inflammatory mediators.[3–6] However, observational studies have revealed variable results for the association between vitamin D and inflammation.[7–11] In addition, randomized controlled trials of vitamin D supplementation have shown inconsistent results, with some trials suggesting a decrease and others concluding no effect on inflammatory biomarkers.[12–16] One of the several factors that can explain these conflicting findings is the possibility that the beneficial effect of vitamin D supplementation is present only in those with lower vitamin D levels and not in those with adequate or higher serum vitamin D levels. We hypothesized that among healthy adults, the favorable association of vitamin D and inflammatory biomarkers exist at relatively lower, but not at higher, serum levels of vitamin D. To test this hypothesis, we studied the relation between 25-hydroxyvitamin D [25(OH)D] and C-reactive protein (CRP), a biomarker of inflammation, in a healthy adult United States population.

Methods

We used publically available data from the continuous National Health and Nutrition Examination Survey (NHANES), an ongoing, multistage, probability sample survey designed to assess the health and nutritional status of the civilian, noninstitutionalized population of the United States. Detailed interviews, physical examinations, and serum samples were obtained from >17,000 subjects in the survey conducted from 2001 to 2006. Details of sampling procedures and data collection techniques have been described previously and are available online (available at: http://www.cdc.gov/nchs/nhanes/about_nhanes.htm, accessed March 10, 2011). To create a large nationally representative sample, the data from three 2-year cycles of continuous NHANES survey were combined from 2001 to 2006. Sample weights were constructed, with rescaling of weights such that sum of weights matched the survey population at the midpoint of each survey period.

The demographic information was ascertained from self-reported responses to the questionnaire administered by trained interviewers. The body mass index was calculated

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by dividing the body weight in kilograms by the height in meters squared. Obesity was defined as a body mass index of $\geq$30 kg/m^2 and overweight if the body mass index was $\geq$25 kg/m^2 but $<$30 kg/m^2. Blood pressure was recorded for $\leq$4 readings. Hypertension was defined as a mean systolic blood pressure of $\geq$140 mm Hg, mean diastolic blood pressure of $\geq$90 mm Hg, a diagnosis of hypertension, or the current use of antihypertensive medications. The glomerular filtration rate was calculated using the Modification of Diet in Renal Disease formula.[17] The serum glucose level was measured using the Beckman Synchron LX20 test (Beckman Coulter, Brea, CA) on refrigerated specimens, and the cholesterol level was measured enzymatically in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. The participants were categorized as smokers if they were currently smoking or had ever smoked $>$100 cigarettes. The Beckman Coulter method was used to determine the white blood cell count combined with automatic diluting and mixing device for sample processing. The DiaSorin (formerly Incstar) radioimmunoassay (Nutritional Biochemistry Branch, Division of Laboratory Sciences, National Center for Environmental Health, Atlanta, Georgia), a 2-step procedure, was used to assay 25(OH)D. The first procedure involved extraction of 25(OH)D and other hydroxylated metabolites from serum with acetonitrile. Next, the treated sample was assayed using an equilibrium radioimmunoassay procedure. Updated and adjusted data files were used for 25(OH)D to address assay drift. CRP was quantified using latex-enhanced nephelometry and a Behring Nephelometer II Analyzer (Immunology Division, Department of Laboratory Medicine, University of Washington Medical Center, Seattle, Washington).[18] CRP was log-transformed to meet the assumptions of residual normality.

All analyses were performed with adjustments for the complex survey sampling method of the NHANES data. In continuous NHANES, primary sampling units represent variance (sampling units used to estimate sampling error) units. These sampling weights were assigned to each subject, reflecting adjustment for the unequal probability of selection and nonresponse and adjustments to independent population controls. The participants were oversampled for certain population subgroups, especially the subgroups of population such as black and Mexican Americans, to ensure reliability and precision of the health estimates indicators in these subgroups. Masked variance units were constructed to protect the confidentiality of data obtained from sample participants. Masked variance units, used to define the strata, were created for each 2-year cycle of the continuous NHANES data, allowing any combination of data cycles without recoding by the users. The sample weights were constructed for 6 years of combined survey data.

To address the hypothesis that the favorable association between vitamin D and serum CRP is present only at lower serum vitamin D level, we introduced a spline in the regression models, with single knot at the population median of 25(OH)D (i.e., 21 ng/ml), hypothesizing that this association exists up to the population median but not above it. Analyses were performed using Stata/IC, version 10.1 (StataCorp, College Station, Texas) using survey-specific commands. Adjusted coefficients and their 95% confidence intervals (CIs) were estimated using univariate and multivariate linear regression models. The analysis was limited to subjects $>$18 years old and adjusted for demographic variables, obesity, hypertension, serum glucose, cholesterol, smoking, white blood cell count, and renal function. A p value of $<$0.05 was considered statistically significant.

Results

After excluding the participants who were $<$18 years old, the population size of the continuous NHANES 2001 to 2006 survey was 17,176. We also excluded participants with missing values for vitamin D (n = 1,993) and CRP (n = 16), leaving 15,167 participants in the sample (Table 1). The participants had a mean $\pm$ SD age of 46.1 $\pm$ 20.4 years, and the median serum 25(OH)D and CRP level was 21 ng/ml (interquartile range 15 to 27) and 0.21 mg/dl (interquartile range 0.08 to 0.5), respectively. In the final data set, 7,849 (52%) were women and 7,318 (48%) were men. We found no difference in the serum 25(OH)D levels between the women and men (mean difference $-$0.23 ng/ml, 95% CI $-$0.61 to 0.15; p = 0.23) in the overall sample. The mean serum 25(OH)D level was significantly higher (p $<$0.001) in whites (25.6 ng/ml) than in blacks (14.5 ng/ml).

In a univariate linear regression model with spline [single knot at 21 ng/ml of 25(OH)D; median in the population], CRP decreased [geometric mean CRP change 0.285 mg/dl for each 10-ng/ml change in 25(OH)D, 95% CI 0.33 to $-$0.23; p $<$0.001] as the serum 25(OH)D level increased $\leq$21 ng/ml. An increase in 25(OH)D beyond its median (21 ng/ml) was not associated with any statistically significant decrease [geometric mean CRP change 0.05 mg/dl for each 10-ng/ml change in serum 25(OH)D, 95% CI 0.11 to 0.005; p = 0.07] in the CRP Level (Table 2 and Figure 1).

The inverse relation between 25(OH)D below its median serum levels and CRP remained unchanged [geometric mean CRP change 0.105 mg/dl for each 10-ng/ml change in serum 25(OH)D, 95% CI 0.162 to $-$0.044; p $<$0.001) on multivariate linear regression analysis. In contrast, we observed a direct relation between a serum 25(OH)D level above the median and CRP [geometric mean CRP change 0.06 mg/dl for each 10-ng/ml change in serum 25(OH)D, 95% CI 0.02 to 0.11; p = 0.005), after adjusting for traditional cardiovascular risk factors in the multivariate linear regression model (Table 2).

Discussion

In the present, large, cross-sectional study of a nationally representative sample of adult United States population, we found an inverse relation between vitamin D and CRP in asymptomatic adults with low vitamin D levels. However, the relation reversed once the vitamin D increased above the population median (i.e., 21 ng/ml), after which any additional increase in vitamin D was not associated with a decrease, but rather an increase, in the CRP levels. Our finding of a direct relation between vitamin D and CRP in the present study suggests that vitamin D levels above the population median might be proinflammatory.

Vitamin D and its analogues inhibit the production of several proinflammatory cytokines that modulates the tissue-

Table 1
Population characteristics

Covariate	Vitamin D (ng/ml)		p Value
	<21 (n = 7,347)	≥21 (n = 7,820)	
Age (years)			0.5
Mean	45.1	45.4	
95% CI	44.6–45.5	45–45.7	
Age group			
≥65 years	1,571 (16%)	1,936 (16%)	0.99
≥45 years but <65 years	1,805 (29.9%)	1,905 (30.8%)	0.45
<45 years	3,971 (54.1%)	3,979 (53.2%)	0.44
Women	3,917 (54.6%)	3,932 (49.8%)	<0.0001
Race			
White	2,223 (53%)	5,341 (84.3%)	<0.0001
Mexican American	1,906 (11.2%)	1,377 (5.5%)	<0.0001
Black	2,619 (22.9%)	596 (3.2%)	<0.0001
Other Hispanics	258 (5.3%)	262 (3.5%)	0.005
Other	341 (7.6%)	244 (3.4%)	<0.0001
Hypertension*	2,839 (37.8%)	2,732 (32.3%)	<0.0001
CRP (mg/dl)			<0.001
Mean	0.22	0.17	
95% CI	0.21–0.23	0.162–0.171	
Serum glucose (mg/dl)			<0.0001
Mean	95.7	91.5	
95% CI	95.2–96.3	91.2–91.9	
Body mass index (>30 kg/m^2)	2,757 (41.4%)	1,895 (25.5%)	<0.0001
Glomerular filtration rate (ml/min/m^2)†			<0.001
Mean	100.1	93.4	
95% CI	99.4–100.7	92.8–94	
Total cholesterol (mg/dl)			0.001
Mean	198.6	201.6	
95% CI	197.6–199.7	200.7–202.5	
White blood cell count (1,000 cells/μL)			0.16
Mean	7.06	6.99	
95% CI	7.02–7.11	6.94–7.04	
Smoker‡	1,296 (22.35%)	1,199 (18.47%)	0.004

* Defined as average systolic blood pressure >140 mm Hg, average diasystolic blood pressure >90 mm Hg, subjects ever told they had hypertension, or if participants were receiving antihypertensive treatment.
† Modification of Diet in Renal Disease equation.
‡ Participants who were current or had ever smoked.

Table 2
β Coefficients with p values and 95% confidence intervals (CIs) log C-reactive protein (CRP) for vitamin D (spline at 21 ng/ml) from univariate and multivariate linear regression models

Log CRP Model	Vitamin D <21 ng/mL			Vitamin D ≥21 ng/mL		
	β Coefficient	p Value	95% CI	β Coefficient	p Value	95% CI
1	−0.34	<0.001	−0.41, −0.26	−0.05	0.07	−0.11, 0.01
2	−0.30	<0.001	−0.37, −0.23	−0.05	0.08	−0.11, 0.01
3	−0.29	<0.001	−0.36, −0.22	−0.04	0.14	−0.09, 0.01
4	−0.26	<0.001	−0.34, −0.19	−0.03	0.34	−0.08, 0.03
5	−0.16	<0.001	−0.23, −0.08	0.05	0.02	0.01, 0.09
6	−0.15	<0.001	−0.23, −0.07	0.06	0.01	0.01, 0.10
7	−0.15	<0.001	−0.23, −0.08	0.05	0.02	0.01, 0.09
8	−0.11	0.003	−0.19, −0.04	0.08	0.001	0.034, 0.12
9	−0.11	0.001	−0.18, −0.05	0.06	0.005	0.02, 0.10

Model 1 was a univariate model with vitamin D spline at 21 ng/ml; model 2 included model 1 plus age, gender, and race; model 3 included model 2 plus hypertension; model 4 included model 3 plus serum glucose; model 5 included model 4 plus obesity (body mass index >30 kg/m^2); model 6 included model 5 plus the glomerular filtration rate; model 7 included model 6 plus total cholesterol; model 8 included model 7 plus smoking status; and model 9 included model 8 plus white blood cell count.

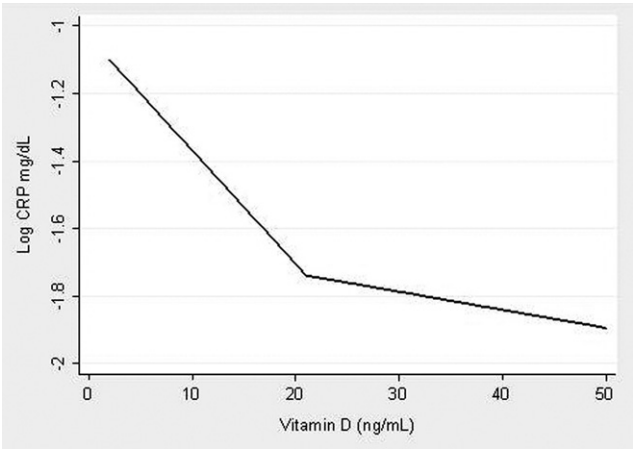

Figure 1. Graph showing relation between log CRP and serum 25(OH)D from univariate regression model spline at population median of vitamin D (i.e., 21 ng/ml).

specific immune response and restrict inflammation.[19–22] The metabolic activities of vitamin D are mediated by its binding to high-affinity vitamin D receptor (VDR) that acts as a ligand-activated transcription factor. Deficiency of both vitamin D and VDR leads to the development of certain autoimmune diseases.[19] It has been suggested that the influence of vitamin D in reducing inflammation is by modulating the expression of several cytokine genes controlled by the VDR.[22–26] Vitamin D, and its analogues, inhibit production of interleukin-2 and interferon-γ and stimulate the effects of T-helper type 2 lymphocytes, which leads to a reduction in matrix metalloproteinase, restricting atherosclerotic plaque progression.[22] By exposing mesenchymal multipotent cells to the active form of vitamin D, Artaza et al[23] observed increased expression and translocation of VDR and decreased profibrotic signaling pathways and gene expression. In human monocytes, VDR produces downregulation of interleukin-10 expression by binding to the promoter region of interleukin-10 transcription site.[24]

Studies conducted in subjects with a wider range of serum vitamin D levels have shown variable results regarding the association of vitamin D and inflammatory cytokines and cardiovascular outcomes.[23,27] In a cross-sectional study, no reduction in subclinical vascular disease and CRP levels was seen among those with an overall mean vitamin D level of 22.3 $\pm$ 6.8 ng/ml. The investigators from the study defined 25(OH)D deficiency at levels $<$20 ng/ml and at 21 to 30 ng/ml; no participant was taking vitamin D supplementation.[11] Jorde et al[12] could not find any reduction in inflammatory cytokines in the subjects taking dual-dose vitamin D supplementation for 1 year (median before supplementation 22.5 ng/ml, interquartile range 6.42 to 57; and median after supplementation 31 ng/ml, interquartile range 6.02 to 67.5). After vitamin D supplementation for 10 weeks to subjects with mean vitamin D levels of 20.5 $\pm$ 8.9 ng/ml at baseline, the investigators failed to report a reduction in tumor necrosis factor when measured at 10- and 20-week intervals (mean vitamin D level 22.9 $\pm$ 22.0 ng/ml at 10 weeks and 19.5 $\pm$ 13.9 ng/ml at 20 weeks).[16] In contrast, Tarcin et al[28] found that 3-month supplementation of vitamin D to patients with very low serum vitamin D levels (mean 10 ng/ml) resulted in improvement in endothelial function and a decrease in the markers of oxidative stress and insulin sensitivity.

From our results, it appears that vitamin D supplementation among asymptomatic subjects with baseline vitamin D values of $\geq$21 ng/ml might have no additional effects on systemic inflammation, as measured by changes in the serum CRP levels. The difference in the relation between vitamin D and CRP levels at $<$21 and $>$21 ng/ml probably speak to the null findings of the anti-inflammatory properties of vitamin D from recent studies.[10–12,27]

The strengths of our study include its large sample size and wide age range (18 to 85 years). The sampling scheme of the NHANES allowed better estimates and adjustments for various ethnic backgrounds. We selected covariates according to previous evidence linking vitamin D deficiency and inflammation. However, given the cross-sectional analysis, our study could not examine a temporal relation between vitamin D and CRP. One potential limitation was that we did not adjust for the geographic location of the participants or factors such as weather and latitude, and the time of the year might have influenced the observed relation. In addition, we used single inflammatory marker to explore the association of vitamin D and inflammation. However, because other biomarkers of inflammation were available in a smaller subset in the data set, which could have limited the number of participants, and given that CRP is probably 1 of the best known inflammatory biomarkers, we decided to focus on CRP only.

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