Relationship of 25-hydroxyvitamin D with all-cause and cardiovascular disease mortality in older community-dwelling adults

Richard D. Semba, M.D., **Denise K. Houston, Ph.D.**, **Stefania Bandinelli, M.D.**, **Kai Sun, M.S.**, **Antonio Cherubini, M.D.**, **Anne R. Cappola, M.D.**, **Jack M. Guralnik, M.D.**, and **Luigi Ferrucci, M.D.**

Department of Ophthalmology, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Section on Gerontology and Geriatric Medicine, Department of Internal Medicine, Wake Forest University, Winston-Salem, NC

Azienda Sanitaria, Florence, Italy

Institute of Gerontology and Geriatrics, University of Perugia, Perugia, Italy

Division of Endocrinology, Diabetes, and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA

Epidemiology and Demography Section, Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Bethesda, MD

Longitudinal Studies Section, National Institute on Aging, Baltimore, MD

Abstract

Background/Objectives—Vitamin D deficiency is associated with cardiovascular disease, osteoporosis, poor muscle strength, falls, fractures, and mortality. Although older adults are at a high risk of vitamin D deficiency, the relationship of serum 25(OH)D with all-cause and cardiovascular disease mortality has not been well characterized in the elderly. We hypothesized that low serum 25(OH)D predicted mortality in older adults.

Subjects/Methods—Serum 25(OH)D and all-cause and cardiovascular disease mortality were examined in 1006 adults, 65 years, who participated in the InCHIANTI study, a population-based, prospective cohort study of aging in Tuscany, Italy. Serum 25(OH)D was measured at enrollment in 1998-1999, and participants were followed for mortality.

Results—During 6.5 years of follow-up, 228 (22.7%) participants died, of whom 107 died from cardiovascular disease. Compared with participants in the highest quartile of serum 25(OH)D (>26.5 ng/mL)(to convert to nmol/L, multiply by 2.496), those in the lowest quartile (<10.5

Correspondence to: Dr. Richard Semba, 550 N. Broadway, Suite 700, Baltimore, MD 21205. Tel. (410) 955-3572, Fax (410) 955-0629, rdsemba@jhmi.edu.

Conflict of interest None of the authors had a conflict of interest.

ng/mL) had increased risk of all-cause mortality (Hazards Ratio [H.R.] 2.11, 95% Confidence Interval [C.I.] 1.22 – 3.64, P = 0.007) and cardiovascular disease mortality (H.R. 2.64, 95% C.I. 1.14 – 4.79, P = 0.02), in multivariate Cox proportional hazards models that adjusted for age, sex, education, season, physical activity, and other potential confounders.

Conclusions—Older community-dwelling adults with low serum 25(OH)D are at higher risk of all-cause and cardiovascular disease mortality.

Keywords

Aging; all-cause mortality; cardiovascular disease mortality; vitamin D

Introduction

Worldwide, the population of persons aged 65 years and older was 420 million in 2000, and it is expected to increase to 973 million by 2030 (Ferrucci *et al*. 2008). By 2030, it is estimated that persons 65 years and older will comprise over 20% of the population of North America (Ferrucci *et al*. 2008). Vitamin D deficiency, as assessed by low serum 25-hydroxyvitamin D (25[OH]D) levels, the standard measure of vitamin D status, is highly prevalent among older, community-dwelling adults (Zadshir *et al*. 2005; Holick, 2006) and has an extremely high prevalence among those who are institutionalized (Gloth *et al*. 1995; Thomas *et al*. 1998). Older adults may be at higher risk of vitamin D deficiency because both the level of outdoor activity (Scragg and Camargo, 2008) and cutaneous production of vitamin D (MacLaughlin and Holick, 1985) decrease with age. Vitamin D deficiency will be a growing public health problem because of the global shifts towards an aged population in much of the world.

Vitamin D deficiency is associated with an increased risk of osteopenia, osteoporosis, muscle weakness, poor physical performance (Houston *et al*. 2007), falls (Bischoff-Ferrari, *et al*. 2004), and fractures (Bischoff-Ferrari *et al*. 2005). Low serum 25(OH)D levels are associated with hypertension (Lind *et al*. 1995; Martins *et al*. 2007), coronary heart disease (Kim *et al*. 2008), myocardial infarction (Giovannucci, *et al*. 2008), heart failure (Zittermann *et al*. 2007; Kim *et al*. 2008), stroke (Poole *et al*. 2006, Pilz *et al*. 2008), and independently predict cardiovascular disease (Wang *et al*. 2008). A recent study showed that patients undergoing coronary angiography at a tertiary care center who had low serum 25(OH)D levels were at higher risk of all-cause and cardiovascular disease mortality (Dobnig *et al*. 2008). Participants, aged 20 years and older, in the Third National Health and Nutrition Examination Survey (NHANES III), who had lower serum 25(OH)D levels were at an increased risk of all-cause but not cardiovascular disease mortality (Melamed *et al*. 2008).

Although vitamin D deficiency is highly prevalent in the elderly, the relationship between low serum 25(OH)D levels and mortality has not been well characterized. We hypothesized that older, community-dwelling men and women with low serum 25(OH)D levels were at higher risk of all-cause and cardiovascular disease mortality. To address this hypothesis, we examined the relationship between serum 25(OH)D and mortality in a population-based sample of older, community-dwelling men and women in Tuscany, Italy.

Subjects and Methods

The study subjects consisted of men and women, aged 65 and older, who participated in the Invecchiare in Chianti, "Aging in the Chianti Area" (InCHIANTI) study, conducted in two small towns in Tuscany, Italy. The rationale, design, and data collection have been described elsewhere, and the main outcome of this longitudinal study is mobility disability (Ferrucci *et al*. 2000). Briefly, in August 1998, 1270 people aged 65 years and older were randomly selected from the population registry of Greve in Chianti (pop. 11,709) and Bagno a Ripoli (pop. 4,704), and of 1,256 eligible subjects, 1,155 (90.1%) agreed to participate. Of the 1,155 participants, 1,043 (90.3%) participated in the blood drawing. During 6.5 years of follow-up, 228 participants died, 34 refused to participate in the study and 16 moved away from the area. Those who refused to participate in follow-up or moved away were known to be alive at the time of censoring of this analysis. Participants received an extensive description of the study and participated after written, informed consent. The study protocol complied with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee and by the Institutional Review Board of the Johns Hopkins University School of Medicine.

Demographic information, including total years of formal education, and information on smoking and medication use were collected using standardized questionnaires. All participants were examined by a trained geriatrician, and diseases were ascertained according to standard, pre-established criteria and algorithms based upon those used in the Women's Health and Aging Study for diabetes mellitus, coronary heart disease, chronic heart failure, stroke, and cancer (Guralnik *et al*. 1995).

Weight was measured using a high-precision mechanical scale. Standing height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Mini-Mental State Examination (MMSE) was administered at enrollment, and an MMSE score <24 was considered consistent with cognitive impairment (Folstein *et al*. 1975). Physical activity in the previous year was based upon responses to multiple questions and was rated as sedentary (inactive or light-intensity physical activity such as walking or light housework <1 h/wk), light physical activity (light physical activity 2-4 h/wk), and moderate-high physical activity (light physical activity >4 h/wk and/or moderate physical activity such as brisk walking, playing soccer, gardening   1 h/wk) (Ruggiero *et al*. 2007). Season of the year (winter: December-February; spring: March-May; summer: June-August; autumn: September-November) was included as a categorical variable to account for seasonal effects on vitamin D levels. Renal insufficiency was defined as estimated glomerular filtration rate of <60 mL/min/1.73 m^2 using the four-variable Modification of Diet in Renal Disease Study equation of Levey and colleagues (Levey *et al*. 1999).

Blood samples were collected in the morning after a 12-h fast. Aliquots of serum and plasma were immediately obtained and stored at -80° C. Serum 25(OH)D was measured at enrollment and the 3-year follow-up visit using a radioimmunoassay (DiaSorin, Stillwater, MN) with intra-assay and inter-assay coefficients of variation (CV) of 8.1% and 10.2%, respectively (Maggio *et al*. 2005). Serum intact PTH levels were measured with a two-site immunoradiometric assay kit (N-tact PTHSP, DiaSorin) with intra-assay and inter-assay

CVs of <3.0% and 5.5%, respectively. Serum creatinine was measured by a standard creatinine Jaffe method (Roche Diagnostics, Mannheim, Germany). Commercial enzymatic tests (Roche Diagnostics) were used for measuring serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol concentrations. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula (Friedewald *et al.* 1972).

Participants were evaluated again for a three-year follow-up visit from 2001-2003 (n = 926) and six-year follow-up visit from 2004-2006 (n = 844). At the end of the field data collection, mortality data of the original InCHIANTI cohort were collected using data from the Mortality General Registry maintained by the Tuscany Region and the death certificates that are deposited immediately after the death at the Registry office of the Municipality of residence. Cardiovascular disease mortality was defined by the death codes 390–459 from the 9th version of the International Classification of Diseases (ICD) (U.S. Department of Health and Human Services, 2006).

Variables are reported as medians (25th, 75th percentiles) or as percentages. Serum 25(OH)D was divided into quartiles: <10.5, 10.5-16.0, 16.1-25.6, >25.6 ng/mL. Characteristics of subjects were compared between groups using Kruskal-Wallis tests. Cox proportional hazards models were used to examine the relationship between serum 25(OH)D and all-cause and cardiovascular disease mortality. We adjusted for possible confounders in multivariate Cox proportional hazards models for mortality using conventional cardiovascular risk factors. The models were tested for possible interactions and collinearity, and no significant interactions or collinearity were found. Pearson correlation was used to examine the correlation between serum 25(OH)D measurements at enrollment and 3 year follow-up. Kaplan-Meier curves were used for survival by quartile of serum 25(OH)D, and survival curves were compared using log-rank tests. The statistical program used was SAS (SAS Institute, Cary, NC). The level of significance used in this study was P <0.05.

Results

The characteristics of participants by quartile of serum 25(OH)D at enrollment are shown in Table 1. Participants in the lower quartiles of serum 25(OH)D were older, more likely to be female, current non-smokers, physically inactive, and more likely to have low education and MMSE scores, heart failure, stroke, or renal insufficiency. Participants in the lower quartiles of serum 25(OH)D were more likely to have had their serum vitamin D measured in the autumn or winter. The participants did not have any significant differences across quartile of serum 25(OH)D by total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides, or by prevalence of hypertension, angina, peripheral artery disease, diabetes mellitus, or cancer.

During 6.5 years of follow-up, 228 (22.7%) of 1,006 participants died. The main causes of death were cardiovascular disease (47.3%), cancer (24.1%), respiratory disease (9.0%), and other (19.2%), and unknown (0.4%). The survival of participants by quartile of serum 25(OH)D for all-cause mortality is shown in Figure 1. Participants in the lowest quartile of serum 25(OH)D had significantly shorter survival than participants in the highest quartile of serum 25(OH)D for all-cause mortality. In multivariate Cox proportional hazards models

adjusting for age, sex, education, and season, and additionally for BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score, and chronic diseases, participants in the lowest quartile of serum 25(OH)D had a significantly increased risk of all-cause mortality compared with participants in the highest quartile of serum 25(OH)D (Table 2). Participants in the second and third quartile of serum 25(OH)D also had a higher risk of all-cause mortality compared with those in the highest quartile of serum 25(OH)D, but the results did not reach statistical significance for those in the second quartile of serum 25(OH)D.

During follow-up, 107 participants died from cardiovascular diseases. The survival of participants by quartile of serum 25(OH)D for cardiovascular disease mortality is shown in Figure 2. Participants in the lowest quartile of serum 25(OH)D had significantly shorter survival than participants in the highest quartile of serum 25(OH)D for cardiovascular disease mortality. In multivariate Cox proportional hazards models adjusting for age, sex, education, and season, and additionally for BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score, and renal insufficiency, participants in the lowest quartile of serum 25(OH)D had a significantly increased risk of cardiovascular disease mortality compared with participants in the highest quartile of serum 25(OH)D (Table 3). Participants in the second and third quartile of serum 25(OH)D also had a higher risk of cardiovascular mortality compared with those in the highest quartile of serum 25(OH)D, but the results did not reach statistical significance for those in the second quartile of serum 25(OH)D. There were 52 deaths from cancer, and quartiles of 25(OH)D were not significantly related with cancer mortality.

Median (25th, 75th percentile) serum PTH concentrations were 27.2 (19.7, 38.3) ng/L among participants who died from all causes compared to 21.6 (15.4, 30.3) ng/L among participants who survived ($P <0.0001$). Addition of PTH and calcium to the multivariate Cox proportional hazards model that also adjusted for age, sex, season, BMI, smoking, aspirin use, physical activity, total and HDL cholesterol, MMSE score, and chronic diseases did not substantially change the relationship between the highest and lowest quartile of serum 25(OH)D and mortality (H.R. 1.97, 95% C.I. 1.11-3.47, $P = 0.02$), or between the highest and second and highest and third quartiles, respectively (H.R. 1.51, 95% C.I. 0.89-2.57, $P = 0.13$; H.R. 1.88, 95% C.I. 1.15-3.09, $P = 0.01$).

Median (25th, 75th percentile) serum PTH concentrations were 28.2 (20.3, 39.8) ng/L among participants who died from cardiovascular diseases compared to 21.6 (15.4, 30.3) ng/L among participants who survived ($P <0.0001$). Addition of PTH and calcium to the multivariate Cox proportional hazards model that also adjusted for age, sex, education, season, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score, and renal insufficiency, did not substantially change the relationship between the highest and lowest quartile of serum 25(OH)D and mortality (H.R. 2.23, 95% C.I. 0.95-5.25, $P = 0.06$), or between the highest and second and highest and third quartiles, respectively (H.R. 1.58, 95% C.I. 0.71-3.53, $P = 0.26$; H.R. 2.11, 95% C.I. 1.01-4.43, $P = 0.049$).

In an alternative analysis, we excluded participants who died within one year of enrollment in the study in order to have a time lag. There were 160 participants who died from all causes after the one year time lag. In a multivariate Cox proportional hazards model for all-cause mortality that adjusted for age, sex, education, season, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score, and chronic diseases as in the final model in Table 2, the H.R. (95% C.I.) for 25(OH)D quartiles of <10.5, 10.5-16.0, 16.1-25.6 ng/mL were 1.82 (1.03, 3.21), 1.38 (0.80, 2.38), and 1.67 (1.01, 2.79), respectively. There were 79 participants who died from cardiovascular disease after the one year time lag. In a multivariate Cox proportional hazards model for cardiovascular mortality that adjusted for age, sex, education, season, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score, and renal insufficiency as in the final model in Table 4, the H.R. (95% C.I.) for 25(OH)D quartiles of <10.5, 10.5-16.0, 16.1-25.6 ng/mL were 2.15 (0.92, 5.04), 1.49 (0.67, 3.34), and 1.78 (0.84, 3.77), respectively.

There were 729 participants who had serum 25(OH)D measurements at both enrollment and the 3 year follow-up visit, of whom 92 (12.6%) died from all causes during the follow-up period from 3 to 6.5 years after enrollment. The Pearson correlation between serum 25(OH)D at enrollment and the 3 year follow-up visit was 0.45 (P <0.0001). Participants were divided into four groups based upon serum 25(OH)D measurements at enrollment and the 3 year follow-up visit: (1) above the median at both enrollment and 3-year follow-up, (2) below the median at enrollment and above the median at the 3-year follow-up, (3) above the median at enrollment and below the median at the 3-year follow-up, and (4) below the median at both enrollment and 3-year follow-up. The mortality rates in the four groups, respectively, were 8.3%, 10.8%, 14.5%, and 17.3% (P = 0.02).

Discussion

This study shows that older, community-dwelling men and women with low serum 25(OH)D levels (lowest quartile, <10.5 ng/mL; <26.2 nmol/L) had a significantly higher risk of both all-cause and cardiovascular disease mortality compared with those with higher serum 25(OH)D concentrations (highest quartile, >25.6 ng/mL; >63.9 nmol/L), after adjusting for demographic and other risk factors. The study corroborates the findings of the Hoorn study (Pilz *et al.* 2009) and extends the association between vitamin D and cardiovascular mortality in a larger study cohort with lower levels of 25(OH)D. Another previous study showed that in a selected group of patients undergoing coronary angiography, low serum 25(OH)D was associated with increased all-cause and cardiovascular disease mortality (Dobnig *et al.* 2008). In the general population of adults, aged 20 years and older in NHANES III, low serum 25(OH)D was an independent predictor of all-cause mortality but the relationship of low serum 25(OH)D and cardiovascular disease mortality did not reach statistical significance (Melamed *et al.* 2008). The results from NHANES III and the present study are not directly comparable due to the differences in age ranges between the two studies.

In the present study, participants who maintained a serum 25(OH)D level above the median at both enrollment and three years later or those who had a serum 25(OH)D level below the median at enrollment but above the median three years later were at a lower risk of

mortality, compared to participants who remained below the median at both enrollment and three year follow-up, or who dropped from above the median at enrollment to below the median at the three year follow-up. These findings suggest that those who maintain a high serum 25(OH)D level over time are at a lower risk of all-cause mortality.

Several biological mechanisms have been proposed that could explain a possible causal relationship between vitamin D deficiency and mortality. The active form of vitamin D, 1,25-dihydroxyvitamin D, has pleiotropic effects, and the vitamin D receptor is widely distributed in tissues (Holick, 2007). Vitamin D plays a role in regulation of the renin-angiotensin axis (Li *et al*. 2002), modulation of cellular proliferation and differentiation (Thorne and Campbell, 2008), cytokine production (Schleithoff *et al*. 2006), and atherosclerosis (Richart *et al*. 2007; Cardus *et al*. 2008). Low serum 25(OH)D levels have been shown to predict the risk of coronary artery calcification (de Boer *et al*. 2009), and vitamin D may also play a role in arterial stiffness (Andrade *et al*. 2008). The wide role that vitamin D plays in different tissues may account for the associations between vitamin D deficiency and cardiovascular disease (Martins *et al*. 2007; Wang *et al*. 2008), cancer (Davis, 2008), and mortality (Dobnig *et al*. 2008; Melamed *et al*. 2008). The effect of vitamin D on mortality may be indirect via hyperparathyroidism secondary to vitamin D insufficiency (Lips, 2001). PTH levels were significantly higher among those who died, however, the addition of PTH to models of 25(OH)D and all-cause and cardiovascular disease mortality did not substantially change the relationship between serum 25(OH)D and mortality.

Vitamin D deficiency is often defined as a serum 25(OH)D level less than 20 ng/mL (50 nmol/L) (Holick, 2007). Using this criterion, the majority of participants in the present study were deficient in vitamin D. The median serum 25(OH)D concentration of 16 ng/mL (40 nmol/L) among participants in the present study is lower than the mean 25(OH)D concentrations of about 28 ng/mL described among participants, aged 60 years and older, in NHANES III (Zadshir *et al*. 2005), but serum 25(OH)D was measured in NHANES III from 89 survey locations across the US and during the warmer months in the northern locations, when sunlight is more abundant (Martins *et al*. 2007).

Vitamin D is found in a limited number of dietary sources such as oily fish, eggs, and vitamin D-fortified milk, cereals, margarine, and yogurts. The current dietary recommendations for adequate daily intake of vitamin D are 400 IU for adults 51 to 70 years of age and 600 IU for adults 71 years of age and older (Food and Nutrition Board, 1997), but recent evidence suggests that the recommended adequate daily intake may need to be increased to 800 IU per day (Holick, 2007). Among the participants 60 years and older in NHANES III, those who participated in daily outdoor activities had mean serum 25(OH)D concentrations that were 30.8 ng/mL (77 nmol/L) (Scragg, *et al*. 2008), well above the cut-off of 50 nmol/L for vitamin D sufficiency. Older adults may require both greater daily outdoor activities and higher dietary intake of vitamin D to reduce the risk of vitamin D deficiency.

This study is limited in that phosphate levels were not collected and could be a potential confounder in the relationship between vitamin D and mortality. There may be other

unmeasured confounding factors in the study, as it is generally not possible to account for all confounders in longitudinal studies. As the study is observational, causality cannot be established. Further studies are also needed to examine the relationship between serum 25(OH) D over time and mortality. In an alternative analysis, PTH was added as a covariate to the model, and it may not be completely accurate to adjust for PTH since PTH might mediate the relationship between 25(OH)D and mortality. The present study may possibly underestimate the relationship between low 25(OH)D and mortality since the reference quartile of 25(OH)D consisted of many participants who were still in the insufficient range of 25(OH)D.

A recent meta-analysis of randomized controlled trials of vitamin D supplementation suggests that vitamin D supplementation may reduce mortality among older adults (Autier *et al*. 2007). Most of the trials in the meta-analysis were conducted among older adults who were at high risk of falls or fractures, and the main outcomes were usually bone density, falls, and clinical fractures, rather than mortality (Autier *et al* 2007). The authors recommended that randomized, placebo controlled clinical trials were needed among people 50 years and older for at least six years with mortality as the primary outcome in order to definitively address the issue of vitamin D supplementation and mortality (Autier *et al*. 2007).

In conclusion, older community-dwelling men and women with low serum 25(OH)D levels are at an increased risk of all-cause and cardiovascular disease mortality.

Acknowledgments

References

Andrade J, Er L, Ignaszewski A, Levin A. Exploration of association of 1,25-OH2D3 with augmentation index, a composite measure of arterial stiffness. Clin J Am Soc Nephrol. 2008; 3:1800–1806. [PubMed: 18922995]

Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med. 2007; 167:1730–1737. [PubMed: 17846391]

de Boer IH, Kestenbaum B, Shoben AB, Michos ED, Sarnak MJ, Siscovick DS. 25-hydroxyvitamin D levels inversely associate with risk for developing coronary artery calcification. J Am Soc Nephrol. 2009; 20:1805–1812. [PubMed: 19443637]

Bischoff-Ferrari HA, Dawson-Hughes B, Willett SC, Staehelin HB, Bazemore MG, Zee RY, et al. Effect of vitamin D on falls: a meta-analysis. JAMA. 2004; 291:1999–2006. [PubMed: 15113819]

Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA. 2005; 293:2257–2264. [PubMed: 15886381]

Cardus A, Panizo S, Encinas M, Dolcet X, Gallego C, Aldea M, et al. 1,25-dihydroxyvitamin D(3) regulates VEGF production through a vitamin D response element in the VEGF promoter. Atherosclerosis. 2008 Aug 29. Epub ahead of print.

Davis CD. Vitamin D and cancer: current dilemmas and future research needs. Am J Clin Nutr. 2008; 88(suppl):565S–569S. [PubMed: 18689403]

Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, et al. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. Arch Intern Med. 2008; 168:1340–1349. [PubMed: 18574092]

Ferrucci L, Bandinelli S, Benvenuti E, Di Iorio A, Macchi C, Harris TB, et al. Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. J Am Geriatr Soc. 2000; 48:1618–1625. [PubMed: 11129752]

Ferrucci L, Giallauria F, Guralnik JM. Epidemiology of aging. Radiol Clin North Am. 2008; 46:643–52. [PubMed: 18922285]

Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State" A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975; 12:189–198. [PubMed: 1202204]

Food and Nutrition Board, National Institute of Medicine. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, D.C.: National Academy Press; 1997.

Friedewald WT, Levy RI, Frederickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparation ultracentrifuge. Clin Chem. 1972; 18:499–502. [PubMed: 4337382]

Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch Intern Med. 168:1174–1180. [PubMed: 18541825]

Gloth FM III, Gundberg CM, Hollis BW, Haddad JG Jr, Tobin JD. Vitamin D deficiency in homebound elderly persons. JAMA. 1995; 274:1683–1686. [PubMed: 7474272]

Guralnik, JM.; Fried, LP.; Simonsick, EM.; Kasper, D.; Lafferty, ME. The Women's Health and Aging Study: Health and Social Characteristics of Older Women with Disability. Bethesda, MD: National Institute on Aging; 1995. NIH Publication No. 95-4009

Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006; 81:353–373. [PubMed: 16529140]

Holick MF. Vitamin D deficiency. N Engl J Med. 2007; 357:266–281. [PubMed: 17634462]

Houston DK, Cesari M, Ferrucci L, Cherubini A, Maggio D, Bartali B, et al. Association between vitamin D status and physical performance: the InCHIANTI study. J Gerontol A Biol Sci Med Sci. 2007; 62:440–446. [PubMed: 17452740]

Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). Am J Cardiol. 2008; 102:1540–1544. [PubMed: 19026311]

Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med. 1999; 130:461–470. [PubMed: 10075613]

Li YC, Kong J, Wei M, Chen ZF, Lui SQ, Cao LP. 1,25-dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002; 110:229–238. [PubMed: 12122115]

Lind L, Hanni A, Lithell H, Hvarfner A, Sorensen OH, Ljunghall S. Vitamin D is related to blood pressure and other cardiovascular disease factors in middle-aged men. Am J Hypertens. 1995; 8:894–901. [PubMed: 8541004]

Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001; 22:477–501. [PubMed: 11493580]

MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D_3. J Clin Invest. 1985; 76:1536–1538. [PubMed: 2997282]

Maggio D, Cherubini A, Lauretani F, Russo RC, Bartali B, Pierandrei M, et al. 25(OH)D serum levels decline with age earlier in women than in men and less efficiently prevent compensatory hyperparathyroidism in older adults. J Gerontol Biol Sci Med Sci. 2005; 60:1414–1419.

Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2007; 167:1159–1165. [PubMed: 17563024]

Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med. 2008; 168:1629–1637. [PubMed: 18695076]

Pilz S, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, et al. Low vitamin D levels predict stroke in patients referred to coronary angiography. Stroke. 2008; 39:2611–2613. [PubMed: 18635847]

Pilz S, Dobnig N, Nijpels G, Heine RJ, Stehouwer CD, Snijder MB, et al. Vitamin D and mortality in older men and women. Clin Endocrinol (Oxf). 2009 Fed; Epub ahead of print.

Poole KES, Loveridge N, Barker PJ, Halsall DJ, Rose C, Reeve J, et al. Reduced vitamin D in acute stroke. Stroke. 2006; 37:243–245. [PubMed: 16322500]

Richart T, Li Y, Staessen JA. Renal versus extrarenal activation of vitamin D in relation to atherosclerosis, arterial stiffening, and hypertension. Am J Hypertens. 2007; 20:1007–1015. [PubMed: 17765144]

Ruggiero C, Cherubini A, Guralnik J, Semba RD, Maggio M, Ling SM, et al. The interplay between uric acid and antioxidants in relation to physical function in older persons. J Am Geriatr Soc. 2007; 55:1206–1215. [PubMed: 17661959]

Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr. 2006; 83:754–759. [PubMed: 16600924]

Scragg R, Camargo CA Jr. Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. Am J Epidemiol. 2008; 168:577–586. [PubMed: 18579538]

Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, et al. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998; 338:777–783. [PubMed: 9504937]

Thorne J, Campbell MJ. The vitamin D receptor in cancer. Proc Nutr Soc. 2008; 67:115–127. [PubMed: 18412986]

United States Department of Health and Human Services. International Classification of Diseases Ninth revision, clinical modification. Washington, D.C.: U S Health and Human Services, Centers for Disease Control and Prevention, Centers for Medicare and Medicaid Services; 2006.

Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008; 117:503–511. [PubMed: 18180395]

Zadshir A, Tareen N, Pan D, Norris K, Martins D. The prevalence of hypovitaminosis D among US adults: data from the NHANES III. Ethn Dis. 2005; 15(suppl 5):S5-97–S5-101.

Zittermann A, Fischer J, Schleitoff SS, Tenderich G, Fuchs U, Koerfer R. Patients with congestive heart failure and healthy controls differ in vitamin D-associated lifestyles factors. Int J Vitam Nutr Res. 2007; 77:280–288. [PubMed: 18271283]

Figure 1.
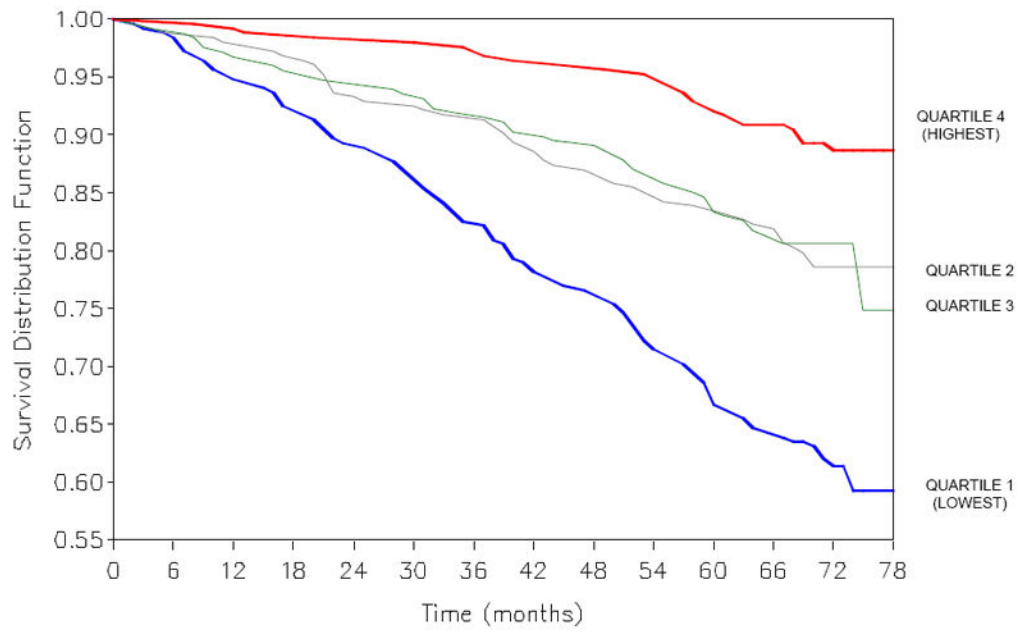Kaplan-Meier plots of all-cause mortality by quartiles of serum 25-hydroxyvitamin D. *P* <0.0001 by log-rank test.
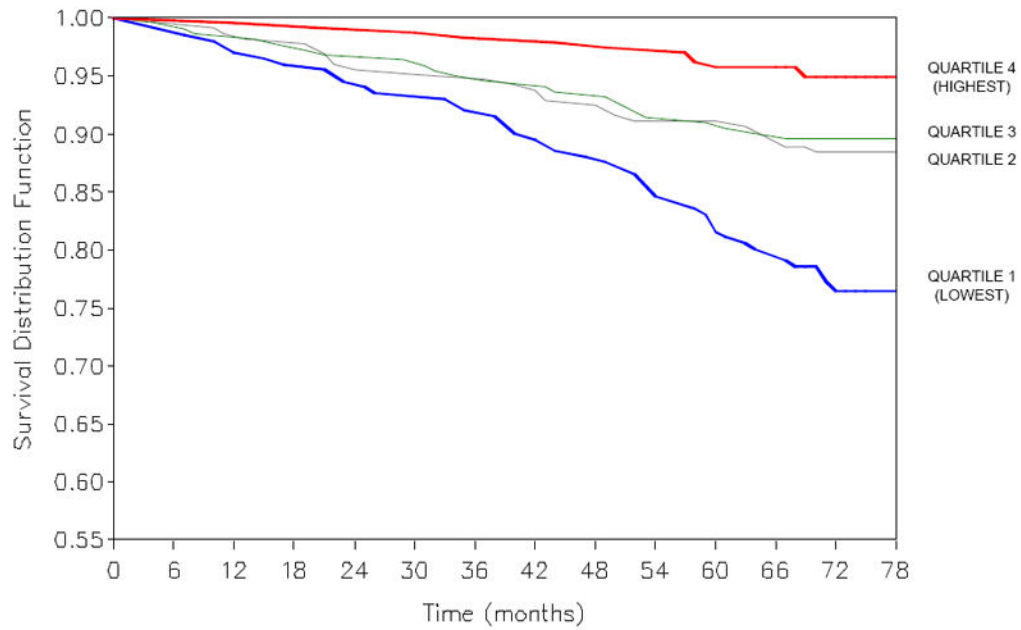
Figure 2.
Kaplan-Meier plots of cardiovascular disease mortality by quartiles of serum 25-hydroxyvitamin D. *P* <0.0001 by log-rank test.

Table 1

Baseline characteristics of adults, aged ≥65 years, in the InCHIANTI study, by quartiles of serum 25(OH)D

Characteristic[1]		25(OH)D Quartiles ng/mL[2]				P
		<10.5 (n = 252)	10.5 – 16.0 (n = 254)	16.1 – 25.6 (n = 247)	>25.6 (n = 253)	
Age, y		78.0 (72.0, 85.0)	75.0 (70.0, 81.0)	72.0 (69.0, 77.0)	71.0 (68.0, 74.0)	<0.0001
Sex, female (%)		32.7	29.5	21.7	16.0	<0.0001
Education, years		5.0 (3.0, 5.0)	5.0 (3.0, 5.0)	5.0 (4.0, 6.0)	5.0 (5.0, 7.0)	<0.0001
Current smoker (%)		9.9	11.0	15.8	19.0	0.01
Aspirin use (%)		36.1	40.2	29.6	25.7	0.002
Body mass index (kg/m²)		27.1 (23.9, 30.0)	27.3 (24.5, 30.1)	27.4 (25.0, 30.1)	26.7 (24.5, 29.1)	0.25
Physical activity (%)	Inactive	45.5	29.5	18.0	6.0	<0.0001
	Low	23.3	27.8	22.8	26.1	
	Moderate-High	13.9	20.0	30.5	35.6	
Parathyroid hormone (ng/L)		30.9 (21.2, 42.8)	24.7 (17.9, 33.4)	20.3 (16.1, 29.0)	16.6 (13.2, 23.5)	<0.0001
Serum calcium (mg/dL)		9.4 (9.1, 9.7)	9.4 (9.2, 9.7)	9.5 (9.2, 9.7)	9.4 (9.2, 9.6)	0.10
Total cholesterol (mg/dL)		212 (186, 240)	217 (186, 242)	212 (187, 245)	217 (191, 244)	0.24
HDL cholesterol (mg/dL)		54 (45, 67)	56 (47, 65)	51 (44, 62)	53 (45, 64)	0.06
LDL cholesterol (mg/dL)		132 (107, 154)	135 (116, 155)	133 (133, 157)	137 (113, 163)	0.19
Triglycerides (mg/dL)		109 (82, 141)	113 (84, 169)	114 (84, 158)	108 (82, 144)	0.52
Mini-Mental Exam Score <24 (%)		42.9	33.4	22.7	18.6	<0.0001
Hypertension (%)		47.6	50.8	44.5	44.7	0.45
Angina (%)		4.8	3.9	5.3	3.9	0.86
Heart failure (%)		9.5	6.7	3.2	2.4	0.001
Peripheral artery disease (%)		5.9	6.7	5.3	6.7	0.89
Stroke (%)		9.1	5.9	3.2	2.4	0.003
Diabetes mellitus (%)		16.7	11.4	11.7	13.4	0.29
Cancer (%)		7.5	7.8	5.7	4.7	0.42
Renal insufficiency (%)		17.7	20.5	14.1	9.5	0.004
Season (%)	Winter	32.7	35.2	21.4	10.7	<0.0001

Characteristic[1]		25(OH)D Quartiles ng/mL[2]				P
		<10.5 (n = 252)	10.5 – 16.0 (n = 254)	16.1 – 25.6 (n = 247)	>25.6 (n = 253)	
	Spring	18.3	20.9	24.3	36.5	
	Summer	17.6	18.5	26.1	37.8	
	Autumn	30.2	27.7	24.8	17.2	

[1] Data are given as median and interquartile range or percentage, as indicated.

[2] To convert to nmol/L, multiply by 2.496.

Table 2

Relationship between serum 25(OH)D and all-cause mortality in separate multivariate Cox proportional hazards models

Covariates in models	25(OH)D Quartiles ng/mL[1,2]			
	<10.5	**10.5 – 16.0**	**16.1 – 25.6**	**>25.6**
Age, sex, education, season	3.53 (2.19, 5.68)	2.15 (1.33, 3.50)	2.22 (1.39, 3.55)	1.00
Age, sex, education, season, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score.	2.20 (1.28, 3.77)	1.57 (0.93, 2.66)	1.96 (1.20, 3.20)	1.00
Age, sex, education, season, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score, and chronic diseases.[3]	2.11 (1.22, 3.64)	1.41 (0.83, 2.40)	1.12 (1.09, 1.15)	1.00

[1] Hazards ratios shown for each quartile of 25(OH) relative to the highest quartile (reference).

[2] To convert to nmol/L, multiply by 2.496.

[3] Chronic diseases include hypertension, diabetes, heart failure, stroke, and renal insufficiency.

Table 3

Relationship between serum 25(OH)D and cardiovascular disease mortality in separate multivariate Cox proportional hazards models

Covariates in models	25(OH)D Quartiles ng/mL[1,2]			
	<10.5	10.5 – 16.0	16.1-25.6	>25.6
Age, sex, education, season	4.15 (2.01, 8.57)	2.63 (1.27, 5.46)	2.65 (1.31, 5.39)	1.00
Age, sex, education, season, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score.	2.57 (1.12, 5.91)	1.76 (0.80, 3.89)	2.28 (1.09, 4.79)	1.00
Age, sex, education, season, BMI, smoking, aspirin use, physical activity, total cholesterol, HDL cholesterol, MMSE score, and renal insufficiency.	2.64 (1.68, 2.19)	1.68 (0.76, 3.72)	2.19 (1.05, 4.60)	1.00

[1]Hazards ratios shown for each quartile of 25(OH) relative to the highest quartile (reference).

[2]To convert to nmol/L, divide by 2.496.