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Circulating 25OHD, Dietary Vitamin D, PTH, and Calcium Associations with Incident Cardiovascular Disease and Mortality: The MIDSPAN Family Study

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Context: Observational studies relating circulating 25-hydroxyvitamin D (25OHD) and dietary vitamin D intake to cardiovascular disease (CVD) have reported conflicting results.

Objective: Our objective was to investigate the association of 25OHD, dietary vitamin D, PTH, and adjusted calcium with CVD and mortality in a Scottish cohort.

Design and Setting: The MIDSPAN Family Study is a prospective study of 1040 men and 1298 women from the West of Scotland recruited in 1996 and followed up for a median 14.4 yr.

Participants: Locally resident adult offspring of a general population cohort were recruited from 1972–1976.

Main Outcome Measures: CVD events (n = 416) and all-cause mortality (n = 100) were evaluated.

Results: 25OHD was measured using liquid chromatography-tandem mass spectrometry in available plasma (n = 2081). Median plasma 25OHD was 18.6 ng/ml, and median vitamin D intake was 3.2 μg/d (128 IU/d). Vitamin D deficiency (25OHD $<$15 ng/ml) was present in 689 participants (33.1%). There was no evidence that dietary vitamin D intake, PTH, or adjusted calcium were associated with CVD events or with mortality. Vitamin D deficiency was not associated with CVD (fully adjusted hazard ratio = 1.00; 95% confidence interval = 0.77–1.31). Results were similar after excluding patients who reported an activity-limiting longstanding illness at baseline (18.8%) and those taking any vitamin supplements (21.7%). However, there was some evidence vitamin D deficiency was associated with all-cause mortality (fully adjusted hazard ratio = 2.02; 95% confidence interval = 1.17–3.51).

Conclusion: Vitamin D deficiency was not associated with risk of CVD in this cohort with very low 25OHD. Future trials of vitamin D supplementation in middle-aged cohorts should be powered to detect differences in mortality outcomes as well as CVD. (***J Clin Endocrinol Metab*** **97: 0000–0000, 2012)**

Many observational studies suggest that low circulating levels of 25-hydroxyvitamin D (25OHD), the circulating storage form of vitamin D, are associated with increased incidence of cardiovascular disease (CVD) (1–6), death (5–8), and noncardiovascular chronic diseases (9), although a recent Institute of Medicine (IOM) report highlights inconsistencies in the literature regarding the definition of vitamin D deficiency and insufficiency (10).

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Abbreviations: CI, Confidence interval; CVD, cardiovascular disease; FEV1, forced expiratory volume in 1 sec; HR, hazard ratio; ICD, International Classification of Diseases; IOM, Institute of Medicine; IQR, interquartile range; 25OHD, 25-hydroxyvitamin D; RCT, randomized controlled trial; SMR, Scotland's morbidity record.

An apparently high prevalence of vitamin D-deficient individuals in many populations (11, 12) has led to calls for widespread supplementation programs to correct vitamin D deficiency, although the IOM report also highlights a lack of data from randomized controlled trials (RCTs) to support these calls (10).

Although several studies have reported no association between 25OHD and CVD (13–15), studies reporting an association have generally been better cited thus far (16). These inconsistencies require more data to clarify associations and reconcile these with findings from RCTs (10). There is also a need to determine the association of 25OHD with CVD and mortality independent of PTH and calcium (17). Although observational data cannot prove causality, estimates of associations with a range of endpoints may guide priority endpoints for future purpose-designed RCTs.

If vitamin D deficiency is an important determinant of CVD or death, one may expect that these associations may be more pronounced in populations with generally low UVB sunlight exposure. With a Northerly latitude, frequent substantial cloud cover, and low levels of processed food fortification with vitamin D, cohorts from Scotland have low levels of 25OHD (12) and are attractive for investigating the association of 25OHD with a range of diseases. The MIDSPAN Family Study is a cohort of 30- to 59-yr-olds living near Paisley in the West of Scotland in 1996. The cohort was recruited from a defined geographical area, mitigating against confounding of vitamin D status by latitude.

Our hypothesis was that, given existing RCT data suggesting that vitamin D deficiency may not be causal in CVD (10), after more complete adjustment for a large range of potential confounders and exclusion of those with chronic baseline disease in the MIDSPAN Family Study, 25OHD would not be associated with CVD or mortality.

Materials and Methods

Recruitment

The MIDSPAN Family Study took place between March and December 1996. The study recruited adult sons and daughters of couples who had participated in the Renfrew/Paisley prospective cohort study between 1972 and 1976, which consisted of 7049 men and 8353 women aged between 45 and 64 yr and who lived in the towns of Renfrew and Paisley (18). Renfrew and Paisley are two similar towns situated on the southwest perimeter of the City of Glasgow within a large postindustrial area. The latitude of Paisley is 55° 84 min N. The aim was to establish a study investigating transgenerational causes of chronic disease as well as to establish a contemporary cohort study. The recruitment process of the MIDSPAN Family Study has been described in detail elsewhere (18, 19). In brief, offspring of the married couples identified within the Renfrew/Paisley cohort, aged 30–59 yr and living locally, formed the eligible population (3202 offspring from 1767 families). In all, 1040 male and 1298 female offspring from 1477 families took part, and all participants were Caucasian, with 99.7% living locally (exclusion of those who did not live locally did not alter reported results). Ineligible offspring (n = 1813) included those too old or too young, those not living locally, those with problematic addresses, or those who died before the study commenced. The original MIDSPAN cohort was representative of the catchment area in being characterized by high levels of socioeconomic deprivation, which was (and is) more severe than Scotland as a whole but less severe than the most deprived areas of Glasgow.

The offspring were invited, in random order, to complete a detailed questionnaire and attend a screening examination, similar to their parents 20 yr previously. All information on physical activity, smoking, occupation, diet, socioeconomic status, and alcohol consumption were based on self-reported answers from standard questionnaires (20, 21). Socioeconomic factors were occupational social class coded according to the Registrar General's classification, highest level of education, and the Carstairs deprivation index (an index of deprivation in a specific postcode based on census data) (22). Measurements of height, weight, waist, hip, forced expiratory volume in 1 sec (FEV1), and blood pressures were obtained by a qualified research nurse who also collected nonfasting venous blood samples. A semiquantitative food frequency questionnaire [modified from that of Yarnell *et al.* (23)] and specifically validated for the antioxidant vitamins against plasma levels was used (24). The frequency of consumption of 60 foods and food groups was reported as one to seven times per week, fortnightly, or rarely/never. Nutrient (including fat, fiber, and vitamin D) intakes were calculated by a computer program, which multiplied the food frequency by standard portion size and by nutrient values from United Kingdom food composition tables, using McCance and Widdowson version 6 (Food Standards Agency), which includes a potency factor for vitamin D metabolites in meat (25). Participants were asked whether they routinely took vitamin supplements; there were seven different possible category codings indicating type of supplement ingestion. All seven categories contained preparations that may have contained vitamin D, and thus in sensitivity analysis, all people who reported taking supplements were excluded from analyses. Participants were asked (yes or no) in the baseline questionnaire whether they had any longstanding illness disability or infirmity and whether this illness or disability limited their activities in any way. If the answer to both questions was affirmative, we defined the individual as having an activity-limiting longstanding illness. Blood samples obtained were spun down, plasma separated, aliquoted, and stored at −80 C for subsequent analysis.

Ethical approval for the study was obtained from the Argyll and Clyde Health Board Local Research Ethics Committee and from Greater Glasgow Health Board Local Research Ethics Committee. Informed written consent was obtained for venipuncture, sample storage, and record linkage.

Events definition

Participants were followed up for a median 14.4 yr. Scotland has a National Health Service (NHS) offering universal healthcare to all citizens, which is free at point of care. Virtually all healthcare (especially emergency care and that relating to

chronic illness) is delivered in NHS institutions. Endpoints were identified by periodic review of the cohort (last review completed up to December 31, 2010) using a national database: the Information Services Division NHS record linkage for Scotland. The Information Services Division-linked database contains information on Scotland's morbidity records (SMRs) for acute specialty day case and inpatient discharges from hospital (SMR01) since January 1981 [with International Classification of Diseases (ICD)-9 codings 390–459 and ICD-10 codings I00–I99 for incident CVD events, *i.e.* any disease of the circulatory system]. Death certificates were obtained from the NHS Central Register where the participants were flagged. For the present paper, the primary CVD endpoint was any CVD event (ICD-10 I00–I99) coded on discharge or on the death certificate. A sensitivity analysis of the CVD endpoint included only events where CVD was the principal reason for hospitalization or death. For coronary heart disease events, an audit of SMR01 shows codings were recorded with 94.2% accuracy [95% confidence interval (CI) = ±3.0%], 93.4% sensitivity, and 99.2% completeness, with corresponding figures for cerebrovascular disease of 94.8% (95% CI = ±4.4%), 91.1%, and 96.0% (26). The secondary endpoint was all-cause mortality, with a sensitivity analysis for deaths that were principally caused by CVD, or principally caused by disease that was not cardiovascular in origin (according to the underlying cause of death on the death certificate).

Circulating biomarker measurement

Measurement of 25OHD was performed on EDTA-anticoagulated plasma via a high-throughput method for the measurement of $25OHD_3$ and $-D_2$ using a gold-standard automated solid-phase extraction procedure with liquid chromatography-tandem mass spectrometry (27). Our method is currently in routine clinical use and is calibrated and controlled using reagents from Chromsystems GmbH (Manchester, UK). Results are reported as total 25OHD ($25OHD_2$ + $25OHD_3$); more than 99% of participants had an undetectable $25OHD_2$, which is commensurate with results observed in routine NHS use. The lower limit of sensitivity was reported as 4 ng/ml for both $25OHD_3$ and total 25OHD. Plasma PTH was measured by electrochemiluminescence on an Elecsys 2010 (Roche Diagnostics, Burgess Hill, UK) using the manufacturer's calibrators and controls. Coefficients of variation were 5.6% at 57 pg/ml and 3.8% at 189 pg/ml. Albumin-corrected calcium was calculated as measured total calcium (millimoles per liter) + 0.02 [40 − serum albumin (grams per liter)]. Serum calcium was measured on fresh samples by automated clinical biochemistry platforms.

Statistical methods

All subjects with available data were used in models fitted for 25OHD, vitamin D intake, PTH, and corrected calcium measures, respectively. Medians and interquartile ranges (IQR) were used to summarize nonnormally distributed data, means and SD for normally distributed data, and frequencies and percentages for summary of categorical data. Demographic measurements were compared between subjects who went on to become CVD cases and noncases using the two-sample *t* test for continuous variables and Fisher's test for categorical variables. Normal distributions were achieved by taking logarithms of positively skewed variables, including 25OHD. Relationships between log 25OHD and baseline continuous characteristics were assessed by age- and sex-adjusted Pearson correlation coefficients. Predictions for 25OHD, vitamin D intake, PTH, and adjusted calcium from a linear regression model adjusted for month were plotted to detect seasonality. Associations of 25OHD, vitamin D intake, PTH, and adjusted calcium with the risk of CVD and mortality were investigated, both by linear and nonlinear models in the form of quadratic curves and penalized regression splines. The associations of these markers with CVD risk and mortality were summarized using hazard ratios (HRs) derived from Cox proportional hazards models (proportional hazard assumptions were met in all cases), using continuous models (HR per 1-SD increase) for circulating 25OHD, vitamin D intake, PTH, and adjusted calcium and using a categorical model for 25OHD deficiency. There is no universally accepted current definition of vitamin D deficiency, so we used a cutoff previously reported to be strongly associated with CVD risk [<15 ng/ml (37.5 mmol/liter)] (2, 4). Cox models were adjusted for classical and nonclassical risk factors in four models as defined in the relevant tables. All available data were used, resulting in smaller complete data sets for models with more complex adjustment due to missing covariate measurements. When analyses were restricted to complete data sets for all models, the estimated associations were unchanged in all models (data not shown).

P values <0.05 were taken to be indicative of a true association. Cox model effect estimates are reported as the HR associated with a 1-SD increase for continuous predictors (on a log scale), or for being vitamin D deficient, with a 95% CI. No adjustments were made for multiple comparisons. Analyses were carried out using R for Windows version 2.12.1.

Results

Baseline characteristics and associations

Two participants were excluded from the analyses because no consent for record linkage was obtained. Due to sample attrition, and the high volume of samples required for 25OHD measurement, total 25OHD was measured in 2081 samples (89% of the total). Data measurements were completed for 2335, 2194, and 2188 individuals for dietary vitamin D intake, PTH, and adjusted calcium, respectively.

Cohort median 25OHD and mean vitamin D dietary intake were generally low, with medians of 18.6 ng/ml and 3.2 μg/d (128 IU/d), respectively, and 689 participants (33.1%) were vitamin D deficient (<15 ng/ml). Estimates of dietary sources of vitamin D intake showed that the primary determinant of dietary vitamin D was fish consumption (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Those who smoked, were deprived, had prevalent coronary heart disease or longstanding illness at baseline, did not meet fiber intake guidelines (18 g/d), consumed more fat, or were sedentary were more likely to be deficient in vitamin D (Table 1). Participants with vitamin D deficiency also had lower creatinine levels and lower FEV1 output. Plasma samples taken during the winter or spring

TABLE 1. Baseline characteristics by vitamin D deficiency

Characteristic	Not deficient (≥15 ng/ml)	Deficient (15 ng/ml)	*P* value
Age			
n	1392	689	0.163
yr	45.2 (6.2)	44.9 (6.1)	
Gender			
n	1392	689	0.073
Male	635 (46%)	285 (42%)	
Female	757 (54%)	404 (58%)	
Smoking			
n	1392	689	<0.001
Never	698 (50%)	277 (40%)	
Former	417 (30%)	171 (25%)	
Current	277 (20%)	241 (35%)	
Systolic BP			
n	1387	688	0.705
mm Hg	125.5 (116.0–136.0)	125.0 (115.5–137.0)	
Total cholesterol			
n	1391	688	0.533
mmol/liter	5.2 (4.6–5.8)	5.2 (4.7–5.8)	
HDL-cholesterol			
n	1203	606	0.651
mmol/liter	1.4 (1.2–1.6)	1.4 (1.1–1.6)	
Triglycerides			
n	1387	687	0.799
mmol/liter	1.2 (0.9–1.9)	1.2 (0.9–1.9)	
Diabetes			
n	1392	689	0.856
Yes	18 (1%)	9 (1%)	
No	1374 (99%)	680 (99%)	
Glucose			
n	1392	687	0.393
mmol/liter	5.1 (4.8–5.5)	5.1 (4.7–5.5)	
BMI			
n	1391	689	0.768
kg/m^2	25.5 (23.2–28.2)	25.6 (22.6–29.2)	
Waist circumference			
n	1392	688	0.456
cm	85.2 (75.7–94.5)	85.3 (74.7–96.2)	
Education			
n	1389	687	0.193
School	662 (48%)	349 (51%)	
Tertiary	727 (52%)	338 (49%)	
Social class			
n	1392	689	0.102
Nonmanual	970 (70%)	455 (66%)	
Manual	422 (30%)	234 (34%)	
Deprivation			
n	1387	689	0.001
1–2	336 (24%)	128 (19%)	
3–5	797 (57%)	394 (57%)	
6–7	254 (18%)	167 (24%)	
CRP			
n	1311	633	0.019
mg/liter	0.8 (0.4–2.0)	0.9 (0.4–2.5)	
Baseline CHD			
n	1390	687	0.001
No	1239 (89%)	577 (84%)	
Yes	151 (11%)	110 (16%)	
Activity-limiting longstanding illness			
n	1392	689	0.004
No	1156 (83%)	535 (78%)	
Yes	236 (17%)	154 (22%)	

(*Continued*)

TABLE 1. Continued

Characteristic	Not deficient (≥15 ng/ml)	Deficient (15 ng/ml)	*P* value
Season			
n	1392	689	<0.001
Winter	104 (7%)	95 (14%)	
Spring	191 (14%)	259 (38%)	
Summer	554 (40%)	219 (32%)	
Autumn	543 (39%)	116 (17%)	
Creatinine			
n	1370	669	<0.001
μmol/liter	98.9 (13.2)	95.4 (12.4)	
Predicted FEV1			
n	1372	662	<0.001
% of predicted	97.8 (13.2)	94.1 (14.3)	
Fiber			
n	1391	688	0.002
≥18 g/d	1058 (76%)	479 (70%)	
<18 g/d	333 (24%)	209 (30%)	
% fat from diet			
n	1311	638	<0.001
%	34.1 (5.8)	35.2 (5.7)	
Alcohol intake			
n	1392	689	0.016
UK U/wk	8.0 (3.0–18.0)	7.0 (1.5–16.0)	
Current medication			
n	1392	689	0.846
No	1376 (99%)	681 (99%)	
Yes	16 (1%)	8 (1%)	
Insulin medication			
n	1392	689	0.512
No	1384 (99%)	687 (100%)	
Yes	8 (1%)	2 (0%)	
Physical activity			
n	1392	689	<0.001
Active	1084 (78%)	470 (68%)	
Sedentary	308 (22%)	219 (32%)	
25OHD			
n	1392	689	<0.001
ng/ml	22.9 (18.6–28.5)	11.0 (8.7–13.0)	
Vitamin D intake			
n	1392	689	<0.001
μg/d	3.3 (2.4–4.7)	3.0 (2.1–4.1)	
PTH			
n	1364	674	<0.001
pg/ml	28.6 (22.4–35.5)	30.9 (24.8–39.2)	
Adjusted calcium			
n	1354	668	0.687
mmol/liter	2.3 (0.1)	2.3 (0.1)	

Values are mean (SD), number (percent), or median (IQR). BP, Blood pressure; BMI, body mass index; CHD, coronary heart disease; CRP, C-reactive protein; HDL, high-density lipoprotein.

were more likely to be below the threshold for deficiency. As expected, circulating 25OHD was considerably lower among those who were deficient, whereas vitamin D consumption was also slightly lower and circulating PTH slightly higher. At baseline, circulating 25OHD was moderately positively correlated with vitamin D intake and inversely correlated with PTH after adjustment for age and sex but was not associated with adjusted calcium (Supplemental Table 1).

Seasonal variation in circulating 25OHD, vitamin D intake, PTH, and adjusted calcium was assessed based on month of blood sample collection (Fig. 1). Month was a significant term in linear models fitted for 25OHD, PTH, and adjusted calcium, indicating evidence of seasonal variation ($P < 0.001$ for all), although there was no evidence dietary vitamin D was associated with month. These trends were unaltered by exclusion of those taking supplements (data not shown). Due to recruitment not taking place in January or February, the median 25OHD of 18.6 ng/ml is likely to be an overestimate of the year-round median within the population this cohort represents.

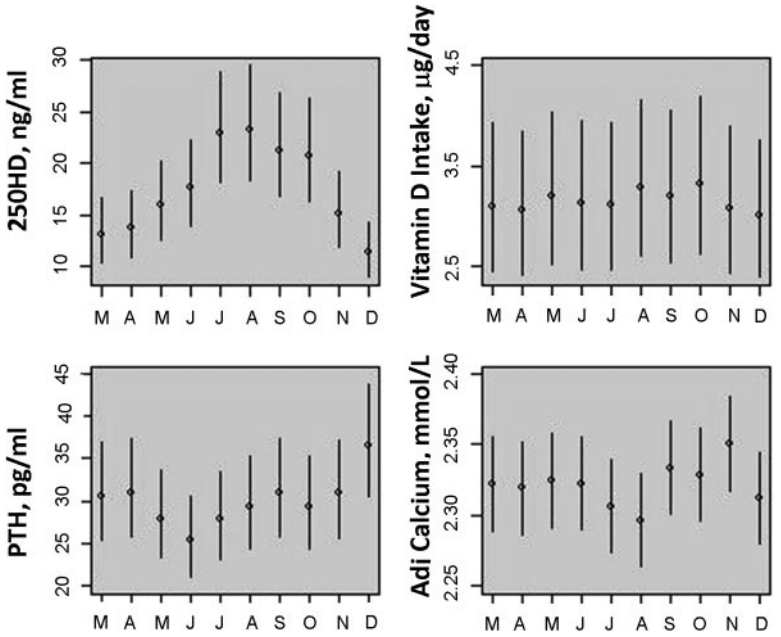

FIG. 1. Mean predicted values (and 95% CI) for circulating 25OHD, vitamin D intake, PTH, and adjusted calcium, from March to December (no baseline samples taken in January or February).

Association with CVD

Classical CVD risk factors generally differed between CVD cases and noncases in the expected directions (Supplemental Table 2). On simple univariable comparison, the median 25OHD concentration was 18.4 ng/ml (IQR = 12.3–25.1 ng/ml) among cases and 18.7 ng/ml (IQR = 13.3–25.5 ng/ml) among noncases (P = 0.32). Estimated vitamin D intake, corrected calcium, and PTH were also no different in CVD cases compared with noncases (Supplemental Table 2).

Both linear and nonlinear associations of 25OHD, vitamin D intake, PTH, and adjusted calcium with CVD were investigated. None of these factors showed strong evidence of a relationship with CVD events in linear models adjusted for age, sex, season, and other CVD risk factors (Table 2) or by nonlinear models using spline or quadratic curves (data not shown). Restriction of the definition of CVD events to those that were the principal reason for hospitalization or death (n = 309 events) did not materially alter results (no association in any model; data not shown).

In analysis adjusted for age, sex, and season only, there was some evidence for an association of vitamin D deficiency (<15 ng/ml) with CVD events (HR = 1.31; 95% CI = 1.07–1.62) (Table 3). However, this association was attenuated to null after adjustment for classical and nonclassical CVD risk factors (HR = 1.00; 95% CI = 0.77–1.31). Restriction of the definition of CVD events to primary cause for hospitalization or death did not materially alter associations (adjusted HR = 0.91; 95% CI = 0.66–1.24).

TABLE 2. Cox proportional hazards models for risk of CVD, with relative associations of 1-SD increases in circulating 25OHD (log scale), dietary vitamin D intake (log scale), PTH (log scale), and adjusted calcium, using various adjustment models

	Model A	Model B	Model C	Model D
25OHD[a]				
HR (95% CI)	0.89 (0.81–0.99)	0.96 (0.85–1.08)	1.06 (0.93–1.21)	1.07 (0.94–1.23)
N	2081	1801	1522	1492
n	416	341	297	293
Vitamin D intake[a]				
HR (95% CI)	0.97 (0.88–1.06)	0.95 (0.86–1.06)	0.95 (0.83–1.07)	0.94 (0.83–1.08)
N	2333	1954	1641	1492
n	462	366	318	293
PTH[a]				
HR (95% CI)	1.06 (0.97–1.17)	1.07 (0.96–1.19)	1.03 (0.92–1.16)	1.05 (0.92–1.18)
N	2194	1903	1609	1492
n	434	358	315	293
Adjusted calcium				
HR (95% CI)	1.02 (0.92–1.12)	0.99 (0.89–1.11)	1.00 (0.89–1.12)	1.01 (0.89–1.13)
N	2188	1904	1635	1492
n	440	362	317	293

Model A was adjusted for age, sex, and season. Model B was additionally adjusted for diabetes, glucose, smoking, systolic blood pressure, total cholesterol, high-density cholesterol, and BMI. Model C was additionally adjusted for triglycerides, waist circumference, creatinine, C-reactive protein, insulin, highest educational level (tertiary level or other), social class, deprivation category, percent fat from diet, alcohol intake, high and low fiber in diet, current medication (ACE inhibitors, antihypertensives, aspirin, insulin, oral hypoglycemics, sartans, and statins), baseline coronary heart disease, low baseline physical activity, and percent predicted FEV1. Model D was additionally adjusted for 25OHD, vitamin D intake, PTH, and adjusted calcium where appropriate. N = number at risk; n = number of events.

[a] Modeled on log scale.

TABLE 3. Cox proportional hazards models for vitamin D deficiency associations with the risk of any CVD event, for all patients with available measures on circulating 25OHD (n = 2074) and for a subgroups of patients

	Model A	Model B	Model C	Model D
Full cohort				
25OHD deficiency (<15 ng/ml), n = 689				
HR (95% CI)	1.31 (1.07–1.62)	1.24 (0.98–1.57)	1.01 (0.78–1.32)	1.00 (0.77–1.31)
N	2081	1801	1522	1492
n	416	341	297	293
Subgroup cohort (no activity-limiting longstanding illness at baseline)				
25OHD deficiency (<15 ng/ml), n = 535				
HR (95% CI)	1.24 (0.96–1.59)	1.16 (0.87–1.54)	0.98 (0.71–1.35)	0.94 (0.68–1.31)
N	1691	1466	1239	1216
n	298	241	206	204

Adjustment models A–D are described in Table 2. N = number at risk; n = number of events.

Association with all-cause mortality

Dietary vitamin D intake, PTH, and adjusted calcium showed no strong evidence of associations with all-cause mortality in linear models (Table 4) or by spline or quadratic curves (figures not shown).

Circulating 25OHD was inversely associated with all-cause mortality, such that higher levels had a borderline association with lower mortality; in fully adjusted linear models of log 25OHD, the HR per 1-SD increase was 0.74 (95% CI = 0.56–0.99). No nonlinear associations were demonstrated by quadratic or spline curves (data not shown). Plasma 25OHD deficiency (<15 ng/ml) was associated with all-cause mortality in both age-, sex-, and season-adjusted (HR = 3.03; 95% CI = 2.01–4.57) and in fully adjusted analyses (HR = 2.02; 95% CI = 1.17–3.51) (Table 5). To reduce the possibility of reverse causality, we excluded those with self-reported activity-limiting longstanding illness at baseline, although this did not alter associations. In addition, exclusion of those taking vitamin supplements at baseline did not materially alter associations.

To elucidate the association of vitamin D deficiency with cause-specific mortality, we investigated the association with principal CVD mortality (CVD the principal cause of death), and mortality where CVD was not the principal cause. Among those who were deficient, the fully adjusted HR for principal CVD mortality (n = 12 events) was 8.13 (95% CI = 1.26–52.7) whereas the risk for principal non-CVD mortality (n =58 events) was 1.82 (95% CI = 0.99–3.35) (Supplemental Table 3).

Discussion

In the MIDSPAN Family Study, median 25OHD was insufficient at 18.6 ng/ml, perhaps compounded by low dietary vitamin D intake. We observed no association of

TABLE 4. Cox proportional hazards models for risk of all-cause mortality, with relative associations of 1-SD increases in circulating 25OHD (log scale), dietary vitamin D intake (log scale), PTH (log scale), and adjusted calcium, using various adjustment models

	Model A	Model B	Model C	Model D
25OHD[a]				
HR (95% CI)	0.57 (0.46–0.70)	0.68 (0.54–0.86)	0.77 (0.59–1.01)	0.74 (0.56–0.99)
N	2081	1801	1522	1492
n	100	79	71	70
Vitamin D intake[a]				
HR (95% CI)	1.01 (0.84–1.22)	1.01 (0.81–1.26)	1.04 (0.80–1.36)	1.05 (0.79–1.41)
N	2333	1954	1641	1492
n	120	90	79	70
PTH[a]				
HR (95% CI)	0.90 (0.74–1.10)	1.05 (0.83–1.31)	0.95 (0.74–1.21)	0.86 (0.66–1.13)
N	2194	1903	1609	1492
n	106	86	78	70
Adjusted calcium				
HR (95% CI)	1.04 (0.86–1.26)	0.98 (0.79–1.22)	0.97 (0.76–1.23)	0.87 (0.67–1.14)
N	2188	1904	1635	1492
n	109	88	79	70

Adjustment models A–D are described in Table 2. N = number at risk; n = number of events.

[a] Modeled on log scale.

TABLE 5. Cox proportional hazards models for vitamin D deficiency associations and the risk of all-cause mortality, in all patients with available measures on circulating 25OHD (n = 2074) and for a subgroup of patients with no activity-limiting longstanding illness reported at baseline (n = 1684)

	Model A	Model B	Model C	Model D
Full cohort				
25OHD deficiency (<15 ng/ml), n = 689				
HR (95% CI)	3.03 (2.01–4.57)	2.42 (1.50–3.90)	2.01 (1.18–3.43)	2.02 (1.17–3.51)
N	2081	1801	1522	1492
n	100	79	71	70
Sensitivity analysis: no activity-limiting longstanding illness at baseline				
25OHD deficiency (<15 ng/ml), n = 535				
HR (95% CI)	3.23 (1.97–5.31)	2.75 (1.55–4.86)	2.49 (1.33–4.65)	2.33 (1.23–4.44)
N	1691	1466	1239	1216
n	69	56	51	50
Sensitivity analysis: patients not taking vitamin supplements at baseline				
25OHD deficiency (<15 ng/ml), n = 579				
HR (95% CI)	3.38 (2.16–5.29)	2.73 (1.63–4.58)	2.05 (1.13–3.73)	2.04 (1.10–3.79)
N	1630	1401	1199	1179
n	86	68	61	60

Adjustment models A–D are described in Table 2. N = number at risk; n = number of events.

vitamin D deficiency (<15 ng/ml) with CVD events in any model. However, death rates were twice as high in those who were deficient, even after exclusion of those with activity-limiting longstanding illness at baseline and those taking supplements, although power to investigate these associations with cause-specific mortality was limited.

It could be argued that the lack of association with CVD we observe may be because so few of our population had optimal 25OHD levels of more than 30 ng/ml, and so we may lack a full range of concentrations with which to compare deficient with sufficient and optimal. However, the association observed between vitamin D deficiency and mortality is in line with other general population cohorts (4–6) and a previous cohort that has reported an association between 25OHD and mortality in Scotland (28). It is also of note that there is evidence both for (29) and against (30–32) an association of 25OHD with carotid artery intima-media thickness (a putative surrogate of CVD risk status). Formal meta-analysis after publication of more observational data will allow assessment of the likelihood of publication bias within the literature as well as to assess the association of vitamin D deficiency with CVD death and nonfatal CVD.

Despite some studies reporting associations of vitamin D deficiency with increased CVD risk (1–6), other (generally smaller) studies have reported no association of circulating 25OHD with CVD (13–15). We note that in our study, vitamin D deficiency was associated with increased all-cause mortality and perhaps with CVD mortality. We have previously shown that other CVD risk markers such as inflammatory markers and obesity are more strongly associated with CVD death than with nonfatal CVD events (33, 34). It is therefore of real interest that several of the best cited studies linking 25OHD to CVD used a fatal CVD endpoint (1–3, 5, 6). Whether vitamin D deficiency renders a CVD event more likely to be fatal (*i.e.* is causal) or whether poor health (and consequently low vitamin D) leads to a reduction in the likelihood of survival of acute CVD requires additional study.

The issue of causality cannot be inferred from an observational study alone. The associations we have observed suggest a strong effect (if the association is causal). However, this finding is in direct contrast to existing data from meta-analysis of randomized trials of vitamin D supplementation, which suggest (primarily in the elderly, where the greatest effect size is likely to be observed due to reduced risk of hospitalization for fractures) a very small effect of supplements on mortality (35). We attempted to reduce the likelihood of confounding and reverse causality by exclusion of those with activity-limiting longstanding illness and adjusting for a wide range of potential confounders. Ultimately, evidence of benefit of higher circulating 25OHD will come from Mendelian randomization studies and supplementation data from trials designed to investigate mortality. Whether or not trials of additional supplementation in countries where vitamin D fortification of foodstuffs is routine (such as VITamin D and OmegA-3 TriaL, VITAL, in the United States) (http://www.vitalstudy.org) will confer benefit is unclear. In this context, more vitamin D supplementation trials in countries where vitamin D fortification in foodstuff is generally low may be additionally informative.

Of interest, dietary vitamin D intake in our study was also not associated with CVD events or with CVD mor-

tality. This is an important observation in a population where comparatively little food is supplemented with vitamin D; average MIDSPAN intake is less than 130 IU/d, whereas the IOM recommends an intake of 600 IU/d in the United States (10). Guidelines in the United Kingdom appear to generally assume vitamin D consumption in the United Kingdom is inadequate (36, 37), which will generally appear to be true compared with the United States where fortification is far more widespread. It is possible that our data extracted from food frequency questionnaires underestimate vitamin D consumption specifically. However, recent data among Scottish mothers (38) and postmenopausal women (39) suggest that, based on food questionnaire data extraction, dietary vitamin D intake in Scotland is typically in the range of 100–150 IU/d.

Strengths of the present study include the use of a well-phenotyped cohort study from a single geographic location to minimize confounding by latitude, in a country where food is not routinely supplemented with vitamin D. Circulating 25OHD was measured using gold-standard liquid chromatography-tandem mass spectrometry methods currently used in an externally quality controlled routine NHS laboratory. Endpoints were identified on follow-up using national record linkage databases, and all routine risk factors showed strong associations with CVD, thereby lending external validity to our findings. Potential weaknesses include the small number of individuals in the study with conventionally optimal circulating 25OHD. Blood samples taken were nonfasting, and diabetes was self-reported, meaning there may have been some undiagnosed baseline diabetes we did not detect and adjust for. Dietary vitamin D intake was estimated from food frequency questionnaires that measured intake of only 60 foods or food groups, although this method has been validated for use for antioxidant vitamins (against circulating plasma levels) (24).

In conclusion, in a cohort of middle-aged Scottish people with low dietary vitamin D intake and low circulating 25OHD, we report no association with CVD events, although there was some evidence that 25OHD deficiency was associated with mortality. These data should help guide the design of trials that, based on our findings, should be sufficiently powered to assess the impact of vitamin D supplementation on all-cause mortality in middle-aged populations.

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