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The inverse association between serum 25-hydroxyvitamin D and mortality may be modified by vitamin A status and use of vitamin A supplements

Schmutz, Einat Avital ; Zimmermann, Michael Bruce ; Rohrmann, Sabine

Abstract: **BACKGROUND** Low serum 25-hydroxyvitamin D [25(OH)D] levels have been associated with higher risk of many diseases that affect mortality, including cardiovascular disease (CVD) and cancer. The inverse association between serum 25(OH)D and mortality may be modified by excess circulating vitamin A, due to interactions of vitamin A at the level of the vitamin D nuclear receptor. In this prospective cohort study, we investigated whether the association of 25(OH)D with all-cause, cancer, and CVD mortality was modified by circulating vitamin A or preformed vitamin A intake from supplements. **METHODS** We analyzed 15,998 adults in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. Mortality data for all-cause (n = 3890), cancer (n = 844), and CVD mortality (n = 1715) were assessed through December 2006. Serum 25(OH)D was measured using a radioimmunoassay kit, vitamin A biomarkers were measured by HPLC, and information on supplement use was obtained by self-report. Multivariable hazard ratios (HRs) and corresponding 95 % confidence intervals (CI) were estimated by proportional hazards regression. **RESULTS** Serum 25(OH)D was significantly inversely associated with all-cause mortality (HR 0.93, 95 % CI 0.89, 0.97, per 10 ng/mL increase) and also with CVD mortality and mortality due to non-cancer/non-cardiovascular causes, but not with cancer mortality. The observed inverse associations remained statistically significant only among participants with serum retinyl esters <7.0 g/dL. High intake (>5000 IU/day) of preformed vitamin A from supplements attenuated the inverse association of 25(OH)D with overall mortality. The observed interactions were not statistically significant. **CONCLUSIONS** 25(OH)D was inversely associated with overall mortality, CVD mortality, and mortality due to non-cancer/non-CVD causes, but not with cancer mortality. A possible interaction between vitamin A exposure and 25(OH)D concentration appears to be associated with an attenuation of the inverse association between risk of death and quartile of 25(OH)D concentration.

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1 **Title**

2 The inverse association between serum 25-hydroxyvitamin D and mortality may be
3 modified by vitamin A status and use of vitamin A supplements

4

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23

24 **ABSTRACT**

25 **Background:** Low serum 25-hydroxyvitamin D [25(OH)D] levels have been associated with
26 higher risk of many diseases that affect mortality, including cardiovascular disease and
27 cancer. The inverse association between serum 25(OH)D and mortality may be modified by
28 excess circulating vitamin A, due to interactions of vitamin A at the level of the vitamin D
29 nuclear receptor. In this prospective cohort study, we investigated whether the association
30 of 25(OH)D with all-cause, cancer and cardiovascular disease mortality was modified by
31 circulating vitamin A or preformed vitamin A intake from supplements.

32 **Methods:** We analyzed 15,998 adults in the Third National Health and Nutrition
33 Examination Survey (NHANES III), 1988-1994. Mortality data for all-cause (n=3890),
34 cancer (n=844) and CVD mortality (n=1715) was assessed through December 2006. Serum
35 25(OH)D was measured using a radio-immunoassay kit, vitamin A biomarkers were
36 measured by HPLC, and information on supplement use was obtained by self-report.
37 Multivariable hazard ratios (HRs) and corresponding 95% confidence intervals (CI) were
38 estimated by proportional hazards regression.

39 **Results:** Serum 25(OH)D was significantly inversely associated with all-cause mortality
40 (HR: 0.93, 95% CI 0.89, 0.97, per 10 ng/mL increase), and also with cardiovascular disease
41 (CVD) mortality and mortality due to non-cancer/non-cardiovascular causes, but not with
42 cancer mortality. The observed inverse associations remained statistically significant only
43 among participants with serum retinyl esters <7.0 µg/dL. High intake (>5000IU/day) of
44 preformed vitamin A from supplements attenuated the inverse association of 25(OH)D with
45 overall mortality. The observed interactions were not statistically significant.

46 **Conclusions:** 25(OH)D was inversely associated with overall mortality, CVD mortality and
47 mortality due to non-cancer/non-CVD causes, but not with cancer mortality. A possible
48 interaction between vitamin A exposure and 25(OH)D concentration appears to be
49 associated with an attenuation of the inverse association between risk of death and quartile
50 of 25(OH)D concentration.
51

52 INTRODUCTION

53 Vitamin D is a fat-soluble steroid molecule that plays a pivotal role in the maintenance of
54 musculoskeletal health. For several years, however, vitamin D is emerging as a critical
55 regulator of the pathogenetic process of a number of non-skeletal diseases such as
56 cardiovascular [1] and autoimmune disorders [2,3], infections [4,5] and several types of
57 cancers [6,7], indicating a possible pleiotropic effect across extraskeletal systems.
58 Accordingly, it was suggested that low levels of vitamin D may increase the risk of death
59 due to the wide ranging anti-inflammatory and immune modulating effects [8,9]. A recently
60 published meta-analysis of observational and trial data relating vitamin D to the risk of all-
61 cause and cause-specific mortality found inverse associations of circulating 25-
62 hydroxyvitamin D [25(OH)D] concentration with risks of death due to cardiovascular
63 disease, cancer, and non-vascular/non-cancer causes [10]. Suggested biological functions in
64 the suspected causal pathway include immune-modulatory properties, induction of cell
65 differentiation, inhibition of angiogenesis and cell proliferation, stimulation of insulin
66 production, and inhibition of rennin production [11,12,7,13-15]. Given the high prevalence
67 of vitamin D deficiency worldwide, this issue is becoming of paramount importance [16].
68 Most of the biologic activities of vitamin D are mediated by its binding to a high-affinity
69 nuclear receptor (VDR) that acts as a ligand-activated transcription factor. A crucial step in
70 the control of gene transcription by VDR involves heterodimerization with the retinoid X
71 receptor (RXR), in order that high affinity binding of the heterodimer (RXR-VDR) to specific
72 DNA sites – the vitamin D response elements (VDREs) – can occur [17]. However,
73 excessively high concentrations of 9-cis-retinoic acid, an active metabolite of vitamin A and
74 the ligand of RXR, can lead to the formation of RXR-RXR homodimers instead of

75 heterodimers with VDR. If this highly regulated heterodimerisation process is interrupted,
76 vitamin D cannot exert its important transcriptional effects in the human body [18]. Recent
77 data from epidemiological studies suggest that the association between 25(OH)D and
78 cancer incidence or mortality is modified by vitamin A, such that excess circulating vitamin
79 A may attenuate a beneficial association [19-22]. Studies on the association of 25(OH)D
80 with mortality other than cancer mortality so far have not considered a potential vitamin D-
81 vitamin A interaction effect. We tested the hypothesis that inverse associations of serum
82 25(OH)D with all-cause, cancer, CVD and non-cancer/non-CVD mortality are modified by
83 circulating levels of vitamin A in a prospective cohort of healthy US adults (NHANES III;
84 1988–1994).

85

86 **SUBJECTS AND METHODS**

87 **Study population**

88 NHANES is a data collection program designed to assess the health and nutritional status of
89 the civilian, non-institutionalized population in the United States. The third NHANES study
90 was conducted by the National Center for Health Statistics (NCHS) between 1988 and 1994
91 using a stratified, multistage probability design. In order to provide reliable estimates for
92 specific subgroups of the US population, young children, older persons (aged 65 or older),
93 black persons, and Mexican Americans were oversampled. NHANES III collected household
94 interview data including demographics and data on health and nutrition for 33,994 (85.6%)
95 of the 39,695 invited participants. Subsequent physical and laboratory examinations in
96 mobile examination centers (MEC) or at home visits were conducted for 30,882 (77.8%)
97 subjects. The NCHS Institutional Review Board approved all procedures, and all subjects

98 were provided a written informed consent sheet. Detailed methods for the NHANES III
99 baseline data collection, including sampling, in-house interview, physical examination,
100 laboratory measurements, mortality linkage, ethics approval, and informed consent have
101 been described elsewhere [23]. All analyses in this report are based on NHANES data
102 extracted from the publicly available NHANES website.

103 Our analysis was restricted to the 20,024 adults, defined as 17 years or older in NHANES III.
104 We excluded those who did not complete both the interview and the subsequent MEC
105 examination including a blood draw (n=1875), women who were pregnant at baseline
106 (n=280), individuals without reported serum 25(OH)D measurement (n=1159) and
107 participants without complete information on the study variables (n=712), resulting in a
108 cohort of 15,998 individuals.

109

110 **Mortality follow-up**

111 The NHANES III linked mortality file provides mortality follow-up data from the date of
112 survey participation (1988-1994) through December 31, 2006. All participants aged 17
113 years or older at baseline were eligible for mortality follow-up. Vital status was assessed
114 based primarily upon the results from a probabilistic match between NHANES III and
115 National Death Index (NDI) death certificate records [24]. The follow-up period was
116 calculated as the time from examination to either a mortal event or censoring date.

117 Underlying cause of death was coded using the 9th revision of the International Statistical
118 Classification of Diseases, Injuries, and Causes of Death (ICD-9) for deaths occurring
119 between 1988 and 1998, and the 10th ICD revision (ICD-10) for deaths occurring between
120 1999 and 2006. All death before 1999 were recoded by the NCHS to ICD-10 codes for

121 comparability. Cardiovascular disease mortality was defined as ICD-10 codes I00–I99,
122 corresponding to the ICD-9 codes 390–434 and 436–459 and cancer mortality as ICD-10
123 codes C00–C34, C37–C41, C43–C49, C50–C52, C54–C65, C67–C80, C82–C85, C88, C90–C95
124 and C97, corresponding to the ICD-9 codes 140–239. Non-cancer/non-CVD mortality
125 included all death with known underlying causes, except CVD and cancer deaths. All-cause
126 mortality included all specified causes of death as well as cases with unknown cause. During
127 the median 14.5 years of follow-up, there were 3890 (24%) deaths in our analytical cohort,
128 including 1715 CVD-related deaths and 844 cancer-related deaths.

129

130 **Covariate assessment**

131 Information on age, sex, race/ethnicity, and socioeconomic status was obtained by self-
132 report from the household interview. Race/ethnicity was reported as non-Hispanic white,
133 non-Hispanic black, Mexican-American (defined as persons of Mexican origin living in the
134 United States), and others (including multiracial). Age was defined as the age in years at
135 time of recruitment. Socioeconomic status was assessed using the poverty income ratio, a
136 calculated variable based on family income and family size, and self-reported years of
137 schooling (less, equal, more than high school). Information on alcohol consumption was
138 divided into 3 categories (none, 1–8, 9+ times/month) and smoking history was classified
139 according to current (1–20 cigarettes/day, >20 cigarettes/day), former or never smokers.
140 The level of physical activity was categorized into none, 1–3, 4+ times of moderate physical
141 activity per week. In women, hormone replacement therapy was also assessed. History of
142 obstructive pulmonary disease (defined as any positive response to one of the diagnoses of
143 asthma, emphysema or chronic bronchitis), as well as myocardial infarction, stroke, heart

144 failure, and cancer were assessed through self-reporting. Intake and type (product label) of
145 mineral and vitamin supplements were recorded from the 30-day supplement interview. As
146 there is no evidence that pro-vitamin A properties arising from dietary intakes of
147 carotenoids contribute to vitamin A-related toxicity [25,26], the present study only
148 considered intake of supplements containing preformed vitamin A in the form of retinol
149 and its esters. Supplement use was divided into quartiles based on information on
150 frequency and quantity (units each time) of consumption in the past month.

151 From physical examination data, height and weight were used to calculate the body mass
152 index (BMI) as kg/m². Hypertension, diabetes mellitus and hypercholesterolemia were
153 defined by history/physician's diagnosis or medication use. Fasting blood samples collected
154 during examination were centrifuged, aliquoted, and frozen to -70°C before transport on
155 dry ice to central laboratories for analysis. Blood collection in mobile unites was performed
156 in two seasonal groups based generally on latitude, with southern collections undertaken
157 during the winter months (November to March), and northern collections during the
158 summer months (April to October). To account for variability of latitude and season, we
159 divided our sample into two groups: winter/ lower latitude and summer/higher latitude.
160 Serum 25(OH)D was measured using a radio-immunoassay kit (Diasorin, Stillwater, MN).
161 Serum levels of retinol and retinyl esters (that is, the sum of retinyl linoleate, retinyl oleate,
162 retinyl palmitate and retinyl stearate) were assayed by isocratic high-performance liquid
163 chromatography with detection at three different wavelengths (Waters, Milford, MA).

164

165 **Statistical Analysis**

166 All analyses were performed using STATA statistical software version 13 (StataCorp. 2013,
167 College Station, Texas, USA). In order to account for the complex survey design of NHANES
168 III all analyses were weighted by using the “survey” command, with the “subpop” option to
169 subset data. A 2-sided P value of 0.05 was the criterion for statistical significance.

170 Continuous variables are expressed as mean +/- standard deviation (SD), categorical
171 variables are presented as proportions. Cox proportional hazard regression models were
172 used to examine the association between 25(OH)D concentrations and mortality, whereby
173 multivariable-adjusted hazard ratios (HR) and 95 % confidence intervals (CI) were
174 estimated for total and cause-specific mortality. Serum 25(OH)D concentration was
175 modeled continuously (per 10 ng/mL) and in quartiles based on the unweighted
176 distribution in the cohort. For all participants, time at entry was the date of physical
177 examination. Time at exit was either date of death or date of censoring, whichever came
178 first. Covariates included in the multivariable models were selected a priori. The primary
179 analysis focused on the association between baseline serum 25(OH)D concentration and all-
180 cause, cancer, CVD, and non-cancer/non-CVD mortality during follow-up. Three different
181 multivariable models were used, specified a priori, to test for the independent effect of
182 serum 25(OH)D on mortality. The first model adjusted for age, sex, race/ethnicity and
183 season. Building on the first model, the second model further adjusted for lifestyle and
184 socioeconomic factors, including BMI, smoking status, alcohol consumption, physical
185 activity, and hormone replacement therapy in women as well as poverty income ratio and
186 education level. The third model added potential mediators in the suspected causal
187 pathway to help explain the observed associations. This model also included hypertension,
188 diabetes mellitus, hypercholesterolemia, obstructive pulmonary disease, and history of

189 myocardial infarction, stroke, and cancer. To examine a potential modifying effect of
190 vitamin A on main effects of serum 25(OH)D we ran stratified analyses by excess circulating
191 vitamin A and preformed vitamin A supplement use. Currently, there is no well-accepted,
192 non-invasive physiological measure of vitamin A excess. Serum retinol is tightly regulated
193 by liver storage and by the production of retinol-binding protein, and is likely a better
194 biomarker of vitamin A deficiency rather than excess [27]. While serum retinol may only
195 slightly be elevated when vitamin A intake is excessive, serum retinyl esters are markedly
196 increased [28]. Therefore, fasting retinyl ester levels have been used as a marker of possible
197 vitamin A toxicity or hypervitaminosis A [29,30]. Under normal conditions retinyl esters
198 account for less than 5% of total serum vitamin A [30] and concentrations ≥ 7.0 $\mu\text{g}/\text{dL}$ have
199 been interpreted as marker of potential toxicity [27]. In our study, excess circulating
200 vitamin A was therefore defined as serum retinyl esters ≥ 7 $\mu\text{g}/\text{dL}$. Data were also stratified
201 by quartiles of serum retinol and preformed vitamin A intake from supplements. Since a
202 definitive cut-off value to indicate excess vitamin A is lacking for both markers, the 75th
203 percentile was used as threshold in the analyses. Effect modification was assessed on a
204 multiplicative scale by using the Wald test to compare adjusted Cox models with and
205 without an interaction term of serum 25(OH)D and the vitamin A stratification variable. To
206 at least partially preclude reverse causation, we conducted additional analyses by excluding
207 cases during the first 5 years of follow-up. Restricted cubic spline models were used to
208 provide evidence of non-linear relations between 25(OH)D and mortality.

209

210 **RESULTS**

211 **Table 1** summarizes demographic characteristics and confounding variables of the
212 weighted NHANES III sample according to serum 25(OH)D quartiles. Median 25(OH)D
213 concentration was 28.3 ng/mL (weighted sample), mean age at baseline was 43.4 years.
214 Individuals with low 25(OH)D concentrations were older, less physically active, and more
215 often female. Blacks and Mexican Americans were overrepresented in the lowest quartile of
216 25(OH)D concentration. Higher 25(OH)D concentration was associated with a higher
217 educational level, higher income, and lower prevalence of diabetes, hypertension, COPD, as
218 well as history of stroke and myocardial infarction.

219 During the 14.5 years of follow-up, 3890 (24.3 %) of the 15,998 study participants died. Of
220 these, 844 (21.7 %) deaths were related to cancer and 1715 (44.1 %) to CVD. A significant
221 association of serum 25(OH)D with all-cause mortality was observed when adjusting for
222 age, sex, race/ethnicity and season (HR: 0.89; 95% CI: 0.85, 0.94 per 10 ng/mL increase in
223 25(OH)D; **Table 2**). The inverse association remained statistically significant even after
224 controlling for potential confounders and intermediate variables (HR: 0.93; 95 % CI: 0.89,
225 0.97 per 10 ng/mL increase in 25(OH)D). A similar pattern was observed in the categorical
226 model, where HRs tended to decrease with increasing quartiles of 25(OH)D. Comparing
227 individuals with a high 25(OH)D concentration of ≥ 40 ng/mL (100 nmol/L) to those with
228 concentrations < 16 ng/mL (25 nmol/L), we observed a HR of 0.70 (95% CI: 0.50, 0.83).

229 Excluding the first five years of follow-up did not materially affect the observed associations
230 (data not shown). Results of the restricted cubic spline models did not indicate a non-linear
231 association between serum 25(OH)D and mortality (P -value > 0.05 ; results not shown).

232 The inverse association between 25(OH)D and all-cause mortality remained statistically
233 significant among participants with serum retinyl ester concentrations < 7.0 $\mu\text{g/dL}$ (HR:

234 0.92; 95% CI 0.88, 0.97 per 10 ng/mL increase in 25(OH)D), but not among those with
235 serum retinyl esters ≥ 7.0 $\mu\text{g}/\text{dL}$ (HR: 0.97, 95% CI 0.85, 1.09; *P*-interaction = 0.59).
236 Similarly, in the categorical model a statistically significant inverse association of serum
237 25(OH)D with all-cause mortality was observed for serum retinyl esters < 7.0 $\mu\text{g}/\text{dL}$ (**Figure**
238 **1**). When data were stratified by preformed vitamin A supplement use, effect estimates
239 were generally lower among participants taking supplements. However, looking at vitamin
240 A supplement use in more detail, the potential protective effect of 25(OH)D on overall
241 mortality held for individuals taking supplements containing preformed vitamin A at
242 amounts ≤ 5000 IU (HR: 0.87, 95% CI 0.77, 0.97 per 10 ng/mL increase in 25(OH)D), but not
243 for those taking high amounts (> 5000 IU) of preformed vitamin A from supplements (HR:
244 1.01, 95% CI 0.72, 1.43; *P*-interaction = 0.53). However, these results should be interpreted
245 with caution due to the low case numbers in the subgroups. When stratifying by serum
246 retinol, HR tended to decrease with increasing quartiles of 25(OH)D concentrations in both
247 strata, with the risk reduction being more pronounced at high concentrations (> 69 $\mu\text{g}/\text{dL}$)
248 of serum retinol (HR: 0.64, 95% CI 0.48, 0.86 for serum retinol > 69 $\mu\text{g}/\text{dL}$ and HR: 0.82,
249 95% CI 0.71, 0.96 for serum retinol ≤ 69 $\mu\text{g}/\text{dL}$; top vs. bottom quartile; *P*-interaction =
250 0.16). Generally, the strength of associations was similar when analyses were stratified by
251 sex (**Supplementary Table 1**).

252 Overall, there was no significant association between serum 25(OH)D and total cancer
253 mortality (**Supplementary Table 2**). Similar to associations seen with all-cause mortality,
254 lower 25(OH)D was associated with increased CVD mortality (HR = 0.79, 95% CI 0.67, 0.94;
255 top vs. bottom quartile, model 3; Supplementary Table 2). At serum retinyl ester
256 concentrations < 7 $\mu\text{g}/\text{dL}$ and serum retinol concentrations > 69 $\mu\text{g}/\text{dL}$, 25(OH)D

257 maintained its inverse association with CVD mortality (HR: 0.76, 95% CI 0.61, 0.95 and HR:
258 0.62, 95% CI 0.44, 0.89, respectively; top vs. bottom quartile). Mortality due to non-
259 cancer/non-CVD causes was significantly inversely associated with 25(OH)D in the
260 multivariable-adjusted model (HR: 0.89, 95% CI 0.82, 0.97 per 10 ng/mL increase in
261 25(OH)D). Lower serum retinyl esters as well as preformed vitamin A supplement use were
262 associated with lower mortality (HR: 0.87, 95% CI 0.78, 0.96 and HR: 0.79, 95% CI
263 0.65, 0.95, respectively, per 10 ng/mL increase in 25(OH)D). Whether differences in
264 preformed vitamin A intake from supplements modify risk estimates for cause-specific
265 mortality could not be assessed due to the low number of cases in the supplementation
266 group.

267

268 **DISCUSSION**

269 In this nationally representative sample of US adults, we observed an inverse association of
270 serum 25(OH)D with all-cause mortality, CVD mortality and mortality due to non-
271 cancer/non-CVD causes. We found these associations to be modified by circulating
272 concentrations of serum retinyl ester, a commonly used biomarker of possible vitamin A
273 excess, in a way that the beneficial associations were attenuated among those with
274 excessively high concentrations (≥ 7 $\mu\text{g}/\text{dL}$). In addition, high preformed vitamin A intake
275 (>5000 IU) from supplements was found to diminish the inverse association of 25(OH)D
276 with overall mortality. However, there was limited statistical evidence of an interaction
277 between 25(OH)D and vitamin A exposure.

278 Our results on the relation of serum 25(OH)D and mortality corroborate earlier findings. A
279 recently published patient level meta-analysis of eight observational studies from Europe

280 and the United States, including the NHANES III survey, has shown that low 25(OH)D is
281 associated with an increase in all-cause and cardiovascular mortality, with a curvilinear
282 inverse association between 25(OH)D concentration and mortality outcomes [31]. These
283 results are similar to previous study level meta-analyses of observational studies, where
284 vitamin D deficiency has been suggested as an independent risk factor for all-cause and CVD
285 mortality [32-34,8,35]. While the association between serum 25(OH)D and CVD mortality
286 appears to be a strong inverse association, findings regarding cancer mortality are
287 heterogeneous. Most prospective studies and meta-analyses have focused on colorectal,
288 breast, and prostate cancer and often yielded different results depending on tumour type
289 [36-43]. Schottker et al. [31] observed an association only among subjects with a history of
290 cancer, and two other recently published meta-analyses showed weak, albeit statistically
291 significant, elevated pooled risk ratios [10,44]. Studies using data of NHANES III have
292 reported an inverse association between circulating concentrations of 25(OH)D and overall
293 [45] as well as cardiovascular disease mortality [46], but the associations with cancer
294 mortality were not entirely clear [47]. These studies differed from our study in that the
295 length of follow-up was shorter (7.3 years [46] and 8.7 years [45]) and one study [46] only
296 included participants aged 65 or older.

297 We further observed that the inverse associations between 25(OH)D and mortality were
298 diminished among those with excess circulating retinyl esters. The fact that this pattern did
299 not hold for serum retinol strata does not conflict with other reports that suggest that
300 retinol concentrations are under tight homeostatic control and may remain constant or
301 decline to compensate for higher retinyl ester concentrations [29,48]. The large differences
302 in risk estimates we observed between strata of vitamin A variables did mostly not result in

303 a significant vitamin D-vitamin A interaction, which may be attributed to small case
304 numbers in the respective subgroups. Previous evidence as to whether vitamin A modifies
305 vitamin D's effect on mortality is limited. To our knowledge, this is the first study to
306 examine a possible vitamin D-vitamin A interaction not only in association with cancer
307 mortality, but also with overall, CVD, and non-cancer/non-CVD mortality. Among the few
308 epidemiological studies investigating the influence of vitamin A on vitamin D-related cancer
309 risks, high intakes of retinol were found to mask a beneficial association of vitamin D with
310 colorectal and pancreatic cancer [22,21]. Several studies on lung cancer have recently been
311 published but results are conflicting. Cheng, Neuhouser [19] reported that the inverse
312 association of 25(OH)D with lung cancer mortality seen in non-smokers was more likely to
313 be observed among those with no sign of excess vitamin A exposure in NHANES III.
314 Similarly, a recent study in postmenopausal women reported suggestive evidence that
315 lower vitamin A intake may be important for a beneficial association of vitamin D
316 supplementation with lung cancer risk [20]. However, statistical evidence to support effect
317 modification by vitamin A was limited in both studies. In the Alpha-Tocopherol, Beta-
318 Carotene Cancer Prevention Study (ATBC) serum retinol was not found to modify the effect
319 of vitamin D on lung cancer risk [49] and results of the Carotene and Retinol Efficacy Trial
320 (CARET) showed that high-dose vitamin A may be important for the protective effect of
321 vitamin D against lung cancer among smokers [50]. Caution should be exercised when
322 comparing our results to these findings. In most studies the primary outcome was cancer
323 incidence rather than mortality. In addition, no study but one [19] used retinyl ester as
324 biomarker of vitamin A excess and studies on vitamin A intake usually included pro-vitamin
325 A carotenoids.

326 Overall, serum 25(OH)D was similarly inversely associated with all-cause and cause-
327 specific mortality when we stratified analyses by sex. Other studies that reported their
328 results for all-cause mortality stratified by sex have found very similar results for both
329 sexes [51,52], stronger associations in men [53], or stronger associations in women [45].
330 One study reported a difference between sexes for cause-specific death [54]. Interestingly,
331 we found the amount of supplemental preformed vitamin A intake to significantly modify
332 the effect of vitamin D on all-cause mortality. Low case numbers among supplement users
333 did not allow for gender-specific analyses for cause-specific mortality.

334 Although we cannot exclude that vitamin D interferes with vitamin A metabolism rather
335 than the other way around, the biological mechanism by which circulating vitamin A is
336 thought to mask a beneficial association of 25(OH)D with mortality seems plausible and has
337 frequently been discussed in the literature [17,55,18]. It involves excessively high
338 concentrations of 9-cis-retinoic acid, an active metabolite of vitamin A, leading to retinoid X
339 receptor (RXR) homodimers (RXR-RXR) instead of VDR-RXR heterodimers. Serum
340 concentrations of 9-cis-retinoic acid are directly related to dietary vitamin A intake [56],
341 but the precise concentration of vitamin A leading to disturbance of heterodimerisation
342 remains unknown. In most developed countries including the U.S., consumption of
343 multivitamin or single supplement products commonly consisting of high-dose preformed
344 vitamin A has increased over time and concerns of subclinical vitamin A toxicity have
345 already been raised [57-59]. In our cohort, 21% of study participants took supplements
346 containing preformed vitamin A and more than 20% had excess circulating vitamin A,
347 defined as retinyl esters ≥ 7.0 $\mu\text{g}/\text{dL}$.

348 The strengths of our study include statistical adjustment for a wide range of factors.
349 Furthermore, NHANES III is a large well-characterized survey, which incorporates a
350 representative sample of the U.S. population. A number of limitations should be considered
351 when interpreting our results. First, serum 25(OH)D was only measured once in NHANES
352 III. Second, serum 25(OH)D data from NHANES III have an inherent season-latitude
353 structure that prevents assessing associations in specific subgroups. In addition, results of
354 sub-analyses should be interpreted with caution because of low number of cases. Moreover,
355 adequate concentrations of vitamin D may reflect a pattern of behavior to minimize threats
356 to one's health and thus be a proxy for a healthy lifestyle. Although we controlled for
357 numerous confounders, potential residual and unmeasured confounding remains a distinct
358 possibility.

359 In this study, inverse associations of 25(OH)D with overall, CVD and non-cancer/non-CVD
360 mortality were found to be diminished if circulating vitamin A was excessively high (retinyl
361 esters ≥ 7.0 $\mu\text{g}/\text{dL}$). The beneficial association between 25(OH)D and all-cause mortality
362 further remained statistically significant only in participants taking preformed vitamin A
363 from supplements in amounts ≤ 5000 IU. If the interaction effect is real, i.e. vitamin A
364 interferes with the action of vitamin D, our findings underscore the need to assess safety of
365 high intakes of preformed vitamin A in order to prevent toxic levels in the body that
366 potentially undermine a protective effect of vitamin D. Other than with preformed vitamin
367 A intake, a diet rich in red and yellow-orange fruits and vegetables such as carrots and
368 sweet potatoes would supply all the carotenoids the body needs to make retinol without the
369 potential for hypervitaminosis A. Further well-designed studies to more clearly identify a

370 potential causal relationship of vitamin D with overall and cause-specific mortality as well
 371 as a potential interaction with vitamin A are warranted.

372

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TABLE 1

Baseline demographic and health-related characteristics of study participants by quartiles of serum 25-hydroxyvitamin D (25(OH)D)

	Total	25(OH)D quartiles			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
Vitamin D (ng/mL)		<17.7	17.7-24.2	24.3-32.0	>32.0
Vitamin D (nmol/L)		<44.2	44.2-60.4	60.5-79.9	>79.9
<i>n</i>	15998	4024	3984	3994	3996
Follow-up time (y, median)	14.5	14.3	14.5	14.7	14.4
Age (y)	43.4±16.5 ¹	45.0±21.2	45.8±18.5	44.1±16.0	40.9±13.0
Female (%)	51.2	66.5	56.5	49.6	43.1
Race/ethnicity (%)					
Non-Hispanic white	76.5	46.5	67.4	81.2	90.4
Non-Hispanic black	10.6	33.8	14.3	6.1	2.3
Mexican American	5.1	7.3	7.0	5.4	2.8
Other	7.9	12.4	11.4	7.3	4.4
Season of blood collection (%)					
Winter/lower latitude	31.5	45.9	35.3	30.3	24.3
Summer/higher latitude	68.5	54.1	64.7	69.7	75.7
Body mass index (kg/m ²)	26.4±5.3	27.9±8.0	27.5±6.3	26.3±4.8	25.2±3.6
Moderate physical activity per week (%)					
≤3	39.1	40.6	43.1	39.9	35.7
>3	46.5	32.8	39.1	46.6	56.1
Never	14.4	26.6	17.8	13.5	8.2
Poverty-income ratio (%)					
Poor	17.5	25.1	19.6	16.4	14.0
Middle income	42.7	41.9	43.4	40.7	44.2
Higher income	33.5	26.6	30.2	36.7	35.8
Missing	6.3	6.4	6.9	6.2	6.0
Education (%)					
Less than high school	26.4	29.3	28.2	27.0	23.9
High school	33.5	37.2	33.8	31.1	33.5
More than high school	40.1	33.4	38.0	41.9	42.7
Smoking status (%)					
Current, ≤20 cigarettes/day	19.9	25.3	19.5	18.0	19.2
Current, >20 cigarettes/day	7.3	6.0	6.2	7.2	8.5

Former	25.3	20.5	24.2	25.8	27.5
Never	47.5	48.1	50.0	49.1	44.8
Alcohol consumption per week (%)					
≤2	26.3	25.4	25.6	26.7	26.8
>2	28.6	21.7	23.7	30.8	32.5
Never	45.1	52.9	50.6	42.6	40.7
Diabetes mellitus (%)	5.2	8.5	6.3	5.5	3.0
Hypertension (%)	18.7	23.1	21.3	18.5	15.6
Hypercholesterolemia (%)	19.5	19.0	20.9	19.1	19.1
Chronic obstructive pulmonary disease (%)	13.0	16.2	13.3	13.1	11.4
History of cancer (%)	3.5	3.1	4.0	3.7	3.3
History of stroke (%)	1.9	2.8	2.3	1.6	1.5
History of myocardial infarction (%)	3.3	3.9	4.0	3.2	2.8

¹Mean±SD (all such variables)

All estimates were weighted to account for the complex survey design of NHANES III

TABLE 2

Hazard ratios with 95% confidence intervals for all-cause and cause-specific mortality in NHANES III (1988–2006)¹

Continuous model ²	All-cause	Cancer	CVD	Non-cancer-non-CVD
<i>n</i> _{cases}	3890	871	1715	1284
Model 1	0.89 (0.85,0.94)	0.98 (0.87,1.09)	0.87 (0.81,0.93)	0.87 (0.79,0.95)
Model 2	0.93 (0.88,0.97)	1.01 (0.90,1.13)	0.91 (0.85,0.98)	0.88 (0.81,0.97)
Model 3	0.93 (0.89,0.97)	1.01 (0.90,1.13)	0.92 (0.86,0.98)	0.89 (0.82,0.97)
Serum retinyl esters				
<i>n</i> _{cases}	963	175	413	312
≥7.0 µg/dL	0.97 (0.85,1.09)	0.94 (0.73,1.21)	0.98 (0.86,1.10)	0.98 (0.83,1.16)
<i>n</i> _{cases}	2927	669	1249	972
<7.0 µg/dL	0.92 (0.88,0.97)	1.04 (0.93, 1.17)	0.90 (0.82,0.98)	0.87 (0.78,0.96)
Serum retinol				
<i>n</i> _{cases}	1269	240	609	413
Above 75th percentile (> 69 µg/dL)	0.89 (0.82,0.96)	0.94 (0.75,1.16)	0.88 (0.78,0.99)	0.86 (0.75,0.98)
<i>n</i> _{cases}	2621	604	1106	871
At or below 75th percentile (≤ 69 µg/dL)	0.95 (0.90,1.01)	1.05 (0.89,1.24)	0.94 (0.86,1.03)	0.90 (0.81,0.99)
Preformed vitamin A supplement use				
<i>n</i> _{cases}	838	187	363	281
Yes	0.89 (0.79,1.00)	0.93 (0.71,1.21)	0.97 (0.84,1.11)	0.79 (0.65,0.95)
<i>n</i> _{cases}	3052	657	1352	1003
No	0.95 (0.89,1.01)	1.04 (0.90,1.19)	0.91 (0.83,0.99)	0.93 (0.84,1.04)
Preformed vitamin A intake from supplements				
<i>n</i> _{cases}	81			
Above 75th percentile (> 5000IU)	1.01 (0.72,1.43)			
<i>n</i> _{cases}	757			
At or below 75th percentile (≤ 5000IU)	0.87 (0.77,0.97)			

¹*n*_{cases}, number of deaths; Model 1 was adjusted for age, sex, race/ethnicity, and season; Model 2 was adjusted for variables of model 1 and for lifestyle and socioeconomic factors (poverty-income ratio, body mass index, physical activity, smoking status, alcohol consumption, education and hormone replacement therapy); Model 3 was adjusted for variables of model 2 and for potential intermediates (hypertension, diabetes mellitus, hypercholesterolemia, history of myocardial infarction, history of stroke, and history of cancer; All P-values for interaction were >0.05

²Per 10 ng/mL increase in 25(OH)D entered as a continuous variable
All estimates were weighted to account for the complex survey design of NHANES III

SUPPLEMENTARY TABLE 1

Sex-specific hazard ratios with 95% confidence intervals for all-cause mortality by quartiles of serum 25-hydroxyvitamin D [25(OH)D] in NHANES III (1988–2006)¹

	Hazard ratios (95% CI) by quartiles of 25(OH)D (ng/mL)				continuous model ²	P-interaction
	Quartile 1 (<17.7)	Quartile 2 (17.7-24.2)	Quartile 3 (24.3-32.0)	Quartile 4 (>32.0)		
Men						
<i>n</i> _{cases}	408	514	578	602		
Model 1	1.00 (reference)	0.83 (0.68,1.01)	0.73 (0.58,0.92)	0.75 (0.61,0.93)	0.95 (0.89, 1.02)	
Model 2	1.00 (reference)	0.88 (0.73,1.07)	0.80 (0.65,1.00)	0.78 (0.64,0.95)	0.95 (0.89,1.01)	
Model 3	1.00 (reference)	0.89 (0.73,1.08)	0.79 (0.64,0.97)	0.81 (0.67,0.98)	0.95 (0.89,1.01)	
Serum retinyl esters						
<i>n</i> _{cases}	51	102	128	163		<i>p</i> _{cat} =0.71, <i>p</i> _{cont} =0.19
≥7.0 µg/dL	1.00 (reference)	0.92 (0.51,1.65)	0.86 (0.45,1.68)	0.94 (0.50,1.78)	1.05 (0.89,1.23)	
<i>n</i> _{cases}	357	412	450	439		
<7.0 µg/dL	1.00 (reference)	0.90 (0.71,1.13)	0.76 (0.59,1.00)	0.78 (0.63,0.95)	0.93 (0.87,0.99)	
Serum retinol						
<i>n</i> _{cases}	101	171	199	248		<i>p</i> _{cat} =0.29, <i>p</i> _{cont} =0.29
Above 75th percentile (> 69 µg/dL)	1.00 (reference)	0.66 (0.43,1.02)	0.62 (0.41,0.95)	0.60 (0.38,0.95)	0.91 (0.81,1.02)	
<i>n</i> _{cases}	307	343	379	354		
At or below 75th percentile (≤ 69 µg/dL)	1.00 (reference)	1.01 (0.83,1.21)	0.83 (0.66,1.05)	0.89 (0.72,1.11)	0.98 (0.90,1.06)	
Preformed vitamin A supplement use						
<i>n</i> _{cases}	39	89	116	154		<i>p</i> _{cat} =0.21, <i>p</i> _{cont} =0.16
Yes	1.00 (reference)	0.75 (0.47,1.22)	0.56 (0.33,0.95)	0.57 (0.35,0.94)	0.85 (0.72,1.00)	
<i>n</i> _{cases}	369	425	462	448		
No	1.00 (reference)	0.90 (0.73,1.10)	0.87 (0.71,1.06)	0.85 (0.70,1.03)	0.98 (0.91,1.05)	
Preformed vitamin A intake from supplements						
<i>n</i> _{cases}	3	11	10	20		<i>p</i> _{cat} =0.04, <i>p</i> _{cont} =0.06
Above 75th percentile (> 5000IU)	1.00 (reference)	3.10 (0.34,28.51)	1.15 (0.15,8.67)	4.93 (0.80,30.40)	1.64 (0.89,3.01)	
<i>n</i> _{cases}	36	78	106	134		
At or below 75th percentile (≤ 5000IU)	1.00 (reference)	0.64 (0.40,1.03)	0.48 (0.27,0.84)	0.47 (0.29,0.76)	0.80 (0.70,0.92)	
Women						
<i>n</i> _{cases}	582	478	411	317		
Model 1	1.00 (reference)	0.75 (0.64,0.87)	0.67 (0.55,0.81)	0.58 (0.48,0.71)	0.84 (0.77,0.91)	
Model 2	1.00 (reference)	0.84 (0.73,0.96)	0.81 (0.68,0.97)	0.73 (0.60,0.88)	0.91 (0.85,0.99)	
Model 3	1.00 (reference)	0.88 (0.77,1.01)	0.82 (0.69,0.97)	0.77 (0.63,0.95)	0.92 (0.86,1.00)	
Serum retinyl esters						
						<i>p</i> _{cat} =0.91, <i>p</i> _{cont} =0.64

n_{cases}	98	161	133	127		
$\geq 7.0 \mu\text{g/dL}$	1.00 (reference)	0.80 (0.52,1.21)	0.71 (0.47,1.07)	0.72 (0.42,1.25)	0.92 (0.76,1.12)	
n_{cases}	484	317	278	190		
$< 7.0 \mu\text{g/dL}$	1.00 (reference)	0.88 (0.74,1.04)	0.82 (0.65,1.03)	0.80 (0.59,1.07)	0.93 (0.84,1.02)	
Serum retinol						$p_{\text{cat}}=0.10, p_{\text{cont}}=0.38$
n_{cases}	136	144	129	141		
Above 75th percentile ($> 69 \mu\text{g/dL}$)	1.00 (reference)	0.95 (0.69,1.31)	0.69 (0.50,0.95)	0.67 (0.45,1.00)	0.87 (0.76,1.00)	
n_{cases}	446	334	282	176		
At or below 75th percentile ($\leq 69 \mu\text{g/dL}$)	1.00 (reference)	0.84 (0.69,1.02)	0.88 (0.72,1.09)	0.78 (0.60,1.03)	0.93 (0.84,1.02)	
Preformed vitamin A supplement use						$p_{\text{cat}}=0.14, p_{\text{cont}}=0.97$
n_{cases}	72	111	125	132		
Yes	1.00 (reference)	0.76 (0.46,1.25)	0.59 (0.41,0.85)	0.66 (0.40,1.09)	0.93 (0.76,1.13)	
n_{cases}	510	367	286	185		
No	1.00 (reference)	0.91 (0.77,1.08)	0.90 (0.72,1.14)	0.77 (0.60,0.99)	0.92 (0.83,1.02)	
Preformed vitamin A intake from supplements						$p_{\text{cat}}=0.50, p_{\text{cont}}=0.16$
n_{cases}	3	6	6	22		
Above 75th percentile ($> 5000\text{IU}$)	1.00 (reference)	0.26 (0.05,1.41)	0.08 (0.00,5.15)	0.69 (0.19,2.57)	0.80 (0.49,1.31)	
n_{cases}	69	105	119	110		
At or below 75th percentile ($\leq 5000\text{IU}$)	1.00 (reference)	0.75 (0.46,1.21)	0.63 (0.43,0.92)	0.68 (0.43,1.09)	0.95 (0.77,1.16)	

¹ n_{cases} , number of deaths; p_{cat} , P-value of test for interaction in the categorical model; p_{cont} , P-value of test for interaction in the continuous model; Model 1 was adjusted for age, sex, race/ethnicity, and season; Model 2 was adjusted for variables of model 1 and for lifestyle and socioeconomic factors (poverty-income ratio, body mass index, physical activity, smoking status, alcohol consumption, education and hormone replacement therapy); Model 3 was adjusted for variables of model 2 and for potential intermediates (hypertension, diabetes mellitus, hypercholesterolemia, history of myocardial infarction, history of stroke, and history of cancer)

²Per 10 ng/mL increase in 25(OH)D entered as a continuous variable
All estimates were weighted to account for the complex survey design of NHANES III

SUPPLEMENTARY TABLE 2

Hazard ratios with 95% confidence intervals for all-cause and cause-specific mortality by quartiles of serum 25-hydroxyvitamin D [25(OH)D] in NHANES III (1988–2006)¹

	Hazard ratios (95% CI) by quartiles of 25(OH)D (ng/mL)				continuous model ²
	Quartile 1 (<17.7)	Quartile 2 (17.7-24.2)	Quartile 3 (24.3-32.0)	Quartile 4 (>32.0)	
All-cause					
<i>n</i> _{cases}	990	992	989	919	
Model 1	1.00 (reference)	0.78 (0.68,0.88)	0.68 (0.59,0.79)	0.66 (0.58,0.75)	0.89 (0.85,0.94)
Model 2	1.00 (reference)	0.84 (0.75,0.94)	0.78 (0.69,0.89)	0.74 (0.65,0.83)	0.93 (0.88,0.97)
Model 3	1.00 (reference)	0.87 (0.77,0.97)	0.78 (0.69,0.89)	0.77 (0.68,0.87)	0.93 (0.89,0.97)
Serum retinyl esters					
<i>n</i> _{cases}	149	263	261	290	
≥7.0 µg/dL	1.00 (reference)	0.85 (0.59,1.23)	0.75 (0.52,1.10)	0.79 (0.53,1.20)	0.97 (0.85,1.09)
<i>n</i> _{cases}	841	729	728	629	
<7.0 µg/dL	1.00 (reference)	0.87 (0.77,1.00)	0.78 (0.65,0.92)	0.77 (0.67,0.88)	0.92 (0.88,0.97)
Serum retinol					
<i>n</i> _{cases}	237	315	328	389	
Above 75th percentile (> 69 µg/dL)	1.00 (reference)	0.82 (0.64,1.06)	0.66 (0.52,0.84)	0.64 (0.48,0.86)	0.89 (0.82,0.96)
<i>n</i> _{cases}	753	677	661	530	
At or below 75th percentile (≤ 69 µg/dL)	1.00 (reference)	0.88 (0.77,1.00)	0.82 (0.71,0.94)	0.82 (0.71,0.96)	0.95 (0.90,1.01)
Preformed vitamin A supplement use					
<i>n</i> _{cases}	111	200	241	286	
Yes	1.00 (reference)	0.76 (0.52,1.12)	0.58 (0.42,0.81)	0.63 (0.43,0.92)	0.89 (0.79,1.00)
<i>n</i> _{cases}	879	792	748	633	
No	1.00 (reference)	0.89 (0.79,1.00)	0.86 (0.74,1.00)	0.80 (0.68,0.94)	0.95 (0.89,1.01)
Preformed vitamin A intake from supplements					
<i>n</i> _{cases}	6	17	16	42	
Above 75th percentile (> 5000IU)	1.00 (reference)	0.86 (0.27,2.75)	0.44 (0.11,1.73)	1.13 (0.48,2.68)	1.01 (0.72,1.43)
<i>n</i> _{cases}	105	183	225	244	
At or below 75th percentile (≤ 5000IU)	1.00 (reference)	0.73 (0.51,1.04)	0.57 (0.41,0.80)	0.59 (0.41,0.85)	0.87 (0.77,0.97)
Cancer					
<i>n</i> _{cases}	201	218	214	211	
Model 1	1.00 (reference)	0.91 (0.69,1.20)	0.83 (0.63,1.10)	0.80 (0.61,1.05)	0.98 (0.87,1.09)
Model 2	1.00 (reference)	1.00 (0.75,1.32)	0.98 (0.73,1.32)	0.92 (0.67,1.25)	1.01 (0.90,1.13)
Model 3	1.00 (reference)	0.99 (0.74,1.32)	0.93 (0.68,1.27)	0.92 (0.66,1.28)	1.01 (0.90,1.13)
Serum retinyl esters					

n_{cases}	22	46	52	55	
$\geq 7.0 \mu\text{g/dL}$	1.00 (reference)	0.81 (0.36,1.86)	0.80 (0.33,1.97)	0.65 (0.25,1.67)	0.94 (0.73,1.21)
n_{cases}	179	172	162	156	
$< 7.0 \mu\text{g/dL}$	1.00 (reference)	1.05 (0.80,1.37)	0.92 (0.69,1.24)	1.04 (0.77,1.42)	1.04 (0.93, 1.17)
Serum retinol					
n_{cases}	42	60	62	76	
Above 75th percentile ($> 69 \mu\text{g/dL}$)	1.00 (reference)	0.84 (0.44,1.61)	0.77 (0.45,1.32)	0.72 (0.36,1.42)	0.94 (0.75,1.16)
n_{cases}	159	158	152	135	
At or below 75th percentile ($\leq 69 \mu\text{g/dL}$)	1.00 (reference)	1.06 (0.76,1.48)	0.99 (0.67,1.44)	1.01 (0.68,1.49)	1.05 (0.89,1.24)
Preformed vitamin A supplement use					
n_{cases}	26	46	52	63	
Yes	1.00 (reference)	0.90 (0.35,2.30)	0.67 (0.27,1.62)	0.73 (0.28,1.94)	0.93 (0.71,1.21)
n_{cases}	175	172	162	148	
No	1.00 (reference)	0.97 (0.72,1.30)	1.01 (0.73,1.39)	0.94 (0.67,1.32)	1.04 (0.90,1.19)
Cardiovascular disease					
n_{cases}	449	433	430	403	
Model 1	1.00 (reference)	0.75 (0.64,0.88)	0.63 (0.54,0.75)	0.65 (0.55,0.77)	0.87 (0.81,0.93)
Model 2	1.00 (reference)	0.80 (0.68,0.95)	0.72 (0.61,0.85)	0.74 (0.63,0.88)	0.91 (0.85,0.98)
Model 3	1.00 (reference)	0.84 (0.71,1.00)	0.74 (0.63,0.86)	0.79 (0.67,0.94)	0.92 (0.86,0.98)
Serum retinyl esters					
n_{cases}	79	127	121	139	
$\geq 7.0 \mu\text{g/dL}$	1.00 (reference)	0.84 (0.56,1.26)	0.73 (0.47,1.14)	0.87 (0.59,1.30)	0.98 (0.86,1.10)
n_{cases}	370	306	309	264	
$< 7.0 \mu\text{g/dL}$	1.00 (reference)	0.84 (0.68,1.04)	0.72 (0.57,0.91)	0.76 (0.61,0.95)	0.90 (0.82,0.98)
Serum retinol					
n_{cases}	118	153	154	184	
Above 75th percentile ($> 69 \mu\text{g/dL}$)	1.00 (reference)	0.85 (0.59,1.22)	0.58 (0.41,0.81)	0.62 (0.44,0.89)	0.88 (0.78,0.99)
n_{cases}	331	280	276	219	
At or below 75th percentile ($\leq 69 \mu\text{g/dL}$)	1.00 (reference)	0.82 (0.66,1.01)	0.83 (0.68,1.01)	0.91 (0.69,1.20)	0.94 (0.86,1.03)
Preformed vitamin A supplement use					
n_{cases}	50	84	104	125	
Yes	1.00 (reference)	0.63 (0.39,1.03)	0.55 (0.34,0.90)	0.71 (0.41,1.24)	0.97 (0.84,1.11)
n_{cases}	399	349	326	278	
No	1.00 (reference)	0.92 (0.76,1.12)	0.82 (0.67,1.01)	0.81 (0.64,1.02)	0.91 (0.83,0.99)
Non-cancer-non-cardiovascular disease					
n_{cases}	328	330	331	295	

Model 1	1.00 (reference)	0.73 (0.58,0.93)	0.65 (0.49,0.86)	0.59 (0.43,0.80)	0.87 (0.79,0.95)
Model 2	1.00 (reference)	0.80 (0.63,1.01)	0.74 (0.56,0.97)	0.63 (0.47,0.84)	0.88 (0.81,0.97)
Model 3	1.00 (reference)	0.82 (0.65,1.04)	0.74 (0.57,0.97)	0.67 (0.51,0.88)	0.89 (0.82,0.97)
Serum retinyl esters					
n_{cases}	47	89	84	92	
≥7.0 µg/dL	1.00 (reference)	0.90 (0.53,1.55)	0.70 (0.40,1.21)	0.81 (0.42,1.55)	0.98 (0.83,1.16)
n_{cases}	281	241	247	203	
<7.0 µg/dL	1.00 (reference)	0.81 (0.62,1.05)	0.76 (0.55,1.03)	0.64 (0.47,0.86)	0.87 (0.78,0.96)
Serum retinol					
n_{cases}	76	100	110	127	
Above 75th percentile (> 69 µg/dL)	1.00 (reference)	0.79 (0.48,1.30)	0.70 (0.45,1.09)	0.63 (0.35,1.11)	0.86 (0.75,0.98)
n_{cases}	252	230	221	168	
At or below 75th percentile (≤ 69 µg/dL)	1.00 (reference)	0.83 (0.64,1.07)	0.72 (0.54,0.97)	0.65 (0.51,0.84)	0.90 (0.81,0.99)
Preformed vitamin A supplement use					
n_{cases}	34	67	82	98	
Yes	1.00 (reference)	0.85 (0.53,1.36)	0.55 (0.33,0.92)	0.52 (0.30,0.89)	0.79 (0.65,0.95)
n_{cases}	294	263	249	197	
No	1.00 (reference)	0.79 (0.61,1.03)	0.81 (0.60,1.08)	0.71 (0.52,0.97)	0.93 (0.84,1.04)

¹ n_{cases} , number of deaths; Model 1 was adjusted for age, sex, race/ethnicity, and season; Model 2 was adjusted for variables of model 1 and for lifestyle and socioeconomic factors (poverty-income ratio, body mass index, physical activity, smoking status, alcohol consumption, education and hormone replacement therapy); Model 3 was adjusted for variables of model 2 and for potential intermediates (hypertension, diabetes mellitus, hypercholesterolemia, history of myocardial infarction, history of stroke, and history of cancer; All P-values for interaction were >0.05

²Per 10 ng/mL increase in 25(OH)D entered as a continuous variable
All estimates were weighted to account for the complex survey design of NHANES III

FIGURE 1

Association between circulating 25(OH)D concentration and all-cause mortality by vitamin A markers in NHANES III (1988–2006); Results are reported as hazard ratios with 95% confidence intervals (CI) per 10 ng/mL increase of 25(OH)D concentration (horizontal bar), adjusted for all variables in model 3¹

(Figure 1 from jpg/png file: *Fig1.jpg* or *Fig 1.png*. MS Excel was used to create figure 1.)

¹Model 3 was adjusted for age, sex, race/ethnicity, and season, lifestyle and socioeconomic factors (poverty-income ratio, body mass index, physical activity, smoking status, alcohol consumption, education and hormone replacement therapy) and for potential intermediates (hypertension, diabetes mellitus, hypercholesterolemia, history of myocardial infarction, history of stroke, and history of cancer). All estimates were weighted to account for the complex survey design of NHANES III

