

Association between fat-soluble vitamins and self-reported health status: a cross-sectional analysis of the MARK-AGE cohort

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Running title: Fat-soluble vitamins and health status

Abstract

Self-rated health (SRH) is associated with higher risk of death. Since low plasma levels of fat-soluble vitamins are related to mortality, we aimed to assess whether plasma concentrations of vitamins A, D and E were associated with SRH in the MARK-AGE study. We included 3158 participants (52% female) aged between 35–75 years. Cross-sectional data were collected via questionnaires. An enzyme immunoassay quantified 25-hydroxyvitamin D and HPLC determined α -tocopherol and retinol plasma concentrations. The median 25-hydroxyvitamin D and retinol concentrations differed significantly ($P<0.001$) between SRH categories, and were lower in the combined fair/poor category versus the excellent, very good, good categories (25-hydroxyvitamin D: 40.8 vs. 51.9, 49.3, 46.7 nmol/l, respectively; retinol: 1.67 vs. 1.75, 1.74, 1.70 μ mol/l, respectively). Both vitamin D and retinol status were independently associated with fair/poor SRH in multiple regression analyses: adjusted ORs (95% CI) for the vitamin D insufficiency, deficiency, severe deficiency categories were 1.33 (1.06–1.68), 1.50 (1.17–1.93), and 1.83 (1.34–2.50) respectively; $P=0.015$, $P=0.001$, $P<0.001$, and for the second/third/fourth retinol quartiles: 1.44 (1.18–1.75), 1.57 (1.28–1.93), 1.49 (1.20–1.84); all $P<0.001$. No significant associations were reported for α -tocopherol quartiles. Lower vitamin A and D status emerged as independent markers for fair/poor SRH. Further insights into the long-term implications of these modifiable nutrients on health status are warranted.

Keywords: α -tocopherol, micronutrients, plasma, retinol, vitamin D

INTRODUCTION

Preserving optimal health and quality of life is becoming vital, particularly in light of the expected increase in global life expectancy, and hence the ageing population^{1, 2}. Accordingly, strategies to reduce the projected future burden on healthcare systems are urgently required². Health status is influenced by a multitude of genetic, environmental and lifestyle factors, with nutrition playing a pivotal role^{3, 4}.

Fat-soluble micronutrient deficiencies have, for instance been associated with various diseases such as cancer, diabetes and cardiovascular disease⁵⁻¹⁰. The specific fat-soluble vitamins A, D and E have a wide array of functions, including important immunomodulatory and inflammatory, and antioxidant-related processes, and deficiencies in these vitamins can contribute to, amongst others, the weakening of the immune system^{5, 8, 11, 12}. Indeed, low micronutrient status, as measured in plasma, has not only been linked to specific diseases as mentioned above, but also to poorer general health status¹³⁻¹⁵. For instance, low plasma α -tocopherol concentrations (a marker for vitamin E status) in older adults have been associated with poor physical and mental health and with greater inflammation¹⁴. In addition, two longitudinal cohorts, the Chinese longitudinal healthy longevity survey (CLHLS) and US National Health and Nutrition Examination Surveys (NHANES) observed older adults to have lower serum 25-hydroxyvitamin D concentrations when their self-rated health status was categorised as poor¹³.

Inadequate micronutrient status, can, to a certain extent, be attributed to the ageing process, given that age has been reported as an independent predictor and main contributor to fat-soluble micronutrient status¹⁶. Nutritional status, however, declines not only with increasing age but also in the setting of chronic health conditions or in those following poor diets, which are age-independent. Indeed, a recent analysis from the 2005–2016 NHANES including 26,282 adults¹⁷ illustrated widespread dietary deficiencies in fat-soluble micronutrients. The following proportions of the population were reported to be below the estimated average requirement for vitamins A, D and E: 45%, 95%, and 84%, respectively¹⁷. Depending on the local fortification strategies, vitamin D status will of course be strongly influenced by sun exposure¹⁸.

Lower nutrient status has been linked to a higher risk of death, as evidenced in a meta-analysis for vitamin D¹⁹ and in a longitudinal study with 15 years of follow-up, which associated both poor diets and poor self-rated health (SRH) with increased mortality²⁰. Of note, SRH has recently been reported to be a significant predictor of mortality in participants with no chronic conditions¹⁵. On account of the biological functions of the fat-soluble vitamins A, D and E, we hypothesised that lower

plasma concentrations of these essential micronutrients are a prevalent risk factor for poorer health status, irrespective of age. Thus, the aim of this cross-sectional analysis was to assess for associations between plasma concentrations of fat-soluble vitamins A, D and E and self-reported health status in 3158 subjects aged between 35–75 years, participating in the European multi-country MARK-AGE study. A previous study using this cohort has previously demonstrated that self-reported health could act as a marker distinguishing people at risk of becoming frail, therefore associating self-reported health status with adverse health outcomes²¹.

PARTICIPANTS AND METHODS

Participants

Data were analysed from the cross-sectional MARK-AGE population study, which aimed to identify a set of biomarkers of ageing²²⁻²⁴. For this analysis, 3158 participants recruited from eight European centers were included. These participants were divided into three main groups. The RASIG group consisted of age-stratified individuals from the general population. This group comprised 2310 women and men aged 35–75 years. This age range, together with the ability to provide written informed consent, represented the main criterion for inclusion.

Descendants from nonagenarians (people aged between 90 and 99) were recruited into the second study group. These 537 men and women were offspring of nonagenarian subjects who had been recruited in another study, the GEHA study (Genetics of Healthy Aging²⁵). Thus this second group was named GO (GEHA offspring). Thirdly, 311 spouses from the recruited GO participants, formed the SGO group (spouses of GO), these subjects were enrolled as a lifestyle control group. Recruitment of GO and SGO participants was carried out in Belgium, Finland, Greece, Italy, the Netherlands and Poland. The exclusion criteria for the MARK-AGE study have been described²³ and included seropositivity for HIV or for the hepatitis B or C viral infections; current treatment for cancer or with glucocorticoids; <50% of lifetime spent in country of residence, and inability to provide informed consent.

Ethics and study procedures

The Local Research Ethics Committees of the respective recruiting centers provided ethical approval for the MARK-AGE project, which conformed to the Declaration of Helsinki and which was registered retrospectively at the German Clinical Trials Register (DRKS00007713). All participants provided written consent prior to participation. The recruitment together with the data collection took

place in the following centers: Tirol/Innsbruck (Austria), Namur (Belgium), Esslingen (Germany), Athens, and other nearby regions (Greece), Bologna (Italy), Warsaw (Poland), Tampere (Finland), and Leiden (The Netherlands). Between November 2008 and June 2012 trained nurses and physicians collected the participant data, for which the standard operating procedures have been published previously²⁶ therefore, only the measurements that are relevant for the present analysis are described below.

Health status

Health status was assessed using a standard self-report five-point scale for rating physical health, which included the following options for the question *In general, would you say your health is:* excellent, very good, good, fair, or poor. This scale is frequently used in self-perceived health statistics which asks participants to provide a subjective assessment of their general health²⁷. Participants also answered questions about falls and hospitalisation in the 12 months preceding participation, current medication intake and the presence of past and current medical problems at the time of the survey.

As per Reichmann et al.²⁸, a comorbidity index was subsequently computed by counting the total number of self-reported current health conditions. The conditions included: elevated blood pressure or cholesterol, heart-related conditions (e.g. angina, heart failure), autoimmune diseases, diabetes, thyroid disease such as hypothyroidism or hyperthyroidism, osteoporosis, arthritis, liver or kidney-related conditions, respiratory conditions, neurological conditions, memory-related or mental health conditions, vision or hearing impairments, chronic pain such as back or leg pain. The total number of comorbidities were grouped into three levels: 0–1, 2–3 and 4+. For this variable, missing values were coded as if the condition was present (worst case scenario) and included in the analyses.

Sociodemographic and lifestyle data

Questionnaire-based data captured sociodemographic factors²¹⁻²³ such as age, sex, marital status, family history, lifestyle behaviours such as nutritional intake, smoking habits and alcohol consumption; and education level. Education was documented based on a seven-point scale and divided into three groups: (1) don't know, never attended school, elementary school unfinished; (2) elementary school finished, first or second stage secondary level, third level education; (3) university education. Body mass index (BMI) data was used that had been calculated from relevant

anthropometry (height and weight), which also included waist circumference (WC). Data from blood pressure measurements was also included.

Fat-soluble vitamins

Plasma measurements of three fat-soluble vitamins were chosen for inclusion: serum 25-hydroxyvitamin D, which is the accepted status marker for vitamin D. For vitamin A, retinol and for vitamin E, α -tocopherol were analysed, since they represent the greatest proportion of these vitamins in blood. High-performance liquid chromatography (HPLC) was used with UV and fluorescence detection to simultaneously measure tocopherols and retinol. For internal quality control (QC), the inter-batch coefficients of variations (CVs) for α -tocopherol and retinol were 6.3% and 3.7%, respectively. The full details on sample preparation and chromatographic conditions have been published¹⁶. Plasma 25-hydroxyvitamin D was quantified using an enzyme immunoassay (OCTEIA, AC57F1, IDS, Boldon, UK)²⁴. The inter-assay variation was 4.7% as determined in five analysis cohorts with two or three QC samples. The intra-assay variation was 1.7% as determined in one assay with eight samples in the first row and eight samples in the last row of the microtiterplate.

Study outcomes and statistics

The primary outcome was to assess for associations between fat-soluble vitamins (A, D, E) and self-rated health (SRH). The Kolmogorov-Smirnov test was used to ascertain the data distributions, and depending on the outcome, either mean \pm standard deviation or median (with interquartile range) was used to report participant characteristics, results and to guide the appropriate statistical tests. Given the very small number of participants in the poor health status group (1.3% of the cohort), this category was combined with the fair category – a procedure used by others, e.g.^{15, 29, 30}. One-way ANOVA or the Kruskal-Wallis test was used to assess differences between the four categories of health status and the continuous variables (age, BMI, WC, blood pressure, fat-soluble vitamin concentrations), followed by the T-test or Mann-Whitney U test, respectively, with Bonferroni correction. The fat-soluble vitamins were subsequently grouped into quartiles and, in the case of vitamin D, also into accepted cut-offs determining vitamin D status, and were then compared, alongside other categorical variables (sex, BMI classification, smoking status, alcohol consumption status, falls, hospital stays over the past year, medications, comorbidities and season) with the categories of health status using Pearson's chi-squared test. As there is no universal consensus regarding accepted cut-offs for vitamin D status, the following were applied to this analysis based on

guideline recommendations and widespread usage in research studies: serum 25-hydroxyvitamin D ≥ 75 nmol/l (optimal/normal), between 74–50 nmol/l (insufficiency), between 49–30 nmol/l (deficiency), < 30 nmol/l (severe deficiency)^{31, 32}. Health status was compared between the three groups of participants included, however given the larger variation in age range for the RASIG group (35–75 years) compared to the GO and SGO groups (mainly 55–75 years), subgroup analyses stratified by age with participants ≥ 55 years only were carried out (depicted in the flow chart in the supplementary materials).

Univariate and multiple logistic regression analysis (with cumulative logit) was used to explore determinants of health status, with results presented as odds ratios (OR) and corresponding 95% confidence intervals (CI). The univariate analysis included the following variables: the standard cut-offs for vitamin D, in addition to the quartiles for retinol and α -tocopherol and adjustments for the following possible confounders: age (categorised into four age-groups: 35–44, 45–54, 55–64, 65–75 years), sex, education (categorised into three groups: elementary school unfinished, school finished, higher education), marital status (four groups: married, divorced, widow, never married), BMI (as covariate), season of blood collection (categorised into the four seasons), comorbidities (categorised into three groups based on number of comorbidities: 0–1, 2–3, and 4+), study group (three categories: RASIG, GO; SGO), supplement intake (yes, no), hospital stays in preceding year (yes, no), smoking status (never, previous, current) and status of alcohol consumption (abstainer, consumer). The dependent variable for the ordinal logistic regression consisted of four categories: excellent, very good, good, and fair/poor, as frequently used in other studies and national surveys^{29, 30}. Only the significant variables from the univariate analysis were entered into the model for multiple logistic regression. The same procedure (using the same independent variables) was carried out for univariate and multiple binary logistic regression. The dependent variable was dichotomised by grouping the fair/poor health status categories and compared to the excellent/very good/good categories, as has been recommended³³ and used in comparable studies^{34, 35}. All statistical analyses were carried out with SPSS 25.0 (IBM, Ehningen, Germany). Statistical significance was determined with two-sided P value ≤ 0.05 . We followed the STROBE guidelines for cross-sectional studies for reporting the results. No participant was excluded based on missing data, given the very low proportion of missing values (indicated in the tables).

RESULTS

Participant characteristics

In total, 3158 MARK-AGE participants (52% women, median age 60 (IQR: 50–66) years) were included in this analysis. The characteristics of the participants stratified by category of health status are presented in Table 1. Participants with self-perceived health status rated as fair or poor, were older than those rating health status as excellent or very good. They also had higher BMI and WC, with fewer participants having BMI in the normal range ($<25 \text{ kg/m}^2$) and a larger proportion in the obese range ($\geq 30 \text{ kg/m}^2$). Moreover, the proportion of non-smokers in the fair/poor group was lower than the other health status categories but percentage of alcohol abstainers was higher. Additionally, the rate of hospitalisation and falls over the past 12 months was higher and percentage of participants with no comorbidities or not taking any medication was lower. All the above differed significantly.

Fat-soluble vitamins: Plasma 25-hydroxyvitamin D and retinol concentrations were significantly lower in participants that rated health status as fair/poor as compared to the excellent, very good, and good ratings (vitamin D) and excellent and very good ratings (retinol; see Figure 1 A, B). No significant differences were observed for α -tocopherol (Figure 1 C). SRH was also compared to the fat-soluble micronutrients after grouping them into quartiles and in the case of vitamin D, into the accepted cut-offs representing vitamin D status (25-hydroxyvitamin D $\geq 75 \text{ nmol/l}$), insufficient vitamin D status (between 74–50 nmol/l), deficient (between 49–30 nmol/l), and severely deficient ($<30 \text{ nmol/l}$)^{31, 32}. There was a statistically significant association between vitamin D cut-off and health status category (chi-square test: $\chi^2(9)=78.97$, $P<0.001$), however, the association was small (Cramer's $V=0.093$). A similar association was reported for vitamin D quartiles ($\chi^2(9)=84.49$, $P<0.001$; Cramer's $V=0.097$). Specifically, a higher proportion of participants with lower vitamin D status also had a lower rating for health status (Figure 2 A, B). As shown in Figure 2 (C, D), retinol quartiles were also significantly associated with category of health status ($\chi^2(9)=23.36$, $P<0.0005$; Cramer's $V=0.051$) whereas α -tocopherol quartiles were not ($\chi^2(9)=6.11$, $P=0.729$; Cramer's $V=0.026$). Specifically, a higher proportion of participants with lower retinol status also reported a more unfavourable health status.

The influence of season on vitamin D status was illustrated in this cohort, with a significant difference between four seasons, when comparing plasma 25-hydroxyvitamin D concentrations in the entire cohort using the Kruskal-Wallis test (Summer: 58 nmol/l, Autumn: 58 nmol/l, Winter: 44 nmol/l, Spring: 47 nmol/l, $P<0.001$). Furthermore, summer and winter seasons were also compared (but not spring or autumn, as they do not represent extremes in or lack of sun exposure in all included

countries) using the categories for vitamin D cut-offs. These chi-square tests corroborated the aforementioned results for vitamin D. When analysing summer season separately a significant difference in the proportion of participants in the different vitamin D categories was still observed for the four groups of health status ($P=0.002$ and $P<0.001$ for vitamin D status and quartiles, respectively). A similar result was also observed when comparing winter season ($P=0.002$ and $P<0.001$, respectively). Neither retinol nor α -tocopherol were significantly associated with SRH category based on winter and summer seasons (all $P>0.05$).

Study groups: Self-reported health status was also evaluated based on the three groups of study participants, RASIG: $n=2310$ (73.1%); GO: $n=537$ (17.1%); SGO: $n=311$ (9.8%). Supplementary Table 1 summarises the number (and percentage) of participants in these groups based on the categories of SRH, which differed significantly ($\chi^2(4)=41.49$, $P<0.001$; Cramer's $V=0.081$). The GO group had the highest percentage of participants rating health status as excellent (14.2%) and the SGO had the highest percentage for self-reported fair/poor health status (14.8%). Given, that the RASIG group included participants from the age of 35 years, a subgroup comparison between the three groups only including participants ≥ 55 years confirmed the above findings ($\chi^2(4)=28.53$, $P<0.001$; Cramer's $V=0.84$).

Country of participation: Supplementary Table 2 summarises the proportion of participants in the four categories of SRH based on country. The highest percentage of participants reporting fair/poor health status was from Poland (26.8% in all ages; 33.7% in those ≥ 55 years), with the lowest reported from Austria with 1.5% (2% in ≥ 55 years). Health status category was significantly associated with country, with similar results obtained when stratifying the cohort according to age and including those 55 years and older (both $P<0.001$).

Vitamin D status associated with dichotomised self-reported health status

To ascertain the effects of fat-soluble vitamins on the likelihood that participants reported fair or poor health status, binary logistic regression was performed, hence health status categories were dichotomised as follows (excellent, very good, good versus fair, poor). The univariate analysis assessed vitamin D status, retinol and α -tocopherol quartiles as well as the following confounders: sex, age group, education, marital status, BMI, smoking and alcohol consumption status, supplement use, number of current comorbidities, number of medications, hospital visits during the preceding 12 months, season of blood sampling, country of residence and study group. Only the significant variables were included in the multiple binary regression analysis. Of these 17 predictor variables

tested in the univariate analysis, 13 were statistically significant (as shown in Supplementary Table 3) and included in multiple binary logistic regression: vitamin D status, retinol quartiles, age, BMI, education, marital status, smoking status, alcohol consumption status, number of current comorbidities, number of medications, hospital visits during the preceding 12 months, season, and country of residence. No multicollinearity was found among the independent variables.

The multiple logistic regression model was statistically significant ($\chi^2(34)=639.78$, $P<0.001$). The model explained 36.9% (Nagelkerke R^2) of the variance in self-reported health status and correctly classified 89.6% of cases. Three of the 13 independent variables included were not significantly associated self-perceived fair/poor health status: age, season and retinol quartiles (Table 2). Thus, from the three fat-soluble vitamins, only vitamin D status demonstrated a significant association with SRH, where for example, participants with a severe vitamin D deficiency (plasma 25-hydroxyvitamin D <30 nmol/l) had 2.07 higher adjusted odds of reporting fair/poor health status than those with more favourable vitamin D status ($P=0.02$).

Association of both vitamin A and D with categories of self-reported health status

Ordinal logistic regression was performed to determine the effect of the three fat-soluble vitamins on the four groups of SRH (excellent, very good, good, fair/good): As with binary logistic regression, the following predictor variables were assessed in a univariate analysis: sex, age group, education, marital status, BMI, smoking and alcohol consumption status, supplement use, number of current comorbidities, number of medications, hospital visits during the preceding 12 months, season of blood sampling, vitamin D status, quartiles for retinol and α -tocopherol, country of residence and study group. Only variables with significant associations were included in the multiple ordinal regression analysis. As with the binary logistic regression, no multicollinearity existed among the independent variables.

The results of the univariate analysis are reported in Supplementary Table 4 and of the multiple regression analysis in Table 2. Overall, 15 of the 17 predictor variables were included in the multiple regression analysis, apart from supplement use and α -tocopherol, because they did not show significant associations with SRH. Vitamin D was significantly associated with SRH. The adjusted odds ratio of being in a lower category of health status (i.e. reporting fair/poor health) for those with insufficient vitamin D levels or with vitamin D deficiency versus optimal vitamin D status was 1.33 (95% CI, 1.06–1.68) and 1.50 (95% CI 1.17–1.93), respectively. Both these results were statistically significant ($P=0.01$ and $P=0.001$, respectively). Participants with severe vitamin D deficiency (<30

nmol/l) displayed a significantly higher odds of reporting a lower category of health status (adjusted OR 1.83, 95% CI, 1.34–2.50, $P < 0.001$) than those with optimal plasma vitamin D concentrations.

Additionally, participants in all three quartiles for retinol showed significantly (all $P < 0.001$) higher odds of reporting a lower category of SRH as compared to those in the first quartile (unlike the binary regression results). Specifically, adjusted OR 1.44, 95% CI, 1.18–1.75 (second quartile); adjusted OR 1.57, 95% CI, 1.28–1.93 (third quartile); adjusted OR 1.49, 95% CI 1.20–1.84 (fourth quartile).

The results for other predictor variables (Table 2) influencing SRH, demonstrated that the number of comorbidities was associated with a higher odds of reporting lower health status, the odds of which increased with a higher level of comorbidities when compared to none or having only one comorbidity. As expected, medication intake (versus no medications) yielded a higher odds for reporting lower health status. Both season and country influenced the odds of reporting a lower health status, specifically spring and winter seasons and the Netherlands and Italy.

DISCUSSION

Poor diets have been associated with a number of non-communicable diseases and adverse health outcomes,³⁶ and both poor nutrition and poor SRH have been associated with an increased risk of death^{19, 20}. The data from the current analysis suggests that SRH is significantly associated with plasma vitamins A and D status. Specifically, both plasma retinol and 25-hydroxyvitamin D but not α -tocopherol were associated with SRH, confirming our hypothesis that low plasma concentrations of fat-soluble vitamins are risk factors for self-perceived poorer health status, irrespective of age.

Ordinal logistic regression analysis demonstrated a higher odds of reporting fair/poor health when plasma vitamin D concentrations were below clinically defined optimal concentrations, supporting previous findings³⁷. Moreover, participants in the lowest category, representing a severe vitamin D deficiency (<30 nmol/l [<12 ng/ml]) had the highest odds, with the odds ratio (adjusted OR 1.83; 95% CI 1.34–2.50) being comparable to that of another cross-sectional study assessing vitamin D concentrations and SRH (OR 1.72; 95% CI 1.16–2.54)³⁷. Binary logistic regression analysis corroborated the findings for the severe vitamin D deficiency category. In another study focussed on young/middle-aged healthy adult males, serum 25-hydroxyvitamin D was significantly associated with SRH in multiple regression analysis (OR 0.91; 95% CI 0.85–0.97; $P = 0.004$). Specifically, every 2.5 nmol/l (1 ng/ml) increase of serum 25-hydroxyvitamin D was associated with 9% reduction in the odds of reporting fair or poor SRH³⁸. Moreover, Rafiq et al. reported participants in the lowest

category of serum 25-hydroxyvitamin D concentrations (<25 nmol/l) to have a lower odds (OR 0.50, 95% CI 0.33-0.76) of scoring a higher SRH compared to participants with vitamin D concentrations between 25-50 nmol/l (OR 0.88, 95% CI 0.67–1.15), a finding that was reported independently of chronic conditions³⁹. Additionally, a recent analysis on vitamin D and SRH which combined the current MARK-AGE cohort with the Health 2000 cohort, reported the association between vitamin D status and SRH to exist regardless of presence or absence of diseases¹⁵. In another study, lower vitamin D concentrations (assessed as continuous variable) were mildly associated with lower SRH³⁴. Of note, however, mean 25-hydroxyvitamin D concentrations were higher than the reported median concentrations in our cohort (59.5 vs. 47.4 nmol/l). Similar associations were observed in two longitudinal cohorts, the CLHLS (China) and NHANES (US) where older adults with SRH categorised as poor had lower plasma 25-hydroxyvitamin D concentrations¹³.

When compared to the highest (first) quartile for retinol, the three quartiles representing lower retinol concentrations displayed increased odds of fair/poor SRH. No significant associations were observed for retinol with binary logistic regression analysis. To the best of our knowledge, no comparable study has been reported, relating plasma retinol with SRH. Similarly, we did not find studies assessing α -tocopherol and SRH, for which we could not find a significant association in the current analysis. A recent study however, assessed the effects of vitamins on mortality, for which vitamin A, together with other vitamins (B₂, B₆, C, E, folic acid) and iron were associated with a lower risk of mortality (hazard ratio 0.69, 95% CI: 0.48–0.99)⁴⁰. Moreover, a study based on the Danish World Health Organisation MONICA surveys, associated poor self-rated health with diets in adults lacking adequate intakes of fruit and vegetables (sources of vitamins A and E)²⁰.

Overall, several features differed significantly in participants from the categories of self-perceived health status with the following observed in those in the fair/poor category: apart from having lower vitamin A and vitamin D plasma levels, they also tended to be older, have a higher BMI and WC, a higher percentage were smokers, were taking medications and had comorbidities. They also had a higher rate of hospitalisations in the year preceding participation in the study. Unexpectedly, the percentage of alcohol abstainers was higher, however this might be explained by the fact that they no longer consumed alcohol as a result of poorer health, or because we categorised the cohort into those who consumed versus those who did not consume alcohol, and thus might have overlooked the suggested benefits of moderate alcohol consumption⁴¹. Logistic regression analyses controlled for the above confounders with the advantage of assessing the four different categories of SRH (as compared to binary logistic regression, which collapsed them into two categories only) and

some of these variables demonstrated a significantly higher odds of reporting fair/poor health status: BMI, smoking status, season, education and marital status, number of medications taken, number of comorbidities and hospitalisations in the past year, and country. These findings corroborate previously reported associations to health status^{34, 42}.

With regard to pathophysiological links between low plasma status of the fat-soluble micronutrients A and D and health, inadequate intake is one likely causal factor^{8, 12}. A recent study in the US using data from the 2005-2016 NHANES, demonstrated that 45%, 95%, and 84% of the population were below the estimated average requirement for vitamins A, D and E, respectively¹⁷. A comparable analysis displayed slightly lower values in Germany, where for example, a national survey reported the following percentage of adults to display inadequate intakes of fat-soluble vitamins (results are reported separately for men and women, respectively): vitamin A: 15% and 10%; vitamin D: 82% and 91%; vitamin E: 48% and 49%⁴³. Nutritional status however, is influenced by other factors other than nutritional intake. For example, vitamin D is primarily obtained from sun exposure and this plays a critical role in vitamin D status, as does the season during which blood is sampled¹⁸. Furthermore, serum 25-hydroxyvitamin D levels naturally decline during the aging process due to a reduction in the cutaneous production of vitamin D and renal production of 1,25-dihydroxyvitamin D⁴⁴. Such a reduction is also observed for vitamins A and E. In our study, α -tocopherol levels did appear to differ significantly between the four age groups but the values increased with increasing age whereas they remained stable for vitamin D (data not shown). Drug-nutrient interactions can also not be ruled out as playing a causative role, as discussed by Weber et al.⁴⁵. Recently, indications of bidirectional interactions between fat-soluble vitamins and the gut microbiome at a molecular and functional level have been reported, with the former affecting gut microbial composition and a dysbiotic microbiome exerting effects on the status, metabolism and function of these vitamins⁴⁶. Mechanisms of actions include modulation of the vitamin D and retinoic acid receptors, vitamin transport systems, thus hindering absorption efficiency, and the biotransformation pathways⁴⁶.

The current study is limited by its cross-sectional design because no inference could be made regarding the time lag between micronutrient status and SRH. Blood sampling for the plasma concentrations of the fat-soluble vitamins were taken at one time point only, thus might not be reflective of the respective nutrient status preceding study participation. A recent study noted that SRH can predict mortality with a time-dependent effect⁴⁷. This concept might also pertain to micronutrient status and subsequent health status rating. Moreover, from our analysis, one cannot

ascertain whether the reported lipophilic micronutrient concentrations observed are a cause or a consequence of the respective SRH. For example, poor SRH will likely also worsen low serum 25-hydroxyvitamin D concentrations, as people who feel unwell might be more reluctant to spend time outdoors. As such vitamin D status might simply reflect a person's way of life, where the two conditions exacerbate each other. A recent study however, reported the effects of vitamin D on SRH to persist even when adjusting for chronic conditions and including healthy participants only¹⁵. The lack of data on physical activity and household income are also confounders that were not controlled for.

Our results might not be applicable to other study populations, since we included a group of participants (the GO group) that were offspring of nonagenarians and thus are considered genetically privileged. In this regard, the GO group had the highest percentage of participants rating health status as excellent. Also, for the present analysis, data from participants taking supplements were included as a predictor variable, but the type of supplements taken was unknown. We nevertheless deemed it valuable to include these participants, since our main outcome was assessing effects of micronutrient status in plasma rather than nutritional intake and because supplement use has been positively associated with SRH in previous studies⁴⁸. The large sample size is a strength of our study, as is the SRH (the main outcome) which has been reported to accurately reflect objective health status and thus has led to its recommendation as a global complementary measure of health⁴⁹.

Overall, these findings suggest that fat-soluble vitamins A and D might be markers of health status. Interventions to improve plasma concentrations of these nutrients could therefore be beneficial in those with fair/poor SRH. Steptoe et al.⁵⁰ reported improvements to SRH after 12 months of an intervention increasing fruit and vegetable consumption, reflected by increases in plasma micronutrient status⁵¹. A meta-analysis demonstrated that people with poor SRH have a twofold higher mortality risk than those with excellent SRH⁴², therefore such targeted interventions for people identified as having fair/poor SRH could provide an opportunity for nutrient status modifications.

IN CONCLUSION: Multiple logistic regression identified both lower plasma vitamin A and D status as being independently associated with fair/poor SRH. Since poorer SRH has been associated with increased mortality, even in the absence of chronic disease, further insights into the long-term implications of these modifiable nutrients on health status are warranted.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions:

The responsibilities and contribution of the authors were as follows: CSS and TG conceived the current study, CSS conducted the analyses and drafted the manuscript, which was critically revised by all authors. DW assisted with the data inclusion and the laboratory analyses and SW with the statistical analyses. WS carried out laboratory (HPLC) and statistical analysis; EJ and METD quantified vitamin D. AB acquired the funding. Recruitment of participants was carried out by BGL and SF (Austrian cohort), OT and FDC (Belgian cohort), JB (German cohort), ESG (Greek cohort), CF and MC (Italian cohort), ESi (Polish cohort), AH (Finish cohort) and ES (Dutch cohort). MMV established the standard operating procedures for biological material and samples distribution, coordinated the database and managed the MARK-AGE project. NB and JF managed the Biobank and contributed to the establishment of standard operating procedures. The final draft submitted has been approved by all authors.

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FIGURE LEGENDS

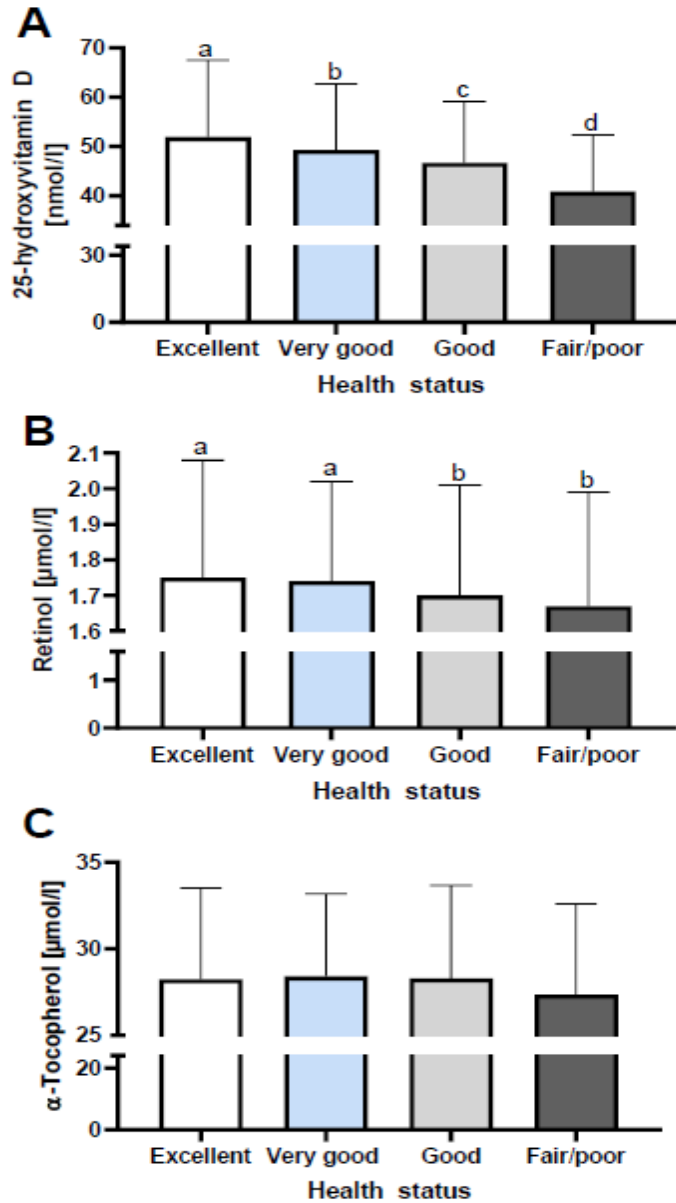


Figure 1. Median and interquartile range for plasma 25-hydroxyvitamin D (**A**), retinol (**B**) and α -tocopherol (**C**) based on self-reported health status. ^{a,b,c,d} Bars with different superscript letters indicates that their medians differ significantly from the others in that figure; conversely, bars sharing a common superscript letter illustrates that their medians are not significantly different from each other ($p < 0.05$), as analysed using Kruskal-Wallis with pairwise comparisons and Tukey's post hoc test. Health status: excellent ($n = 369$); very good ($n = 1112$); good ($n = 1292$); fair/poor ($n = 385$).

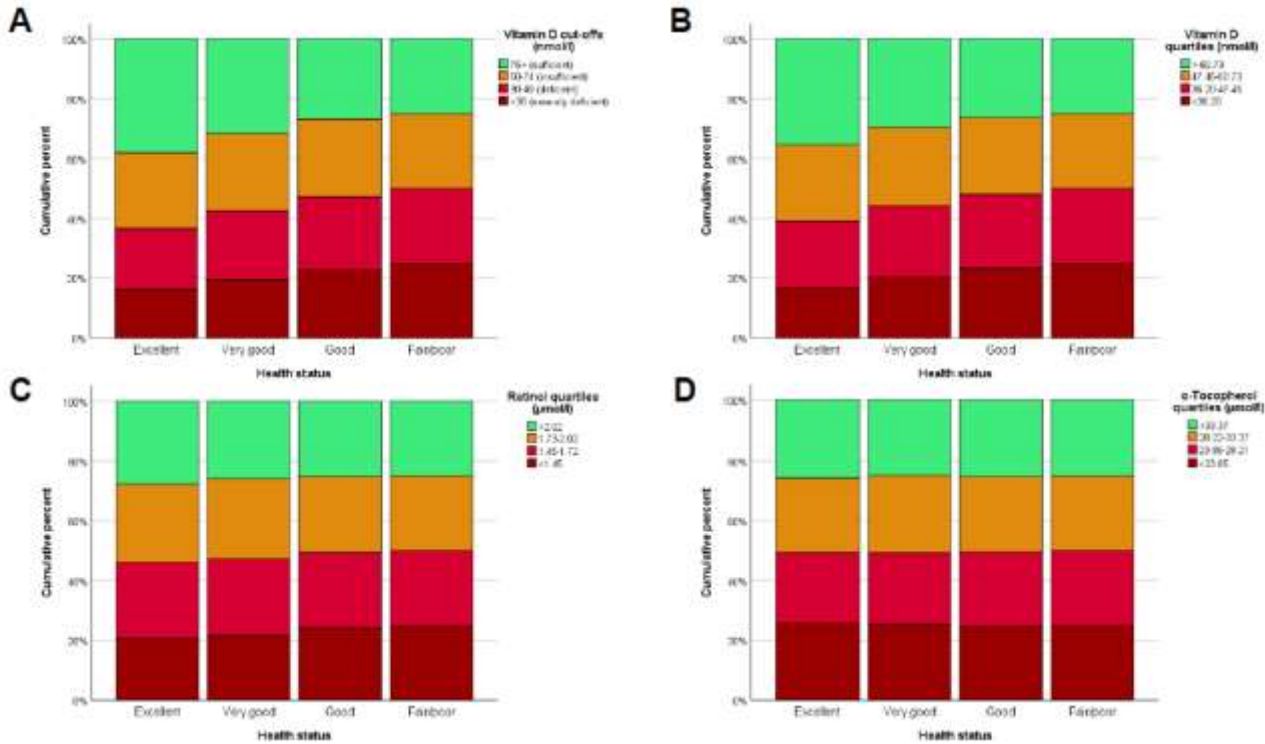


Figure 2. When comparing the proportion of participants in the different vitamin D groups with the categories of health status, a statistically significant association was demonstrated between categories for vitamin D status: $\chi^2(9)=78.97$, $P<0.001$ (A) and for vitamin D quartiles: $\chi^2(9)=84.49$, $P<0.001$ (B). Retinol quartiles also illustrated a significant association with category of health status: $\chi^2(9)=23.36$, $P<0.0005$ (C) but α -tocopherol quartiles did not: $\chi^2(9)=6.11$, $P=0.729$ (D).

Table 1. Baseline participant characteristics stratified by self-rated health status

	All 3158 (100)	Excellent 369 (11.4)	Very Good 1112 (35.2)	Good 1292 (40.9)	Fair/Poor 385 (12.2)	P
<i>Socio-demographic data</i>						
Sex, F/M (%)	1654/1504 (52.4/47.6)	170/199 (46.1/53.9)	570/542 (51.3.0/48.7)	705/587 (54.6/45.4)	209/176 (54.3/45.7)	0.023
Age (years)	60 (50 – 66)	57 (45 – 65) ^a	57 (47 – 65) ^a	60 (52 – 67) ^b	63 (57 – 68) ^c	< 0.001
<i>Anthropometry</i>						
Body mass index [BMI] (kg/m ²)	26 (23 – 29)	25 (23 – 27) ^a	25 (23 – 28) ^b	26 (24 – 29) ^c	28 (25 – 31) ^d	< 0.001
< 25 kg/m ² , n (%)	1331 (42.2)	203 (55.0)	531 (47.8)	485 (37.6)	112 (29.1)	< 0.001
25 – 30 kg/m ² , n (%)	1238 (39.2)	142 (38.5)	423 (38.0)	533 (41.3)	140 (36.4)	
≥ 30 kg/m ² , n (%)	588 (18.6)	24 (6.5)	158 (14.2)	273 (21.1)	133 (34.5)	
Waist circumference [WC] (cm)	92 (84 – 100)	89 (80 – 96) ^a	90 (83 – 98) ^b	94 (85 – 102) ^c	98 (87 – 105) ^d	< 0.001
Systolic blood pressure (mm Hg)	133 (120 – 147)	130 (120 – 142) ^a	131 (120 – 145) ^a	134 (122 – 149) ^b	138 (125 – 151) ^b	< 0.001
Diastolic blood pressure (mm Hg)	80 (74 – 88)	80 (72 – 85) ^a	80 (74 – 89) ^b	80 (74 – 89) ^a	80 (73 – 90) ^a	0.010
<i>Lifestyle factors</i>						

Non-smoker, <i>n</i> (%)*	2626 (83.2)	319 (86.4)	964 (86.7)	1052 (81.4)	291 (75.6)	< 0.001
Alcohol abstainer, <i>n</i> (%)	455 (14.4)	33 (8.9)	118 (10.6)	209 (16.2)	95 (24.7)	< 0.001
<i>Factors related to health status</i>						
Hospitalisation, <i>n</i> (%) [†]	355 (11.0)	26 (7.0)	103 (9.3)	150 (11.6)	76 (19.7)	< 0.001
Falls, <i>n</i> (%) [‡]	344 (10.9)	28 (7.6)	100 (9.0)	142 (11.0)	74 (19.3)	< 0.001
No comorbid conditions, <i>n</i> (%)	1014 (32.1)	210 (56.9)	442 (39.7)	327 (25.3)	35 (9.1)	< 0.001
No medications taken, <i>n</i> (%)	1400 (44.3)	233 (63.1)	604 (54.3)	491 (38.0)	72 (18.7)	< 0.001
<i>Season of blood collection</i>						
Winter <i>n</i> (%)	687 (21.8)	89 (24.1)	257 (23.1)	289 (22.4)	52 (13.5)	< 0.001
Spring <i>n</i> (%)	664 (21.0)	65 (17.6)	208 (18.7)	285 (22.1)	106 (27.5)	
Summer <i>n</i> (%)	784 (24.8)	84 (22.8)	276 (24.8)	314 (24.3)	110 (28.6)	
Autumn <i>n</i> (%)	1023 (32.4)	131 (35.5)	371 (33.4)	404 (31.3)	117 (30.4)	
<i>Plasma fat-soluble vitamins</i>						
25-hydroxyvitamin D	47.4 (36.2 –	51.9 (41.2 –	49.3 (37.7 –	46.7 (35.5 –	40.8 (31.5 –	<

(nmol/l)	60.7)	67.5) ^a	62.6) ^b	59.1) ^c	52.4) ^d	0.001
α -Tocopherol (μ mol/l)	28.2 (23.9 – 33.4)	28.2 (23.8 – 33.5)	28.4 (23.9 – 33.2)	28.3 (24.1 – 33.6)	27.3 (23.2 – 32.6)	0.344
Retinol (μ mol/l)	1.72 (1.45 – 2.02)	1.75 (1.50 – 2.08) ^a	1.74 (1.50 – 2.02) ^a	1.70 (1.43 – 2.01) ^b	1.67 (1.36 – 1.99) ^b	0.001

Significant *p*-values are highlighted in **bold**. Significant results for Kruskal-Wallis pairwise comparisons indicated by a different superscript letter (i.e. sharing same superscript indicates no statistically significant difference).

*The 'non-smoker' category represents current non-smokers and includes those who were previous smokers.

[†]Hospitalisation was defined as being hospitalised (with an overnight stay) within the last 12 months.

[‡]Falls was defined as one or more falls in the last 12 months.

The following variables contained missing values (ordered as presented in table): BMI (n=1), Hospitalisation status (n=6), 25-hydroxyvitamin D (n=140), retinol (n=136)

Table 2. Logistic regression analysis for self-reported health status as the dependent variable

Independent variables	Binary regression OR (95% CI)	Ordinal regression OR (95% CI)	Independent variables	Binary regression OR (95% CI)	Ordinal regression OR (95% CI)
Sex (reference: female)		0.97 (0.83, 1.13)	Hospitalised past 12 months	1.72 (1.21, 2.44) **	1.35 (1.08, 1.69) **
Age group: 35 – 44 years	Reference	Reference	Season: Summer	Reference	Reference **
45 – 54 years	1.28 (0.69, 2.37)	0.98 (0.82, 1.17)	Autumn	1.09 (0.72, 1.63)	1.22 (0.98, 1.51)
55 – 64 years	1.38 (0.79, 2.48)	0.92 (0.74, 1.15)	Winter	1.02 (0.67, 1.57)	1.37 (1.12, 1.67) **
65 – 75 years	1.37 (0.75, 2.51)	1.24 (0.96, 1.61)	Spring	1.38 (0.91, 2.12)	1.49 (1.20, 1.84) ***
Education: Higher education	Reference **	Reference ***	Vitamin D status (nmol/l): Optimal (≥ 75)	Reference ***	Reference **
Finished school	1.66 (1.22, 2.27) **	1.16 (0.54, 2.52)	Insufficiency (74–50)	0.83 (0.46, 1.47)	1.33 (1.06, 1.68) **
Elementary school unfinished	3.31 (1.16, 9.47) *	1.78 (0.81, 3.88)	Deficiency (49–30)	1.21 (0.68, 2.13)	1.50 (1.17, 1.93) **
Marital status: Married	Reference	Reference **	Severe deficiency (<30)	2.08 (1.11, 3.90) *	1.83 (1.34, 2.50) ***
Divorced	1.03 (0.70, 1.51)	0.52 (0.35, 0.76) **	Retinol quartiles (μmol): First (>2.02)	Reference	Reference ***

Widow	0.71 (0.42, 1.19)	0.75 (0.54, 1.04)	Second (2.02 – 1.73)	0.89 (0.61, 1.31)	1.44 (1.18, 1.75) ***
Never	1.65 (1.04, 2.62) *	0.72 (0.50, 1.04)	Third (1.72 – 1.45)	1.01 (0.69, 1.47)	1.57 (1.28, 1.93) ***
BMI (kg/m ²)	1.04 (1.01, 1.07) **	0.96 (0.94, 0.97) ***	Fourth (<1.45)	1.45 (0.99, 2.12)	1.49 (1.20, 1.84) ***
Alcohol (reference: no)	0.63 (0.45, 0.87) **	0.75 (0.61, 0.93) **	Country: Germany	Reference ***	Reference ***
Smoking status: Never	Reference ++	Reference ***	Austria	0.20 (0.08, 0.54) **	1.13 (0.78, 1.63)
Previous	1.12 (0.82, 1.51)	1.44 (1.15, 1.78) **	Belgium	0.93 (0.49, 1.76)	0.70 (0.52, 0.94) *
Current	1.88 (1.32, 2.69) **	1.71 (1.39, 2.10) ***	Finland	2.73 (1.43, 5.21) **	1.11 (0.78, 1.58)
Medications: 0	Reference ***	Reference ***	Greece	2.18 (1.16, 4.09) *	0.28 (0.20, 0.39) ***
1 – 2	1.28 (0.88, 1.87)	1.70 (1.25, 2.30) **	Italy	1.37 (0.74, 2.54)	2.07 (1.50, 2.85) ***
3 – 4	2.64 (1.74, 4.01) ***	3.16 (2.37, 4.20) ***	Netherlands	0.91 (0.40, 2.10)	2.70 (1.90, 3.83) ***
5+	4.69 (4.69, 7.37) ***	4.02 (2.98, 5.42) ***	Poland	4.36 (2.47, 7.69) ***	1.04 (0.74, 1.45)

<i>Number of</i>	Reference ***	Reference ***	<i>Study group: RASIG</i>	Reference	Reference *
<i>comorbidities: 0 – 1</i>					
2 – 3	1.58 (0.98, 2.54)	2.86 (2.37, 3.45) ***	GO	-	1.45 (1.09, 1.92) *
4+	5.86 (3.66, 9.39) ***	5.05 (4.05, 6.31) ***	SGO	-	1.35 (1.01, 1.80) *

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

Significant P-values: *P=0.05 – 0.01; **P=0.01 – 0.001; ***P < 0.001

Independent variables included in multiple binary logistic regression analyses: vitamin D status (4 categories), retinol quartiles (4 categories), age group (4 categories), BMI, education (3 categories), marital status (4 categories), smoking status (3 categories), alcohol consumption status, number of current comorbidities (3 categories), number of medications (4 categories), hospital visits during the preceding 12 months, season of blood sampling (4 categories), country of residence (8 categories).

Independent variables included in multiple ordinal logistic regression analyses: vitamin D status (4 categories), retinol quartiles (4 categories), sex, age group (4 categories), BMI, education (3 categories), marital status (4 categories), smoking status (3 categories), alcohol consumption status, number of current comorbidities (3 categories), number of medications (4 categories), hospital visits during preceding 12 months, season of blood sampling (4 categories), country of residence (8 categories), study group (3 categories).

The following variables contained missing values (ordered as presented in table): BMI (n=1), Hospitalisation status (n=6), 25-hydroxyvitamin D (n=140), retinol (n=136)