



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

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### 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 and 75 years. Cross-sectional data were collected via questionnaires. An enzyme immunoassay quantified 25-hydroxyvitamin D and HPLC determined  $\alpha$ -tocopherol and retinol plasma concentrations. The median 25-hydroxyvitamin D and retinol

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concentrations differed significantly ( $P < 0.001$ ) between SRH categories and were lower in the combined fair/poor category *v.* the excellent, very good and good categories (25-hydroxyvitamin D: 40.8 *v.* 51.9, 49.3, 46.7 nmol/l, respectively; retinol: 1.67 *v.* 1.75, 1.74, 1.70  $\mu\text{mol/l}$ , respectively). Both vitamin D and retinol status were independently associated with fair/poor SRH in multiple regression analyses: adjusted OR (95% CI) for the vitamin D insufficiency, deficiency and severe deficiency categories were 1.33 (1.06–1.68), 1.50 (1.17–1.93) and 1.83 (1.34–2.50), respectively;  $P = 0.015$ ,  $P = 0.001$  and  $P < 0.001$ , and for the second/third/fourth retinol quartiles: 1.44 (1.18–1.75), 1.57 (1.28–1.93) and 1.49 (1.20–1.84); all  $P < 0.001$ . No significant associations were reported for  $\alpha$ -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.

**Key words:**  $\alpha$ -tocopherol: Micronutrients: Plasma: Retinol: Vitamin D

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<sup>(1,2)</sup>. Accordingly, strategies to reduce the projected future burden on healthcare systems are urgently required<sup>(2)</sup>. Health status is influenced by a multitude of genetic, environmental and lifestyle factors, with nutrition playing a pivotal role<sup>(3,4)</sup>.

Fat-soluble micronutrient deficiencies have, for instance, been associated with various diseases such as cancer, diabetes and CVD<sup>(5–10)</sup>. 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<sup>(5,8,11,12)</sup>. 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 status<sup>(13–15)</sup>. For instance, low plasma  $\alpha$ -tocopherol concentrations (a marker for vitamin E status) in older adults have been associated with poor physical and mental health and with greater inflammation<sup>(14)</sup>. 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 (SRH) status was categorised as poor<sup>(13)</sup>.

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 status<sup>(16)</sup>. 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 adults<sup>(17)</sup> 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%, respectively<sup>(17)</sup>. Depending on the local fortification strategies, vitamin D status will of course be strongly influenced by sun exposure<sup>(18)</sup>.

Lower nutrient status has been linked to a higher risk of death, as evidenced in a meta-analysis for vitamin D<sup>(19)</sup> and in a longitudinal study with 15 years of follow-up, which associated both poor diets and poor SRH with increased mortality<sup>(20)</sup>. Of note, SRH has recently been reported to be a significant predictor of mortality in participants with no chronic conditions<sup>(15)</sup>. 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 and 75 years, participating in the European multi-country MARK-AGE study. A previous study using this cohort has 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 outcomes<sup>(21)</sup>.

## Participants and methods

### Participants

Data were analysed from the cross-sectional MARK-AGE population study, which aimed to identify a set of biomarkers of ageing<sup>(22–24)</sup>. For this analysis, 3158 participants recruited from 8 European centres 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 years) 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 Aging<sup>(25)</sup>). Thus, this second group was named GO (GEHA offspring). Thirdly, 311 spouses from the recruited GO participants formed the SGO group (spouses of GO), and 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 described<sup>(23)</sup> 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 centres 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 centres: 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 previously<sup>(26)</sup>; 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 health<sup>(27)</sup>. Participants also answered questions about falls and hospitalisation in 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.*<sup>(28)</sup>, a co-morbidity 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 and 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 co-morbidities 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 factors<sup>(21–23)</sup> 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 and elementary school unfinished; (2) elementary school finished, first or second stage secondary level, and third-level education; and (3) university education. BMI data were used that had been calculated from relevant anthropometry (height and weight), which also included waist circumference. Data from blood pressure measurements were 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,  $\alpha$ -tocopherol were analysed, since they represent the greatest proportion of these vitamins in blood. HPLC was used with UV and fluorescence detection to simultaneously measure tocopherols and retinol. For internal quality control, the inter-batch CV for  $\alpha$ -tocopherol and retinol were 6.3% and 3.7%, respectively. The full details on sample preparation and chromatographic conditions have been published<sup>(16)</sup>. Plasma 25-hydroxyvitamin D was quantified using an enzyme immunoassay (OCTEIA, AC57F1, IDS, Boldon, UK)<sup>(24)</sup>. The inter-assay variation was 4.7% as determined in five analysis cohorts with two or three quality control 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 and E) and 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<sup>(15,29,30)</sup>. 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, waist circumference, blood pressure and 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, co-morbidities and season) with the categories of health status using Pearson's  $\chi^2$  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  $\geq$  75 nmol/l (optimal/normal), between 74 and 50 nmol/l (insufficiency), between 49 and 30 nmol/l (deficiency), and  $<$  30 nmol/l (severe deficiency)<sup>(31,32)</sup>. 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 with the GO and SGO groups (mainly 55–75 years), subgroup analyses stratified by age with participants  $\geq$  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 OR and corresponding 95% CI. The univariate analysis included the following variables: the standard cut-offs for vitamin D, in addition to the quartiles for retinol and  $\alpha$ -tocopherol and adjustments for the following possible confounders: age (categorised into four age groups: 35–44, 45–54,

55–64 and 65–75 years), sex, education (categorised into three groups: elementary school unfinished, school finished and higher education), marital status (four groups: married, divorced, widow and never married), BMI (as covariate), season of blood collection (categorised into the four seasons), co-morbidities (categorised into three groups based on number of co-morbidities: 0–1, 2–3 and 4+), study group (three categories: RASIG, GO and SGO), supplement intake (yes and no), hospital stays in preceding year (yes and no), smoking status (never, previous and 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<sup>(29,30)</sup>. 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 with the excellent/very good/good categories, as has been recommended<sup>(33)</sup> and used in comparable studies<sup>(34,35)</sup>. All statistical analyses were carried out with SPSS 25.0 (IBM). Statistical significance was determined with two-sided *P*-value  $\leq 0.05$ . 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 waist circumference, 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 co-morbidities 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 with the excellent, very good and good ratings (vitamin D) and excellent and very good ratings (retinol; see Fig. 1(a) and (b)). No significant differences were observed for  $\alpha$ -tocopherol (Fig. 1(c)). SRH was also compared with 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: optimal/normal (25-hydroxyvitamin D  $\geq 75 \text{ nmol/l}$ ), insufficient vitamin D status

(between 74 and 50 nmol/l), deficient (between 49 and 30 nmol/l) and severely deficient ( $< 30 \text{ nmol/l}$ )<sup>(31,32)</sup>. There was a statistically significant association between vitamin D cut-off and health status category ( $\chi^2(9) = 78.97$ ,  $P < 0.001$ ); 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 (Fig. 2(a) and (b)). As shown in Fig. 2(c) and (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  $\alpha$ -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 and 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  $\chi^2$  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  $\alpha$ -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%); and 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  $\geq 55$  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 and 33.7% in those  $\geq 55$  years), with the lowest reported from Austria with 1.5% (2% in  $\geq 55$  years). Health status category was significantly associated with country, with similar results



**Table 1.** Baseline participant characteristics stratified by self-rated health status (Number and percentages)§

	All 3158 (100)		Excellent 369 (11.4)		Very good 1112 (35.2)		Good 1292 (40.9)		Fair/poor 385 (12.2)		P
	n	%	n	%	n	%	n	%	n	%	
<b>Sociodemographic data</b>											
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	<b>0.023</b>
Age (years)											
Median	60		57		57		60		63		< <b>0.001</b>
IQR	50–66		45–65 <sup>a</sup>		47–65 <sup>a</sup>		52–67 <sup>b</sup>		57–68 <sup>c</sup>		
<b>Anthropometry</b>											
BMI (kg/m <sup>2</sup> )											
Median	26		25		25		26		28		< <b>0.001</b>
IQR	23–29		23–27 <sup>a</sup>		23–28 <sup>b</sup>		24–29 <sup>c</sup>		25–31 <sup>d</sup>		
< 25 kg/m <sup>2</sup>	1331	42.2	203	55.0	531	47.8	485	37.6	112	29.1	< <b>0.001</b>
25–30 kg/m <sup>2</sup>	1238	39.2	142	38.5	423	38.0	533	41.3	140	36.4	
≥ 30 kg/m <sup>2</sup>	588	18.6	24	6.5	158	14.2	273	21.1	133	34.5	
<b>Waist circumference (WC) (cm)</b>											
Median	92		89		90		94		98		< <b>0.001</b>
IQR	84–100		80–96 <sup>a</sup>		83–98 <sup>b</sup>		85–102 <sup>c</sup>		87–105 <sup>d</sup>		
<b>Systolic blood pressure (mm Hg)</b>											
Median	133		130		131		134		138		< <b>0.001</b>
IQR	120–147		120–142 <sup>a</sup>		120–145 <sup>a</sup>		122–149 <sup>b</sup>		125–151 <sup>b</sup>		
<b>Diastolic blood pressure (mm Hg)</b>											
Median	80		80		80		80		80		<b>0.010</b>
IQR	74–88		72–85 <sup>a</sup>		74–89 <sup>b</sup>		74–89 <sup>a</sup>		73–90 <sup>a</sup>		
<b>Lifestyle factors</b>											
Non-smoker*	2626	83.2	319	86.4	964	86.7	1052	81.4	291	75.6	< <b>0.001</b>
Alcohol abstainer	455	14.4	33	8.9	118	10.6	209	16.2	95	24.7	< <b>0.001</b>
<b>Factors related to health status</b>											
Hospitalisation†	355	11.0	26	7.0	103	9.3	150	11.6	76	19.7	< <b>0.001</b>
Falls‡	344	10.9	28	7.6	100	9.0	142	11.0	74	19.3	< <b>0.001</b>
No co-morbid conditions	1014	32.1	210	56.9	442	39.7	327	25.3	35	9.1	< <b>0.001</b>
No medications taken	1400	44.3	233	63.1	604	54.3	491	38.0	72	18.7	< <b>0.001</b>
<b>Season of blood collection</b>											
Winter	687	21.8	89	24.1	257	23.1	289	22.4	52	13.5	< <b>0.001</b>
Spring	664	21.0	65	17.6	208	18.7	285	22.1	106	27.5	
Summer	784	24.8	84	22.8	276	24.8	314	24.3	110	28.6	
Autumn	1023	32.4	131	35.5	371	33.4	404	31.3	117	30.4	
<b>Plasma fat-soluble vitamins</b>											
<b>25-hydroxyvitamin D (nmol/l)</b>											
Median	47.4		51.9		49.3		46.7		40.8		< <b>0.001</b>
IQR	36.2–60.7		41.2–67.5 <sup>a</sup>		37.7–62.6 <sup>b</sup>		35.5–59.1 <sup>c</sup>		31.5–52.4 <sup>d</sup>		
<b>α-Tocopherol (μmol/l)</b>											
Median	28.2		28.2		28.4		28.3		27.3		0.344
IQR	23.9–33.4		23.8–33.5		23.9–33.2		24.1–33.6		23.2–32.6		
<b>Retinol (μmol/l)</b>											
Median	1.72		1.75		1.74		1.70		1.67		< <b>0.001</b>
IQR	1.45–2.02		1.50–2.08 <sup>a</sup>		1.50–2.02 <sup>a</sup>		1.43–2.01 <sup>b</sup>		1.36–1.99 <sup>b</sup>		

Fat-soluble vitamins and health status

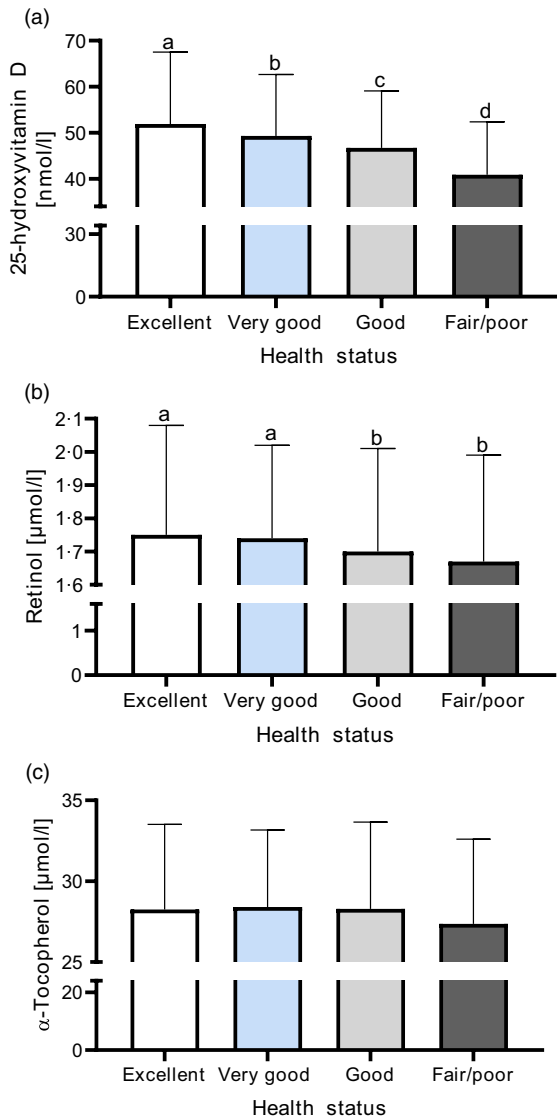
Significant *P*-values are highlighted in bold. Significant results for Kruskal–Wallis pairwise comparisons indicated by a different superscript letter (i.e. sharing same superscript indicates no statistically significant difference).

\* 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) and retinol (*n* 136).



**Fig. 1.** Median and interquartile range for plasma 25-hydroxyvitamin D (a), retinol (b) and  $\alpha$ -tocopherol (c) based on self-reported health status. <sup>a,b,c,d</sup> Bars with different superscript letters indicate that their medians differ significantly from the others in that figure; conversely, bars sharing a common superscript letter illustrate 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).

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 *v.* fair and poor). The univariate analysis assessed vitamin D status, retinol and  $\alpha$ -tocopherol quartiles as well as the following confounders: sex, age group, education, marital status, BMI,

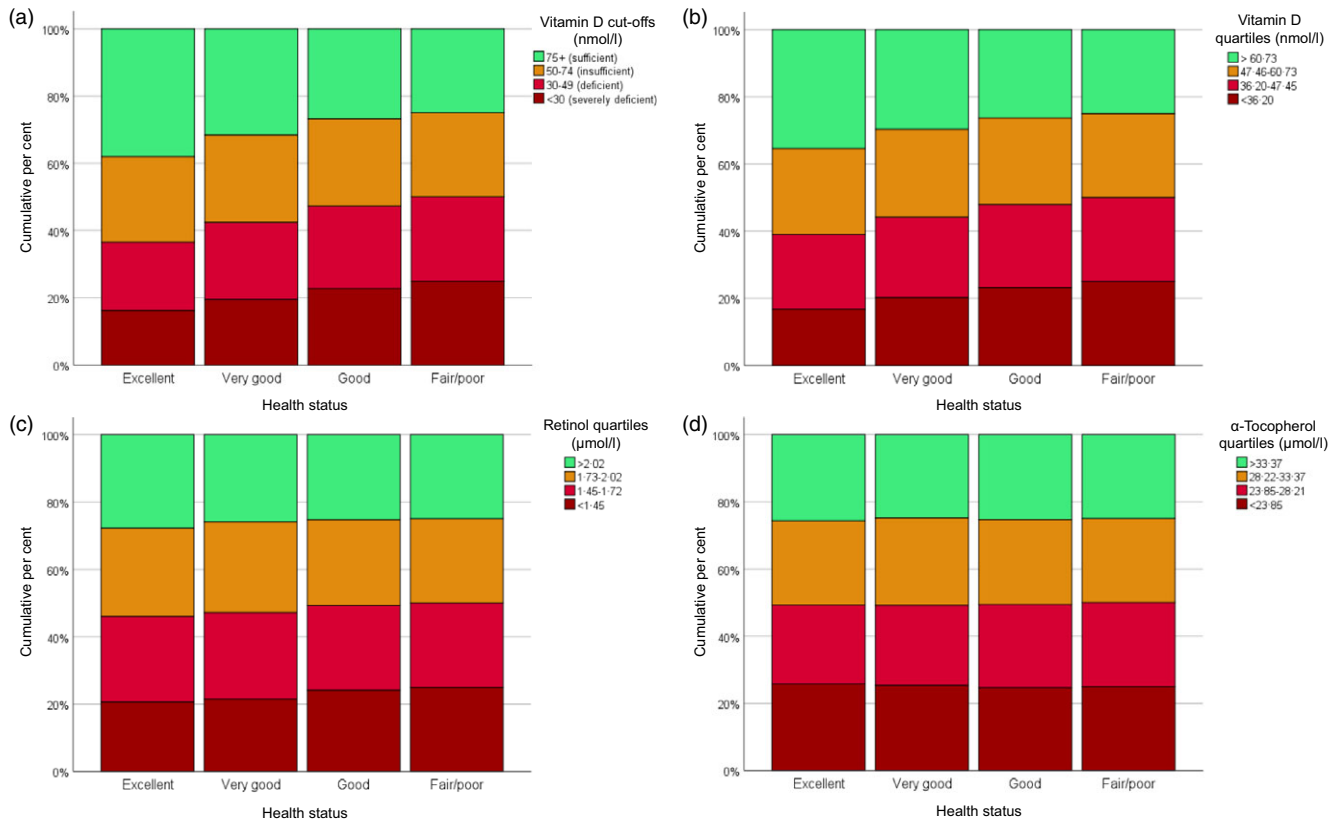
smoking and alcohol consumption status, supplement use, number of current co-morbidities, number of medications, hospital visits during the preceding 12 months, season of blood sampling, country of residence and study group. Only the significant variables were included in the multiple binary regression analysis. Of these seventeen predictor variables tested in the univariate analysis, thirteen were statistically significant (as shown in online 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 co-morbidities, 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<sup>2</sup>) of the variance in self-reported health status and correctly classified 89.6% of cases. Three of the thirteen independent variables included were not significantly associated with 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 and 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 co-morbidities, number of medications, hospital visits during the preceding 12 months, season of blood sampling, vitamin D status, quartiles for retinol and  $\alpha$ -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, fifteen of the seventeen predictor variables were included in the multiple regression analysis, apart from supplement use and  $\alpha$ -tocopherol, because they did not show significant associations with SRH. Vitamin D was significantly associated with SRH. The adjusted OR 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 *v.* 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



**Fig. 2.** When comparing the proportion of participants in the different vitamin D groups with the categories of self-reported 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  $\alpha$ -tocopherol quartiles did not:  $\chi^2(9) = 6.11$ ,  $P = 0.729$  (d).

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 with 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) and 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 co-morbidities was associated with a higher odds of reporting lower health status, the odds of which increased with a higher level of co-morbidities when compared with none or having only one co-morbidity. As expected, medication intake (*v.* 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<sup>(36)</sup>, and both poor nutrition and poor SRH have been associated with an increased risk of death<sup>(19,20)</sup>. The data from the current analysis suggest that SRH is

significantly associated with plasma vitamins A and D status. Specifically, both plasma retinol and 25-hydroxyvitamin D but not  $\alpha$ -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 findings<sup>(37)</sup>. Moreover, participants in the lowest category representing a severe vitamin D deficiency ( $< 30$  nmol/l ( $< 12$  ng/ml)) had the highest odds, with the OR (adjusted OR 1.83; 95 % CI 1.34, 2.50) being comparable with that of another cross-sectional study assessing vitamin D concentrations and SRH (OR 1.72; 95 % CI 1.16, 2.54)<sup>(37)</sup>. 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 SRH<sup>(38)</sup>. 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 with participants with vitamin D concentrations

**Table 2.** Logistic regression analysis for self-reported health status as the dependent variable (Odd ratio and 95 % confidence intervals)†

Independent variables	Binary regression		Ordinal regression		Independent variables	Binary regression		Ordinal regression	
	OR	95 % CI	OR	95 % CI		OR	95 % CI	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 <sup>2</sup> )	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***	The 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
Number of co-morbidities: 0–1	Reference ***		Reference***		Study group: RASIG	Reference		Reference*	
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*

Significant *P*-values:

\* *P* = 0.05–0.01;

\*\* *P* = 0.01–0.001;

\*\*\* *P* < 0.001.

† Ident variables included in multiple binary logistic regression analyses: vitamin D status (four categories), retinol quartiles (four categories), age group (four categories), BMI, education (three categories), marital status (four categories), smoking status (three categories), alcohol consumption status, number of current co-morbidities (three categories), number of medications (four categories), hospital visits during the preceding 12 months, season of blood sampling (four categories) and country of residence (eight categories).

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



between 25 and 50 nmol/l (OR 0.88, 95 % CI 0.67, 1.15), a finding that was reported independently of chronic conditions<sup>(39)</sup>. 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 diseases<sup>(15)</sup>. In another study, lower vitamin D concentrations (assessed as continuous variable) were mildly associated with lower SRH<sup>(34)</sup>. Of note, however, mean 25-hydroxyvitamin D concentrations were higher than the reported median concentrations in our cohort (59.5 *v.* 47.4 nmol/l). Similar associations were observed in two longitudinal cohorts, the CLHLS (China) and NHANES (USA) where older adults with SRH categorised as poor had lower plasma 25-hydroxyvitamin D concentrations<sup>(13)</sup>.

When compared with 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  $\alpha$ -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 (B<sub>2</sub>, B<sub>6</sub>, C, E and folic acid) and Fe were associated with a lower risk of mortality (hazard ratio 0.69, 95 % CI 0.48, 0.99)<sup>(40)</sup>. Moreover, a study based on the Danish WHO MONICA surveys associated poor SRH with diets in adults lacking adequate intakes of fruit and vegetables (sources of vitamins A and E)<sup>(20)</sup>.

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 waist circumference, a higher percentage were smokers, were taking medications and had co-morbidities. 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 *v.* those who did not consume alcohol, and thus might have overlooked the suggested benefits of moderate alcohol consumption<sup>(41)</sup>. Logistic regression analyses controlled for the above confounders with the advantage of assessing the four different categories of SRH (as compared with 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 co-morbidities and hospitalisations in the past year, and country. These findings corroborate previously reported associations to health status<sup>(34,42)</sup>.

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<sup>(8,12)</sup>. A recent study in the USA 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, respectively<sup>(17)</sup>. 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 %<sup>(43)</sup>. 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 sampled<sup>(18)</sup>. Furthermore, serum 25-hydroxyvitamin D levels naturally decline during the ageing process due to a reduction in the cutaneous production of vitamin D and renal production of 1,25-dihydroxyvitamin D<sup>(44)</sup>. Such a reduction is also observed for vitamins A and E. In our study,  $\alpha$ -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.*<sup>(45)</sup> 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 vitamins<sup>(46)</sup>. Mechanisms of actions include modulation of the vitamin D and retinoic acid receptors, vitamin transport systems, thus hindering absorption efficiency, and the biotransformation pathways<sup>(46)</sup>.

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 effect<sup>(47)</sup>. 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 only<sup>(15)</sup>. 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 as been positively associated with SRH in previous studies<sup>(48)</sup>. 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 health<sup>(49)</sup>.

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.*<sup>(50)</sup> reported improvements to SRH after 12 months of an intervention increasing fruit and vegetable consumption, reflected by increases in plasma micronutrient status<sup>(51)</sup>. A meta-analysis demonstrated that people with poor SRH have a twofold higher mortality risk than those with excellent SRH<sup>(42)</sup>; therefore, such targeted interventions for people identified as having fair/poor SRH could provide an opportunity for nutrient status modifications.

### 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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The responsibilities and contribution of the authors were as follows: C. S. S. and T. G. conceived the current study, C. S. S. conducted the analyses and drafted the manuscript, which was critically revised by all authors. D. W. assisted with the data inclusion and the laboratory analyses and S. W. with the statistical analyses. W. S. carried out laboratory (HPLC) and statistical analysis; E. J. and METD quantified vitamin D. A. B. acquired the funding. Recruitment of participants was carried out by B. G. L. and SF (Austrian cohort), O. T. and FDC (Belgian cohort), J. B. (German cohort), E. S. G. (Greek cohort), C. F. and M. C. (Italian cohort), ESi (Polish cohort), A. H. (Finish cohort) and E. S. (Dutch cohort). M. M. V. established the standard operating procedures for biological material and samples distribution, coordinated the database and managed the MARK-AGE project. N. B. and J. F. managed the Biobank and contributed to the establishment of standard operating procedures. The final draft submitted has been approved by all authors.

The authors of the present study have nothing to declare regarding conflict of interest.

### Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114521004633>

### References

1. United Nations (2019) World Population Ageing 2019: Highlights. Department of Economic and Social Affairs. Population Division. New York: United Nations. <https://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2019-Highlights.pdf> (accessed August 2020).
2. Chang AY, Skirbekk VF, Tyrovolas S, *et al.* (2019) Measuring population ageing: an analysis of the Global Burden of Disease Study 2017. *Lancet Public Health* **4**, e159–e167.
3. Rask-Andersen M, Karlsson T, Ek WE, *et al.* (2017) Gene–environment interaction study for BMI reveals interactions between genetic factors, physical activity, alcohol consumption, socioeconomic status. *PLoS Genet* **13**, e1006977.
4. Govindaraju D, Atzmon G & Barzilay N (2015) Genetics, lifestyle and longevity: lessons from centenarians. *Appl Transl Genom* **4**, 23–32.
5. Lewis ED, Meydani SN & Wu D (2019) Regulatory role of vitamin E in the immune system and inflammation. *IUBMB Life* **71**, 487–494.
6. Cantorna MT, Snyder L & Arora J (2019) Vitamin A and vitamin D regulate the microbial complexity, barrier function, and the mucosal immune responses to ensure intestinal homeostasis. *Crit Rev Biochem Mol Biol* **54**, 184–192.
7. Sassi F, Tamone C & D'Amelio P (2018) Vitamin D: nutrient, hormone, and immunomodulator. *Nutrients* **10**, 1656.
8. Mora JR, Iwata M & von Andrian UH (2008) Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol* **8**, 685–698.
9. Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* **80**, 1678S–1688S.
10. Stephensen CB (2001) Vitamin A, infection, and immune function. *Annu Rev Nutr* **21**, 167–192.
11. Weber D, Kochlik B, Demuth I, *et al.* (2020) Plasma carotenoids, tocopherols and retinol - Association with age in the Berlin Aging Study II. *Redox Biol* **32**, 101461.
12. Charoenngam N & Holick MF (2020) Immunologic effects of vitamin D on human health and disease. *Nutrients* **12**, 2097.
13. Wei J, Zhu A & Ji JS (2019) A comparison study of vitamin D deficiency among older adults in China and the United States. *Sci Rep* **9**, 19713.
14. Capuron L, Moranis A, Combe N, *et al.* (2009) Vitamin E status and quality of life in the elderly: influence of inflammatory processes. *Br J Nutr* **102**, 1390–1394.
15. Kananen L, Enroth L, Raitanen J, *et al.* (2021) Self-rated health as an indicator of bodily states - associations with biomarkers in individuals with, without disease in three populations. *Sci Rep* **11**, 6139.
16. Stuetz W, Weber D, Dolle ME, *et al.* (2016) Plasma carotenoids, tocopherols, and retinol in the age-stratified (35–74 years) general population: a cross-sectional study in six European countries. *Nutrients* **8**, 614.
17. Reider CA, Chung RY, Devarshi PP, *et al.* (2020) Inadequacy of immune health nutrients: intakes in US adults, the 2005–2016 NHANES. *Nutrients* **12**, 1735.
18. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.

19. Schöttker B, Jorde R, Peasey A, *et al.* (2014) Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ* **348**, g3656.
20. Osler M, Heitmann BL, Gerdes LU, *et al.* (2001) Dietary patterns and mortality in Danish men and women: a prospective observational study. *Br J Nutr* **85**, 219–225.
21. Rietman ML, Spijkerman AMW, Wong A, *et al.* (2019) Antioxidants linked with physical, cognitive and psychological frailty: analysis of candidate biomarkers and markers derived from the MARK-AGE study. *Mech Ageing Dev* **177**, 135–143.
22. Bürkle A, Moreno-Villanueva M, Bernhard J, *et al.* (2015) MARK-AGE biomarkers of ageing. *Mech Ageing Dev* **151**, 2–12.
23. Capri M, Moreno-Villanueva M, Cevenini E, *et al.* (2015) MARK-AGE population: from the human model to new insights. *Mech Ageing Dev* **151**, 13–17.
24. Jansen E, Beekhof P, Cremers J, *et al.* (2015) Quality control data of physiological and immunological biomarkers measured in serum and plasma. *Mech Ageing Dev* **151**, 54–59.
25. Franceschi C, Bezrukov V, Blanche H, *et al.* (2007) Genetics of healthy aging in Europe: the EU-integrated project GEHA (Genetics of Healthy Aging). *Ann NY Acad Sci* **1100**, 21–45.
26. Moreno-Villanueva M, Capri M, Breusing N, *et al.* (2015) MARK-AGE standard operating procedures (SOPs): a successful effort. *Mech Ageing Dev* **151**, 18–25.
27. Eurostat (2019) Statistics Explained: Self-Perceived Health Statistics. [https://ec.europa.eu/eurostat/statistics-explained/index.php/Self-perceived\\_health\\_statistics](https://ec.europa.eu/eurostat/statistics-explained/index.php/Self-perceived_health_statistics) (accessed August 2020).
28. Reichmann WM, Katz JN, Kessler CL, *et al.* (2009) Determinants of self-reported health status in a population-based sample of persons with radiographic knee osteoarthritis. *Arthritis Rheum* **61**, 1046–1053.
29. Wu CF, Wang MS & Eamon MK (2014) Employment hardships and single mothers' self-rated health: evidence from the panel study of income dynamics. *Soc Work Health Care* **53**, 478–502.
30. Thomas SB, Sansing VV, Davis A, *et al.* (2010) Racial differences in the association between self-rated health status and objective clinical measures among participants in the BARI 2D trial. *Am J Public Health* **1**, S269–S276.
31. Amrein K, Scherkl M, Hoffmann M, *et al.* (2020) Vitamin D deficiency 2.0: an update on the current status worldwide. *Eur J Clin Nutr* **74**, 1498–1513.
32. Holick MF, Binkley NC, Bischoff-Ferrari HA, *et al.* (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **96**, 1911–1930.
33. Manor O, Matthews S & Power C (2000) Dichotomous or categorical response? Analysing self-rated health and lifetime social class. *Int J Epidemiol* **29**, 149–157.
34. Gallagher JE, Wilkie AA, Corder A, *et al.* (2016) Factors associated with self-reported health: implications for screening level community-based health and environmental studies. *BMC Public Health* **16**, 640.
35. Barger SD (2006) Do psychological characteristics explain socioeconomic stratification of self-rated health? *J Health Psychol* **11**, 21–35.
36. Cecchini M, Sassi F, Lauer JA, *et al.* (2010) Tackling of unhealthy diets, physical inactivity, and obesity: health effects and cost-effectiveness. *Lancet* **376**, 1775–1784.
37. Schöttker B, Saum KU, Perna L, *et al.* (2014) Is vitamin D deficiency a cause of increased morbidity and mortality at older age or simply an indicator of poor health? *Eur J Epidemiol* **29**, 199–210.
38. Tepper S, Dabush Y, Shahar DR, *et al.* (2016) Vitamin D status and quality of life in healthy male high-tech employees. *Nutrients* **8**, 366.
39. Rafiq R, Swart KM, van Schoor NM, *et al.* (2014) Associations of serum 25-hydroxyvitamin D concentrations with quality of life and self-rated health in an older population. *J Clin Endocrinol Metab* **99**, 3136–3143.
40. Wang W, Gao J, Li N, *et al.* (2020) Dietary iron and vitamins in association with mortality. *Clin Nutr* **40**, 2401–2409.
41. Fernandez-Sola J (2015) Cardiovascular risks and benefits of moderate and heavy alcohol consumption. *Nat Rev Cardiol* **12**, 576–587.
42. DeSalvo KB, Bloser N, Reynolds K, *et al.* (2006) Mortality prediction with a single general self-rated health question. A meta-analysis. *J Gen Intern Med* **21**, 267–275.
43. Max Rubner-Institut (2008) (Hrsg.) Nationale Verzehrsstudie II E, Teil 2. Karlsruhe. [https://www.mri.bund.de/fileadmin/MRI/Institute/EV/NVSII\\_Abschlussbericht\\_Teil\\_2.pdf](https://www.mri.bund.de/fileadmin/MRI/Institute/EV/NVSII_Abschlussbericht_Teil_2.pdf) (accessed August 2020).
44. Gallagher JC (2013) Vitamin D and aging. *Endocrinol Metab Clin North Am* **42**, 319–332.
45. Weber D, Kochlik B, Stuetz W, *et al.* (2020) Medication intake is associated with lower plasma carotenoids and higher fat-soluble vitamins in the cross-sectional MARK-AGE Study in older individuals. *J Clin Med* **9**, 2072.
46. Stacchiotti V, Rezzi S, Eggersdorfer M, *et al.* (2021) Metabolic and functional interplay between gut microbiota and fat-soluble vitamins. *Crit Rev Food Sci Nutr* **61**, 3211–3232.
47. Lorem G, Cook S, Leon DA, *et al.* (2020) Self-reported health as a predictor of mortality: a cohort study of its relation to other health measurements, observation time. *Sci Rep* **10**, 4886.
48. Radimer K, Bindewald B, Hughes J, *et al.* (2004) Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999–2000. *Am J Epidemiol* **160**, 339–349.
49. Wu S, Wang R, Zhao Y, *et al.* (2013) The relationship between self-rated health and objective health status: a population-based study. *BMC Public Health* **13**, 320.
50. Steptoe A, Perkins-Porras L, Hilton S, *et al.* (2004) Quality of life and self-rated health in relation to changes in fruit and vegetable intake and in plasma vitamins C and E in a randomised trial of behavioural and nutritional education counselling. *Br J Nutr* **92**, 177–184.
51. Steptoe A, Perkins-Porras L, McKay C, *et al.* (2003) Behavioural counselling to increase consumption of fruit and vegetables in low income adults: randomised trial. *BMJ* **326**, 855.