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Associations of fat-soluble micronutrients and redox biomarkers with frailty status in the FRAILOMIC initiative

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Abstract

Background A poor fat-soluble micronutrient (FMN) and a high oxidative stress status are associated with frailty. Our aim was to determine the cross-sectional association of FMNs and oxidative stress biomarkers [protein carbonyls (PrCarb) and 3-nitrotyrosine] with the frailty status in participants older than 65 years.

Methods Plasma levels of vitamins A (retinol), D_3, E (α-tocopherol and γ-tocopherol) and carotenoids (α-carotene and β-carotene, lycopene, lutein/zeaxanthin, and β-cryptoxanthin), PrCarb, and 3-nitrotyrosine were measured in 1450 individuals of the FRAILOMIC initiative. Participants were classified into robust, pre-frail, and frail using Fried's frailty criteria. Associations between biomarkers and frailty status were assessed by general linear and logistic regression models, both adjusted for cohort, season of blood sampling, gender, age, height, weight, and smoking.

Results Robust participants had significantly higher vitamin D_3 and lutein/zeaxanthin concentrations than pre-frail and frail subjects; had significantly higher γ-tocopherol, α-carotene, β-carotene, lycopene, and β-cryptoxanthin concentrations than frail subjects, and had significantly lower PrCarb concentrations than frail participants in multivariate linear models. Frail subjects were more likely to be in the lowest than in the highest tertile for vitamin D_3 (adjusted odds ratio: 2.15; 95% confidence interval: 1.42–3.26), α-tocopherol (2.12; 1.39–3.24), α-carotene (1.69; 1.00–2.88), β-carotene (1.84; 1.13–2.99), lycopene (1.94; 1.24–3.05), lutein/zeaxanthin (3.60; 2.34–5.53), and β-cryptoxanthin (3.02; 1.95–4.69) and were more likely to be in the highest than in the lowest tertile for PrCarb (2.86; 1.82–4.49) than robust subjects in multivariate regression models.

Conclusions Our study indicates that both low FMN and high PrCarb concentrations are associated with pre-frailty and frailty.

Keywords Fat-soluble micronutrients; Carotenoids; Frail; Protein carbonyls; 3-Nitrotyrosine

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Introduction

Frailty, a geriatric syndrome caused by an age-related dynamic process affecting multiple physiological systems[1,2], is associated with a higher risk for falls, hospitalization, disability, and death[3] and its prevalence increases with age and is more common in women.[3–6] Age-associated oxidative stress (OS) and impairments in redox homeostasis as well as

impairments in muscle structure, function, and performance are key factors in the development of frailty.[7–9] Fortunately, frailty might be reversed by exercise and decelerated by nutritional interventions.[10]

Higher fruit and vegetable consumption and higher adherence to a Mediterranean diet were associated with a lower risk for frailty in older individuals.[11–13] Additionally, a suboptimal vitamin and carotenoid intake and/or status as well as a micronutrient pattern low in vitamins A and E were associated with a higher prevalence and risk for frailty.[14–17] Furthermore, a suboptimal vitamin D (VD) status was shown to be related with low physical activity, weakness, and slowness, which are main constituents of the frailty syndrome,[18,19] with reduced muscle mass and poor physical performance in frail subjects[20] and with a higher prevalence and incidence of frailty.[19,21] Moreover, VD is linked to redox homeostasis as shown in both VD deficient rat muscles and VD-treated murine myoblast C2C12 cells.[22]

Some fat-soluble micronutrients (FMN) can counteract OS, which is associated with several age-related diseases and the aging process itself. OS can be monitored by biomarkers such as protein carbonyls (PrCarb) and 3-nitrotyrosine (3-NT),[23–25] and some OS biomarkers have been shown to be elevated in frail subjects.[26] Both higher OS and lower antioxidant parameters are associated with frailty.[27]

To the best of our knowledge, no study explored the association of frailty with FMN and OS biomarkers simultaneously in a large cohort so far. We therefore investigated the cross-sectional relationship of plasma vitamins A, D_3, E, carotenoids, PrCarb, and 3-NT with frailty status (robust, pre-frail, and frail) of participants aged >65 years in the European FRAILOMIC initiative.

Materials and methods

Study population and cohorts

In this study, we investigated 1450 individuals out of 1636 participants from the FRAILOMIC database by excluding subjects with missing values for frailty status (n = 114) and VD_3 (n = 74). The FRAILOMIC initiative aims to identify and validate classical and 'omics'-based biomarkers that predict the risk of frailty, detect frailty, and assess the progression of frailty.[28] Participants of the FRAILOMIC initiative come from four population-based European cohorts of older adults: the Bordeaux sample of the Three-City Study (France),[29] the Aging Multidisciplinary Investigation cohort (Gironde, France),[30] the Toledo Study for Healthy Aging (TSHA, Toledo, Spain),[31] and the Invecchiare in Chianti (InCHIANTI) study (Chianti geographical area, Tuscany, Italy).[32] These cohorts were described more in detail elsewhere.[14] Study protocols of all cohorts were approved by Ethical Committees according to the principles of the Declaration of Helsinki and all participants signed a written consent. Three-City Study, Aging Multidisciplinary Investigation, TSHA, and InCHIANTI were approved by the Ethical Committee of the University Hospital of Kremlin-Bicêtre,[29] Ethics Committee of the University Hospital of Bordeaux,[30] Clinical Research Ethics Committee of the University Hospital of Toledo,[31] and Ethical Committee of the Italian National Research Council on Aging,[32] respectively.

Frailty classification

Participants were classified into robust, pre-frail, and frail using criteria by Fried *et al.*[3] The harmonization of criteria across cohorts was described in detail elsewhere.[14,31] Briefly, participants exhibiting ≥3 of the five following criteria were considered as frail: slowness, low energy expenditure, shrinking, weakness, and self-reported exhaustion; while those exhibiting 1 to 2 of these criteria were considered as pre-frail.

Participant characteristics

Participants' information included gender, age (years), weight (kg), height (cm), body mass index (BMI; kg/m^2), smoking (current smoker), and global cognitive performance (Mini-Mental State Examination). The assessment of characteristics is described elsewhere.[14,29–32]

Biomarker analyses

All analyses were carried out at the Department of Nutritional Toxicology (University of Jena, Germany) between 2010 and 2013.

Vitamin D_3 in plasma samples was measured by the high-performance liquid chromatography (HPLC) method described by Pilleron *et al.*[14] VD_3 was detected in all samples; in contrary, only 20/1430 samples (1.4%) revealed values above the limit of detection for VD_2. Therefore, only VD_3 is described and included in the statistical analyses. Analysis of retinol, α-tocopherol and γ-tocopherol, α-carotene and β-carotene, lycopene, lutein/zeaxanthin, and β-cryptoxanthin in plasma samples was performed by the HPLC method described by Stuetz *et al.*[33] and Weber *et al.*[34] PrCarbs and protein bound 3-NT in plasma samples were measured by non-commercial in-house ELISA methods as described by Weber *et al.*[34,35]

Statistical analyses

Demographic characteristics are described using means ± standard deviation for continuous variables (age, weight, height, BMI, and Mini-Mental State Examination) and frequencies (%) for categorical variables (gender, frailty status,

and smoking). Differences in characteristics between frailty groups and between cohorts were assessed by general linear models (GLMs; continuous variables) and Pearson's χ^2 test (categorical variables). When necessary, biomarker concentrations were transformed to achieve normal distribution using logarithmic (LN) transformation and are described by geometric means with 95% confidence intervals. Differences of biomarkers between frailty groups were assessed by simple (frailty status as only factor) and adjusted (covariates included cohort, season of blood sampling, gender, age, height, weight, and smoking) GLMs. Additionally, simple [odds ratio (OR); frailty status as only factor] and multiple adjusted [adjusted OR (AOR); covariates included cohort, season of blood sampling, gender, age, height, weight, and smoking] logistic regression analysis using tertiles (Supporting Information, *Table* S1) of FMN and OS biomarkers were applied: ORs and AORs of the lowest tertile and the median tertile vs. the highest tertiles for FMN and, vice versa for OS markers, in pre-frail and frail compared with control groups were calculated. For a clearer presentation of the results, ORs and AORs are not shown for median vs. highest tertiles for FMN, and vice versa for OS biomarkers. Statistically significant differences were considered to be present at $P < 0.05$. All statistical analyses were carried out using SPSS software (SPSS Inc., Chicago, IL, USA; Version 20.0.0). Figures were prepared by using SPSS Version 20.0.0 and Microsoft Office Power Point 2007.

Results

Study characteristics for both the total sample and the three frailty groups are shown in *Table* 1. In our study, 41.7% and 22.1% of the participants were pre-frail and frail, respectively, and 65.7% of the frail participants were women. Frail participants (81.4 ± 6.3 years) were significantly older than pre-frail (78.0 ± 6.0 years) and robust participants (74.6 ± 5.9 years), and also significantly lighter ($P = 0.012$) and shorter ($P < 0.001$) than robust participants, while BMI was not associated with frailty status. Additionally, characteristics and biomarker concentrations were different between the individual FRAILOMIC cohorts (Supporting Information, *Tables* S2 and S3).

Fat-soluble micronutrient and OS concentrations differed significantly between the frailty groups (*Table* 2 and *Figure* 1). Significantly higher VD_3 (*Figure* 1A) and lutein/zeaxanthin (*Figure* 1B) concentrations were observed in robust participants compared with pre-frail and frail participants, and in pre-frail compared with frail participants (all $P < 0.01$), in simple GLMs. Furthermore, robust and pre-frail participants had higher γ-tocopherol, α-carotene, β-carotene, lycopene, and β-cryptoxanthin (*Figure* 1C) concentrations than frail subjects (all $P \leq 0.02$); pre-frail participants had higher α-tocopherol concentrations than frail subjects ($P = 0.002$); and robust subjects had significantly lower PrCarb concentrations than frail and pre-frail participants ($P < 0.001$; *Figure* 1D). In multivariate analyses, β-cryptoxanthin was higher in robust than in pre-frail and frail participants and in pre-frail compared with frail participants. Furthermore, VD_3 and β-carotene concentrations were higher in robust than in pre-frail and frail participants (all $P \leq 0.001$); lycopene and lutein/zeaxanthin concentrations were higher in robust and pre-frail participants compared with frail subjects (all $P \leq 0.02$); α-tocopherol concentrations were higher in pre-frail participants than in frail subjects ($P = 0.013$); and lower PrCarb concentrations were found in robust compared with frail and pre-frail participants ($P = 0.006$). No association was observed between retinol or 3-NT and frailty status.

Results from GLMs were confirmed by logistic regression analyses (*Table* 3). Pre-frail and frail participants were more likely to be in the lowest than in the highest tertile for VD_3 and lutein/zeaxanthin than robust participants. Frail participants were more likely to be in the lowest than in the highest tertile for α-tocopherol, γ-tocopherol, α-carotene, β-carotene, lycopene, and β-cryptoxanthin than robust participants. Subsequently, multivariable adjustment showed that pre-frail and frail compared with robust participants were more likely to be in the lowest than in the highest tertiles for VD_3 (AOR: 1.98; 95% confidence interval: 1.42–2.76 and 2.15; 1.42–3.26), β-carotene (1.56; 1.06–2.28 and 1.84; 1.13–2.99), and especially for lutein/zeaxanthin (1.38; 1.00–

Table 1 Study characteristics by frailty groups

	Total	Robust	Pre-frail	Frail	*P*
N, % (*n*)	100 (1450)	36.1 (524)	41.7 (605)	22.1 (321)	—
Females, % (*n*)	55.9 (811)	48.1 (252)	57.5 (348)	65.7 (211)	<0.001[#]
Age, years	77.5 ± 6.5	74.6 ± 5.9[a]	78.0 ± 6.0[b]	81.4 ± 6.3[c]	<0.001
Weight, kg	70.6 ± 13.7	72.0 ± 12.0[a]	69.9 ± 14.0[b]	69.7 ± 15.5[b]	0.012
Height, cm	160.0 ± 9.6	161.5 ± 9.5[a]	160.0 ± 9.5[b]	157.6 ± 9.6[c]	<0.001
BMI, kg/m^2	27.6 ± 4.6	27.6 ± 3.9	27.3 ± 4.6	28.0 ± 5.6	0.066
Smoker, % (*n*)	5.0 (73)	5.6 (29)	5.6 (34)	3.1 (10)	0.209[#]
MMSE, points	25.6 ± 4.1	26.5 ± 3.1[a]	26.1 ± 3.3[a]	22.9 ± 5.7[b]	<0.001

All results reported as means ± standard deviation or % (*n*). BMI, body mass index; MMSE, Mini-Mental State Examination. Superscript letters indicate statistical significant differences between frailty groups by unadjusted GLM.
[#]Differences between frailty groups determined by Pearson's χ^2 test. $P < 0.05$.

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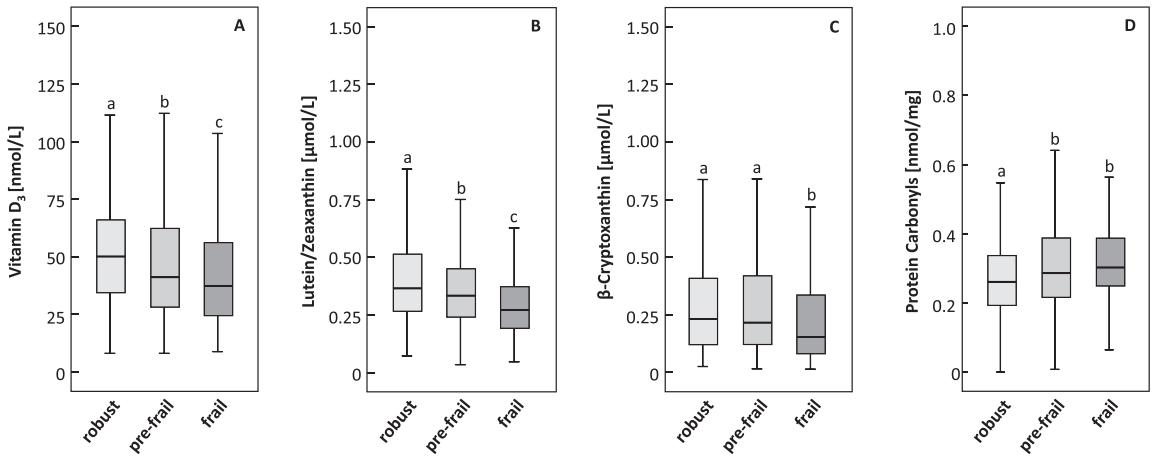
Table 2 Plasma concentrations of biomarkers by frailty groups

Biomarker	FRAILOMIC	Robust	Pre-frail	Frail	*P*
Vitamin D_3, nmol/L	42.0 (40.8–43.2)	47.2 (45.0–49.5)[a]	40.9 (39.1–42.7)[b]	36.6 (34.4–38.9)[c]	<0.001
adjusted		46.6 (44.0–9.5)[a]	39.5 (37.2–41.8)[b]	37.5 (34.9–40.2)[b]	<0.001
Retinol, μmol/L	1.88 (1.85–1.91)	1.87 (1.82–0.91)	1.90 (1.86–1.94)	1.87 (1.82–1.93)	0.577
adjusted		1.84 (1.78–0.90)	1.87 (1.81–1.93)	1.86 (1.79–1.93)	0.757
α-Tocopherol, μmol/L	29.4 (29.0–29.9)	29.2 (28.5–9.9)[a,b]	30.3 (29.6–30.9)[a]	28.3 (27.4–29.2)[b]	0.002
adjusted		28.8 (27.9–0.7)[a,b]	29.4 (28.5–30.3)[a]	27.2 (26.1–28.3)[b]	0.013
γ-Tocopherol, μmol/L	1.19 (1.16–1.22)	1.21 (1.16–1.26)[a]	1.22 (1.18–1.27)[a]	1.10 (1.04–1.16)[b]	0.005
adjusted		1.18 (1.12–1.25)	1.17 (1.11–1.24)	1.12 (1.05–1.19)	0.405
α-Carotene, μmol/L	0.12 (0.12–0.13)	0.13 (0.12–0.14)[a]	0.12 (0.12–0.14)[a]	0.11 (0.10–0.12)[b]	0.020
adjusted		0.12 (0.11–0.13)[a]	0.10 (0.10–0.11)[a,b]	0.10 (0.09–0.11)[b]	0.018
β-Carotene, μmol/L	0.41 (0.40–0.43)	0.44 (0.41–0.47)[a]	0.43 (0.40–0.46)[a]	0.35 (0.32–0.39)[b]	<0.001
adjusted		0.42 (0.39–0.45)[a]	0.36 (0.34–0.39)[b]	0.34 (0.31–0.37)[b]	0.001
Lycopene, μmol/L	0.36 (0.34–0.37)	0.40 (0.37–0.42)[a]	0.36 (0.34–0.39)[a]	0.29 (0.26–0.31)[b]	<0.001
adjusted		0.35 (0.32–0.38)[a]	0.33 (0.30–0.35)[a]	0.28 (0.25–0.30)[b]	0.002
Lutein/zeaxanthin, μmol/L	0.33 (0.32–0.34)	0.36 (0.35–0.38)[a]	0.33 (0.32–0.34)[b]	0.27 (0.25–0.28)[c]	<0.001
adjusted		0.34 (0.32–0.36)[a]	0.32 (0.30–0.33)[a]	0.27 (0.25–0.29)[b]	<0.001
β-Cryptoxanthin, μmol/L	0.20 (0.19–0.21)	0.22 (0.21–0.24)[a]	0.21 (0.20–0.23)[a]	0.16 (0.14–0.18)[b]	<0.001
adjusted		0.21 (0.20–0.23)[a]	0.17 (0.16–0.19)[b]	0.14 (0.13–0.16)[c]	<0.001
Protein carbonyls, nmol/mg	0.31 (0.30–0.31)	0.26 (0.25–0.27)[a]	0.30 (0.29–0.32)[b]	0.31 (0.30–0.33)[b]	<0.001
adjusted		0.26 (0.25–0.27)[a]	0.27 (0.25–0.28)[a]	0.30 (0.28–0.32)[b]	0.006
3-Nitrotyrosine, pmol/mg	7.22 (6.94–7.51)	7.64 (7.15–8.16)	6.98 (6.56–7.42)	7.01 (6.45–7.63)	0.103
adjusted		7.49 (6.90–8.14)	7.93 (7.32–8.60)	7.13 (6.45–7.88)	0.202

All results reported as geometric means (95% confidence interval). Superscript letters indicate statistical significant differences between frailty groups by GLM (unadjusted; *adjusted* for cohort, season of blood sampling, gender, age, height, weight, and smoking status); unadjusted model: $n = 1448$, except PrCarb ($n = 1446$), 3-NT ($n = 1441$), and VD_3 ($n = 1450$); *adjusted* model: $n = 1448$, except PrCarb ($n = 1429$), 3-NT ($n = 1441$), and VD_3 ($n = 1450$). $P < 0.05$.

Figure 1 Plasma concentrations of (A) vitamin D_3, (B) lutein/zeaxanthin, (C) β-cryptoxanthin, and (D) protein carbonyls by frailty groups. Superscript letters indicate statistical significant differences between frailty groups by unadjusted GLM; $P < 0.05$.



1.91 and 3.60; 2.34–5.53) and β-cryptoxanthin (1.70; 1.20–2.41 and 3.02; 1.95–4.69). Furthermore, frail participants were more likely to be in the lowest than in the highest tertile for α-tocopherol (2.12; 1.39–3.24), α-carotene (1.69; 1.00–2.88), and lycopene (1.94; 1.24–3.05) than robust participants. In contrast, for OS markers, ORs and AORs to be in the highest than in the lowest tertiles were calculated. Pre-frail and frail participants were associated with a higher likelihood to be in the highest than in the lowest tertile for PrCarb. For frail participants, this association was confirmed in the adjusted logistic regression model (2.86; 1.82–4.49). No associations were found between retinol and 3-NT tertiles and frailty status in both logistic regression models.

Discussion

In our study, we aimed to determine cross-sectional associations of FMNs and OS biomarkers simultaneously with the

Table 3 Odds ratios of pre-frail and frail participants (robust participants as reference) related to biomarker tertiles

	Pre-frailty	Frailty
Vitamin D_3	1.96 (1.46–2.63)*	2.83 (1.99–4.01)*
adjusted	1.98 (1.42–2.76)*	2.15 (1.42–3.26)*
Retinol	0.88 (0.66–1.17)	0.98 (0.70–1.37)
adjusted	0.93 (0.67–1.28)	0.82 (0.55–1.22)
α-Tocopherol	0.82 (0.62–1.09)	1.54 (1.09–2.19)*
adjusted	1.12 (0.81–1.54)	2.12 (1.39–3.24)*
γ-Tocopherol	0.95 (0.72–1.27)	1.62 (1.15–2.28)*
adjusted	1.04 (0.76–1.42)	1.46 (0.98–2.19)
α-Carotene	0.96 (0.72–1.28)	1.48 (1.05–2.07)*
adjusted	1.40 (0.92–2.13)	1.69 (1.00–2.88)
β-Carotene	1.10 (0.82–1.46)	1.75 (1.24–2.47)*
adjusted	1.56 (1.06–2.28)*	1.84 (1.13–2.99)*
Lycopene	1.28 (0.96–1.71)	2.36 (1.66–3.36)*
adjusted	1.18 (0.83–1.68)	1.94 (1.24–3.05)*
Lutein/zeaxanthin	1.58 (1.18–2.12)*	4.09 (2.84–5.87)*
adjusted	1.38 (1.00–1.91)*	3.60 (2.34–5.53)*
β-Cryptoxanthin	1.11 (0.83–1.49)	2.09 (1.48–2.93)*
adjusted	1.70 (1.20–2.41)*	3.02 (1.95–4.69)*
Protein carbonyls	1.83 (1.37–2.44)*	2.90 (2.00–4.21)*
adjusted	1.21 (0.87–1.68)	2.86 (1.82–4.49)*
3-Nitrotyrosine	0.79 (0.59–1.05)	0.72 (0.52–1.02)
adjusted	1.31 (0.94–1.82)	0.90 (0.59–1.36)

All results reported as odds ratios (95% confidence interval). Multivariate logistic regression models (unadjusted; *adjusted* for cohort, season of blood sampling, gender, age, height, weight, and smoking status); T3 was the reference for vitamin D_3, retinol, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin, and T1, was the reference for PrCarb and 3-NT; unadjusted model: $n = 1448$, except PrCarb ($n = 1445$), 3-NT ($n = 1441$), and VD_3 ($n = 1450$); *adjusted* model: $n = 1431$, except PrCarb ($n = 1432$), 3-NT ($n = 1428$), and VD_3 ($n = 1433$).
*$P < 0.05$.

frailty status of participants older than 65 years, and we demonstrated both differences in concentrations between robust, pre-frail, and frail individuals and associations of FMN and PrCarb with pre-frailty and frailty.

The prevalences of pre-frailty (41.7%) and frailty (22.1%) are in accordance to previously shown prevalences of 42.3% and 17.0%, respectively, in community-dwelling individuals from 10 European countries.[4] Our findings confirm previously published associations of frailty with age and female gender.[4–6]

A higher risk for frailty has previously been related to a low intake of micronutrients and possibly resulting lower micronutrient status. Low intakes of vitamin E or VD led to higher ORs to be frail than non-frail, after adjusting for several confounders, in 802 subjects (>65 years) from the InCHIANTI cohort.[15] Women (>65 years) in the lowest quartile of serum carotenoids had a higher risk of becoming frail during a 3 year follow-up period in the Women's Health and Aging Study I (WHAS I).[17] Lower VD, retinol, α-carotene, β-carotene, lycopene, lutein/zeaxanthin, and β-cryptoxanthin plasma concentrations were found in frail compared with non-frail women of the WHAS I and WHAS II, and the ORs of being frail were significantly higher for women in the lowest quartile compared with the top three quartiles for total carotenoids, α-tocopherol, and VD.[16] The strongest association was found for β-carotene, lutein/zeaxanthin, and total carotenoids, after adjusting for age, sociodemographic status, smoking, and BMI.[16] Using FRAILOMIC initiative data, Pilleron *et al*. observed a significant relationship between a circulating micronutrient pattern, which is low in vitamins A and E, and high in carotenoid concentrations, and a higher prevalence of frailty.[14] However, all these studies did not make the distinction between frail and pre-frail, considering pre-frail as robust.

The lack of association between retinol and frailty status, found in our study, was previously reported in participants of the FRAILOMIC initiative and the InCHIANTI cohort.[14,15] This might be due to the homeostatically regulated retinol metabolism, and furthermore, circulating retinol is considered a poor relevant marker of vitamin A status.[36]

Beside low micronutrient concentrations possibly leading to frailty, VD itself may play a role in the pathogenesis of frailty. Vitamin D, beside its endocrine/indirect effects on muscle function, can act directly on skeletal muscle via the VD receptor, contributing to a normal muscle structure and metabolism.[37] A low VD status was related to a reduced muscle mass and an impaired physical performance in 127 pre-frail and frail Dutch participants (>65 years).[20] Vogt *et al*. observed that participants (>65 years) having baseline VD levels <37.5 nmol/L compared with ≥75 nmol/L were more likely to become pre-frail (2.4; 1.17–5.03) and pre-frail/frail combined (2.5; 1.23–5.22), after a 3 year follow-up.[19] Our findings of an association between low VD_3 and pre-frailty and frailty prevalence differ from those found by Pilleron *et al*.[14] who observed no association between VD_3 and frailty status in the same sample cohort. This was possibly due to the fact that Pilleron *et al*. used different cut-offs for VD_3 and included the pre-frail participants in the robust group. In our analyses, tertiles of VD_3 were chosen to be able to compare groups with similar sample size. The lowest VD_3 tertile (<33.7 nmol/L) in our study meets the definition of a deficient or even severely deficient VD_3 status, depending on the references used.[38]

Low FMN concentrations might be due to a low intake of fruits, vegetables, nuts, seeds, and oils, which could potentially originate from a decreased ability for older persons to go shopping or prepare meals themselves. A low VD status may occur due to less exposure to sunlight and less physical activity, which might be a result of frailty itself. The reason for low concentrations of some carotenoids may lie in their food source. Elderly persons may experience physiological changes in the gastrointestinal tract leading to reflux, heartburn, or constipation that may result in a decreased intake of fruits and vegetables. Our observations might just reflect a low intake of these fruits and vegetables; unfortunately, we are not able to adjust our models for dietary intake.

Inadequate micronutrient intake and plasma concentrations may result in higher OS leading to an impaired muscle

function and performance and contributing to frailty. A low dietary intake of a combination of vitamins A, E, B_6, and B_{12}, folate, selenium, and zinc led to a lower oxidative capacity and reduced muscle function and physical activity in aged male C57/BL6J mice.[39] In contrast, a high intake of fruits and vegetables was previously associated with low biomarkers of OS in 296 healthy, middle-aged men[40] and a lower risk of frailty in older individuals (>60 years).[13] In a previous analysis of the TSHA cohort, higher PrCarb concentrations were observed in frail compared with non-frail individuals but no association was observed with age.[26] In contrast, higher plasma PrCarb were previously observed in older adults (61–85 years) compared with young subjects (21–40 years)[24] supported by an age-dependent increase in PrCarb levels in 80 healthy persons (18–85 years).[25] In our study, there was a significant positive correlation between age and PrCarb ($r = 0.196$; $P < 0.001$; not shown) but also a negative correlation between age and 3-NT ($r = -0.225$; $P < 0.001$; not shown) in robust participants. In frail subjects, no correlations between age and OS markers were found. In addition, an association between higher OS levels and frailty was reported,[27] but only one study on frailty used PrCarb as OS marker, and therefore, no comparisons with other studies are possible. However, PrCarb was two-fold lower and 3-NT was two-fold higher compared with subjects from a general population in the MARK-AGE study.[41] The FMN concentrations on the other hand were comparable with those found in the MARK-AGE study, except for lycopene that was two-fold higher in MARK-AGE.[33]

Due to the cross-sectional design of our study, we cannot conclude whether frailty leads to a low micronutrients/high PrCarb status or if a low micronutrients/high PrCarb status leads to frailty. Furthermore, data regarding socio-economic status and income were not available and, therefore, are missing in the multivariate-adjusted models. Strengths of our study are the large sample size including frailty classification into robust, pre-frail, and frail and the harmonized frailty criteria used in all four cohorts. Especially the possibility to include the pre-frail group is a novel feature in a study with such a large sample size. Additionally, participants from different European countries were analyzed; thus, our study results reflect a broad range of the society and different lifestyles. Furthermore, the broad spectrum of parameters, including nutritional biomarkers, antioxidants, and OS biomarkers, are a unique feature of this study. The high-quality blood analyses were performed by the same trained persons in one laboratory, thus limiting variability related to operators, methods, and analytical instruments.

From our study, we conclude that both low concentrations of several single FMN (VD_3, β-carotene, lutein/zeaxanthin, and β-cryptoxanthin) and high concentrations of PrCarb are associated with pre-frailty and frailty in four European cohorts of adults aged 65 years and older. Thus, we suggest that following a diet rich in FMN, subsequently leading to higher micronutrient and lower OS concentrations, may support the prevention of frailty. Further large-scale longitudinal and intervention studies are needed to investigate the role of FMN on the frailty risk and the potential mediating effect of OS.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Tertiles of fat-soluble micronutrients and oxidative stress markers

Table S2 Study characteristics by individual FRAILOMIC cohorts

Table S3 Plasma concentrations of biomarkers by the FRAILOMIC cohorts

Conflict of interest

C.F. received fees for conferences from Danone Research and Nutricia not related to the present work. No further conflicts of interest are declared.

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