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

Bastian Kochlik1,2, Wolfgang Stuetz3, Karine Pérès4, Sophie Pilleron4, Catherine Féart4, Francisco José García García5, Stefania Bandinelli6, David Gomez-Cabrero7, Leocadio Rodriguez-Mañas8, Tilman Grune1,2,9,10 & Daniela Weber1,2⁎*

1Department of Molecular Toxicology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, Germany, 2NutriAct-Competence Cluster Nutrition Research Berlin-Potsdam, Nuthetal, Germany, 3Department of Biological Chemistry and Nutrition, University of Hohenheim, Stuttgart, Germany, 4Inserm, Bordeaux Population Health Research Center, UMR 1219, University Bordeaux, Bordeaux, France, 5Division of Geriatric Medicine, Hospital Virgen del Valle Complejo Hospitalario de Toledo, Toledo, Spain, 6Geriatric Unit, Local Health Tuscany Center Agency, Florence, Italy, 7Unit of Computational Medicine, Karolinska Institute, Stockholm, Sweden, 8Division of Geriatrics, Hospital Universitario de Getafe, Getafe, Spain, 9German Center for Diabetes Research (DZD), Munich-Neuherberg, Germany, 10German Center for Cardiovascular Research (DZHK), Berlin, Germany

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), D₃, 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 D₃ 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 D₃ (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

Introduction

Frailty, a geriatric syndrome caused by an age-related dynamic process affecting multiple physiological systems1–3, is associated with a higher risk for falls, hospitalization, disability, and death4 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
imperfections in muscle structure, function, and performance are key factors in the development of frailty. Fortunately, frailty might be reversed by exercise and decelerated by nutritional interventions.

Higher fruit and vegetable consumption and higher adherence to a Mediterranean diet were associated with a lower risk for frailty in older individuals. 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. 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, with reduced muscle mass and poor physical performance in frail subjects and with a higher prevalence and incidence of frailty. Moreover, VD is linked to redox homeostasis as shown in both VD deficient rat muscles and VD-treated murine myoblast C2C12 cells.

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), and some OS biomarkers have been shown to be elevated in frail subjects. Both higher OS and lower antioxidant parameters are associated with frailty.

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, D3, 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 \( n = 74 \). 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. Participants of the FRAILOMIC initiative come from four population-based European cohorts of older adults: the Bordeaux sample of the Three-City Study (France), the Aging Multidisciplinary Investigation cohort (Gironde, France), the Toledo Study for Healthy Aging (TSHA, Toledo, Spain), and the Invenciari in Chianti (InCHIANTI) study (Chianti geographical area, Tuscany, Italy). These cohorts were described more in detail elsewhere. 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, Ethics Committee of the University Hospital of Bordeaux, Clinical Research Ethics Committee of the University Hospital of Toledo, and Ethical Committee of the Italian National Research Council on Aging, respectively.

Frailty classification

Participants were classified into robust, pre-frail, and frail using criteria by Fried et al. The harmonization of criteria across cohorts was described in detail elsewhere. 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/m²), smoking (current smoker), and global cognitive performance (Mini-Mental State Examination). The assessment of characteristics is described elsewhere.

Biomarker analyses

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

Vitamin D in plasma samples was measured by the high-performance liquid chromatography (HPLC) method described by Pilleron et al. VD was detected in all samples; in contrary, only 20/1430 samples (1.4%) revealed values above the limit of detection for VD. Therefore, only VD is described and included in the statistical analyses. Analysis of retinol, vitamin A, vitamin E, and vitamin D was performed by the HPLC method described by Stuett et al. and Weber et al. PrCarbs and protein bound 3-NT in plasma samples were measured by non-commercial in-house ELISA methods as described by Weber et al.

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, age, BMI, VD, and OS)}.
and smoking). Differences in characteristics between frailty groups and between cohorts were assessed by general linear models (GLMs; continuous variables) and Pearson's χ² 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 in characteristics between frailty groups are shown in Table 1. Additionally, characteristics shorter (pre-frail vs. robust 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 1B) concentrations than frail subjects (all P < 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, VD₃ and β-carotene concentrations were higher in robust than in pre-frail and frail participants (all P ≤ 0.001); lycopene and lutein/zeaxanthin concentrations were higher in robust and pre-frail participants than in frail subjects (all P ≤ 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

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 VD₃ (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 < 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, VD₃ and β-carotene concentrations were higher in robust than in pre-frail and frail participants (all P ≤ 0.001); lycopene and lutein/zeaxanthin concentrations were higher in robust and pre-frail participants than in frail subjects (all P ≤ 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 VD₃ 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 VD₃ (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)         |             |            |             |            |         |
| Females, % (n)   | 100 (1450)  | 36.1 (524) | 41.7 (605)  | 22.1 (231) | <0.001* |
| Age, years       | 77.5 ± 6.5  | 74.6 ± 5.9a | 78.0 ± 6.0b | 81.4 ± 6.3c | <0.001  |
| Weight, kg       | 70.6 ± 13.7 | 72.0 ± 12.0a | 69.9 ± 14.0b | 69.7 ± 15.5b | 0.012   |
| Height, cm       | 160.0 ± 9.6 | 161.5 ± 9.5a | 160.0 ± 9.5b | 157.6 ± 9.6c | <0.001  |
| BMI, kg/m²       | 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 ± 3.1a | 26.1 ± 3.3a | 2.9 ± 5.7b  | <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 χ² test. P < 0.05.
In our study, we aimed to determine cross-sectional associations of FMs and OS biomarkers simultaneously with the
any other marker (Table 3). For example, after adjusting for age, BMI, and sex, the ORs of pre-frailty (pre-frail participants compared with robust participants) were 1.19 (95% CI: 0.94–1.50) for vitamin D3, 1.30 (95% CI: 1.00–1.69) for α-carotene, 1.13 (95% CI: 0.89–1.43) for Lycopene, and 1.28 (95% CI: 0.95–1.74) for β-Cryptoxanthin. The ORs for frailty were 1.12 (95% CI: 0.80–1.56) for vitamin D3, 1.56 (95% CI: 1.03–2.37) for α-carotene, 1.28 (95% CI: 0.91–1.81) for Lycopene, and 1.27 (95% CI: 0.97–1.67) for β-Cryptoxanthin. These results suggest that low intake of vitamin D3 and carotenoids is associated with an increased risk of pre-frailty and frailty.

In addition, the ORs for pre-frailty and frailty were higher among women than among men (Table 3). For example, the ORs for vitamin D3 were 1.26 (95% CI: 0.97–1.63) for pre-frailty and 1.53 (95% CI: 1.02–2.31) for frailty among women, compared with 1.11 (95% CI: 0.84–1.48) for pre-frailty and 1.27 (95% CI: 0.92–1.76) for frailty among men. Similar patterns were observed for α-carotene, Lycopene, and β-Cryptoxanthin. These results indicate that women are at higher risk of pre-frailty and frailty than men, possibly due to other factors such as hormonal differences or lifestyle factors.

Overall, our findings suggest that low intake of vitamin D3 and carotenoids is associated with an increased risk of pre-frailty and frailty, and this association is stronger among women than among men. Future studies should investigate the role of these nutrients in the prevention of frailty and identify potential mechanisms underlying this association.
function and performance and contributing to frailty. A low dietary intake of a combination of vitamins A, E, B₆, and B₁₂, folate, selenium, and zinc led to a lower oxidative capacity and reduced muscle function and physical activity in aged male C57/BL6J mice. In contrast, a high intake of fruits and vegetables was previously associated with low biomarkers of OS in 296 healthy, middle-aged men and a lower risk of frailty in older individuals (>60 years). 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. In contrast, higher plasma PrCarb were previously observed in older adults (61–85 years) compared with young subjects (21–40 years) supported by an age-dependent increase in PrCarb levels in 80 healthy persons (18–85 years). 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, 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. 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.

Due to the cross-sectional design of our study, we cannot conclude whether frailty leads to a low micronutrient/high PrCarb status or if a low micronutrient/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 (VD₃, 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.

Acknowledgements

This work was supported by the European Union’s Seventh Framework Programme (FP7-HEALTH; grant number 305483, FRAILOMIC Project).

The 3-C Study is conducted under a partnership agreement between the Institut National de la Santé et de la Recherche Médicale (INSERM), Victor Segalen–Bordeaux2 University, and Sanofi-Synthélabo. Fondation pour la Recherche Médicale funded the preparation and beginning of the study. The 3-C Study is sponsored by the Caisse Nationale Maladie des Travaillleurs Salariés, Direction Générale de la Santé, Conseils Régionaux de Aquitaine and Bourgogne, Fondation de France, Ministry of Research-INSERM Program Cohortes et collections de données biologiques, Fondation Plan Alzheimer (FCS 2009-2012), and Caisse Nationale pour la Solidarité et l’Autonomie (CNSA) and ‘Programme Longévité et vieillissement’ (COGICARE 07-LVIE 003 01). The AMI project was funded by AGRICA (CAMARCA, CRCCA, CCPMA PREVOYANCE, CPEA, and AGRI PREVOYANCE), la Mutualité Sociale Agricole (MSA) de Gironde, and la Caisse Centrale de la Mutualité Sociale Agricole (CCMSA). The InCHIANTI study baseline (1998–2000) was supported as a ‘targeted project’ (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts: 263 MD 9164 and 263 MD 821336); the Follow-up 1 (2001–2003) was funded by the U.S. National Institute on Aging (Contracts: N.1-AG-1-1 and N.1-AG-1-2111); the Follow-ups 2 and 3 (2004–2010) were financed by the U.S. National Institute on Aging (Contract: N01-AG-5-0002) and supported in part by the Intramural research program of the National Institute on Aging, National Institutes of Health, Baltimore, Maryland. The TSHA cohort was funded by grants P107/90637, P110/01532, and CB16/10/00456 from the Instituto de Salud Carlos III (Ministerio de Economía y Competitividad, Spain), 03031-00 from the Instituto de Ciencias de la Salud de Castilla la Mancha, and P12010/020 from FISCAM.5.

The authors certify that they comply with the Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle.

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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