Journal of Gerontology: MEDICAL SCIENCES
2006, Vol. 61A, No. 6, 600–607

Nutrition and Aging

Vitamin and Carotenoid Status in Older Women: Associations With the Frailty Syndrome

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Objective. We investigated the relationship of micronutrient deficiencies with the frailty syndrome in older women living in the community.

Methods. Frailty status and serum micronutrients were assessed in a cross-sectional study of 754 women, 70–80 years old, from the Women's Health and Aging Studies I and II.

Results. Among nonfrail, prefrail, and frail women, respectively, geometric mean serum concentrations were 1.842, 1.593, and 1.376 μmol/L for total carotenoids ($p < .001$); 2.66, 2.51, and 2.43 μmol/L for retinol ($p = .04$); 50.9, 47.4, and 43.8 nmol/L for 25-hydroxyvitamin D ($p = .019$); 43.0, 35.8, and 30.9 nmol/L for vitamin B_6 ($p = .002$); and 10.2, 9.3, and 8.7 ng/mL for folate ($p = .03$). Frail women were more likely to have at least two or more micronutrient deficiencies ($p = .05$). The age-adjusted odds ratios of being frail were significantly higher for those participants whose micronutrient concentrations were in the lowest quartile compared to the top three quartiles for total carotenoids, α-tocopherol, 25-hydroxyvitamin D, and vitamin B_6. The association between nutrients and frailty was strongest for β-carotene, lutein/zeaxanthin, and total carotenoids (odds ratio ranging from 1.82 to 2.45, $p = .05$), after adjusting for age, sociodemographic status, smoking status, and body mass index.

Conclusion. Frail women are more likely to have relatively low serum carotenoid and micronutrient concentrations and are more likely to have multiple micronutrient deficiencies. Future longitudinal studies are needed to examine the relationships between micronutrient concentrations and frailty in older women.

FRAILTY is a geriatric syndrome characterized by a multisystem reduction in physiological reserve and vulnerability to stressors. It has been shown to predict adverse health outcomes in older adults, including hospitalization, institutionalization, disability, falls, and death (1). Phenotypic characteristics associated with a syndrome of frailty have recently been described in a population-based study of older adults (2,3). A validated definition describes frailty as having at least three of five criteria that include weight loss, weakness, low exercise tolerance, slow walking speed, and low physical activity.

Although previous studies (4–8) have focused primarily on the roles of weight loss and sarcopenia in frailty, micronutrient malnutrition is also thought to play an important role. Deficiencies of micronutrients are known to be common among older adults (9), but the role that micronutrient deficiencies may play in the frailty syndrome has not been characterized. Micronutrient deficiencies have also been associated with many conditions and factors associated with the frailty syndrome, including an increased risk of chronic diseases (10), impaired immune function (11), decreased antioxidant activity (12), an increased risk of osteoporosis (13), as well as peripheral vascular disease and atherosclerosis (14), and a more rapid aging process (6,15).

The goal of our study was to determine if decreased micronutrient concentrations, measured either as defined deficiencies or relatively low serum concentrations, are associated with prevalent frailty. Because micronutrients are generally enzyme cofactors responsible for critical steps in metabolism, or have antioxidant activity, such research is a first step in gaining insight into the potential contribution of micronutrient malnutrition to the frailty syndrome and potential pathophysiological mechanisms (3–7). Such hypothesized pathways could include increased oxidative stress from low antioxidant micronutrients and problems such as osteoporosis, disability, and falls associated with vitamin D deficiency. We hypothesized that micronutrient malnutrition is associated with frailty and that multiple deficiencies are more common in frail women.

Methods

Participants

Participants in this study were women, aged 70–80 years, who participated in the Women's Health and Aging Studies (WHAS) I and II, two complementary, population-based studies designed to evaluate the causes and course of

Table 1. Baseline Characteristics of Women Aged 70–80 Years Who Participated and Did Not Participate in the Blood Draws for WHAS I and WHAS II Studies

Characteristic	Blood Drawn $N = 756$	No Blood Drawn $N = 108$	p
Age, y*	74.4	74.8	.228
White race, %	78	71	.133
Marital status widowed, %	49	61	.143
Educational level, y*	11.7	10.3	.006
Income in US$/y*	21815	11604	.001
Never smokers, %	52	46	.268
Drink at least once a week, %	26	12	.012
Body mass index (weight/height^2)*	28.4	28.5	.216
Self-reported weight loss past year, %	21	20	.985
Appetite fair/poor, %	15	29	.001
Wear dentures, %	61	70	.100
Problems chewing/swallowing, %	10	15	.090
Multivitamin users, %	20	18	.558
Self-perceived health fair/poor, %	25	61	.001
Domains of disability, %			.001
0	40	7	
1 or 2	40	39	
3 or 4	20	54	
Health status, %			.001
Nonfrail	48	28	
Prefrail	42	50	
Frail	10	22	

*Results presented as means.

physical disability in older women living in the community. WHAS I was recruited from an age-stratified random sample of women aged 65 years or older selected from Medicare enrollees residing in 12 contiguous ZIP code areas in Baltimore (16). Women were screened to identify self-reported physical disability that was categorized into four domains by report difficulty with tasks in the following areas: (a) mobility, (b) upper extremity function, (c) higher functioning household management, and (d) self-care. WHAS I enrolled the one-third most disabled women age 65 or older, which were those women with disability in two or more domains. Of the 1409 women who met study eligibility criteria, 1002 agreed to participate in the study in 1992. There were no major differences in sociodemographic or reported health characteristics between eligible participants and those who declined. Standardized questionnaires were administered in the participant's home by trained interviewers. Two weeks later, a trained registered nurse conducted an examination of each study participant in her home, using a standardized protocol that included physical performance measures and a directed physical examination. Approximately 75% of women also consented to phlebotomy performed during a separate visit by a trained phlebotomist who followed a standardized protocol.

WHAS II was specifically designed to be a companion study for WHAS I and includes a cohort of women, aged 70–79 years, selected to be representative of the two-thirds least disabled women living in the community. Participants were selected via age-stratified random samples from the same sampling frame as in WHAS I, and were screened using the same four domains of physical function. Eligible women had either no disability or disability in only one domain. In 1994, 880 women were found eligible for WHAS II, and 436 consented to participate. Those agreeing were more highly educated and reported more diseases that those who refused, but did not differ significantly in disability characteristics. An interview standardized to that performed in WHAS I was administered in The Johns Hopkins Functional Status Laboratory. Trained technicians then conducted a standardized examination that included a directed physical examination and physical performance measures. Phlebotomy was performed in 93% of WHAS II participants by a trained phlebotomist following the same protocol as that used in WHAS I. Details on the study methods and sampling design of the WHAS studies are published elsewhere (16,17).

For our analyses, we used a combined sample linking the two WHAS studies with a methodology that has been developed by the WHAS research team (18). The sample consists of women participating in WHAS I or WHAS II who are 70–80 years of age ($n = 864$). Appropriate weights have been calculated to adjust for differential selection probability with respect to age and disability status from the sampling frame. Of the 864 women in the combined sample, 756 had blood samples available for analysis of micronutrients. Two individuals did not have complete information for health status classification and were excluded from our analysis.

Definition and Classification of Frailty

Individuals were categorized as nonfrail, prefrail, or frail according to criteria recently developed by Fried and colleagues (2). These criteria describe a frailty phenotype, validated in a population-based sample of older adults and based on the presence or absence of five measurable characteristics: (a) shrinking (reported unintentional weight loss of 10 pounds or more in the prior year), (b) weakness (measured grip strength in the lowest quintile at baseline), (c) poor endurance and energy (self-report of exhaustion), (d) slowness (the slowest quintile on test of timed walking speed), and (e) low physical activity level (lowest quintile of physical activity scale). This frailty phenotype was originally developed in the Cardiovascular Health Study (2) and was also assessed in WHAS I and II (Table 1). Individuals were defined as frail by the presence of three or more of the five components. Individuals with none of the components were categorized as nonfrail, and those with one or two components as prefrail, based on other work indicating that this group is at high risk of progression to frailty (2). In the Cardiovascular Health Study cohort, individuals categorized as frail had a significantly increased 7-year hazard ratio for incident falls, worsening mobility, hospitalization, and death (2). The hazard ratios were lower for those individuals categorized as nonfrail, and intermediate for those categorized as prefrail.

Components of the frailty phenotype were measured using standardized questions (weight loss and exhaustion) and protocols. Physical activity level was determined using the modified Minnesota Leisure Time Activities Questionnaire (16). A weighted score of kilocalories expended per week was calculated from questions on frequency and dura-

tion of walking for exercise, dancing, bowling, performing moderately strenuous household and outdoor chores, and participating in any regular exercise program. Weakness was measured by level of maximal grip strength. Data on maximal grip strength were obtained using a JAMAR handheld dynamometer (model BK-7498; Fred Sammons, Inc., Burr Ridge, IL) and was measured three times with each hand. The best measure in the dominant hand was used. Walking speed was measured using a standardized protocol for timed walking. The participant could use a cane, walker, or other walking aide, but not the aid of another person. The length of walk in meters divided by the time in seconds was used to calculate the walking speed (16). Cutoff points in each measure that met the criteria for frailty were standardized to those used for women in the Cardiovascular Health Study (2).

Demographic characteristics, self-rated health, and information about appetite and eating were measured in the WHAS questionnaires. Chronic diseases were adjudicated by WHAS co-investigators on the basis of the questionnaire, physical examination, and physician contact (16). Physical function and disability status were assessed by the questionnaires and have been described in detail elsewhere (16). Participants were questioned about 15 distinct physical tasks in four functional domains: mobility, upper extremity function, self-care tasks, and higher functioning skills. For each task the participant was asked, ''By yourself, that is, without help from another person or special equipment, do you have any difficulty <performing task>?'' The responses were dichotomized into ''yes'' difficulty versus ''no'' difficulty.

Functional groups were derived from several standardized scales (Activities of Daily Living [ADL], Instrumental Activities of Daily Living [IADL], Rosow-Breslau) (16,17). Individual tasks were grouped as follows: 1) mobility (walking 2–3 blocks, climbing 10 steps, getting in or out of bed or chairs, and heavy housework); 2) upper extremity function (raising arms, grasping, and lifting up to 10 pounds); 3) higher functioning tasks (IADL) (using telephone, light housekeeping, meal preparation, and personal shopping); and 4) self-care tasks (ADL) (bathing and showering, getting in and out of bed or chair, dressing, eating, and using the toilet).

A woman was defined as having a physical disability in a particular group if she reported difficulty in any of the tasks specific to that category. However, individuals often reported difficulty for tasks in several groups. Therefore, study participants were further differentiated into six mutually exclusive domains of disability derived from the original four groups: upper extremity only, mobility only, both mobility and upper extremity, higher functioning, self-care, and higher functioning and self-care. The latter three generally included people who had mobility and/or upper extremity difficulty in addition to their higher functioning and/or self-care difficulty (16,17).

Laboratory Analysis

Nonfasting blood samples were obtained by venipuncture between 9:00 AM and 2:00 PM. Processing, aliquoting, and freezing were carried out at the Core Genetics Laboratory of The Johns Hopkins University School of Medicine following a standardized protocol. Blood samples were delivered to Quest Diagnostics Laboratories (Teterboro, NJ) on the day of blood drawing for assays conducted by this commercial laboratory. Plasma carotenoids, retinol, and α-tocopherol were determined by high performance liquid chromatography in the laboratory of one of the investigators (R.D.S.) (19). Total carotenoids were calculated as the sum of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene (in micromoles per liter). The other serum biochemical measurements were performed by Quest Diagnostics Laboratories. Serum concentration of 25-hydroxyvitamin D [25(OH)D] was measured using a radioreceptor assay (20). Vitamin B_6 status was assessed by pyridoxal 5-phosphate measurements using high performance liquid chromatography (21). Serum vitamin B_{12} and folate were measured by radioimmunoassay (RIA) (22). Within-run and between-run coefficients of variation were 10.7% and 23.9% for α-carotene, 7.0% and 19.1% for β-carotene, 4.7% and 8.5% for β-cryptoxanthin, 4.1% and 4.6% for lutein/zeaxanthin, 10.0% and 14.0% for lycopene (in micromoles per liter), 4.1% and 9.7% for α-tocopherol, and 7.5% and 9.6% for 25-hydroxyvitamin D.

Micronutrient deficiencies were defined using standard cutoffs: for serum retinol < 1.05 μmol/L; for vitamin E, α-tocopherol < 11.6 μmol/L; for vitamin D, 25(OH)D < 30 nmol/L; for vitamin B_6 (plasma pyridoxal 5′-phosphate) $<$ 30 nmol/L; for vitamin $B_{12} < 300$ pg/mL; and for folate $<$ 5.0 ng/mL (23). Cutoffs for carotenoids have not been established and therefore were not included in the analysis of prevalence of deficiencies. A serum creatinine concentration >1.4 mg/dL was considered consistent with renal disease. Elevated liver enzymes (alanine aminotransferase >74 U/L, alkaline phosphatase >154.5 U/L, and/or aspartate aminotransferase >68 U/L) were considered consistent with significant hepatic disease. In seasonal analyses, seasons were defined as summer (June–August), autumn (September–November), winter (December–February), and spring (March–May), with summer as the reference category.

Statistical Analysis

Probability weights specific to WHAS I and II were used in the analysis to make inferences about community-dwelling women aged 70–80 years based on our study sample. The procedures used to calculate the weights for the WHAS samples are described elsewhere (16,18). Descriptive statistics were used to characterize the study population, give the distribution of biochemical measurements of micronutrients, and calculate the prevalence of deficiencies. In addition to described deficiencies, for all the micronutrients, the population was categorized into quartiles based on micronutrient concentration. This was done because deficiencies are described for the general population and may not necessarily be meaningful in older women, and we hypothesized that low levels may have physiological significance, even if they did not reach the level of ''deficiency.'' For the carotenoids, which do not have described deficiencies, quartiles were considered the

Table 2. Baseline Characteristics of Study Participants by Frailty Status

Characteristics	Health Status			p
	Nonfrail N = 331 (%)	Prefrail N = 337 (%)	Frail N = 86 (%)	
Mean age, y (95% CI)	74.1 (73.7–74.4)	74.4 (74.1–74.7)	75.8 (75.1–76.5)	.001
White race, %	81	76	68	.025
Marital status widowed, %	45	50	61	.027
Educational level, y (95% CI)	12.5 (12.1–12.9)	11.2 (10.5–12.0)	9.5 (8.7–10.3)	.014
Income < US $9,000, %	20	39	48	.001
Never smokers, %	56	51	39	.006
Drink at least once a week, %	35	20	9	.001
Drink >8 drinks per week, %	9	5	1	.016
Mean body mass index (95% CI)	26.2 (25.5–26.8)	28.5 (27.8–29.2)	29.2 (27.6–30.8)	.001
Self-reported weight loss past year, %	17	23	29	.013
Appetite fair/poor, %	8	18	31	.001
Wear dentures, %	57	63	70	.036
Problems chewing/swallowing, %	6	11	27	.001
Multivitamin users, %	21	21	14	.422
Creatinine >1.4 mg/dL, %	3	5	7	.247
Elevated liver enzymes, %	3	3	9	.015
Self-perceived health fair/poor, %	12	32	59	.001
Domains of disability, %				.001
0	64	22	3	
1 or 2	30	53	37	
3 or 4	6	26	60	

Note: CI = confidence interval.

appropriate method of analysis. All the measurements of micronutrients had a skewed distribution and were analyzed using logarithmic transformation and presented as geometric means and 95% confidence intervals.

We first compared individuals who had blood tests and those who did not, using the Pearson chi-square test. Chi-square and trend tests were used to examine the associations between micronutrients and dichotomized covariates, i.e., frailty status, and means. Values of $p \leq .05$ were considered statistically significant. Binomial logistic regression models were used to examine associations between low micronutrient concentrations and frailty. Low micronutrient concentrations were defined as the lowest quartile of the distribution of each micronutrient. Women were categorized in two groups as frail versus not (prefrail and nonfrail groups combined). The odds ratio (OR) for being frail was then calculated for the lowest quartile of each micronutrient's distribution, using all other quartiles combined as the reference. Initial models were adjusted for age. Sequential adjustment was done adding variables known to be associated with both micronutrient deficiencies and frailty status. Models were adjusted for age and sociodemographic characteristics (race [black, white], income [in tertiles], education [completed high school vs not]); then smoking status (current smoker vs not) was added; and finally, body mass index (BMI, calculated from measured height and weight).

Trends across frailty subgroups were examined using generalized linear models for continuous variables and the chi-square test for categorical variables. Trends across frailty subgroups were analyzed for differences in the baseline characteristics of the participants, mean concentrations of micronutrients, and prevalence of micronutrient deficiencies. The statistical programs used were SAS (SAS Institute, Cary, NC) (24) for data management and Stata (Stata Corporation, College Station, TX) (25) for weighted analyses.

Results

Participants with ($n = 756$) and without ($n = 108$) blood drawing in the study are compared in Table 1. Those without blood drawing had lower educational and income levels than did those who participated in the blood drawing. Those without blood drawing were less likely to report drinking at least once a week, but more likely to report fair or poor self-rated health and appetite. Disability and frailty were more prevalent among those participants who did not participate in blood drawing.

Social and demographic characteristics were compared by frailty status (Table 2). Women classified as frail were older, had a lower income, and had fewer years of education. The frail group had a higher proportion of African Americans, widows, current or former smokers, and a lower proportion of weekly alcohol drinkers than did the other groups. It also had a higher proportion with self-reported weight loss, fair or poor appetite, denture use, and problems chewing or swallowing. The mean BMI was lower in the nonfrail than in the prefrail or frail subgroups, although the prevalence of obesity (BMI $\geq$ 30) was higher among the nonfrail participants. A higher proportion of frail individuals reported their self-perceived health as fair or poor and had three or four domains of disability. All of these trends were significant across the frailty groups from nonfrail to frail. However, the proportion of women who reported use of multivitamins did not differ among the three groups.

The unadjusted means of serum micronutrient concentrations are presented in Table 3. Mean serum concentrations of all micronutrients were lower in the frail than in

Table 3. Mean Serum Concentration of Micronutrients by Frailty Status

Micronutrients	Health Status		
	Nonfrail $N = 331$	Prefrail $N = 337$	Frail $N = 86$
Carotenoids (μmol/L)			
α-carotene	0.097 (0.088–0.107)	0.075 (0.068–0.083)	0.058 (0.048–0.070)
β-carotene	0.440 (0.401–0.485)	0.363 (0.329–0.400)	0.296 (0.249–0.352)
β-cryptoxanthin	0.136 (0.126–0.147)	0.111 (0.101–0.121)	0.090 (0.077–0.106)
Lutein/zeaxanthin	0.410 (0.388–0.434)	0.345 (0.326–0.365)	0.323 (0.288–0.363)
Lycopene	0.589 (0.549–0.633)	0.545 (0.509–0.583)	0.460 (0.397–0.532)
Total carotenoids	1.842 (1.741–1.949)	1.593 (1.505–1.687)	1.376 (1.249–1.515)
Vitamins			
Retinol (μmol/L)	2.66 (2.53–2.80)	2.51 (2.40–2.62	2.43 (2.25–2.63)
α-tocopherol (μmol/L)	22.27 (21.37–23.20)	21.88 (20.84–22.98)	19.61 (18.11–21.23)
25(OH) D (nmol/L)	50.90 (48.32–53.62)	47.43 (44.28–50.81)	43.85 (38.11–50.46)
Vitamin B_6 (nmol/L)	43.09 (38.79–47.86)	35.86 (32.03–40.16)	30.99 (26.47–36.29)
Vitamin B_{12} (pg/mL)	452.94 (426.79–480.69)	428.24 (404.40–453.49)	417.20 (373.60–465.8)
Folate (ng/mL)	10.20 (9.47–10.99)	9.29 (8.64–9.98)	8.68 (7.44–10.13)

Note: Results presented as geometric means and 95% confidence intervals.

the other groups. The trend toward lower serum micronutrient concentrations in the frail group was statistically significant for each of the carotenoids, retinol, 25(OH)D, vitamin B_6, and folate. The trend was not statistically significant for α-tocopherol and vitamin B_{12}. In analyses of micronutrient concentrations by season, significant differences by season were found only for retinol, 25-hydroxyvitamin D, and vitamin B_6, with the generally the highest concentrations noted in the summer season.

The prevalence of specific vitamin deficiencies by frailty status is shown in Figure 1. The overall prevalence of vitamin deficiencies was: vitamin B_6 (41.6%), vitamin B_{12} (19.4%), vitamin D (14.2%), folate (12.1%), vitamin E (3.4%), and vitamin A (1.1%). For all vitamins, there was a trend toward higher prevalence of deficiencies among the frail and a lower prevalence of deficiencies among the nonfrail group; this trend was statistically significant for α-tocopherol, vitamin D, and vitamin B_{12}. Using the criteria for micronutrient deficiencies (23), we found that 43% of women had no micronutrient deficiencies, 33% had one deficiency only, and 24% had two or more deficiencies. In the frail group there was a lower proportion of individuals with no deficiencies and a higher proportion with two or more deficiencies than other groups; this trend was statistically significant (Figure 2).

Table 4 shows the results of logistic regression models of the associations between frailty and low serum micronutrient concentrations, including low carotenoid concentrations. In these models, nonfrail and prefrail data were combined to focus on frailty and to create conservative models. In all models specified, whether adjusted only for age or for multiple variables, the likelihood of being frail (vs nonfrail or prefrail), as described by the OR, was always higher for those with low micronutrient concentrations (the lowest quartile of the distribution of serum micronutrients compared to the upper quartiles combined). For example, in

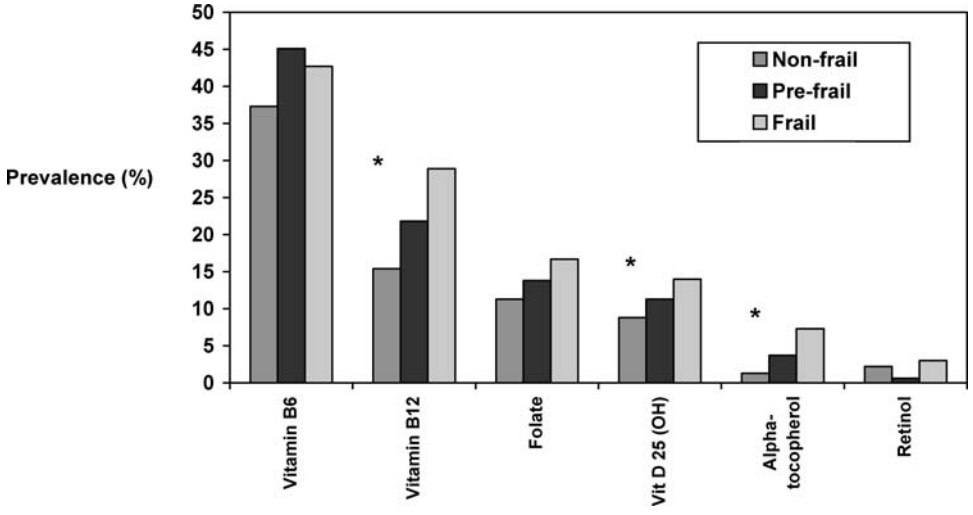

Figure 1. Prevalence of specific vitamin deficiencies by frailty status. *$p < .05$ by Mantel–Haenszel chi-square.

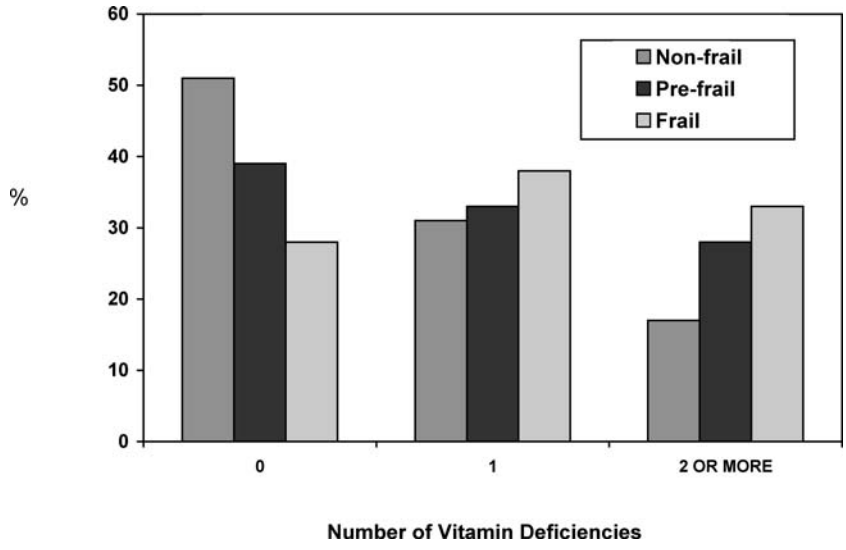

Figure 2. Number of vitamin deficiencies by frailty status. *$p < .05$ by Mantel–Haenszel chi-square.

the age-adjusted model for α-carotene, the OR for being frail was 2.24 for the group in the lowest quartile of α-carotene distribution compared to the group in the upper quartiles. The strongest associations of low micronutrient concentration with frailty were noted for the carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and total carotenoids), and these associations were statistically significant in age-adjusted models, with α-tocopherol, 25(OH)D, vitamin B_6, and folate reaching borderline significance.

In general, after adjustment for socioeconomic variables, the OR describing the association between frailty and low micronutrient concentrations became attenuated. However, associations for several carotenoid measures were persistently significant, including total carotenoids, lutein/zeaxanthin, β-carotene, and β-cryptoxanthin. No major differences in the associations were observed after adjustment for smoking status and BMI, except for β-cryptoxanthin, which lost statistical significance. Additional multivariate models were run which adjusted for both seasonality and multivitamin use; these did not change the significance of the results shown the main multivariate models in Table 4.

Discussion

Our study has shown a stepwise association of decreasing serum micronutrient concentration with frailty status in older women, i.e., nonfrail, prefrail, and frail. Although this trend was noted for every micronutrient tested, the strongest trends were found for serum carotenoids. In addition, low concentrations of serum carotenoids showed the strongest associations with frailty status, compared to the other micronutrients studied, and this association persisted even after socioeconomic characteristics, smoking status, and BMI were taken into account. Carotenoids have strong antioxidant activity and are thought to protect against free radical damage to tissues (19). A higher dietary intake or serum concentrations of carotenoids are associated with decreased risk of cardiovascular disease (26), cancer (27),

Table 4. Odds Ratio for Being Frail vs. Not Frail (Pre-Frail and Non-Frail Combined) at the Lower Quartile of the Distribution of Serum Nutrients Compared to the Upper Quartiles

Micronutrients	Age-adjusted*	Adjusted for Age/SES	Adjusted for Age/SES/Smoking	Adjusted for Age/SES/ Smoking/BMI
α-carotene	2.24 (1.34–3.74)	1.31 (0.70–2.47)	1.26 (0.67–2.36)	1.28 (0.67–2.45)
β-carotene	2.38 (1.41–4.01)	1.81 (1.03–3.17)	1.70 (0.97–2.96)	1.82 (1.03–3.21)
β-cryptoxanthin	2.34 (1.38–3.99)	1.77 (0.98–3.19)	1.60 (0.87–2.92)	1.63 (0.87–3.08)
Lutein/zeaxanthin	2.92 (1.75–4.88)	2.62 (1.49–4.61)	2.48 (1.38–4.46)	2.45 (1.32–4.53)
Lycopene	1.39 (0.82–2.37)	1.21 (0.63–2.30)	1.20 (0.62–2.32)	1.20 (0.61–2.36)
Total Carotenoids	2.50 (1.51–4.14)	1.87 (1.06–2.32)	1.76 (0.99–3.14)	1.91 (1.06–3.46)
Retinol	1.31 (0.77–2.20)	1.03 (0.58–1.80)	1.07 (0.61–1.88)	1.16 (0.65–2.07)
α-tocopherol	1.64 (0.95–2.84)	1.23 (0.64–2.36)	1.26 (0.65–2.44)	1.27 (0.64–2.52)
25 (OH) D	1.71 (1.00–2.94)	1.53 (0.83–2.81)	1.54 (0.84–2.82)	1.57 (0.86–2.86)
Vitamin B_6	1.79 (0.99–3.24)	1.45 (0.74–2.81)	1.45 (0.75–2.83)	1.69 (0.85–3.36)
Vitamin B_{12}	1.28 (0.74–2.23)	1.34 (0.75–2.39)	1.46 (0.81–2.61)	1.35 (0.74–2.45)
Folate	1.62 (0.92–2.83)	1.44 (0.76–2.73)	1.41 (0.76–2.63)	1.45 (0.76–2.76)

*Odds ratio (95% confidence interval).

macular degeneration (28), and sarcopenia (19). These known links may possibly explain the strength and persistence of the associations between low carotenoid levels and frailty status noted in the present study. Among the other micronutrients, all had sequentially decreasing levels in nonfrail, prefrail, and frail women, with only two (vitamin B_{12} and α-tocopherol) not reaching statistical significance for the trend.

The prevalence of vitamin deficiencies was higher among the frail compared to the nonfrail and prefrail women. The trends across frailty status were significant for vitamin B_{12}, α-tocopherol, and 25(OH)D deficiencies, and there was a similar but not statistically significant trend for folate. Measurement of folate status may provide limited insight because low serum folate concentrations are considered to indicate negative folate balance but not necessarily depleted body stores associated with functional changes (29). The trend of increasing deficiency prevalence with frailty is biologically plausible because deficiencies of B vitamins (B_6, B_{12}, and folate) are associated with homocysteine elevation and subsequent increased risk of cardiovascular disease (30) and decreased cognitive function (31). Cardiovascular disease has been linked to the frailty syndrome (32). Such a biological mechanism, while plausible, needs further investigation.

The increasing prevalence of 25(OH)D deficiency in a dose-response type of relationship from nonfrail to frail is also biologically plausible. A high prevalence of vitamin D deficiency among older adults has been observed in other epidemiological studies (33). Vitamin D deficiency is associated with osteoporosis, myopathy (34), disability (35), and falls (36), all of which are associated with the frailty syndrome.

Vitamin B_6 showed the highest prevalence of deficiency in our total sample. Lower vitamin B_6 concentrations were observed among the prefrail and frail, but no significant trend in the prevalence of deficiency across the frailty subgroups was observed. This high prevalence of vitamin B_6 deficiency has also been observed in other epidemiological studies (9,37), although the significance of these findings for older populations is not well established. Rich dietary sources of vitamin B_6 include fortified cereals, white potatoes and other starchy vegetables, noncitrus fruits, poultry, and beef. Vitamin B_6 deficiency is known to affect humoral and cellular immune responses in older adults (38).

In summary, the association between low micronutrient levels and frailty was increased for every micronutrient studied, and was particularly strong and persistent for the carotenoids. When sociodemographic variables were included in multivariate models, the associations of frailty (vs nonfrail and prefrail) with decreased α-carotene, β-cryptoxanthin, and lycopene were no longer statistically significant although the associations with β-carotene, lutein/zeaxanthin, and total carotenoids, although attenuated, persisted. Further adjustment for smoking status and BMI did not substantially change our findings. The carotenoids comprise a major portion of circulating antioxidants, and oxidative stress is associated with aging, multiple comorbidities, and disruption in the inflammatory system. This study raises the potential of an independent association of carotenoids with frailty.

Our study has some limitations. First, 13% of the women in our sample refused blood draws. Those who refused had lower educational and income levels, and were more likely to be disabled and to be classified as frail. However, because the most impaired women in the sample may have not participated in the blood studies, the associations that we have found are likely to underestimate associations that would have been found if all the women had been available for study. Second, serum measurements of micronutrients tend to have high within-person variability, particularly when single measures and nonfasting samples are used, as was the case in our study (39). Single measurements can lead to misclassification, and the cutoff points routinely used to define deficiency may identify an acute rather than a chronic deficiency syndrome (39). Repeated measures could improve the precision of the estimates and strengthen the observed associations. Third, this is a cross-sectional study, and the direction of the associations cannot be deduced from the study design. Women who are frail may have more difficulty shopping and preparing vitamin- and carotenoid-rich foods at home, or the lack of carotenoids and vitamins could contribute to the pathogenesis of frailty as discussed above. Disability has also been associated with financial difficulty in acquiring food among older women (40). Fourth, the study did not include dietary assessment, thus, it relies solely on biochemical measurements of nutritional status.

Further investigations are needed to elucidate the potential biological pathways by which micronutrient deficiencies could increase the risk of frailty. Longitudinal studies may help determine whether preexisting micronutrient deficiencies in nonfrail individuals increase the risk of subsequently developing frailty. Such future studies may provide insight into the role of micronutrients in the frailty syndrome and identify potential strategies to prevent frailty. Such strategies may include micronutrient supplementation and improvement in quality of dietary intake in older adults, but further evidence may be needed from large controlled clinical trials.

Acknowledgments

This work was supported by National Institute on Aging (NIA) Grant RO1 AG027012, National Institutes of Health-National Center for Research Resources, Outpatient Unit-General Clinical Research Center grant RR00722, RO1 AI41956, R37 AG19905, and NIA Contract NO1-AG12112. Dr. Michelon was supported by a post-doctoral scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)-Brazil. Dr. Blaum was supported by NIA-K08 AG00749-02.

We thank Dana Totin Moncrief and Barbara Dancheck for carotenoid and vitamin E analyses.

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Received February 11, 2004
Accepted June 21, 2004
Decision Editor: John E. Morley, MB, BCh