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Low total plasma carotenoids are independent predictors of mortality among older persons

The InCHIANTI study

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Abstract *Background* Plasma carotenoids are considered a valid biological marker for fruit and vegetable dietary intake. Recent studies show that low carotenoid levels are associated with a high risk of inflammation, cancer, and cardiovascular disease. *Aim of the study* To determine whether low plasma carotenoids are associated with increased mortality among older adults. *Methods* Longitudinal study among 1,043 adults, 65 years and older, in the InCHIANTI study, a population-based cohort of adults living in the community in the Tuscany region, Italy. *Results* Mean total carotenoid concentration was 1.80 $\mu\text{mol/l}$. During eight years of follow-up, 310 (29.7%) of participants died. Eight-year survival was lower in the lowest compared with the highest tertile of total serum carotenoids ($P < 0.0001$ by Mantel-Haenszel chi-square). In a multivariate Cox proportional hazards model adjusted for age, education, smoking, body mass index, energy intake, and chronic

diseases, adults in the highest tertile of plasma carotenoids at enrollment had lower mortality compared to those in the lowest tertile (Hazards Ratio obtained by considering carotenoids level as an ordinal variable 0.81, 95% CI 0.65–0.99; P for trend = 0.046). *Conclusions* Low plasma carotenoids are an independent risk factor for mortality among older adults living in the community.

Key words aging – carotenoids – fruits – mortality – vegetables

Introduction

Epidemiologic studies suggest that a diet high in fruit and vegetable intake is protective against cardiovas-

cular disease [19], stroke [15], and cancer [30]. Fruits and vegetables are high in bioactive compounds such as carotenoids, flavonoids, and other plant polyphenols. Carotenoids are powerful antioxidants and have been shown to protect against damage caused by

oxidative stress. The reduction in oxidative damage is related to the decreased risk of all-cause, cancer and cardiovascular disease mortality [12]. Carotenoids act as free radical scavengers, modulate immune responses, and play an important role in the redox regulation involved in inflammation [25]. Flavonoids account for about two-thirds of polyphenols, the most abundant group of dietary antioxidants. Polyphenols reduce oxidative stress and inflammation and appear to be vasoprotective [24]. Carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, and lycopene) occur in a wide variety of fruits and vegetables. Plasma carotenoids can be reliably quantified and are considered a valid biological marker for vegetable and fruit intake [10].

The relationship between plasma carotenoids and mortality has not been well characterized. Low total serum carotenoids were an independent predictor of mortality among older women, aged 70–79 years, living in the community in Baltimore, Maryland [29]. Low plasma α - and β -carotene concentrations were associated with a higher risk of death among men and women, aged 70–75 years, in a multicenter study in Europe [2].

We hypothesized that low plasma carotenoid concentrations were associated with increased mortality in older adults. In order to address this hypothesis, we examined the relationship between plasma carotenoid levels and mortality in the InCHIANTI study, a population-based cohort of older adults living in the community in Tuscany, Italy.

Methods

■ Study population

The study participants consisted of men and women, aged 65 and older, who participated in the Invecchiare in Chianti, “Aging in the Chianti Area” (InCHIANTI) study, conducted in two small towns in Tuscany, Italy. The rationale, design, and data collection have been described elsewhere, and the main outcome of this longitudinal study is mobility disability [6]. Briefly, in August 1998, 1,270 people aged 65 years and older were randomly selected from the population registry of Greve in Chianti (pop. 11,709) and Bagno a Ripoli (pop. 4,704), and of 1,256 eligible subjects, 1,155 (90.1%) agreed to participate. Of the 1,155 participants, 1,043 (90.3%) participated in the blood drawing. During the eight-years of follow-up study, 310 participants died, 22 refused to participate to the study, and eight moved away from the area. Participants received an extensive description of the study and participated after written, informed consent. The

study protocol complied with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee. Participants were evaluated again for a three-year follow-up visit from 2001–2003 ($n = 926$) and six-year follow-up visit from 2004–2006 ($n = 844$) at which time they underwent repeated phlebotomy and laboratory testing and assessment of physical performance. At the end of the field data collection, we collected data on mortality of the original InCHIANTI cohort, using data from the Mortality General Registry maintained by the Tuscany Region and the death certificates that are deposited immediately after the death at the Registry office of the Municipality of residence.

Demographic information and information on smoking and medication use were collected using standardized questionnaires. Smoking history was determined from self-report and dichotomized in the analysis as “current smoking” versus “ever smoked” and “never smoked”. Education was recorded as years of school. Average daily intakes of energy (kcal), was estimated using the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire, validated in the InCHIANTI population [27]. All participants were examined by a trained geriatrician, and diseases were ascertained according to standard, pre-established criteria and algorithms based upon those used in the Women’s Health and Aging Study for coronary heart disease, chronic heart failure, stroke and cancer [14]. Weight was measured using a high-precision mechanical scale. Standing height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight/height² (kg/m²). Mini-mental status examination (MMSE) was administered at enrollment [9].

■ Laboratory analyses

Blood samples were collected in the morning after a 12-h fast. Aliquots of serum and plasma were immediately obtained and stored at -80° . Carotenoids have been shown to be stable for at least 15 years under these conditions [3]. Aliquots of plasma were shipped on dry ice to Dr. Semba’s laboratory for measurements of plasma carotenoids. The laboratory protocol was originated by Schleicher at the Centers for Disease Control in Atlanta and is described in better detail in the paper by Dorgan [5]. Carotenoids were measured using high performance liquid chromatography (HPLC) [22]. Total carotenoids were calculated as the sum of α -carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, and lycopene in $\mu\text{mol/l}$. Within-run and between-run coefficients of variation, respectively, were 7.3 and

9.6% for α -carotene, 4.5 and 5.4% for β -carotene, 2.7 and 3.5% for β -cryptoxanthin, 2.6 and 7.1% for lutein, 6.2 and 6.8% for zeaxanthin, and 7.5 and 7.8% for lycopene.

Statistical analysis

Variables are reported as means (standard deviations) for normally distributed parameters or as percentages. Characteristics of subjects according to their vital status were compared using *t* test for continuous variables and chi-square tests for categorical variables. Plasma carotenoids was analyzed both as a continuous variable and as tertiles, where the tertiles for carotenoids were defined as 1.466, 1.467–1.971 > 1.971 $\mu\text{mol/l}$. Survival analysis (Kaplan Meier) and Cox proportional hazards models adjusted for age, sex, education, BMI, total energy intake, coronary heart disease, chronic heart failure, stroke and cancer were used to examine the relationship between plasma carotenoids and mortality (Table 3, model 1 and 2). Covariates for adjustment were selected by using the univariate Cox proportional hazards models as those that were associated with mortality with a *P*-value < 0.10 (Table 2). Kaplan Meier survival curves were compared using log-rank test. All analyses were performed using SAS (v. 8.2, SAS Institute, Inc., Cary, NC) with a statistical significance level set at *P* < 0.05.

Results

Mean total carotenoid concentration was 1.80 (0.69) $\mu\text{mol/l}$. The mean plasma level of total carotenoids with aging is significantly lower with aging (*P*-for trend = 0.0004) (Fig. 1). During the eight years of follow-up, 310 (29.7%) of participants died. The demographic and disease characteristics of the participants who were alive versus those who died during

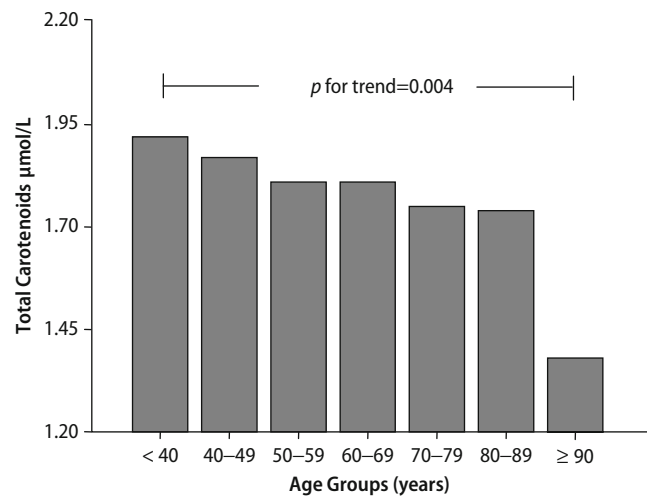


Fig. 1 Distribution of the mean total carotenoids ($\mu\text{mol/l}$) across age groups

the eight years of follow up are shown in Table 1. Adults who died were older, more likely to be men, more likely to have had a lower plasma total carotenoids and had higher baseline prevalence of congestive heart failure and stroke compared to those who survived over eight years of follow-up.

Univariate Cox proportional hazards models were used to examine the relationship between plasma carotenoids concentrations at baseline and mortality. Age, sex, lower education, higher BMI, lower total energy, current smoking, congestive heart failure, and stroke, were significant risk factors for mortality (Table 2).

Eight year survival was lower in the lowest tertile compared with the highest tertile of total carotenoids (*P* < 0.0001 by Mantel-Haenszel chi-square) (Fig. 2). From the highest to the lowest tertile of total carotenoids 74 (21.2%), 105 (30.2%) and 131 (37.8%) participants died after 8 years of follow-up, respectively.

In Cox hazards models adjusted for age and sex, participants with the highest tertile of plasma

Table 1 Characteristics the study population at enrollment and at eight-years follow-up

Characteristic	All Participants (n = 1,043)	Survived (n = 733)	Died (n = 310)	<i>p</i> ^b
Age (years)	75.53 (7.42)	72.92 (5.58)	81.71 (7.58)	<0.001
Sex (% female)	591 (56.66)	431 (58.80)	160 (51.61)	0.03
Education (years) ^a	5.32 (3.39)	5.67 (3.28)	4.47 (3.15)	0.59
Current smokers (%)	140 (13.42)	96 (13.10)	94 (14.19)	0.64
Body mass index (kg/m ²)	27.47 (4.09)	27.68 (4.06)	26.88 (4.10)	0.66
Plasma carotenoids ($\mu\text{mol/l}$) ^a	1.80 (0.69)	1.88 (0.68)	1.61 (0.69)	<0.001
Total energy intake (kcal/day) ^a	1,905 (567)	1,946 (579)	1,807 (523)	0.14
Coronary heart disease (%)	46 (4.41)	29 (3.96)	17 (5.48)	0.27
Congestive heart failure (%)	56 (5.37)	16 (2.18)	40 (12.90)	<0.0001
Cancer (%)	65 (6.23)	41 (5.59)	24 (7.74)	0.19
Stroke	55 (5.27)	19 (2.59)	36 (11.61)	<0.0001

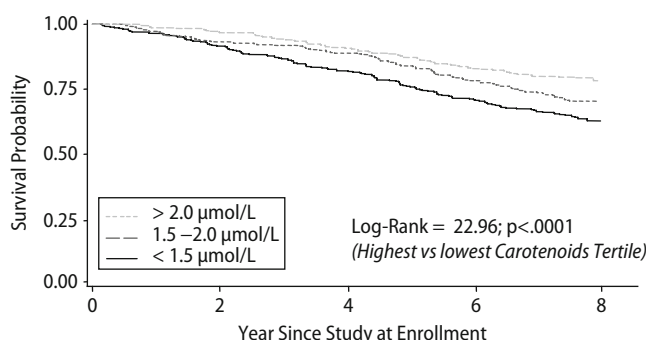
^aMean (SD) for continuous variables or percentages as noted

^bAge-adjusted ANCOVA

Table 2 Univariate relationship between carotenoids and other risk factors with all-cause mortality in older adults

Characteristic	HR	95% CI	P
Plasma carotenoids ($\mu\text{mol/l}$)		P^a for trend	<0.0001
Tertile 1	1.00	—	—
Tertile 2	0.712	0.520–0.977	0.04
Tertile 3	0.445	0.318–0.623	<0.0001
Age (years)	1.206	1.177–1.236	<0.001
Sex (female)	0.747	0.572–0.976	0.03
Education (years)	0.867	0.822–0.915	<0.001
Body mass index (kg/m^2)	0.951	0.917–0.987	0.008
Total energy intake (kcal/day)	0.956	0.932–0.980	0.0004
Current smoking	1.098	0.747–1.612	0.64
Congestive heart failure	1.628	1.389–1.908	<0.001
Coronary heart disease	1.409	0.762–2.603	0.27
Cancer	1.416	0.840–2.388	0.19
Stroke	2.311	1.726–3.095	<0.0001

^aP for trend was obtained by considering selenium quartile level as an ordinal variable

**Fig. 2** Kaplan Meier survival curves of older adults by tertiles of plasma carotenoids at enrollment

carotenoids at enrollment had lower mortality compared to those in the lowest tertile (Hazard Ratio 0.63, 95%; CI 0.47–0.84; $P = 0.002$) (Table 3, Model 1). After adjusting for age, sex, education, smoking, body mass index, energy intake, and chronic diseases, adults in the highest tertile of plasma carotenoids at enrollment had lower mortality compared to those in the lowest tertile although the association was slightly attenuated (Hazard Ratio obtained by considering carotenoids level as an ordinal variable 0.81, 95%; CI 0.65–0.99; P for trend = 0.046) (Table 3, Model 2).

When IL-6 (log) was included in the model as covariates, in addition to the covariates already in model 2, adults in the highest tertile of plasma carotenoids at enrollment had lower mortality compared to those in the lowest tertile (Hazard Ratio obtained by considering carotenoids level as an ordinal variable 0.73, 95%; CI 0.52–0.98; P for trend = 0.042).

Table 3 Age- and sex-adjusted and multivariate relationship between carotenoids and other risk factors with all-cause mortality in older adults

Characteristic	HR	95% CI	P
Model 1			
Plasma carotenoids ($\mu\text{mol/l}$)		P^a for trend	0.0005
Tertile 1	1.00	—	—
Tertile 2	0.881	0.681–1.140	0.34
Tertile 3	0.627	0.470–0.836	0.002
Age (years)	1.146	1.130–1.163	<0.001
Sex (female)	0.648	0.517–0.812	0.0002
Model 2			
Plasma carotenoids ($\mu\text{mol/l}$)		P^a for trend	0.04
Tertile 1	1.00	—	—
Tertile 2	0.985	0.731–1.327	0.92
Tertile 3	0.716	0.514–0.995	0.04
Age (years)	1.152	1.129–1.175	<0.001
Sex (female)	0.589	0.446–0.779	0.0002
Education (years)	1.152	1.129–1.175	0.18
Body mass index (kg/m^2)	1.152	1.129–1.175	0.91
Total energy intake (kcal/day)	1.004	0.977–1.032	0.77
Congestive heart failure	1.310	1.137–1.510	0.0002
Coronary heart disease	1.072	0.643–1.787	0.79
Cancer	1.748	1.099–2.779	0.02
Stroke	1.561	1.318–1.850	<0.0001
Current smoking	1.500	1.020–2.205	0.04

^aP for trend was obtained by considering carotenoids level as an ordinal variable

Discussion

The present study suggests that low total plasma carotenoids, a marker for fruit and vegetable intake, are an independent predictor of 8 year, all-cause mortality among older persons. These results are consistent with some previous findings. It was recently shown in the women's health and aging study (WHAS) that total carotenoids are a strong predictor of mortality among older women living in the community [29]. A study of Dutch elderly men and women living in the city of Arnhem also showed that there was an inverse association between all-cause mortality and total serum carotenoids [32]. Recently, low plasma carotenoid levels were significantly associated with all-cause mortality in men but not in women in the EVA study [1]. We reported the results that total plasma carotenoid levels are significantly lower with aging, although our mean plasma levels were similar to those reported by other authors [26, 28].

The underlying biological mechanism by which carotenoids (or other substances found in fruits and vegetables) could contribute to an increased risk of mortality may be related to increased oxidative stress and inflammation. A low-grade inflammatory state is common among older adults and is characterized by increased concentrations of cytokines and acute phase proteins [7]. The age-related pro-inflammatory state is associated with the metabolic syndrome, athero-

sclerosis, cardiovascular disease, and cerebrovascular disease. Recent studies suggest that a high intake of fruits and vegetables reduces biomarkers of inflammation such as C-reactive protein, IL-6, and IL-18 and protects against cardiovascular disease [23]. The findings from the present study are also consistent with recent observations that the Mediterranean diet, characterized by a higher intake of fruits, vegetables, and nuts, is associated with decreased mortality in older men and women [21, 31].

An earlier study relating low serum β -carotene and 7-year all cause mortality found that serum β -carotene was inversely related to markers of inflammation in men only [16]. However, more recent studies suggest that specific antioxidant nutrients mechanistically decrease levels of hydrogen peroxide and lipid peroxides to suppress up-regulation of IL-6 in disabled older women [33]. The present study showed a relationship between plasma total carotenoids and mortality in older persons in this cohort, even after adjustment for IL-6 at enrollment. This is consistent with the present findings that an increased level of plasma total carotenoids is protective against mortality independent of inflammatory markers.

In our study, the prevalence of smoking and alcohol use were much higher among men than among women. Plasma carotenoid levels are lower in smokers, as carotenoids are involved in the quenching of free radicals that are produced by smoking [4]. Alcohol consumption has been associated with lower serum β -carotene [11] and lutein/zeaxanthin concentrations [13].

A limitation of the present study is that the specific causes of death among the 310 participants who died have not yet been ascertained, and that this study reports only all-cause mortality. Among the 89 women who died in WHAS, the major causes of death were heart disease, cancer, stroke, infection, and chronic obstructive pulmonary disease [29]. Even if specific causes of death were known in the InCHI-ANTI study, currently the power to detect a rela-

tionship between plasma carotenoid levels and specific causes of mortality may be limited because of the sample size of deaths within specific categories. Causality is difficult to prove in an observational study such as this, and there could be unmeasured differences between the various carotenoid quantities that could explain the results.

Previous observational studies have examined the relationship between serum β -carotene, one of the six major dietary carotenoids, and mortality. The relationship between circulating β -carotene concentrations and mortality has not been consistent [8]. A pooled analysis of nine cohorts also found inconclusive evidence of a reduction in coronary heart disease. This was attributed to difficulty in adjusting for nondietary confounding factors [20]. In the Japan Collaborative Cohort Study of 39,140 people, ages 40–79, a lower risk of death from lung cancer was significant or of marginal significance for the highest serum concentrations of α -carotene, β -carotene, β -cryptoxanthin, zeaxanthin/lutein, canthaxanthin, total carotenoids, vitamin E, and total cholesterol, when compared to the lowest concentrations [18]. In a study involving nearly 12 years of follow-up, high levels of alpha-carotene, β -carotene, and lycopene were associated with a significantly decreased risk of cardiovascular mortality [17].

In conclusion, these results suggest that low total plasma carotenoids are an independent predictor of mortality among older adults living in the community. This work further shows the important relationship between antioxidant nutrients and mortality among older persons. Further work is needed to identify the sub-groups in the population which might be helped by interventions that reduce mortality in older populations.

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