Circulating carotenoids and subsequent risk of rheumatoid arthritis in women

Y. Hu[1,2], J. Cui[2,3], J.A. Sparks[2,3]
S. Malspeis[2], K.H. Costenbader[2,3]
E.W. Karlson[2,3], B. Lu[2,3]

[1]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA; [2]Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Boston, MA; [3]Department of Medicine, Harvard Medical School, Boston, MA, USA.

Yang Hu, MSc
Jing Cui, MD, PhD
Jeffrey A. Sparks, MD
Susan Malspeis, MSc
Karen H. Costenbader, MD, PhD
Elizabeth W. Karlson, MD
Bing Lu, MD, DrPH

Please address correspondence to:
Bing Lu, MD, DrPH,
Section of Clinical Sciences,
Division of Rheumatology,
Immunology & Allergy,
Brigham & Women's Hospital
and Harvard Medical School,
75 Francis Street PBB-B3,
Boston, MA 02115, USA.
E-mail: blu1@partners.org

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ABSTRACT

Objective. *The aim of the present study was to examine the associations between circulating carotenoids and future risk of rheumatoid arthritis (RA).*

Methods. *We conducted a nested case-control study consisting of 227 incident RA cases and 671 matched controls with prospectively measured plasma carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene and lutein/zeaxanthin) levels in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS II). Each incident RA case was matched with 3 healthy controls. Serologic phenotype of RA was determined by rheumatoid factor or anti-citrullinated peptide antibody (ACPA) obtained by chart review. Multivariable logistic regressions were used to estimate odds ratios (OR) and 95% confident intervals (95% CI) for RA risk associated with each circulating carotenoid after adjusting for matching factors and other covariates.*

Results. *The median time from blood draw until RA diagnosis was 8.6 years. In the multivariable models, no significant associations were found between any plasma carotenoids and risk of RA. We further examined the associations for two subtypes of RA, and found associations of circulating α-carotene and β-carotene with reduced risk of seronegative RA. After correction for multiple comparisons using the Bonferroni method, the findings did not reach statistical significance.*

Conclusion. *Circulating carotenoids levels are not associated with reduced risk of RA. Further investigations using large prospective cohorts are needed to confirm our findings.*

Introduction

Antioxidants such as carotenoids are of particular interest in RA prevention because of their anti-inflammatory properties. Reports from population-based studies consistently suggested that RA patients have generally lower plasma antioxidant levels than people who are free of the disease (1-3), but evidence regarding whether antioxidant supplement intake is able to reduce the future risk of RA generates mixed results (4-6). Most of these studies used FFQ-based consumption as an indirect measure of the antioxidant level, so it might be subject to multiple sources of measurement errors and potential misclassification. Moreover, since genetic factors and some lifestyle factors differ in association between seropositive and seronegative RA phenotypes (7, 8), it is also of interest to explore whether effects of antioxidants on risk of two serologic phenotypes differ.

Our goal of the current analysis was to further examine the hypothesis that dietary supplement of carotenoids is associated with reduced risk of incident RA. We took advantage of a nested case-control study to investigate the association between plasma carotenoids levels and incident RA risk with subject measurement of circulating carotenoids using two large well-characterised prospective cohorts, the Nurse's Health Study (NHS) and the Nurse's Health Study II (NHS II).

Materials and methods

Study design and participants

From 1989 to 1990, 32,826 participants in the NHS (aged 43–70 years) provided plasma samples in heparinised tubes that were transported and processed at a central laboratory within 24 hours of blood draw, and stored in liquid nitrogen freezers (<-130°C). From 1996 through 1999, 29,611 participants in the NHS II (ages 32–51 years) provided blood samples with the same standardised procedures. The baseline of the current study was 1986 in NHS and 1995 in NHS II for most of the participants. Women who developed RA were identified from self-reports and were subsequently validated by medical record review. 1987 American College of Rheumatology (ACR) criteria were used to ascertain the RA case. Detailed RA case validation procedures have been described elsewhere (9). The serostatus of RA was determined by results of testing for rheumatoid factor (RF) and/or anti-cyclic citrullinated protein (CCP) antibodies recorded in the medical records. The participants in our cohort were enrolled from the general population. The proportion of seropositive RA may be lower than cohorts that directly enrol RA patients from

academic hospitals. After excluding all prevalent RA cases at the time of blood collection, we included 227 incident RA cases (134 seropositive; 93 seronegative) and 671 matched controls with a stored blood sample collected at least 3 months prior to the date of the first RA symptom documented in the medical record. Three healthy controls were randomly selected to match each case for age, menopausal status, postmenopausal hormone use, time, day and fasting status at blood sample collection. The participants in both cohorts were followed biennially and responded to a questionnaire about their body weight, height, lifestyle practices, and medical histories in each follow up cycle. This study was approved by the Partners' Healthcare institutional review board.

Frozen plasma samples were sent to the Micronutrient Analysis Laboratory in the Department of Nutrition at the Harvard School of Public Health. Plasma antioxidant levels (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, retinol, α-tocopherol, γ-tocopherol) were assessed by reversed-phase highperformance liquid chromatography (HPLC) methods. The overall CV % ranged from 4.3% to 5.2%. Detailed methods of the assay have been published previously (10).

Assessment of covariates

Cigarette smoking is a strong environmental risk factor for RA (11). We adjusted for smoking status (never, past, current) in the analysis. Information on height was collected at baseline and weight was updated every two years through self-reported questionnaire. Body mass index was calculated as weight (kg) divided by squared height (m^2). Other potential confounders included the time between blood collection and RA onset, parity and duration of breastfeeding, menopausal status, and oral contraceptive use. All of these covariates were ascertained in the questionnaire prior to the blood collection.

Statistical analysis

Baseline characteristics by different phenotypes of RA cases and controls were expressed as either mean ± SD or number (%). Logistic regression was used to examine the association between each carotenoid and risk of total RA and seropositive and seronegative RA in separate models (removing cases with the other phenotype). Carotenoid levels were included in the model as quartiles. The values of trans-lycopene and cis-lycopene were summed up to represent the total lycopene level and trans β-carotene and cis β-carotene were added up to reflect the total carotene level. Likewise, all measured carotenoids were summed up to calculate the total carotenoids level. We reported results of multivariable models adjusting for matching factors, BMI (<25, 25-29.9, ≥30) and smoking status (never, past, current). P values for linear trend were obtained by examining the linear association of median concentration of the quartile for each carotenoid in the logistic models. Because we tested the associations for multiple outcomes, the Bonferroni method was employed to correct the significant level. The nominal significant level was 0.05/10=0.005 to account for 10 antioxidant groups for each outcome. All *p*-values were two-sided. Data were analysed with the SAS software, v. 9.3 (SAS Institute, Inc., Cary, North Carolina).

Results

The median time from blood draw until RA diagnosis was 8.6 years (range from 0.33 year to 19 years). The baseline characteristics of study sample are presented in Table I. Age at blood draw, alcohol consumption were similar between cases and controls, while RA cases generally had higher prevalence of obesity and current smoker status than controls. Physical activity was

Table I. Baseline characteristics of study participants in the Nurses' Health Study (1986) and the Nurses' Health Study II (1995).

Variables	Controls	Total RA cases	Seropositive cases	Seronegative cases
n.	671	227	134	93
Age at blood draw, years, mean ± SD	51.9 ± 8.1	51.9 ± 8.0	51.6 ± 7.7	52.4 ± 8.4
Physical activity, median(IQR),mets/week	10.2 (3.7-21.5)	10.1 (3.8-20.2)	10.9 (3.9-19.9)	8.8 (3.6-20.2)
Alcohol consumption, median(IQR),g/day	1.8 (0.27-7.5)	1.9 (0.3-6.2)	1.9 (0.3-5.3)	1.9 (0-6.6)
Cohort origin, NHS	456 (67.7)	152 (67.0)	90 (67.2)	62 (66.7)
Body mass index				
<25 kg/m^2	400 (59.4)	113 (49.8)	65 (48.5)	48 (51.6)
25-30 kg/m^2	191 (28.3)	78 (34.4)	49 (36.6)	29 (31.2)
≥30 kg/m^2	83 (12.3)	36 (15.8)	20 (14.9)	16 (17.2)
Menopausal status and hormone use				
Premenopausal	241 (35.8)	82 (36.1)	52 (38.8)	30 (32.3)
Post-menopausal hormone non-user	143 (21.2)	47 (20.7)	24 (17.9)	23 (24.7)
Post-menopausal hormone user	206 (30.6)	69 (30.4)	37 (27.6)	32 (34.4)
Unknown	84 (12.5)	29 (12.8)	21 (15.7)	8 (8.6)
Fasting status				
Fasting ≥ 8 hours	459 (68.1)	152 (67.0)	88 (65.7)	64 (68.8)
No fasting or unknown	215 (31.9)	75 (33.0)	46 (34.3)	29 (31.2)
Smoking status				
Never smoker	346 (51.3)	100 (44.1)	56 (41.8)	44 (47.3)
Past smoker	235 (34.9)	91 (40.1)	56 (41.8)	35 (37.6)
Current smoker	93 (13.8)	36 (15.8)	22 (16.4)	14 (15.1)

Values are number (%) or otherwise indicated. IQR: Interquartile range.

Table II. Odds ratio (95% confidence intervals) of incident RA by circulating carotenoids*.

Antioxidants	Q1	Q2	Q3	Q4	*p* for trend
			All RA (n=227)		
Total carotenoids¥	1.00	0.75 (0.48,1.16)	1.08 (0.71,1.65)	0.89 (0.57,1.39)	0.97
Total lycopene	1.00	1.31 (0.85,2.02)	1.22 (0.79,1.89)	1.14 (0.74,1.77)	0.71
trans lycopene	1.00	1.47 (0.95,2.28)	1.32 (0.85,2.05)	1.27 (0.82,1.98)	0.47
cis lycopene	1.00	1.44 (0.94,2.23)	1.25(0.81,1.94)	1.20 (0.77,1.87)	0.64
Total β-carotene	1.00	1.13 (0.74,1.73)	1.01 (0.65,1.58)	1.10 (0.70,1.74)	0.81
trans β-carotene	1.00	1.18 (0.77,1.81)	0.99 (0.63,1.55)	1.18 (0.75,1.87)	0.62
cis β-carotene	1.00	0.66 (0.42,1.02)	0.92 (0.60,1.42)	0.95 (0.60,1.49)	0.70
α-carotene	1.00	0.69 (0.44,1.06)	0.83 (0.54,1.29)	0.84 (0.54,1.31)	0.77
Lutein/zeaxanthin	1.00	1.23 (0.80,1.88)	1.09 (0.70,1.70)	1.03 (0.65,1.62)	0.89
β-cryptoxanthin	1.00	0.87 (0.57,1.32)	0.77 (0.49,1.19)	0.88 (0.56,1.37)	0.58
			Seropositive RA (n=134)		
Total carotenoids¥	1.00	1.26 (0.72,2.22)	1.68 (0.97,2.92)	1.49 (0.84,2.66)	0.14
Total lycopene	1.00	1.42 (0.83,2.45)	1.47 (0.86,2.52)	1.18 (0.68,2.06)	0.65
trans lycopene	1.00	1.81 (1.03,3.16)	1.71 (0.97,2.99)	1.50 (0.85,2.64)	0.31
cis lycopene	1.00	1.77 (1.02,3.07)	1.62 (0.93,2.81)	1.35 (0.76,2.39)	0.49
Total β-carotene	1.00	1.57 (0.91,2.71)	1.13 (0.63,2.03)	1.79 (1.01,3.17)	0.10
trans β-carotene	1.00	1.54 (0.89,2.67)	1.16 (0.65,2.09)	1.82 (1.03,3.21)	0.08
cis β-carotene	1.00	0.76 (0.43,1.33)	0.95 (0.54,1.67)	1.55 (0.89,2.69)	0.03
α-carotene	1.00	0.89 (0.52,1.55)	1.08 (0.63,1.87)	1.24 (0.71,2.14)	0.31
Lutein/zeaxanthin	1.00	1.03 (0.59,1.80)	1.26 (0.73,2.18)	1.31 (0.76,2.27)	0.26
β-cryptoxanthin	1.00	0.89 (0.52,1.53)	0.99 (0.58,1.71)	1.19 (0.68,2.06)	0.44
			Seronegative RA (n=93)		
Total carotenoids¥	1.00	0.39 (0.21,0.74)	0.63 (0.35,1.13)	0.44 (0.23,0.84)	0.04
Total lycopene	1.00	1.13 (0.62,2.08)	0.89 (0.47,1.69)	1.02 (0.55,1.89)	0.90
trans lycopene	1.00	1.13 (0.61,2.07)	0.91 (0.48,1.70)	1.00 (0.54,1.84)	0.83
cis lycopene	1.00	1.07 (0.58,1.98)	0.85 (0.45,1.59)	1.00 (0.54,1.84)	0.86
Total β-carotene	1.00	0.76 (0.42,1.39)	0.87 (0.48,1.58)	0.49 (0.24,0.99)	0.07
trans β-carotene	1.00	0.87 (0.48,1.57)	0.80 (0.43,1.47)	0.59 (0.29,1.17)	0.13
cis β-carotene	1.00	0.55 (0.30,1.01)	0.83 (0.46,1.48)	0.37 (0.17,0.77)	0.03
α-carotene	1.00	0.49 (0.27,0.90)	0.60 (0.33,1.09)	0.47 (0.24,0.90)	0.06
Lutein/zeaxanthin	1.00	1.43 (0.80,2.56)	0.83 (0.43,1.60)	0.65 (0.33,1.31)	0.08
β-cryptoxanthin	1.00	0.81 (0.46,1.44)	0.51 (0.27,0.98)	0.55 (0.28,1.06)	0.05

*Multivariable analysis adjusted for matching factors (age at blood draw, menopausal status and hormone use, time of blood draw, cohort origin), smoking status (never, past, current) and body mass index (<25, 25-30.9, 30-34.9, ≥30).
¥Total carotenoids were the sum of lutein/zeaxanthin, β-cryptoxanthin, total lycopene, α-carotene andtotal β-carotene.

similar between controls and seropositive RA cases and was greater than the values within seronegative RA cases. Moreover, seropositive RA cases had lower prevalence of post-menopausal hormone use than controls and seronegative RA cases.

In the multivariable models, no significant associations were found between any plasma carotenoids and risk of RA (for total carotenoids, $OR_{Q4\ vs\ Q1}$=0.87, 95% CI: 0.56, 1.37, *p* for trend 0.93). No significant associations were found for seropositive RA in either total carotenoids or any individual components. However, we found women in the highest quartile of total carotenoids had 57% reduced risk of seronegative RA (OR=0.43, 95% CI: 0.23-0.83, *p*=0.012) compared with those in the lowest quartile (*p* for trend 0.04). Assessing individual carotenoids, we found higher levels of circulating β-carotene and β-cryptoxanthin were associated with reduced risk of seronegative RA (for cis β-carotene, *p* trend=0.02; for β-cryptoxanthin p trend=0.05), but the associations did not reached nominal statistical significant level after the Bonferroni correction.

Discussion

In this prospective nested case-control study using population from two large cohorts, we did not find that circulating carotenoid levels is associated with reduced risk of RA. However, some significant signals observed for seronegative RA may suggest the effect of antioxidants may be different between two phenotypes of RA.

In a previous study of dietary intake in the NHS cohorts, we did not find significant associations between dietary antioxidant intake and risk of developing either RA or systemic lupus erythematosus in NHS and NHSII (5). However, correlations between dietary carotenoids and plasma levels were low in these cohorts, ranging from 0.21 to 0.48 (12). Dietary antioxidant intake based on self-reported FFQ may suffer from misclassification bias. The FFQ is designed to be self-administered in manageable amount of time, therefore it aims to capture habitual dietary intake. It is not feasible to include all consumed food items and highly accurate portion sizes, which leads to potential measurement error. The estimate of each antioxidant may collectively result in misclassification for the total

antioxidant intake. Furthermore, this study did not examine the association for the different serologic phenotypes of RA. Compared to seronegative RA, seropositive RA consisting the majority of RA cases, has been more strongly associated with environmental risk factors such as cigarette smoking and oral contraceptive use, while seronegative RA has been associated obesity. A prospective cohort study found no associations between any carotenoids and risk of RA except for β-cryptoxanthin (6). Since the antioxidants level was assessed using FFQ, multiple sources of random measurement errors might attenuate the associations towards the null. In addition, this study also did not examine the associations in two serologic types of RA separately. In our current study, we found a non-significant dose-response relationship between cis β-carotene and increased risk of seropositive RA which was in the opposite direction of association observed for seronegative RA. Although the detailed mechanism was unknown, β-carotene might play different roles in etiology between two serologic types of RA. Given relative modest number of cases, we could not rule out the possibility of chance findings.

The strengths of the current study include an objective measurement of plasma carotenoids and a prospective nested case-control design. However, the blood collection occurred at one point in time, and we could not study repeated antioxidant levels in the same subject over time prior to development of RA. The study population only included female health professionals; whether these results could be generalised to men and general population remains unknown. Additionally, given the exploratory feature of this study with limited sample size, we have limited power to correct potential multiple hypothesis testing, but the results were consistent across the types of carotenoids and the total carotenoid level.

In conclusion, our study showed that plasma concentrations of is not associated with reduced risk of RA. The non-significant inverse associations found for seronegative RA merits further investigations and more studies are needed to confirm our findings.

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