The relationship between total plasma carotenoids and risk factors for chronic disease among middle-aged and older men

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Individual plasma carotenoids have been associated with various chronic diseases but little is known about the relationship between total plasma carotenoids and risk factors for chronic diseases. In the Physicians' Health Study, we examined 492 men free of CVD and cancer for the relationship between total plasma carotenoids (the sum of α-carotene, β-carotene, lycopene, zeaxanthin, lutein and β-cryptoxanthin) and a wide variety of factors that predict chronic disease. Multivariate linear and logistic regression was performed to calculate parameter estimates (95 % CI) and OR (95 % CI) for total plasma carotenoids. In linear regression models, BMI, hypertension, alcohol intake and plasma levels of each lipid parameter and α-tocopherol significantly predicted levels of total plasma carotenoids. Upon adjustment for multiple chronic disease risk factors, the OR for levels of total plasma carotenoids greater than or equal to the median ($\geq 1 \cdot 301 \, \mu mol/l$) was statistically significant for current smoking (OR 0·21; 95 % CI 0·06, 0·77), weekly alcohol ingestion (OR 2·30; 95 % CI 1·06, 4·99), daily alcohol ingestion (OR 2·46; 95 % CI 1·29, 4·67), each 100 mg/l increase in total cholesterol (OR 0·73; 95 % CI 0·58, 0·91), LDL-cholesterol (OR 1·48; 95 % CI 1·17, 1·89) and HDL-cholesterol (OR 1·58; 95 % CI 1·26, 1·99), each 100 mg/ml increase in intercellular adhesion molecule-1 (OR 0·70; 95 % CI 0·53, 0·93) and each 10 μmol/l increase in α-tocopherol (OR 1·33; 95 % CI 1·12, 1·57), using logistic regression. Few lifestyle and clinical risk factors appear to be related to levels of total plasma carotenoids; however, levels of biomarkers such as plasma lipids and α-tocopherol may be strongly related.

Carotenoids: Risk factors: Chronic disease

Higher levels of fruit and vegetable intake have been associated with a decreased risk of various chronic medical conditions[1]. The antioxidant properties of carotenoids, commonly found in fruits and vegetables, are one explanation for this finding. Increased dietary carotenoid consumption and levels of individual plasma carotenoids may be associated with the decreased risk of cancer and CVD[2,3]. In addition, levels of individual plasma carotenoids have been associated with specific risk factors for chronic medical conditions such as diet, exercise and cholesterol[4–7]. Some authors have suggested using total plasma carotenoids as a marker of a diet high in fruits and vegetables[8]. However, there are comparatively fewer data regarding total plasma carotenoids and the frequency of both traditional and more novel risk factors for chronic medical conditions. Therefore, we examined the association between various traditional and more novel risk factors with total plasma carotenoids in a cross-sectional sample of middle-aged and older men.

Methods

Study population

The Physicians' Health Study (PHS) has previously been described in detail[9]. Briefly, the PHS began in 1982 as a randomised, double-blind, placebo-controlled, 2×2 factorial trial of β-carotene and aspirin in 22 071 initially healthy male US physicians in the primary prevention of cancer and CVD. All protocols were approved by the Institutional Review Board of Brigham and Women's Hospital.

Blood collection

A follow-up blood collection was conducted among 18 764 randomised men still alive starting in December 1995. Blood kits with vacuum tubes containing EDTA, instructions for the blood draws, and coldpacks were mailed to all 18 764 participants. Physicians returned blood samples in

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Abbreviations: CRP, C-reactive protein; HDL-C, HDL-cholesterol; ICAM-1, intercellular adhesion molecule-1; LDL-C, LDL-cholesterol; PHS, Physicians' Health Study.

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provided coldpacks by prepaid overnight courier. Upon receipt, each sample was centrifuged, divided into samples, and stored at $-82°C$. Follow-up blood kits were received from 11 718 (62 %) of 18 764 participants alive at the time of the second blood collection beginning in 1995. A total of 492 middle-aged and older men who contributed blood and were identified as free of CVD and cancer were selected for a nested case–control study of plasma carotenoids and CVD[10]. We performed a cross-sectional analysis on these men identified as controls for this previous study.

Blood samples were analysed for lycopene, β-crypto-xanthin, α-carotene, β-carotene, lutein, zeaxanthin, α-toco-pherol and γ-tocopherol at Our Lady of Mercy Medical Center (Bronx, NY, USA), a laboratory that has participated in the US Quality Assurance Program. All assays were quantified by reversed-phase HPLC after extraction and concentration by conventional methods[11]. Mean intra-assay CV based on blinded quality-control samples ranged from 7·6 % for α-carotene to 11·9 % for α-tocopherol. Plasma lipids, including total cholesterol, HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were also assayed using commercially available diagnostic kits (Sigma-Aldrich Chemical Co., St Louis, MO, USA) and conventional methods[12,13]. Levels of C-reactive protein (CRP) were assayed using a validated, high-sensitivity assay (Denka Seiken Company, Tokyo, Japan). Levels of IL-6 and intercellular adhesion molecule-1 (ICAM-1) were assayed using commercially available enzyme-linked immunoadsorbent assays (R&D Systems; Minneapolis, MN, USA).

Baseline characteristics

In addition to returning a blood sample, participants completed a comprehensive questionnaire that included information on self-reported clinical and lifestyle characteristics. For those who did not return a comprehensive questionnaire at the same time as their blood sample, we used questionnaires that were completed closest to the time of blood collection, within a few years, to ensure the complete assessment of potential confounding for our analyses. Questionnaires provided information on age (years), weight and height (converted to BMI; kg/m^2), smoking status (categorised as current, former, or never), alcohol use (categorised as rarely or never, monthly, weekly, or daily), frequency of exercise (categorised as rarely or never, <3 d per week, 3–4 d per week, or ≥ 5 d per week), history of hypertension (yes or no), history of hyperlipidaemia (yes or no), history of diabetes mellitus (yes or no) and systolic blood pressure (mmHg).

Data analysis

Total plasma carotenoids were defined as the sum of plasma α-carotene, β-carotene, lycopene, zeaxanthin, lutein and β-cryptoxanthin. We defined quartiles of total plasma carotenoids based upon their distribution among controls: $<0·995$, 0·995–1·300, 1·301–1·715 and $>1·715 \mu mol/l$. We first compared participants for clinical and lifestyle characteristics by quartile of total plasma carotenoids. Crude Spearman correlation tests were first calculated for total and individual carotenoids, followed by adjustment for age and total cholesterol. Linear regression models then calculated the parameter

estimate and 95 % CI for levels of total plasma carotenoids and each variable after adjustment for total cholesterol. Subsequent models further adjusted for three classes of variables – clinical, lifestyle and biomarkers – as well as a model that included all covariates. We also considered forward, backward and stepwise selection procedures to identify which combination of variables provided the best and most efficient fit of the data.

Next, we used logistic regression models to calculate the OR and 95 % CI for having total plasma carotenoids that are above *v.* below the median value (1·301 μmol/l). We followed a similar model-building strategy as described above. We considered full multivariate models comparing the upper quartile of total plasma carotenoids ($>1·715 \mu mol/l$) with the bottom quartile of total plasma carotenoids ($<0·950 \mu mol/l$). Finally, we considered the use of a multivitamin in our multivariate models. All statistical analyses were performed using SAS (version 9.1; Cary, NC, USA).

Results

For all 492 men, the mean age in this analysis was 69·6 (SD 8·0) years. For individual carotenoids, the mean levels of each individual carotenoid were as follows: lycopene, 0·18 (SD 0·09) μmol/l; β-cryptoxanthin, 0·20 (SD 0·15) μmol/l; lutein, 0·28 (SD 0·12) μmol/l; zeaxanthin, 0·09 (SD 0·05) μmol/l; α-carotene, 0·14 (SD 0·13) μmol/l; β-carotene, 0·56 (SD 0·51) μmol/l. The mean level of total plasma carotenoids for the study population was 1·45 (SD 0·71) μmol/l. Table 1 presents the characteristics of the population by quartiles of total plasma carotenoids. Increasing levels of total plasma carotenoids were not associated with age or BMI. Significantly fewer men had a diagnosis of hypertension, smoked or drank alcohol with increasing levels of total plasma carotenoids. Total cholesterol, LDL-C and HDL-C each significantly increased with higher quartiles of total plasma carotenoids. Finally, as would be expected, individual plasma carotenoids significantly increased with increasing levels of total plasma carotenoids.

Table 2 presents Spearman correlations among individual and total plasma carotenoids after controlling for total cholesterol and age. No significant differences were found for crude *v.* adjusted Spearman correlation coefficients. Individual plasma carotenoids were significantly correlated with one another as well as total plasma carotenoids. After zeaxanthin and lutein, the next highest Spearman correlation among individual carotenoids occurred between α-carotene and β-carotene (r 0·66; $P<0·01$).

Forward, backward and stepwise selection methods were used to generate and compare linear regression models that may predict total plasma carotenoids. The maximum r^2 in each selection process was 0·17 with total cholesterol, LDL-C, HDL-C, BMI, daily alcohol ingestion, weekly alcohol ingestion, current smoking, diagnosis of hypertension, ICAM-1, α-tocopherol and γ-tocopherol statistically significant in each model for total plasma carotenoids (data not shown). Table 3 presents results from the linear regression models for total plasma carotenoids. Among the clinical variables, diagnoses of diabetes mellitus and hyperlipidaemia were not significant predictors of increased total plasma carotenoids in any model, whereas a diagnosis of hypertension and

Table 1. Characteristics of 492 middle-aged and older male physicians by quartiles of total plasma carotenoids*

(Mean values and standard deviations for 123 subjects per quartile)

Quartiles of total plasma carotenoids…	1 (<0·995 μmol/l)		2 (0·995–1·300 μmol/l)		3 (1·301–1·715 μmol/l)		4 (>1·715 μmol/l)		P for trend
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Subjects (n)	123		123		123		123		
Age (years)	69·6	8·2	70·5	7·6	68·9	8·7	69·5	7·6	0·29
BMI (kg/m^2)	26·3	3·7	25·2	3·0	25·5	3·0	25·7	14·3	0·88
Systolic blood pressure (mmHg)	131·8	13·5	128·9	12·3	130·3	12·4	127·2	12·1	0·03
Diagnosis of hypertension (%)	67·5		59·4		60·2		48·8		<0·01
Diagnosis of diabetes mellitus (%)	5·7		5·0		1·6		4·1		0·30
Diagnosis of hyperlipidaemia (%)	16·7		14·3		19·7		13·2		0·74
Smoking status (%)									<0·01
Never	43·1		43·1		52·9		55·3		
Former	44·7		49·6		45·5		43·9		
Current	12·2		7·3		1·6		0·8		
Exercise (%)									0·14
Rarely or never	38·2		41·5		33·3		30·1		
< 3 d/week	21·1		15·5		19·5		23·6		
3–4 d/week	27·6		22·0		31·7		26·8		
≥ 5 d/week	13·0		21·1		15·5		19·5		
Alcohol consumption (%)									<0·01
Rarely/never	35·3		43·9		48·8		53·7		
Monthly	17·2		13·8		13·0		17·9		
Weekly	23·8		26·8		22·8		20·3		
Daily	23·8		15·5		15·5		8·1		
Plasma total cholesterol (mg/l)	2043	413	2179	429	2227	398	2199	469	<0·01
Plasma LDL-C (mg/l)	1312	332	1428	367	1471	367	1452	401	<0·01
Plasma HDL-C (mg/l)	426	121	481	157	490	135	491	131	<0·01
Plasma CRP (mg/l)	3·2	4·9	2·9	3·7	2·8	4·3	2·0	2·7	0·05
Plasma ICAM-1 (ng/ml)	324·2	123·5	299·8	89·3	291·3	67·1	281·5	59·2	<0·01
Plasma IL-6 (pg/ml)	2·27	1·58	2·55	3·51	1·88	1·45	2·24	3·21	0·68
α-Tocopherol (μmol/l)	34·6	18·4	35·8	14·9	36·7	14·2	39·5	15·9	0·03
γ-Tocopherol (μmol/l)	4·27	3·02	4·48	3·11	4·09	2·39	4·23	3·24	0·51
Plasma lycopene (μmol/l)	0·13	0·06	0·19	0·09	0·20	0·07	0·22	0·10	<0·01
Plasma β-cryptoxanthin (μmol/l)	0·10	0·06	0·17	0·10	0·23	0·13	0·31	0·19	<0·01
Plasma lutein (μmol/l)	0·19	0·06	0·27	0·08	0·30	0·10	0·36	0·16	<0·01
Plasma zeaxanthin (μmol/l)	0·06	0·02	0·09	0·04	0·10	0·04	0·13	0·07	<0·01
Plasma α-carotene (μmol/l)	0·06	0·04	0·10	0·06	0·14	0·07	0·25	0·20	<0·01
Plasma β-carotene (μmol/l)	0·22	0·11	0·35	0·13	0·52	0·17	1·13	0·70	<0·01

LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1.
* For details of procedures, see Methods.

BMI were significant predictors in all three models. Among the lifestyle variables, daily and weekly alcohol intakes were significant predictors of increased total plasma carotenoids in all three models. Among the biomarkers, ICAM-1, HDL-C and α-tocopherol were significant predictors of increased total plasma carotenoids in all three models.

Table 4 presents results from total cholesterol-adjusted, class-adjusted and multivariate-adjusted logistic regression models comparing those above v. below the median level of total plasma carotenoids. Among clinical variables, no variable was associated with increased levels of total plasma carotenoids in the overall multivariate-adjusted model.

Table 2. Spearman correlations for plasma levels of each individual carotenoid and total carotenoids controlled for total cholesterol and age among middle-aged and older male physicians*

	β-Crypto-xanthin		Lycopene		α-Carotene		β-Carotene		Lutein		Zeaxanthin	
	r	P	r	P	r	P	r	P	r	P	r	P
Lycopene	0·16	<0·01	–		–		–		–		–	
α-Carotene	0·27	<0·01	0·25	<0·01	–		–		–		–	
β-Carotene	0·35	<0·01	0·24	<0·01	0·66	<0·01	–		–		–	
Lutein	0·37	<0·01	0·22	<0·01	0·28	<0·01	0·17	<0·01	–		–	
Zeaxanthin	0·36	<0·01	0·22	<0·01	0·27	<0·01	0·17	<0·01	>0·99	<0·01	–	
Total plasma carotenoids	0·58	<0·01	0·38	<0·01	0·71	<0·01	0·86	<0·01	0·49	<0·01	0·49	<0·01

* For details of procedures and subjects, see Methods and Table 1.

Table 3. Parameter estimates for total plasma carotenoids from total cholesterol-adjusted, class-adjusted and overall multivariate-adjusted linear regression models*

(Parameter estimates and 95 % confidence intervals)

	Parameter estimate					
	Total cholesterol-adjusted	95 % CI	Class-adjusted†	95 % CI	Overall multivariate-adjusted‡	95 % CI
Clinical variables						
Age (per 10 years)	−0·01	−0·09, 0·07	0·01	−0·07, 0·10	0·03	−0·06, 0·12
BMI (per 5 kg/m^2)	0·05	0·01, 0·09	0·07	0·02, 0·11	0·08	0·04, 0·12
Systolic blood pressure (per 10 mmHg)	−0·06	−0·11, −0·01	−0·04	−0·10, 0·02	−0·02	−0·08, 0·04
Diagnosis of hypertension	−0·22	−0·35, −0·10	−0·20	−0·35, −0·05	−0·20	−0·35, −0·06
Diagnosis of diabetes mellitus	−0·03	−0·34, 0·29	−0·07	−0·41, 0·28	−0·04	−0·38, 0·30
Diagnosis of hyperlipidaemia	−0·07	−0·24, 0·10	−0·08	−0·25, 0·10	−0·15	−0·33, 0·03
Lifestyle variables						
Smoking						
Never smoked		Reference		Reference		Reference
Quit smoking	−0·09	−0·22, 0·04	−0·07	−0·19, 0·06	−0·11	−0·24, 0·03
Current smoking	−0·52	−0·79, −0·24	−0·44	−0·73, −0·16	−0·28	−0·59, 0·02
Exercise						
Rarely or never exercise		Reference		Reference		Reference
Exercise <3 d per week	0·07	−0·11, 0·24	0·05	−0·13, 0·22	0·06	−0·12, 0·24
Exercise 3−4 d per week	0·07	−0·09, 0·23	0·03	−0·13, 0·19	0·08	−0·09, 0·24
Exercise ≥5 d per week	0·11	−0·07, 0·29	0·06	−0·12, 0·24	0·08	−0·11, 0·27
Alcohol intake						
Rare or never alcohol ingestion		Reference		Reference		Reference
Monthly alcohol ingestion	0·09	−0·11, 0·29	0·06	−0·14, 0·26	0·11	−0·10, 0·33
Weekly alcohol ingestion	0·28	0·06, 0·51	0·25	0·03, 0·48	0·36	0·12, 0·60
Daily alcohol ingestion	0·26	0·08, 0·44	0·21	0·03, 0·40	0·28	0·08, 0·47
Biomarkers						
Total cholesterol (per 100 mg/l)	0·02	0·00, 0·03	−0·09	−0·15, −0·03	−0·09	−0·16, −0·03
LDL-C (per 100 mg/l)	0·01	−0·04, 0·06	0·11	0·04, 0·17	0·11	0·04, 0·18
HDL-C (per 100 mg/l)	0·06	0·01, 0·11	0·12	0·06, 0·18	0·14	0·08, 0·21
CRP (per 10 mg/l)	−0·20	−0·36, −0·04	−0·15	−0·31, 0·02	−0·10	−0·26, 0·07
ICAM-1 (per 100 ng/ml)	−0·10	−0·17, −0·03	−0·08	−0·15, −0·01	−0·08	−0·15, 0·00
IL-6 (per 100 pg/ml)	−1·29	−3·68, 1·11	−0·76	−3·18, 1·66	−0·85	−3·34, 1·64
α-Tocopherol (per 10 μmol/l)	0·04	0·00, 0·08	0·09	0·04, 0·14	0·10	0·05, 0·15
γ-Tocopherol (per 1 μmol/l)	−0·01	−0·03, 0·01	0·02	−0·01, 0·04	0·03	0·00, 0·05

LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1.
* For details of procedures and subjects, see Methods and Table 1.
† Each multivariate model controlled for total cholesterol and each variable listed within each variable class.
‡ Each multivariate model controlled for total cholesterol and every other variable listed.

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Table 4. Odds ratios for above *v.* below the median value (1·301 μmol/l) of total plasma carotenoids from total cholesterol-adjusted, class-adjusted and overall multivariate-adjusted logistic regression models*

(Odds ratios and 95 % confidence intervals)

	OR					
	Total cholesterol-adjusted	95 % CI	Class-adjusted†	95 % CI	Overall multivariate-adjusted‡	95 % CI
Clinical variables						
Age (per 10 years)	0·90	0·72, 1·12	0·90	0·70, 1·15	0·94	0·70, 1·25
BMI (per 5 kg/m^2)	0·98	0·87, 1·10	1·00	0·88, 1·12	1·04	0·90, 1·19
Systolic blood pressure (per 10 mmHg)	0·91	0·79, 1·06	0·96	0·81, 1·14	1·03	0·85, 1·24
Diagnosis of hypertension	0·69	0·48, 1·00	0·75	0·49, 1·14	0·71	0·44, 1·13
Diagnosis of diabetes mellitus	0·57	0·22, 1·47	0·58	0·21, 1·62	0·63	0·21, 1·96
Diagnosis of hyperlipidaemia	1·08	0·66, 1·76	1·06	1·01, 1·11	1·01	0·57, 1·79
Lifestyle variables						
Smoking						
Never smoked		Reference		Reference		Reference
Quit smoking	0·72	0·50, 1·05	0·78	0·53, 1·13	0·72	0·47, 1·09
Currently smoking	0·10	0·03, 0·36	0·13	0·04, 0·45	0·21	0·06, 0·77
Exercise						
No exercise		Reference		Reference		Reference
Exercise <1 d per week	1·44	0·88, 2·38	1·37	0·82, 2·29	1·40	0·80, 2·47
Exercise 2−3 d per week	1·47	0·93, 2·31	1·34	0·84, 2·15	1·39	0·81, 2·38
Exercise ≥4 d per week	1·26	0·75, 2·12	1·09	0·64, 1·86	1·05	0·57, 1·93
Alcohol intake						
Rare alcohol ingestion		Reference		Reference		Reference
Monthly alcohol ingestion	1·43	0·79, 2·59	1·33	0·72, 2·44	1·48	0·75, 2·95
Weekly alcohol ingestion	1·84	0·96, 3·53	1·67	0·85, 3·26	2·30	1·06, 4·99
Daily alcohol ingestion	2·27	1·32, 3·88	1·98	1·14, 3·45	2·46	1·29, 4·67
Biomarkers						
Total cholesterol (per 100 mg/l)	1·06	1·01, 1·10	0·77	0·64, 0·93	0·73	0·58, 0·91
LDL-C (per 100 mg/l)	1·06	0·92, 1·23	1·40	1·14, 1·72	1·48	1·17, 1·89
HDL-C (per 100 mg/l)	1·17	1·02, 1·35	1·42	1·17, 1·72	1·58	1·26, 1·99
CRP (per 5 mg/l)	0·68	0·42, 1·11	0·83	0·49, 1·41	1·16	0·66, 2·05
ICAM-1 (per 100 ng/ml)	0·65	0·50, 0·84	0·68	0·53, 0·88	0·70	0·53, 0·93
IL-6 (per 100 pg/ml)	0·01	<0·01, 14·14	0·02	<0·01, 39·47	0·03	<0·01, 54·71
α-Tocopherol (per 10 μmol/l)	1·10	0·98, 1·24	1·28	1·11, 1·48	1·33	1·12, 1·57
γ-Tocopherol (per 1 μmol/l)	0·96	0·91, 1·03	1·03	0·96, 1·11	1·05	0·97, 1·14

LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1.
* For details of procedures and subjects, see Methods and Table 1.
† Each multivariate model controlled for total cholesterol and each variable listed within each variable class.
‡ Each multivariate model controlled for total cholesterol and every other variable listed.

Among the lifestyle variables, the OR and 95 % CI were statistically significant in all three models for current smoking (OR 0·10 (95 % CI 0·03, 0·36), OR 0·13 (95 % CI 0·04, 0·45) and OR 0·21 (95 % CI 0·06, 0·77), respectively) and daily alcohol ingestion (OR 2·27 (95 % CI 1·32, 3·88), OR 1·98 (95 % CI 1·14, 3·45) and OR 2·46 (95 % CI 1·29, 4·67), respectively). No other lifestyle variables had a significant association in any model. Significant positive associations with total plasma carotenoids were found for each 100 mg/l difference in LDL-C and 10 μmol/l difference in α-tocopherol in the class-adjusted and overall multivariate-adjusted models only. However, the OR for each 100 mg/l difference in HDL-C was statistically significant in all three models (OR 1·17 (95 % CI 1·02, 1·35), OR 1·42 (95 % CI 1·17, 1·72) and OR 1·58 (95 % CI 1·26, 1·99), respectively). Including the use of a multivitamin in the overall multivariate model did not significantly alter these results (data not shown).

We also compared men in the highest quartile of total plasma carotenoids with men in the lowest quartile using multivariate-adjusted logistic regression models. The only statistically significant OR were for 10 mmHg difference in systolic blood pressure (OR 0·66; 95 % CI 0·47, 0·93), daily alcohol ingestion (OR 11·38; 95 % CI 3·37, 38·45), weekly alcohol ingestion (OR 9·45; 95 % CI 2·45, 36·43), monthly alcohol ingestion (OR 4·57; 95 % CI 1·28, 16·28), 100 mg/l increase in total cholesterol (OR 0·54; 95 % CI 0·36, 0·80), 100 mg/l increase in LDL-C (OR 2·21; 95 % CI 1·43, 3·42), 100 mg/l increase in HDL-C (OR 2·41; 95 % CI 1·62, 3·61), 100 ng/ml increase in ICAM-1 (OR 0·53; 95 % CI 0·29, 0·95) and 10 μmol/l increase in α-tocopherol (OR 1·83; 95 % CI 1·39, 2·41). Including the use of a multivitamin in the overall multivariate model did not significantly alter these results (data not shown).

Discussion

In the present cross-sectional study examining potential determinants of total plasma carotenoids in middle-aged and older men, few lifestyle and clinical risk factors appear to be related to levels of total plasma carotenoids. Only current smoking and daily alcohol ingestion appeared to be significantly associated with total plasma carotenoid levels. On the other hand, plasma lipids and α-tocopherol were strongly associated with total plasma carotenoid levels. More novel inflammatory biomarkers such as CRP, ICAM-1 and IL-6 were not associated with total plasma carotenoids when considered simultaneously with lipids and tocopherols. Of the individual carotenoids, levels of β-carotene and α-carotene correlated the most with levels of total plasma carotenoids.

The noted inverse correlation between smoking and levels of total plasma carotenoid has been observed in other studies[14,15]. Data from the 'β-Carotene and Retinol Efficacy Trial' (CARET) showed that for every ten cigarettes smoked per d, serum β-carotene concentration was 5·4 % lower[16]. Proposed mechanisms for the inverse relationship between total plasma carotenoid levels and cigarette smoking include decreased absorption of dietary carotenoids among active smokers and increased clearance of plasma carotenoids as a consequence of their interaction with free radicals produced as a result of cigarette smoking[17].

Dietary characteristics may partially explain the observed associations among HDL-C, α-tocopherol and total plasma carotenoids. Studies have shown that diets high in unsaturated fat may increase the absorption of individual and total carotenoids[18–20]. Dietary sources of unsaturated fats also tend to be sources of tocopherols. In addition, diets high in unsaturated fats are associated with increased levels of plasma HDL-C[21]. Another possible explanation for the relationship between carotenoids and tocopherols is that physicians may be more inclined to take multivitamin supplements that include both carotenoids and tocopherols. Unfortunately, we did not have information on the components of individual diets or multivitamin supplements for this analysis.

Previous studies have shown an inverse correlation between inflammatory markers and levels of carotenoids[19,20,22,23]. The Mediterranean diet may also be associated with increased serum levels of carotenoids and decreased serum levels of inflammatory markers such as CRP[19,20]. One study found a statistically significant inverse correlation between serum levels of ICAM-1 and both lutein and lycopene as well as between CRP and β-carotene[23]. In our analysis, serum levels of each inflammatory marker decreased with increasing levels of total plasma carotenoids. However, in multivariate analyses, we did not find CRP, IL-6 or ICAM-1 to be statistically significant predictors for levels of total plasma carotenoids when we simultaneously considered other biomarkers such as lipids and tocopherols.

The present cross-sectional study examines plasma carotenoids with a comprehensive assortment of variables, but some potentially important limitations remain. First, this analysis is cross-sectional from which no causal relationships can be directly assessed. Second, we did not have specific information on diet or supplements. Third, we did not have information regarding the number of cigarettes consumed or the amount of alcohol consumed by these men. Though we relied upon self-reports from our physician participants, healthcare providers have been shown to be accurate reporters of their health status[24]. Finally, our analyses were limited to predominantly white, apparently healthy, middle-aged and older male physicians and therefore may not apply to women, men with poor health, younger men, or men at other socioeconomic levels. There may be differences in plasma carotenoids according to sex, ethnic and cultural groups[4–7] that warrant additional research.

In conclusion, total plasma carotenoid levels were primarily influenced by smoking, alcohol ingestion, lipid parameters and α-tocopherol. We found that current smoking was associated with lower levels of total plasma carotenoids, while daily alcohol ingestion, increasing plasma levels of LDL-C, HDL-C and α-tocopherol were each significantly associated with increasing levels of total plasma carotenoids. Understanding the predictors of total plasma carotenoids may improve our understanding of risk factors for chronic conditions such as CVD and cancer.

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