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Original article

Total carotene plasma concentrations are inversely associated with atherosclerotic plaque burden: A post-hoc analysis of the DIABIMCAP cohort

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SUMMARY

Background and aims: Atherosclerosis is the major risk factor for cardiovascular disease (CVD), the first cause of death worldwide. Chronic low-grade inflammation and a sustained oxidative milieu are causatively related to atherosclerosis onset and progression, and therefore, dietary patterns rich in bioactive compounds with anti-inflammatory and antioxidant activities might likely contribute to revert or slowing the progression of atherosclerosis. The aim of this study is to analyse the association between fruit and vegetables intake, quantitatively measured through carotene plasma concentrations, and atherosclerotic burden, as a surrogate biomarker of CVD, in free-living subjects from the DIABIMCAP cohort study.

Methods: The 204 participants of the DIABIMCAP Study cohort (Carotid Atherosclerosis in Newly Diagnosed Type 2 Diabetic Individuals, ClinicalTrials.gov Identifier: NCT01898572), were included in this cross-sectional study. Total, α-, and β-carotenes were quantified by HPLC-MS/MS. Lipoprotein analysis in serum was performed by 2D- 1H NMR- DOSY, and atherosclerosis and intima media thickness (IMT) were measured through standardized bilateral carotid artery ultrasound imaging.

Results: Subjects with atherosclerosis (n = 134) had lower levels of large HDL particles than subjects without atherosclerosis. Positive associations were found between α-carotene and both large and medium HDL particles, and inverse associations were found between β- and total carotene, and VLDL and its medium/small particles. Subjects with atherosclerosis presented significantly lower plasma concentrations of total carotene compared with subjects without atherosclerosis. Plasma concentrations of carotene decreased as the number of atherosclerotic plaques increased, although after multivariate adjustment, the inverse association between β- and total carotene with plaque burden remained significant only in women.

Abbreviations: CVD, cardiovascular disease; IMT, intima media thickness; EDTA, ethylenediaminetetraacetic acid; 1H NMR, nuclear magnetic resonance; DOSY, diffusion ordered spectroscopy; HPLC-MS/MS, liquid chromatography–tandem mass spectrometry; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance.

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Conclusions: A diet rich in fruit and vegetables results in higher plasmatic carotene concentrations, which are associated with a lesser atherosclerotic plaque burden.

1. Introduction

Atherosclerosis is the major risk factor for cardiovascular disease (CVD), the first cause of death worldwide [1]. Chronic low-grade inflammation and a sustained oxidative milieu are causatively related with atherosclerosis onset and progression, and therefore, dietary patterns rich in bioactive compounds with anti-inflammatory and antioxidant activities might likely contribute to revert or slowing the progression of atherosclerosis.

A large body of literature supports the antioxidant and anti-inflammatory effect of carotenes, both in animal and cellular models, suggesting a protective role of carotene intake in the progression of atherosclerosis and other risk factors of CVD [2,3]. However, studies in humans show inconclusive and apparently contradictory results [4]. In one of the largest follow-up studies [5] higher serum β-carotene concentrations were associated with lower overall and cardiovascular death. Nevertheless, this study was performed only in Finish men with active smoking habits at inclusion, and therefore, the association between total carotene intake, understood as a mixture of α-, β-, and other carotenes naturally contained in plant-derived foods, and CVD is not yet fully elucidated for both men and women. Oppositely, a recent meta-analysis of randomised controlled trials concluded that β-carotene supplementation had no beneficial effects on CVD incidence and was even associated with increased risk of CVD and all-cause mortality [6]. In this line, dietary supplementation with multivitamins and/or antioxidants have shown null or even detrimental effects on CVD and all-cause mortality [7].

In diabetic subjects, we previously observed that plasma lycopene concentrations derived from foods were associated with lower atherosclerotic plaque burden [8]. Considering that lycopene is contained almost exclusively in tomato and derivatives, it does not reflect overall carotene intake. In this context, total carotene intake, measured through the quantification of objective biomarkers in plasma, might better reflect the dietary pattern and total fruit and vegetables consumption. Therefore, the aim of this study is to analyse the association between fruit and vegetables intake, quantitatively measured through carotene plasma concentrations, and atherosclerotic burden, as a surrogate biomarker of CVD, in free-living subjects from the Carotid Atherosclerosis in Newly Diagnosed Type 2 Diabetic Individuals (DIABIMCAP) cohort study.

2. Materials and methods

2.1. Study population

In this cross-sectional study, the whole DIABIMCAP Study cohort (ClinicalTrials.gov Identifier: NCT01898572), consisting of 204 participants, was included [9]. The protocol of the study was approved by the institutional ethics committee, in agreement with the Declaration of Helsinki. Subjects were included in the study between January and December 2012.

The inclusion criteria for this study were adult men and women between 40 and 75 years of age. The exclusion criteria were history of previous cardiovascular events or congestive heart failure (class III-IV); chronic diseases such as cancer, renal failure, liver disease, or other debilitating diseases; short life expectancy; history of chronic alcohol or drug abuse or dependence; and major psychiatric disease. After a first physical examination at their primary health care centre, all included subjects were selected according to the inclusion and exclusion criteria and signed an informed consent. A validated Mediterranean Diet adherence questionnaire [10], and a validated 137-item semiquantitative food-frequency questionnaire [11] were administered to 180 subjects of the total of 204 individuals at inclusion in the study, because 24 participants declined to respond the questions for personal reasons. Assessment of intake of fruit and vegetables was inferred by the number and size of serving of each food item, ranging from never or almost never to >6 times a day.

2.2. Clinical and laboratory measurements

Data about the subjects' age, sex, clinical, sociodemographic, and anthropometric characteristics were documented at inclusion in the study. Blood pressure was measured with an Omron HEM-7223-E (Hoofddorp, The Netherlands), the same day that the carotid ultrasound was conducted, and results are given as the mean of 3 measurements after 10 min in supine position. Due to the high percentage of individuals undergoing statin treatment and this being known to modulate plaque evolution, a statin score for statistical adjustment was calculated as the years of cholesterol-lowering treatment per average dose of these drugs normalized to simvastatin. Thus, the statin score accounted for both intensity and lifelong exposure to hypolipidemic treatment.

Fasting blood samples were collected in ethylenediaminetetraacetic acid (EDTA) or serum tubes. Blood was centrifuged at 3000 rpm for 10 min at 4 °C, within 30 min after extraction, and plasma and serum were stored at −80 °C until analyses. Biochemical measurements were performed at the Biomedical Diagnostic Centre, Hospital Clinic, Barcelona, Spain, as previously described [8].

Lipoprotein analysis in serum was performed by two-dimensional (2D) diffusion-ordered proton nuclear magnetic resonance (1H NMR) spectroscopy (DOSY), as reported in detail in references [8,12].

The extraction and isolation of carotenes from human plasma was carried out avoiding light exposure. Total, α-, and β-carotene were identified and quantified by high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) according to previous studies [8,13,14]. Results are expressed as μmol/L of plasma.

2.3. Carotid atherosclerosis assessment

Atherosclerosis and intima media thickness (IMT) were measured in a second visit after inclusion through standardized bilateral carotid artery ultrasound imaging with an Acuson X300 ultrasound system (Siemens AG, Erfurt, Germany) equipped with a VF10–5 linear transducer (5–10 MHz frequency range) [9], as previously explained in detail [8,15,16]. Plaque presence was defined as focal wall thickening encroaching into the arterial lumen by at least 50% of the surrounding IMT value or with thickness of at least 1.5 mm as measured from the media adventitia interference to the intima-lumen surface. In addition, plaque burden was obtained

by the sum of maximum heights of all plaques, either longitudinal or transversal, as appropriate, measured in common, bulb, and internal carotid segments.

2.4. Statistical analyses

Statistical analysis was carried out with the IBM SPSS Statistics (version 25.0) software, and statistical significance was set at the $p < 0.05$ level in all cases.

The normal distribution of the variables was calculated with the Kolmogorov–Smirnov test for one sample. For quantitative variables, descriptive analyses are presented as mean ± standard deviation (SD) and its associated p-value, using either the *t*-test for independent samples for variables following a normal distribution or the Mann–Whitney test for variables following a non-parametric distribution. For categorical variables, descriptive analyses are presented as absolute frequencies and percentages, alongside with its associated p-value, obtained with the chi-square test.

Bivariate correlations using Spearman's correlation coefficient were carried out to explore the association between the three carotene species (α-carotene, β-carotene, and total carotene), and other variables such as atherosclerotic plaque parameters, the lipid profile, or the adherence to the Mediterranean Diet.

Linear, logistic, and multinomial regression models were performed to determine the associations between plasma carotene and the lipid profile or atherosclerosis burden, adjusting by potential confounders such as age, sex, diabetes, and statin treatment. Multiple testing corrections were performed with the use of the Bonferroni post-hoc test.

3. Results

3.1. Subjects' characteristics

The 204 participants of the DIABIMCAP cohort were included in this study. As shown in Table 1, atherosclerotic plaque presence was more common in men, older people, and subjects with elevated triglycerides and insulin resistance. In addition, subjects with atherosclerotic plaque had lower levels of HDL, and were in a higher proportion undergoing statin treatment than those without atherosclerotic plaque, although differences in statin score did not reach statistical significance.

Regarding lipoprotein particles, Table 2 shows that subjects with atherosclerosis had higher levels of VLDL particles and lower levels of large HDL than subjects without atherosclerosis. However, differences in VLDL were no longer significant after multivariate adjustment. On the other hand, no differences in LDL particles were observed between subjects with and without atherosclerosis.

As expected and as per definition, subjects with atherosclerotic plaque had significantly higher IMT in all of the three measured zones of the carotid artery (internal, common, and bulb), as shown in Table 3.

3.2. Association between carotene intake and lipoprotein profile

Carotene intake was assessed through the quantification of plasma concentrations of total, α- and β-carotene. Table 4 shows the association between lipoproteins and carotene plasma concentrations in the total studied cohort. Independently of atherosclerosis presence and after multivariate adjustment, positive associations were found between α-carotenes and both large and medium HDL particles. On the other hand, inverse associations were found between β- and total carotene, and VLDL and its medium/small particles. After multivariate adjustment, no associations were found neither between HDL and β- or total carotene plasma concentrations, nor between LDL or triglycerides and plasma carotenes.

3.3. Association between carotene intake and atherosclerotic plaque

As can be seen in Table 5, subjects with atherosclerosis presented significantly lower plasma concentrations of total, α- and β-carotene compared with subjects without atherosclerosis.

However, after adjustment for potential confounders, the inverse association between plasma carotene and atherosclerotic plaque presence remained significant only for total carotenes, although for β-carotene the association was close to statistical significance (Table 6).

In addition, as depicted in Fig. 1, plasma concentrations of carotene decreased as the number of atherosclerotic plaques increased. Therefore, an inverse negative association was found between carotene plasma concentrations and number of plaques. After adjusting for potential confounders, the inverse association between carotenes in plasma and number of plaques was no longer significant. However, the inverse association between β- and total carotene with plaque burden remained significant only in women (Supplemental Table 1). No significant correlations were found between carotene plasma concentrations and plaque heights.

3.4. Association between plasma carotene and adherence to the Mediterranean Diet

Subjects with higher scores of adherence to the Mediterranean Diet presented significantly higher plasma concentrations of α-, β-, and total carotene, as shown in Supplemental Table 2. This association remained significant after multivariate adjustment (data not shown). As expected, subjects with higher intake of fruit and vegetables also showed higher adherence to the Mediterranean Diet and higher plasma concentrations of α-, β-, and total carotene (Supplemental Table 3).

4. Discussion

In this manuscript, the association between carotene intake and atherosclerotic burden in the DIABIMCAP cohort was analysed. The main findings of our study are that atherosclerotic burden was inversely associated with plasma concentrations of total, α- and β-carotene compared with subjects without atherosclerosis.

Elevated levels of carotene plasma concentrations have been reported in dietary patterns rich in fruit and vegetables such as the Mediterranean Diet [17]. Consistent with this finding, in our study, a higher adherence to a Mediterranean dietary pattern was significantly associated with increased α-, β- and total carotene plasma concentrations. It is worth mentioning that in our cohort, only 10 subjects were under antioxidant supplements, and therefore, it is plausible to assume that plasma concentrations of carotene are mainly achieved through the intake of foods (i.e., fruit and vegetables or eggs to a lesser extent). This is important on the grounds that β-carotene supplementation has shown null [6,18] or even detrimental effects on CVD [19].

There is a large body of evidence demonstrating the anti-atherosclerotic activity of carotenes both in vitro and in animal models through several pathways [20–23]. They possess strong antioxidant activity and also anti-inflammatory activity by modulating NF-κB, PI3K/Akt/mTOR and Nrf2 signalling pathways, mainly by interacting with nuclear receptors, scavenging/quenching reactive oxygen and nitrogen species, by protein retinoylation and by modulating protein kinases (reviewed in [14,24]). However,

Table 1
Characteristics of the 204 subjects included in the study.

	Without plaque (n = 70)	With plaque (n = 134)	*P*
Men [n (%)]	31 (44)	79 (59)	0.046
Age (years)	58.07 ± 8.87	61.83 ± 6.45	0.007
Diabetes [n (%)]	30 (43)	75 (56)	0.075
Hypertension [n (%)]	36 (51)	74 (55)	0.606
Smokers [n (%)]	12 (17)	28 (21)	0.522
BMI (kg/m^2)	29.02 ± 5.15	29.88 ± 4.79	0.157
Waist (cm)	99.31 ± 12.52	102.16 ± 13.13	0.129
Systolic blood pressure (mmHg)	127.40 ± 16.42	130.32 ± 16.32	0.227
Diastolic blood pressure (mmHg)	81.76 ± 10.06	81.25 ± 9.5	0.721
Insulin (μU/mL)	15.15 ± 12.70	17.29 ± 10.93	0.025
HOMA-IR	4.63 ± 4.87	5.36 ± 3.88	0.008
Glucose (mg/dL)	114.24 ± 25.04	123.07 ± 36.66	0.086
HbA1C (%)	6.11 ± 1.22	6.63 ± 1.68	0.027
Triglycerides (mg/dL)	119.66 ± 77.07	134.44 ± 70.71	0.023
Total cholesterol (mg/dL)	206.99 ± 33.52	203.53 ± 40.74	0.543
HDL (mg/dL)	58.65 ± 16.34	51.97 ± 12.03	0.009
LDL (mg/dL)	126.48 ± 29.68	125.44 ± 33.04	0.960
ApoA (mg/dL)	140.24 ± 25.44	137.41 ± 21.12	0.563
ApoB (mg/dL)	94.45 ± 24.88	104.07 ± 27.50	0.103
Metformin [n (%)]	9 (12.86)	29 (21.64)	0.126
Statin treatment [n (%)]	20 (28.60)	57 (42.50)	0.051
Statin score[1]	80.04 ± 89.21	73.79 ± 75.53	0.762
Creatinine (mg/dL)	0.82 ± 0.12	0.86 ± 0.17	0.285
Leukocytes (10^3 cells/μL)	6.80 ± 2.12	7.05 ± 1.95	0.243
Platelets (10^3 cells/μL)	272.83 ± 68.25	265.84 ± 64.29	0.412
Lymphocytes (10^3 cells/μL)	2.20 ± 0.73	2.35 ± 1.03	0.316

Data are expressed as mean ± SD or n (%) where appropriate. *P* from the comparison between subjects with and without carotid atherosclerosis (*t*-test or Mann–Whitney test for quantitative and chi-square test for categorical variables). [1]Statin score was calculated as the product of the duration of treatment in years by the average dose received of statin drugs standardized to simvastatin. BMI indicates body mass index; and HOMA-IR, homeostatic model assessment for insulin resistance.

Table 2
Concentration of lipoprotein particles of the subjects included in the study according to atherosclerotic plaque presence.

Particle number (nmol/L)	Without plaque (n = 70)	With plaque (n = 134)	*P*	P^1
HDL, total	28.4 ± 7.31	28.77 ± 6.46	0.727	0.880
Large HDL	1.79 ± 1.64	1.25 ± 1.18	0.014	0.024
Medium HDL	8.55 ± 4.16	7.35 ± 3.84	0.052	0.053
Small HDL	18.07 ± 9.16	20.17 ± 8.06	0.135	0.222
LDL, total	1596.49 ± 340.50	1596.87 ± 402.61	0.995	0.784
Large LDL	197.42 ± 89.19	184.47 ± 106.75	0.275	0.264
Medium LDL	616.44 ± 201.15	618.08 ± 263.24	0.402	0.881
Small LDL	782.63 ± 219.26	794.31 ± 237.74	0.862	0.440
VLDL, total	61.7 ± 47.93	70.17 ± 40.92	0.038	0.176
Large VLDL	2.00 ± 3.50	2.53 ± 2.62	0.010	0.220
Medium VLDL	9.54 ± 10.94	11.26 ± 9.35	0.049	0.208
Small VLDL	50.16 ± 34.22	56.39 ± 29.88	0.055	0.175

Data are expressed as mean ± SD. *P* from the *t*-test or Mann–Whitney test comparing subjects with and without carotid atherosclerosis, and P^1 from logistic regression analyses, adjusted by sex, age, diabetes, and statin score. HDL stands for high density lipoprotein particle; LDL, low density lipoprotein particle; and VLDL, very low density lipoprotein particle.

Table 3
Atherosclerotic plaque characteristics in the 204 subjects included in the study.

IMT (mm)	Without plaque (n = 70)	With plaque (n = 134)	*P*	P^1
Common carotid artery				
Mean	0.71 ± 0.11	0.81 ± 0.14	<0.001	<0.001
Mean of maximums	0.79 ± 0.12	0.92 ± 0.18	<0.001	<0.001
Maximum	0.84 ± 0.14	0.99 ± 0.22	<0.001	<0.001
Carotid artery bulb				
Mean	0.85 ± 0.14	1.15 ± 0.26	<0.001	<0.001
Mean of maximums	1.04 ± 0.16	1.48 ± 0.43	<0.001	<0.001
Maximum	1.14 ± 0.21	1.70 ± 0.62	<0.001	<0.001
Intern carotid artery				
Mean	0.67 ± 0.12	0.91 ± 0.35	<0.001	<0.001
Mean of maximums	0.82 ± 0.15	1.19 ± 0.53	<0.001	<0.001
Maximum	0.90 ± 0.19	1.40 ± 0.69	<0.001	<0.001

Data are expressed as mean ± SD. *P* from the t-test or or Mann–Whitney test analyses comparing between subjects with and without carotid atherosclerosis, and P^1 from logistic regression analyses, adjusted by sex, age, diabetes, and statin score. IMT stands for intima-media thickness; mean indicates the mean of the heights of the IMT measurements of the left and right carotid artery; mean of maximums denotes the mean of the highest IMT measurements of the left and right carotid artery; and maximum, the highest IMT measurement, either from the left or right carotid artery.

Table 4
Association between plasma carotene and lipoprotein particles.

Particle number (nmol/L)	α-Carotene		β-Carotene		Total Carotene	
	B	*P*	B	*P*	B	*P*
HDL						
Unadjusted	0.056	0.451	0.019	0.810	0.047	0.520
Multivariate model[1]	0.037	0.625	0.030	0.700	0.041	0.578
Multivariate model[2]	0.043	0.578	0.033	0.679	0.046	0.541
Large HDL						
Unadjusted	0.295	<0.001	0.077	0.322	0.148	0.044
Multivariate model[1]	0.229	0.001	0.061	0.404	0.111	0.107
Multivariate model[2]	0.190	0.006	0.012	0.871	0.069	0.307
Medium HDL						
Unadjusted	0.262	<0.001	0.056	0.471	0.124	0.091
Multivariate model[1]	0.188	0.007	0.044	0.541	0.088	0.200
Multivariate model[2]	0.152	0.026	0.002	0.975	0.050	0.454
Small HDL						
Unadjusted	−0.124	0.092	−0.025	0.748	−0.044	0.548
Multivariate model[1]	−0.094	0.206	−0.008	0.919	−0.026	0.719
Multivariate model[2]	−0.067	0.371	0.023	0.765	0.002	0.980
LDL						
Unadjusted	0.087	0.239	0.026	0.739	0.031	0.671
Multivariate model1	0.031	0.675	0.015	0.846	0.004	0.955
Multivariate model2	−0.002	0.981	−0.016	0.835	−0.027	0.707
Large LDL						
Unadjusted	0.096	0.195	0.009	0.911	0.052	0.482
Multivariate model[1]	0.062	0.409	0.012	0.881	0.039	0.591
Multivariate model[2]	0.047	0.532	0.002	0.981	0.029	0.694
Medium LDL						
Unadjusted	0.041	0.580	0.010	0.902	0.005	0.950
Multivariate model[1]	−0.003	0.963	0.003	0.967	−0.015	0.837
Multivariate model[2]	−0.028	0.707	−0.017	0.828	−0.036	0.621
Small LDL						
Unadjusted	0.059	0.425	0.029	0.713	0.024	0.744
Multivariate model[1]	0.028	0.712	0.016	0.840	0.005	0.943
Multivariate model[2]	0.006	0.934	−0.009	0.911	−0.019	0.798
VLDL						
Unadjusted	−0.177	0.016	−0.212	0.006	−0.183	0.012
Multivariate model1	−0.160	0.032	−0.219	0.005	−0.178	0.015
Multivariate model2	−0.127	0.089	−0.188	0.015	−0.148	0.043
Large VLDL						
Unadjusted	−0.144	0.050	−0.170	0.028	−0.129	0.080
Multivariate model[1]	−0.130	0.083	−0.179	0.021	−0.125	0.091
Multivariate model[2]	−0.097	0.193	−0.149	0.053	−0.096	0.192
Medium VLDL						
Unadjusted	−0.168	0.023	−0.191	0.014	−0.162	0.027
Multivariate model[1]	−0.137	0.065	−0.196	0.010	−0.151	0.038
Multivariate model[2]	−0.109	0.143	−0.170	0.028	−0.126	0.084
Small VLDL						
Unadjusted	−0.178	0.015	−0.218	0.005	−0.190	0.009
Multivariate model[1]	−0.166	0.027	−0.225	0.004	−0.186	0.011
Multivariate model[2]	−0.132	0.078	−0.192	0.013	−0.155	0.034
Triglycerides						
Unadjusted	−0.160	0.024	−0.168	0.025	−0.155	0.028
Multivariate model[1]	−0.150	0.036	−0.177	0.018	−0.153	0.029
Multivariate model[2]	−0.108	0.123	−0.129	0.080	−0.108	0.113

Data are presented as a standardized linear regression coefficient (B) for the association between subjects' lipid profile and carotene plasma concentrations (α-, β-, and total carotene). *P* from multiple linear regression analyses; Multivariate model[1]: adjusted by sex and age; and Multivariate model[2]: adjusted by sex, age, diabetes, and statin score.

Table 5
Carotene plasma concentrations according to atherosclerosis in the 204 subjects included in the study.

μmol/L	Without plaque (n = 70)	With plaque (n = 134)	*P*
α-Carotene	0.12 ± 0.14	0.09 ± 0.09	0.011
β-Carotene	60.56 ± 59.31	36.82 ± 56.49	0.001
Total Carotene	70.84 ± 76.22	42.10 ± 70.12	0.002

Data are expressed as mean ± SD. *P* for the comparison between subjects with and without carotid atherosclerosis (Mann–Whitney test).

Table 6
Association between plasma carotene and plaque presence.

	α-Carotene		β-Carotene		Total Carotene	
	B	*P*	B	*P*	B	*P*
Unadjusted	−3.161	0.037	−0.007	0.018	−0.006	0.017
Multivariate model[1]	−3.002	0.052	−0.006	0.028	−0.005	0.023
Multivariate model[2]	−2.610	0.088	−0.006	0.053	−0.005	0.045

Data are presented as a standardized logistic regression coefficient (B) for the association between plaque presence and carotene plasma concentrations (α-, β-, and total carotene), with its 95% confidence interval. *P* from the logistic regression analyses; Multivariate model[1]: adjusted by sex and age; and Multivariate model[2]: adjusted by sex, age, diabetes, and statin score.

randomised clinical trials in humans have achieved a disparity of outcomes [25]. In our study, subjects with atherosclerotic plaque presence showed significantly lower (almost half of) plasma concentrations of total, α- and β-carotenes than those without atherosclerotic plaque. However, this association was attenuated after multivariate adjustment, and remained significant only for total carotenes, suggesting that the potential atheroprotective effects of carotenes might be synergistical and not given by a single chemical specie (α- or β-), as we previously observed for lycopenes in subjects with diabetes [8]. In fact, this synergistical effect was demonstrated by Zou and colleagues [26], who observed higher reductions of carotid artery IMT after the combination of lutein and lycopene supplementation than when supplemented separately. A cross-sectional study from a Chinese population showed that higher dietary and serum levels of total carotenoids and of α-carotene and β-carotene, were associated with lower values of carotid IMT [27]. In addition, inverse associations between the progression of IMT max and plasma concentrations of lycopene, α-carotene, and β-carotene after 7 years follow-up have been observed in Finnish men [28]. In our cohort, an inverse negative association was found between carotene plasma concentrations and number of plaques. After adjusting for potential confounders, the inverse association between carotenes in plasma and number of plaques was no longer significant. However, the inverse association between β- and total carotene with plaque burden remained significant only in women, in contrast with previous findings [28]. This might be partially explained by the fact that in the DIABIMCAP cohort women without atherosclerosis showed about 60% higher plasma concentrations of carotene (and higher adherence to a Mediterranean dietary pattern) compared with men without atherosclerosis, and that a lower prevalence of atherosclerosis was also observed in women. Nonetheless, potential sex divergences in carotene bioavailability and/or biological effects in atherosclerosis might not be overlooked. Maugeri et al., also observed that antioxidant intake, computed as the composite dietary zinc, selenium, vitamin A, vitamin C, vitamin E, and carotenoids, was inversely associated with the carotid IMT in women but not in men [29]. A potential explanation to these observations is reported in a recent meta-analysis [30], in which authors state that the prevalence of smoking habits and harmful use of alcohol is higher in men, and this might have an effect in the carotene's cardioprotective efficacy.

As expected, subjects with atherosclerosis presented more cardiovascular risk factors than subjects without atherosclerosis. However, no significant differences were observed in LDL or VLDL and its subparticle levels between subjects with or without plaque, probably because a larger proportion of subjects with atherosclerosis were under statin treatment. Subjects with atherosclerosis presented lower concentrations of large HDL particles. HDL particle number is a strong inverse predictor of incident cardiovascular events [31], and both small and large LDL and VLDL particles have been shown to have a high atherogenic potential [32]. We observed a negative association between plasma carotenes and a lipoprotein

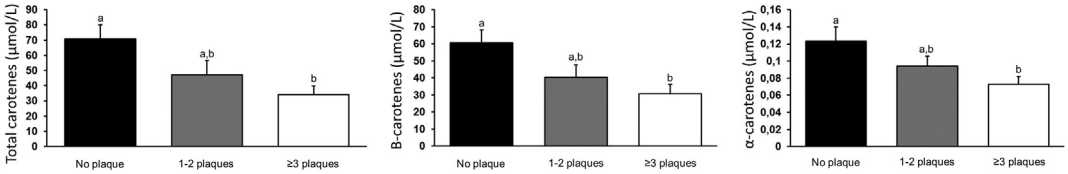

Fig. 1. Concentration of plasma carotene according to atherosclerotic plaque burden. Results are expressed as mean + sd. Columns with different superscript letters denote significant differences (from the ANOVA analysis with the Bonferroni post-hoc test). No plaque, n = 70; 1–2 plaques, n = 81; and ≥3 plaques, n = 53.

subparticle proatherogenic profile, according to the subjects' characteristics. In fact, we observed positive associations between α-carotenes and both large and medium HDL particles. Additionally, we also observed inverse associations between β- and total carotenes, and VLDL and its medium/small particles. Whereas the association between large/medium HDL particles and CVD are not yet clear [33,34], remnant cholesterol (non-HDL or LDL cholesterol) is strongly associated with increased risk of major cardiovascular events [35], overall suggesting a potential mechanism by which carotenes may protect against CVD.

The major strengths of our study are that atherosclerosis was determined by gold-standard imaging techniques, and carotenoid intake was quantitatively measured through plasma concentrations which are objective biomarkers of intake. In addition, only 10 subjects received antioxidant supplements and thus, the observed concentrations are almost exclusively derived from food. However, there are some limitations in our study. These include the relatively small sample size and the observational nature that prevents from establishing causality.

In conclusion, a diet rich in fruit and vegetables results in higher plasmatic carotene concentrations, which are associated with a lesser atherosclerotic plaque burden. Further studies are needed in order to approach the causality of the inverse associations between carotene plasma concentrations and atherosclerotic plaque presence.

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Author contributions

A.J., E.O., G.C-B.: Conceptualization; F.B., R.G., R.L-R., M.M-H.: Data curation; Z.H., M.C., M.P.: Formal analysis; E.O., G.C-B.: Funding acquisition; All authors: Investigation; F.B., G.C-B.; Writing - original draft; All authors: Writing - review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.05.005.

References

[1] ESC cardiovascular realities. 2022.
[2] Ciccone MM, Cortese F, Gesualdo M, Carbonara S, Zito A, Ricci G, et al. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediat Inflamm 2013;2013.
[3] Soleti R, Coué M, Trenteseaux C, Hilairet G, Fizanne L, Kasbi-Chadli F, et al. Carrot supplementation improves blood pressure and reduces aortic root lesions in an atherosclerosis-prone genetic mouse model. Nutrients 2021;13:1181 [Internet]. 2021 Apr 2 [cited 2023 Jan 24];13(4):1181. Available from: https://www.mdpi.com/2072-6643/13/4/1181/htm.
[4] Yao Y, Goh HM, Kim JE. The roles of carotenoid consumption and bioavailability in cardiovascular health. Antioxidants 2021;10. MDPI.
[5] Huang J, Weinstein SJ, Yu K, Männistö S, Albanes D. Serum beta carotene and overall and cause-specific mortality [cited 2023 Jan 24] Circ Res [Internet] 2018;123(12):1339–49. Available from: https://pubmed.ncbi.nlm.nih.gov/30566060/.
[6] Yang J, Zhang Y, Na X, Zhao A. β-Carotene supplementation and risk of cardiovascular disease: a systematic review and meta-analysis of randomized controlled trials [cited 2023 Jan 24] Nutrients [Internet] 2022;14(6):1284. Available from: https://www.mdpi.com/2072-6643/14/6/1284/htm.
[7] Jenkins DJA, Spence JD, Giovannucci EL, Kim Y, Josse R, Vieth R, et al. Supplemental vitamins and minerals for CVD prevention and treatment [cited 2023 Jan 24] J Am Coll Cardiol [Internet] 2018;71(22):2570–84. Available from: https://pubmed.ncbi.nlm.nih.gov/29852980/.
[8] Chiva-Blanch G, Jiménez C, Pinyol M, Herreras Z, Catalán M, Martínez-Huélamo M, et al. 5- cis-, trans- and total lycopene plasma concentrations inversely relate to atherosclerotic plaque burden in newly diagnosed type 2 diabetes subjects [cited 2023 Jan 24] Nutrients [Internet] 2020;12(6):1–11. Available from: https://pubmed.ncbi.nlm.nih.gov/32517202/.
[9] Catalan M, Herreras Z, Pinyol M, Sala-Vila A, Amor AJ, de Groot E, et al. Prevalence by sex of preclinical carotid atherosclerosis in newly diagnosed type 2 diabetes [cited 2023 Jan 24] Nutrition, Metabolism and Cardiovascular Diseases [Internet] 2015;25(8):742–8. Available from: http://www.nmcd-journal.com/article/S0939475315001155/fulltext.
[10] Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk [cited 2023 Jan 25] Nutr Metab Cardiovasc Dis [Internet] 2006;16(8):559–68. Available from: https://pubmed.ncbi.nlm.nih.gov/17126772/.
[11] Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain [cited 2023 Apr 27] Br J Nutr [Internet] 2010;103(12):1808–16. Available from: https://pubmed.ncbi.nlm.nih.gov/20102675/.
[12] Mallol R, Amigó N, Rodríguez MA, Heras M, Vinaixa M, Plana N, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy [cited 2023 Jan 24] J Lipid Res [Internet] 2015;56(3):737–46. Available from: http://www.jlr.org/article/S0022227520356017/fulltext.
[13] Arranz S, Martínez-Huélamo M, Vallverdu-Queralt A, Valderas-Martinez P, Illán M, Sacanella E, et al. Influence of olive oil on carotenoid absorption from tomato juice and effects on postprandial lipemia. Food Chem 2015;168:203–10.
[14] Bonet ML, Ribot J, Galmés S, Serra F, Palou A. Carotenoids and carotenoid conversion products in adipose tissue biology and obesity: pre-clinical and human studies. Biochim Biophys Acta Mol Cell Biol Lipids 2020;(11):1865.
[15] de Groot E, van Leuven SI, Duivenvoorden R, Meuwese MC, Akdim F, Bots ML, et al. Measurement of carotid intima–media thickness to assess progression and regression of atherosclerosis. Nat Clin Pract Cardiovasc Med 2008;5:5 [Internet]. 2008 Mar 11 [cited 2023 Jan 24];5(5):280–8. Available from: https://www.nature.com/articles/ncpcardio1163.
[16] Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). Cerebrovasc Dis 2012;34:290–6.

[17] Marhuenda-Muñoz M, de Alvarenga JFR, Hernáez Á, Tresserra-Rimbau A, Martínez-González MÁ, Salas-Salvadó J, et al. High fruit and vegetable consumption and moderate fat intake are associated with higher carotenoid concentration in human plasma. Antioxidants 2021;10:473 [Internet]. 2021 Mar 17 [cited 2023 Feb 6];10(3):473. Available from: https://www.mdpi.com/2076-3921/10/3/473/htm.
[18] Corbi G, Ali S, Intrieri M, Modaferri S, Calabrese V, Davinelli S, et al. Association between beta-carotene supplementation and mortality: a systematic review and meta-analysis of randomized controlled trials. Front Med (Lausanne) 2022;9:2040.
[19] An P, Wan S, Luo Y, Luo J, Zhang X, Zhou S, et al. Micronutrient supplementation to reduce cardiovascular risk [cited 2023 Jan 26] J Am Coll Cardiol [Internet] 2022 Dec;80(24):2269–85. Available from: https://www.jacc.org/doi/10.1016/j.jacc.2022.09.048.
[20] Zhou F, Wu X, Pinos I, Abraham BM, Barrett TJ, von Lintig J, et al. β-Carotene conversion to vitamin A delays atherosclerosis progression by decreasing hepatic lipid secretion in mice [cited 2023 Feb 6] J Lipid Res [Internet] 2020;61(11):1491–503. Available from: http://www.jlr.org/article/S0022227520437356/fulltext.
[21] Amengual J, Coronel J, Marques C, Aradillas-García C, Morales JMV, Andrade FCD, et al. β-Carotene oxygenase 1 activity modulates circulating cholesterol concentrations in mice and humans. J Nutr 2020;150(8):2023–30.
[22] Varghese R, George Priya Doss C, Kumar RS, Almansour AI, Arumugam N, Efferth T, et al. Cardioprotective effects of phytopigments via multiple signaling pathways [cited 2023 Feb 6] Phytomedicine [Internet] 2022;95:153859. Available from: https://pubmed.ncbi.nlm.nih.gov/34856476/. Available from:.
[23] Harari A, Melnikov N, Kfir MK, Kamari Y, Mahler L, Ben-amotz A, et al. Dietary β-carotene rescues vitamin A deficiency and inhibits atherogenesis in apolipoprotein E-deficient mice. Nutrients 2020;12:1625 [Internet]. 2020 Jun 1 [cited 2023 Feb 6];12(6):1625. Available from: https://www.mdpi.com/2072-6643/12/6/1625/htm.
[24] Saini RK, Prasad P, Lokesh V, Shang X, Shin J, Keum YS, et al. Carotenoids: dietary sources, extraction, encapsulation, bioavailability, and health benefits—a review of recent advancements. Antioxidants 2022;11:795 [Internet]. 2022 Apr 18 [cited 2023 Apr 21];11(4):795. Available from: https://www.mdpi.com/2076-3921/11/4/795/htm.
[25] Martini D, Negrini L, Marino M, Riso P, del Bo C, Porrini M. What is the current direction of the research on carotenoids and human health? An overview of registered clinical trials [cited 2023 Feb 6] Nutrients [Internet] 2022;14(6):1191. Available from: https://www.mdpi.com/2072-6643/14/6/1191/htm.
[26] Zou ZY, Xu XR, Lin XM, Zhang H Bin, Xiao X, Ouyang L, et al. Effects of lutein and lycopene on carotid intima–media thickness in Chinese subjects with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial [cited 2023 Feb 8] Br J Nutr [Internet] 2014;111(3):474–80. Available from: https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/effects-of-lutein-and-lycopene-on-carotid-intimamedia-thickness-in-chinese-subjects-with-subclinical-atherosclerosis-a-randomised-doubleblind-placebocontrolled-trial/0575676F514A732B27FA7636E75CFCE6.
[27] Wang C, Qiu R, Cao Y, Ouyang WF, Li H Bin, Ling WH, et al. Higher dietary and serum carotenoid levels are associated with lower carotid intima–media thickness in middle-aged and elderly people [cited 2023 Feb 8] Br J Nutr [Internet] 2018;119(5):590–8. Available from: https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/higher-dietary-and-serum-carotenoid-levels-are-associated-with-lower-carotid-intimamedia-thickness-in-middleaged-and-elderly-people/E84BE0AE87FAFF30BFCEC3EB0954D46B.
[28] Karppi J, Kurl S, Ronkainen K, Kauhanen J, Laukkanen JA. Serum carotenoids reduce progression of early atherosclerosis in the carotid artery wall among eastern Finnish men [cited 2023 Feb 8] PLoS One [Internet] 2013;8(5):e64107. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0064107.
[29] Maugeri A, Hruskova J, Jakubik J, Kunzova S, Sochor O, Barchitta M, et al. Dietary antioxidant intake decreases carotid intima media thickness in women but not in men: a cross-sectional assessment in the Kardiovize study. Free Radic Biol Med 2019;131:274–81.
[30] Yang J, Zhang Y, Na X, Zhao A. β-Carotene supplementation and risk of cardiovascular disease: a systematic review and meta-analysis of randomized controlled trials [cited 2023 Feb 8] Nutrients [Internet] 2022;14(6):1284. Available from: https://www.mdpi.com/2072-6643/14/6/1284/htm.
[31] Khera AV, Demler OV, Adelman SJ, Collins HL, Glynn RJ, Ridker PM, et al. Cholesterol efflux capacity, high-density lipoprotein particle number, and incident cardiovascular events: an analysis from the JUPITER trial (justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin) [cited 2023 Apr 27] Circulation [Internet] 2017;135(25):2494–504. Available from: https://pubmed.ncbi.nlm.nih.gov/28450350/.
[32] Ma S, Xia M, Gao X. Biomarker discovery in atherosclerotic diseases using quantitative nuclear magnetic resonance metabolomics. Front Cardiovasc Med 2021;8:819.
[33] McGarrah RW, Ferencik M, Giamberardino SN, Hoffmann U, Foldyna B, Karady J, et al. Lipoprotein subclasses associated with high-risk coronary atherosclerotic plaque: insights from the PROMISE clinical trial [cited 2023 Feb 8] J Am Heart Assoc [Internet] 2023;12(1). Available from: https://pubmed.ncbi.nlm.nih.gov/36565187/.
[34] Bortnick AE, Buzkova P, Otvos JD, Jensen MK, Tsai MY, Budoff MJ, et al. High-density lipoprotein and long-term incidence and progression of aortic valve calcification: the multi-ethnic study of atherosclerosis [cited 2023 Feb 8] Arterioscler Thromb Vasc Biol [Internet] 2022;42(10):1272–82. Available from: https://pubmed.ncbi.nlm.nih.gov/35979837/.
[35] Castañer O, Pintó X, Subirana I, Amor AJ, Ros E, Hernáez Á, et al. Remnant cholesterol, not LDL cholesterol, is associated with incident cardiovascular disease [cited 2023 Feb 8] J Am Coll Cardiol [Internet] 2020;76(23):2712–24. Available from: https://pubmed.ncbi.nlm.nih.gov/33272365/.