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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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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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 atherogenic 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 carotenoids 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 interface to the intima-lumen surface. In addition, plaque burden was obtained
were carried out to explore the association between the three 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 [5,18] or even detrimental effects on CVD [19].

There is a large body of evidence demonstrating the antiatherosclerotic 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,
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). †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.

<table>
<thead>
<tr>
<th>Particle number (nmol/L)</th>
<th>Without plaque (n = 70)</th>
<th>With plaque (n = 134)</th>
<th>P</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL total</td>
<td>28.4 ± 7.31</td>
<td>28.77 ± 6.46</td>
<td>0.727</td>
<td>0.880</td>
</tr>
<tr>
<td>Large HDL</td>
<td>1.79 ± 1.64</td>
<td>1.25 ± 1.18</td>
<td>0.014</td>
<td>0.024</td>
</tr>
<tr>
<td>Medium HDL</td>
<td>8.55 ± 4.16</td>
<td>7.35 ± 3.84</td>
<td>0.052</td>
<td>0.053</td>
</tr>
<tr>
<td>Small HDL</td>
<td>18.07 ± 9.16</td>
<td>20.17 ± 8.06</td>
<td>0.135</td>
<td>0.222</td>
</tr>
<tr>
<td>LDL total</td>
<td>1596.49 ± 340.50</td>
<td>1569.87 ± 402.61</td>
<td>0.995</td>
<td>0.784</td>
</tr>
<tr>
<td>Large LDL</td>
<td>197.42 ± 89.19</td>
<td>184.47 ± 106.75</td>
<td>0.275</td>
<td>0.264</td>
</tr>
<tr>
<td>Medium LDL</td>
<td>616.44 ± 201.15</td>
<td>618.08 ± 263.24</td>
<td>0.402</td>
<td>0.881</td>
</tr>
<tr>
<td>Small LDL</td>
<td>782.63 ± 219.26</td>
<td>794.31 ± 237.74</td>
<td>0.862</td>
<td>0.440</td>
</tr>
<tr>
<td>VLDL total</td>
<td>61.7 ± 47.93</td>
<td>70.17 ± 40.92</td>
<td>0.038</td>
<td>0.176</td>
</tr>
<tr>
<td>Large VLDL</td>
<td>2.00 ± 3.50</td>
<td>2.53 ± 2.62</td>
<td>0.010</td>
<td>0.220</td>
</tr>
<tr>
<td>Medium VLDL</td>
<td>9.54 ± 10.94</td>
<td>11.26 ± 9.35</td>
<td>0.049</td>
<td>0.208</td>
</tr>
<tr>
<td>Small VLDL</td>
<td>50.16 ± 34.22</td>
<td>56.39 ± 29.88</td>
<td>0.055</td>
<td>0.175</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. *P from the t-test or Mann–Whitney test comparing subjects with and without carotid atherosclerosis, and †P 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.

<table>
<thead>
<tr>
<th>IMT (mm)</th>
<th>Without plaque (n = 70)</th>
<th>With plaque (n = 134)</th>
<th>P</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common carotid artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.71 ± 0.11</td>
<td>0.81 ± 0.14</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean of maximums</td>
<td>0.79 ± 0.12</td>
<td>0.92 ± 0.18</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.84 ± 0.14</td>
<td>0.99 ± 0.22</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carotid artery bulb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.85 ± 0.14</td>
<td>1.15 ± 0.26</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean of maximums</td>
<td>1.04 ± 0.16</td>
<td>1.48 ± 0.43</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum</td>
<td>1.14 ± 0.21</td>
<td>1.70 ± 0.62</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intern carotid artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.67 ± 0.12</td>
<td>0.91 ± 0.35</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean of maximums</td>
<td>0.82 ± 0.15</td>
<td>1.19 ± 0.53</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.90 ± 0.19</td>
<td>1.40 ± 0.69</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. *P from the t-test or or Mann–Whitney test comparing between subjects with and without carotid atherosclerosis, and †P 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.
The association between plasma carotene and lipoprotein particles.

Data are presented as a standardized linear regression coefficient (B). Unadjusted analyses; Multivariate model1: adjusted for sex and age; and Multivariate model2: adjusted for sex, age, diabetes, and statin score.

The concentration of total, α and β-carotene), and the potential atheroprotective effect of carotenes might be of importance. In fact, the synergistic 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 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 DIABIMICAP 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 carotene and lipid particle concentrations (α-, β-, and total carotene), and remained significant only for total carotene, suggesting that the potential atheroprotective effects of carotenes might be synergistic 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 synergistic effect was demonstrated by Zou and colleagues [26], who observed higher...
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 caroten 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 caroten 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.: Fund- ing 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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Fig. 1. Concentration of plasma carotene according to atherosclerotic plaque burden. Results are expressed as mean ± sd. Columns with different superscript letters denote signifi- cant 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.

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


