TITLE

Plasma carotenoids and risk of depressive symptomatology in a population-based cohort of older adults.

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Running Head

Plasma carotenoids and risk of depressive symptomatology

LIST OF ABBREVIATIONS

3C: Three-City, ATC: Anatomical Therapeutic Chemical, BMI: Body Mass Index, CES-D: Center for Epidemiologic Studies-Depression, CI: Confidence Interval, DS: Depressive Symptomatology, HPLC: High-Performance Liquid Chromatography, IL-6: Interleukin-6, IL-1β: Interleukin-1β, LLQ: Limit of Quantification, LOD: Limit of Detection, MMSE: Mini Mental State Examination, OR: Odd Ratio, SD: Standard Deviation, TC: Total Cholesterol, TG: Triglycerides, TNF- α: Tumor Necrosis Factor-α.

ABSTRACT

Background: As part of a healthy diet, higher carotenoid intakes have been associated with a reduced risk of depression, mainly in adults, while prospective studies on plasma carotenoids in older adults are lacking. The aim of this study was to assess the prospective association between plasma carotenoids and the risk of Depressive Symptomatology (DS) in older adults.

Methods: The study sample was based on the Three-City cohort of adults aged 65y+ free from DS at enrollment in 1999. Plasma carotenoids were measured at baseline. DS was assessed every 2-3 years over 17 years and defined by a Center for Epidemiologic Studies-Depression Scale score ≥16 and/or by antidepressant use. The association between plasma carotenoids or carotenoid/lipids (cholesterol and triglycerides) ratio and the risk for DS was assessed through multiple random-effect logistic regression.

Results: The study sample was composed of 1,010 participants (mean age 74 y (+/- 4.9), 58% of women) followed-up during a median time of 13.4 years. Plasma zeaxanthin and ratios of zeaxanthin/lipids, lutein+zeaxanthin/lipids and β-carotene/lipids were independently associated with a significant reduced risk of DS over time (Odds ratio (OR)=0.81, 95% Confidence Interval (CI) [0.67;0.99], OR=0.79 [0.67;0.98], OR=0.79 [0.64;0.94] and OR=0.80 [0.66;0.97] for +1 standard deviation of each exposure respectively).

Limitations: Plasma carotenoids were only available at study baseline.

Conclusion: Focusing on circulating carotenoids and considering lipids levels, the present results suggested an association between higher levels of plasma zeaxanthin, combined lutein+zeaxanthin and β-carotene and a decreased risk of DS over time in older adults.

Key words: Depressive symptomatology, plasma carotenoids, cohort, older adults, Three-City.

INTRODUCTION

Depression is a common mental disorder that affected more than 280 millions of people worldwide in 2019 (Feigin et al., 2021). In recent decades, an increased prevalence has been observed and exacerbated by the COVID-19 pandemic (Santomauro et al., 2021). Depression is characterized by a feeling of sadness often associated with anhedonia and by a set of secondary significant symptoms (including severe fatigue, feeling guilty and unworthy, loss of appetite, sleep disorders and suicidal thoughts) (Malhi and Mann, 2018). Older adults have a higher risk of depression compared with other age groups, somewhat in response to higher age-related risk factors for depression, such as social isolation and loss of autonomy (Maier et al., 2021; Solmi et al., 2022; Vink et al., 2008; Worrall et al., 2020). Antidepressant and psychotherapy are the two main existing therapeutic options for treating the depression. However, antidepressant treatments have side effects and add to the polymedication of older adults, leading to potential withdrawal of treatments (Dattani et al., 2021). Moreover, in older adults antidepressant seem to be effective in only 1 out of 2 treated patients (Gałecki et al., 2022). Therefore, increasing research on preventive strategies is relevant to reduce the risk of depression in this vulnerable population. In this line, physical activity, good social support and a better self-rated health have already been identified as protective factors for depression (Worrall et al., 2020). Additionally, nutrition is a modifiable and lifelong exposure that also appears as a promising approach. Indeed, it has been observed that healthy dietary behaviors, including higher consumption of fruits and vegetables had beneficial effect on depression risk across several studies (Bardinet et al., 2022a; Lassale et al., 2019; Liu et al., 2016; Psaltopoulou et al., 2013). Among main healthy nutrients provided by fruits and vegetables, carotenoids are natural pigments synthetized by plants and microorganisms (Kumar et al., 2021; Manochkumar et al., 2021). Less than 20 carotenoids out of >1,000 have been identified in human plasma, with α-carotene, β-carotene, lycopene, lutein, zeaxanthin and β-

cryptoxanthin representing >95% of the total plasma carotenoids (Kumar et al., 2021; Manochkumar et al., 2021). Higher carotenoid intakes have been associated with lower odds of depression or depressive symptomatology (DS) in several studies (Johnson et al., 2013; Lai et al., 2016; Milaneschi et al., 2012; Nguyen et al., 2017; Oishi et al., 2009; Payne et al., 2012). However, the generalization of such results was limited by the cross-sectional design of most studies, the short follow-up of the few longitudinal studies and the use of dietary assessment of carotenoids, biological assessment being an objective measure closer to their effective bioavailability. To our knowledge, only two prospective studies investigated the association between plasma carotenoids and depression in older adults (Lai et al., 2016; Milaneschi et al., 2012). The first study observed a significant inverse association between plasma lutein and zeaxanthin and depressive symptoms in older men (Lai et al., 2016) and the second study reported that a higher plasma level of total carotenoids was associated with a reduced risk of depressive symptomatology (DS) in older adults (Milaneschi et al., 2012). These studies reported some associations but present methodological limitations, including the sample size, and the short duration.

Therefore, the aim of the present study was to examine the prospective association between plasma carotenoids and carotenoid/lipids ratio (total carotenoids and each of the 6 main carotenoids) and the risk of DS in older men and women enrolled in the Three-City (3C) cohort, followed for up to 17 years.

METHODS

Study sample

The study population was derived from the 3C cohort, a population-based cohort of 9,294 not institutionalized French individuals aged 65 years and over in 1999-2001. Community-dwelling participants were recruited from electoral lists of the three following French cities: Bordeaux (n=2,104), Dijon (n=4,931) and Montpellier (n=2,259). Every two to three years, face-to-face interviews were administered at home or in a study center until 2018 (up to 8 visits). Ethics approval was granted by the Advisory Committee for the Protection of Persons Participating in Biomedical Research of the Centre Hospitalier Universitaire of Kremlin-Bicêtre and a free and informed consent was signed by each participant. The entire protocol and methodology of the 3C cohort have been previously detailed elsewhere (3C Study Group, 2003).

A case-control study nested in the 3C cohort was carried out from the biobank constituted at baseline (95% of participants accepted to have blood sampling) to investigate the relationship between some biological markers and 3 diseases including dementia (the design of the nested case-control study is detailed elsewhere (Berr et al., 2016)). In addition, for an ancillary study of 3C, ALIENOR study (Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes), additional blood samples were analyzed in Bordeaux (Delcourt et al., 2010). Thus, the present study sample was based on participants from these Bordeaux and Montpellier 3C sub-cohorts as following:

Eligible people were all participants i) with available information on plasma carotenoids, plasma lipids and an assessment of DS at baseline as ascertained by the Center for Epidemiological Studies-Depression (CES-D) scale (detailed further) and by their

antidepressant use, ii) without prevalent DS at baseline, and iii) reviewed at least once during the follow-up and without missing data on DS incidence.

Plasma carotenoids and lipids

At baseline, fasting blood samples were collected through heparinized vacutainers, centrifuged at 1,000g for 15 minutes and stored (-80°C) until plasma biomarkers determination. The concentration of plasma carotenoids was assessed by high-performance liquid chromatography (HPLC) analytical methods developed at the R&D Analytical Research Center of DSM Nutritional Products Ltd (Feart et al., 2016). Plasma carotenoids measured (in μg/L convert into μmol/L) were the most common in humans: α-carotene, β-carotene and lycopene (from the carotene group) and lutein, zeaxanthin and β-cryptoxanthin, (from xanthophyll group) (Kumar et al., 2021). Total carotenoids, total carotenes, total xanthophylls and combined lutein and zeaxanthin were also calculated (as previously published (Feart et al., 2016; Merle et al., 2021)). In addition, plasma lipid concentrations (i.e., triglycerides (TG) and total cholesterol (TC) in mmol/L) were also measured through routine enzymatic methods, centralized and performed by the Biochemistry Laboratory of the University Hospital of Dijon (Berr et al., 2016). TG and TC are the main carotenoid carriers and have a significant role in the absorption and bioavailability of carotenoids (Bohn et al., 2021). Therefore, the carotenoid/lipids ratios (i.e. carotenoid/(TG+TC) ratios) were calculated for each carotenoid and for the total carotenoids, total carotenes, total xanthophylls and for combined lutein and zeaxanthin, and considered as additional relevant exposures.

Depressive Symptomatology

Depressive Symptomatology (DS) was measured with the CES-D scale, administered through interviews conducted by a neuropsychologist at each follow-up visit. This tool consists of 20 items, each assessing the frequency of depressive symptoms during the previous week, from

« never » to « always » (rated from 0 to 3), leading to a total score ranging from 0 to 60 (higher score indicating higher level of DS). As did other studies, the validated, widely used international cut-off of 16 and over has been used to define a DS (Bardinet et al., 2022a, 2022b; Vilagut et al., 2016). In addition, the use of antidepressant treatment was considered at each visit through the "N06A" code of the World Health Organization's Anatomical Therapeutic Chemical (ATC) classification recorded from the inventory of all prescribed and consumed medication ("WHOCC - ATC/DDD Index," n.d.). The cut-off CES-D score ≥16 and/or the use of antidepressant treatment were combined to assess incident DS at each visit during the follow-up.

Other variables

Sociodemographic, lifestyle and health characteristics were collected at baseline, including age, gender, living condition (living alone, in couple or cohabitation), monthly income (<1,500€, 1,500-2,250€, ≥2,250€ or no answer), educational level (no study or elementary without degree, elementary with degree or secondary, high school or university), body mass index (BMI) (<25, 25-30 or ≥30 kg/m²), physical activity (poor, intermediate or optimal level, considered with daily leisure activities and sport), smoking status (non-smoker, ex-smoker or current smoker), alcohol consumption (in number of weekly glasses), Mini Mental State Examination (MMSE) score and multimorbidity (Boyd and Fortin, 2010). This last variable was defined as ≥5 medication use and/or ≥2 health disorders among hypertension, diabetes, angina, cardiac rhythm disorders, arteritis, cardiac failure, myocardial infarction, hospitalization for stroke, asthma, Parkinson's disease, dyspnea, osteoporosis and thyroid diseases. All these health disorders were self-reported, except for hypertension (defined by a clinical measure or antihypertensive treatment use) and for diabetes (considered as anti-diabetic treatment use or blood glucose >7 mmol/L).

Statistical Analysis

All statistical analyses were performed using R Software (R version 4.1.1) and statistical significance was set at $p<0.05$. Baseline sociodemographic, lifestyle and clinical information of the study sample were described according to the quartiles of total plasma carotenoids using chi-square tests for qualitative variables and analysis of variance for quantitative variables (non-parametric tests for quantitative variables with asymmetric distribution). Then, each plasma carotenoid and plasma carotenoid/lipids ratio was described.

Association between plasma carotenoids and risk of DS

The associations between baseline plasma carotenoids and the risk of incident DS over time were estimated using a random effect logistic regression, controlled for the following relevant confounders: age (included as time-scale in the model), sex, living condition, educational level, season of the blood drawing, smoking status, alcohol consumption, physical activity, BMI, TG, TC, multimorbidity and study center. All these confounders were selected based on available literature and using a Directed Acyclic Graph. Random effect logistic regression is adapted to repeated measures of DS over time in the present study, thus considering individual potential change during the follow-up (Stiratelli et al., 1984). Indeed, DS could fluctuate over time, the mixed model seemed to be the most appropriate in the study of the DS regarding our aim. Two random effects were introduced into the model, a random intercept assuming the heterogeneity of participants at baseline and a random slope, assuming variability in the response of individual DS over time (Commenges and Jacqmin-Gadda, 2015). The choice of the number of random effects was based on the Akaike criteria and all the assumptions of the model were checked. The function gamm4 of the R-package *gam* was used to estimate the studied associations.

In a first model, the association was estimated between each plasma carotenoid concentration at baseline (per 1 Standard Deviation (SD) increase) and the risk of DS over time adjusted for all selected confounders. Then, total carotenoids, total carotenes, total xanthophylls and combined lutein+zeaxanthin levels (per 1SD increase) were considered as additional exposures.

In an alternative model, the association was examined between each baseline plasma carotenoid/lipids ratio (per 1 SD increase) and the risk of DS over time adjusted for the same confounders, excepted for TG and TC to avoid over adjustment. Again, total carotenoids/lipids, total carotenes/lipids and total xanthophylls/lipids and lutein+zeaxanthin/lipids ratios were examined in relation to the risk of DS over time.

Additional analyses

Alternative definitions of DS were explored in additional analyses. Firstly, incident DS was only identified using the CES-D threshold ≥16 (irrespective of treatment use). Secondly, DS was assessed using an alternative relevant CES-D thresholds, i.e. ≥20 increasingly used to identify DS, in addition to the antidepressant treatment use (Vilagut et al., 2016).

Missing data

Some carotenoids with very low circulating concentrations could not be quantified (n=163) and have been replaced by the threshold value for quantification (**Supplementary Materials Table S1**).

Data were missing for n=115 participants for physical activity, n=1 for educational level, n=1 for living conditions, n=9 for season of blood drawing, n=20 for alcohol consumption and n=5 for BMI. Thus, we performed a multiple imputation via chained equation on the missing values of the potential confounders using the *mice* package (Buuren et al., 2021). This method assumes that data are missing at random (it has been checked when comparing participants with and without missing data) and each variable is associated with an imputation model conditionally to the other variables of the dataset.

RESULTS

Sample selection

Among the 4,363 participants from the baseline Bordeaux and Montpellier samples, 3,024 individuals had no information about plasma carotenoids, 1 about TG levels and 14 participants about DS at baseline; 309 participants had DS at baseline (i.e. CES-D score≥16 and/or antidepressant treatment use); and 5 participants did not have information about incident DS during the follow-up, leading to a final study sample of 1,010 individuals (**Figure 1**). Among the 1,010 included individuals, 355 (35.1%) participated in the last follow-up visit, while 474 (46.9%) deceased during the follow-up (median of follow-up 13.4 years (min 1.6 and max 18.7)).

Descriptive characteristics

The study sample was composed of 585 women (57.9%) who were aged 74.0 years (+/- 4.9) on average. Sociodemographic, lifestyle and health characteristics have been described according to increasing quartiles of total plasma carotenoids at baseline in **Table 1**. Eighty-five percent of participants lived in Bordeaux, 61.3% lived as a couple, 444 participants (44.0%) had a high level of education (high school or university) and 368 participants (36.4%) reported a monthly income lower than 1,500€ at baseline. Sixty percent of the study sample were non-smokers and the consumption of alcohol was 11 glasses per week on average. Participants had a mean CES-D score of 5.2 (+/- 4.3) at baseline. In a few words, participants in the highest quartile of total plasma carotenoids were more represented by women, consumed less tobacco and alcohol and were less often living with overweight or obesity.

Plasma carotenoids and lipids levels

The level of total plasma carotenoids was 1.99 µmol/L (+/- 1.01) on average in the total study sample (**Table 2**). Plasma β-carotene was the highest carotenoid with a concentration of

0.71 µmol/L (+/- 0.56) on average and plasma zeaxanthin was the lowest with a concentration of 0.07 µmol/L (+/-0.04) on average. Regarding plasma carotenoid/lipids ratios, the mean β-carotene/lipids ratio was the highest and the mean zeaxanthin/lipids ratio was the lowest on average in the total study sample.

Depressive Symptomatology over time

In the study sample, 436 participants (43.2%) were identified with an incident DS over time (i.e. CES-D score≥16 and/or antidepressant treatment at least once during the follow-up): 235 participants (23.3%) had a CES-D score≥16, 97 participants (9.6%) used an antidepressant treatment and 104 participants (10.3%) had both a CES-D score≥16 and used an antidepressant treatment, at least once during the follow-up.

Association between plasma carotenoids and the risk of DS over time

The association between baseline plasma carotenoids and the risk of DS over time revealed that each additional SD increase in plasma zeaxanthin (one SD=0.04 µmol/L) was associated with significant reduced odds of DS by 19% after adjustment on potential confounders (OR=0.81, 95% CI [0.67;0.99]) (**Table 3**). No significant association was observed with the other plasma carotenoids. However, controlling for the same potential confounders excepted TG and TC, the plasma ratios of β-carotene/lipids and zeaxanthin/lipids were independently associated with significant reduced odds of DS over time (OR=0.79, 95% CI=[0.64;0.98] and OR=0.79, 95% CI=[0.67;0.94], for 1 SD increase respectively). In addition, the ratios of lutein+zeaxanthin/lipids, total xanthophylls/lipids and total carotenoids/lipids were also independently and significantly associated with a reduced risk of DS over time by a magnitude of up to 22% (Table 3).

Additional analyses

Firstly, using only the CES-D score≥16 to define DS, we identified 339 participants (33.6%) with incident DS over time. With this definition, each additional SD increase in plasma β-cryptoxanthin and total carotenoids were significantly associated with reduced odds of DS of up to 19% over time after adjustment (**Supplementary Materials Table S2**). No significant association was observed between the other plasma carotenoids and the risk of DS. Regarding plasma carotenoid/lipids ratios, each additional SD increase in the lipids ratios of plasma lycopene, β-cryptoxanthin, total carotenes, total xanthophylls and total carotenoids was associated with reduced odds of DS of up to 24% over time after adjustment for potential confounders (Supplementary Materials Table S2).

Secondly, using alternative CES-D thresholds (i.e. CES-D score ≥20) and/or the use of antidepressant treatment as an alternative definition of DS, we identified 351 participants (34.8%) with incident DS over time. No significant association was observed between plasma carotenoids and the risk of DS. Regarding plasma carotenoid/lipids ratios, each additional SD increase in the lipids ratios of plasma lutein and zeaxanthin was associated with reduced odds of DS of up to 28% over time after adjustment for potential confounders (**Supplementary Materials Table S3**).

DISCUSSION

In this prospective study conducted on French older adults aged ≥65 years, we observed that higher levels of plasma zeaxanthin, and mainly lipids ratios of plasma total carotenoids, total xanthophylls, β-carotene and zeaxanthin (combined with lutein or not) were significantly associated with a 19 to 22% reduced odds of DS over more than 17 years of follow-up. Using alternative definitions to identify the incident DS over time, our results confirmed that higher total carotenoids (as a ratio of lipids or not), total xanthophylls/lipids, zeaxanthin/lipids and lutein/lipids ratios were associated with a decreased odds of DS over time.

Regarding the available literature, potential association between β-carotene or zeaxanthin (combined or not with lutein) and depression have been previously observed (Beydoun et al., 2013; Ge et al., 2020; Li and Li, 2019; Lin and Shen, 2021; Park et al., 2021; Prohan et al., 2014; Y. Zhang et al., 2022). However, most of these studies were carried out on adults or in subgroups such as men or women. Although older adults are the most affected by depression, epidemiological research has rarely been conducted in this vulnerable subgroup. Our literature review revealed that 6 observational cross-sectional studies examined the association between carotenoids (from diet or in plasma) and depression prevalence in adults aged 55 and over (Johnson et al., 2013; Lai et al., 2016; Milaneschi et al., 2012; Nguyen et al., 2017; Oishi et al., 2009; Payne et al., 2012). To our knowledge, only two longitudinal studies explored the prospective association through plasma carotenoids, which is more accurate than dietary surveys and none of them have investigated the association through carotenoid/lipids ratio. Precisely, in the first prospective study based on 111 Australian adults (55-85 years), men with higher combined plasma lutein and zeaxanthin levels had less occurrence of severe depressive symptoms compared to whose with the lowest level (Lai et al., 2016). In the second study based on 858 Italian older adults (≥65 years), a doubled risk to develop DS over 6 years of follow-up was observed in participants having the lowest plasma total carotenoids levels compared with

the highest levels, but no significant association was observed for each individual plasma carotenoid (Milaneschi et al., 2012). Our results are in line with these two prospective studies; however, DS was only assessed two or three times over the 6-year follow-up periods in the latter studies, whereas up to 8 measurement times over 17 follow-up years were analyzed in the present study. In addition, these previous studies focused on smaller sample sizes than the present one, or used more conventional logistic regression models, not taking into account the repeated measures of DS over the follow-up (potential change from one follow-up to the next). Finally, the association with carotenoid to lipids ratios were not previously estimated, although lipids have a major role in the bioavailability of the plasma carotenoids (Bohn et al., 2021). Indeed, carotenoids are lipophilic compounds: the dietary lipids mediate their transport and cellular uptake contributing to their bioavailability (Abdel-Aal et al., 2013; Demmig-Adams et al., 2020; Murillo et al., 2019; Tudor and Pintea, 2020). These properties could in part explain additional results from the present study when plasma carotenoid/lipids ratios were exploited.

Although there are still few observational epidemiological studies, the relevance of carotenoids to mental health is noted by many of them (Beydoun et al., 2013; Ge et al., 2020; Johnson et al., 2013; Lai et al., 2016; Li and Li, 2019; Lin and Shen, 2021; Milaneschi et al., 2012; Nguyen et al., 2017; Oishi et al., 2009; Park et al., 2021; Payne et al., 2012; Prohan et al., 2014; Y. Zhang et al., 2022), with a specific focus on lutein and zeaxanthin which have already been explored as nutritional supplements in clinical trials. Interestingly, two small clinical trials investigated the efficacy of lutein and zeaxanthin on mood. A first preventive trial including 59 healthy young adults (aged 18-25 years) evidenced a beneficial effect of a 12-month supplementation in carotenoids (13 or 27 mg of zeaxanthin, meso-zeaxanthin, and lutein) on mood, reporting a decrease in depressive symptoms assessed with the Beck Depression Inventory, not observed in the placebo group (Stringham et al., 2018). However, a second therapeutic trial on 90 adults aged 40-75 years with self-reported cognitive complaints observed

no improvement in the Profile Of Mood States after 6 months of 10 mg lutein and 2 mg zeaxanthin supplementation (Lopresti et al., 2022).

The biological plausibility of putative benefits of carotenoids (i.e. β-carotene, lutein and zeaxanthin) on depression was also explored in animal models (Kim et al., 2016; Zhou et al., 2018; Dhingra and Bansal, 2014; Badgujar and Saraf, 2015; Zeni et al., 2019). For example, two preclinical studies reported an improvement in depressive-like behaviors in mice or diabetic rats, as well as a decrease in inflammatory biomarkers (such as interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor- α (TNF-α)) after β-carotene or zeaxanthin supplementation (Kim et al., 2016; Zhou et al., 2018). On the other hand, two studies also evidenced a reduction in depressive-like behaviors in rodents and an antioxidant activity: a decrease oxidative stress by reduction of the plasma nitrite levels after β-carotene supplementation (Dhingra and Bansal, 2014) and a decrease in spinal reactive oxygen species after administration of lutein and zeaxanthin (isolated or combined) (Badgujar and Saraf, 2015). Altogether, the benefit of carotenoids on depression may be explained by their antioxidant and anti-inflammatory properties (Manochkumar et al., 2021), especially for xanthophylls, such as lutein and zeaxanthin, thanks to their polarity and molecular structures (Manochkumar et al., 2021; Murillo et al., 2019). Additionally, crocin, a derivate of zeaxanthin from saffron, had also repeatedly shown beneficial effects on depressive-like behaviors in animal models in part attributed to its anti-inflammatory and antioxidant activities (El Midaoui et al., 2022; Xiao et al., 2020; F. Zhang et al., 2022; Zhang et al., 2018). The crocin concentration has not been explored in the present study, but its structural similarity with zeaxanthin supports the present findings in favor of a benefit of zeaxanthin in relation to depression (El Midaoui et al., 2022).

This study has some limitations. Plasma carotenoids were only available at baseline, assuming that carotenoid concentrations were stable throughout life whereas diet or seasonality, among others, could modify the plasma levels over time. However, a previous study underlined that a

single assessment of plasma carotenoids sufficiently and accurately defined the usual plasma level of carotenoids for a large group of persons (van Kappel et al., 2001). Although a clinical diagnosis of depression was not available, depressive symptoms were assessed by neuropsychologists using the CES-D scale, a validated tool widely used in epidemiological studies (Vilagut et al., 2016). Despite limitations, some strengths could also be underlined regarding the study conception, as the prospective design, the long follow-up and the exclusion of prevalent case of DS at baseline (limiting the potential inverse causality bias) and the large sample size. In addition, carotenoids were measured through plasma concentration and carotenoid to lipids ratios (taking into account bioavailability) and alternative definitions of DS were used in sensitivity analyses to support main results, with significant results for total carotenoids/lipids and total xanthophylls/lipids without taking into account antidepressant use in the definition of DS and for zeaxanthin/lipids with an alternative CES-D threshold (i.e. ≥20). Finally, regarding the statistical method, we used a mixed logistic regression model on repeated measures to take into account potential changes in DS over time for each individual, with adjustment for a wide range of potential confounders.

Conclusion

In the present study, higher plasma zeaxanthin, lipids ratios of plasma zeaxanthin (combined or not with lutein) and β-carotene were associated with a significant reduced risk of DS over time in older adults followed for up to 17 years and controlled for potential confounders. As β-carotene is mainly provided by yellow and orange fruits and vegetables whereas lutein and zeaxanthin are mainly provided by green leafy vegetables (Rao and Rao, 2007), adopting a large variety of colorful fruits and vegetables as part of healthy dietary habits might prevent depressive symptoms.

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Author Statement Contributors

JB, CP and CF designed research; JB, conducted research; CB, SE, CH and CD provided essential materials; JB analyzed data or performed statistical analysis; JB, CP and CF wrote paper; JB had primary responsibility for final content; and all authors have read and approved the final manuscript.

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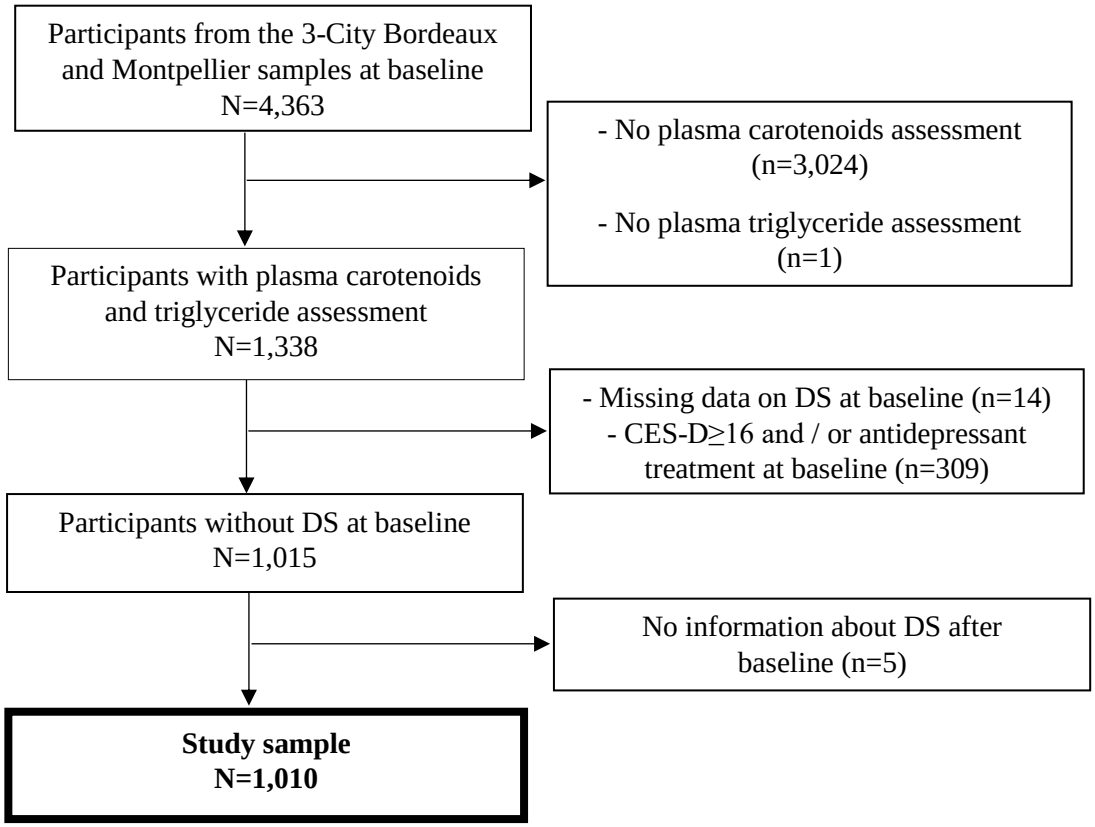
FIGURES AND TABLES



DS=Depressive Symptomatology ; CES-D=Center for Epidemiologic Studies-Depression

Figure 1: *Flow chart for the study of association between plasma carotenoids and depressive symptomatology, 3-City Bordeaux and Montpellier samples, 1999-2018*

Table 1: *Baseline sociodemographic, lifestyle and health characteristics of the study sample according to quartiles of total plasma carotenoids, from the 3C Bordeaux and Montpellier samples, 1999-2018 (N=1,010)*

	Overall (n=1,010)	Q1 - Low concentration of total carotenoids (n=253) *≤1.28µmol/L*	Q2 - Low to moderate concentration of total carotenoids (n=253) *]1.28µmol/L;1.81µmol/L]*	Q3 - Moderate concentration of total carotenoids (n=251) *]1.81µmol/L; 2.57µmol/L]*	Q4 - High concentration of total carotenoids (n=253) *>2.57µmol/L*	*p-value*[1]
	N (%) / Mean +/- SD	N (%) / Mean +/- SD	N (%) / Mean +/- SD	N (%) / Mean +/- SD	N (%) / Mean +/- SD	
Women	585 (57.9)	115 (45.5)	134 (53.0)	153 (61.0)	183 (72.3)	*<0.001*
Age (years)	74.0 +/- 4.9	74.6 +/- 5.1	73.9 +/- 4.8	73.8 +/- 4.9	73.7 +/- 4.7	*0.105*
Bordeaux center	860 (85.1)	211 (83.4)	214 (84.6)	214 (85.3)	221 (87.4)	*0.648*
Living conditions [a]						*0.575*
- Alone	341 (33.8)	93 (36.8)	85 (33.6)	82 (32.7)	81 (32.1)	
- In couple	619 (61.3)	151 (59.7)	159 (62.8)	153 (61.0)	156 (61.9)	
- Cohabitation (family or not)	49 (4.9)	9 (3.6)	9 (3.6)	16 (6.4)	15 (6.0)	
Educational level [b]						*0.174*
- No study or elementary without degree	97 (9.6)	30 (11.9)	28 (11.1)	21 (8.4)	18 (7.1)	
- Elementary with degree or secondary	468 (46.4)	124 (49.0)	116 (45.8)	110 (43.8)	118 (46.8)	
- High school	230 (22.8)	51 (20.2)	65 (25.7)	63 (25.1)	51 (20.2)	
- University	214 (21.2)	48 (19.0)	44 (17.4)	57 (22.7)	65 (25.8)	
Monthly income						*0.171*
- <1,500 €	368 (36.4)	105 (41.5)	95 (37.5)	91 (36.3)	77 (30.4)	
- ≥1,500 and <2,250 €	272 (26.9)	71 (28.1)	71 (28.1)	62 (24.7)	68 (26.9)	
- ≥2,250 €	325 (32.2)	69 (27.3)	79 (31.2)	85 (33.9)	92 (36.4)	
- No answer	45 (4.5)	8 (3.2)	8 (3.2)	13 (5.2)	16 (6.3)	
Smoking status						*<0.001*
- Non-smoker	608 (60.2)	123 (48.6)	142 (56.1)	156 (62.2)	187 (73.9)	
- Ex-smoker	341 (33.8)	102 (40.3)	94 (37.2)	89 (35.5)	56 (22.1)	
- Current smoker	61 (6.0)	28 (11.1)	17 (6.7)	6 (2.4)	10 (4.0)	

	Overall (n=1,010)	Q1 - Low concentration of total carotenoids (n=253) *≤1.28µmol/L*	Q2 - Low to moderate concentration of total carotenoids (n=253) *]1.28µmol/L;1.81µmol/L]*	Q3 - Moderate concentration of total carotenoids (n=251) *]1.81µmol/L; 2.57µmol/L]*	Q4 - High concentration of total carotenoids (n=253) *>2.57µmol/L*	*p-value*[1]
	N (%) / Mean +/- SD	N (%) / Mean +/- SD	N (%) / Mean +/- SD	N (%) / Mean +/- SD	N (%) / Mean +/- SD	
Alcohol consumption (number of glasses/week) [c]	10.9 +/- 12.7	13.6 +/- 14.4	12.2 +/- 14.1	10.5 +/- 11.9	7.5 +/- 8.9	*<0.001*
Energy intake (kcal/day) [d]	1,744 +/- 563	1,788 +/- 583	1,767 +/- 566	1,735 +/- 589	1,685 +/- 513	*0.283*
Regular physical activity [e]						*0.253*
- Poor	297 (33.2)	87 (37.5)	77 (34.4)	72 (33.0)	61 (27.6)	
- Intermediate	308 (34.4)	81 (34.9)	79 (35.3)	71 (32.6)	77 (34.8)	
- Optimal level	290 (32.4)	64 (27.6)	68 (30.4)	75 (34.4)	83 (37.6)	
BMI (kg/m²) [f]						*<0.001*
- <25	416 (41.4)	67 (26.7)	90 (35.9)	115 (45.8)	144 (57.1)	
- ≥25 and <30	451 (44.9)	129 (51.4)	128 (51.0)	113 (45.0)	81 (32.1)	
- ≥30	138 (13.7)	55 (21.9)	33 (13.1)	23 (9.2)	27 (10.7)	
MMSE Score [g]	27.6 +/- 2.0	27.4 +/- 2.0	27.5 +/- 2.1	27.6 +/- 2.0	27.8 +/- 1.8	*0.104*
Multimorbidity[2]	398 (39.4)	99 (39.1)	94 (37.2)	99 (39.4)	106 (41.9)	*0.752*
CES-D score	5.2 +/- 4.3	5.2 +/- 4.4	5.0 +/- 4.3	5.5 +/- 4.3	5.0 +/- 4.2	*0.600*

SD=Standard Deviation; BMI=Body Mass Index; MMSE=Mini Mental State Examination; CES-D=Center for Epidemiologic Studies-Depression

[a]Missing data for n=1, [b]Missing data for n=1, [c] Missing data for n=20, [d] Missing data for n=205, [e] Missing data for n=115, [f] Missing data for n=5, [g] Missing data for n=3

[1]p-value from Chi-square tests for qualitative variables and from Analysis of Variance for quantitative variables

[2]2 out of 13 health disorders among hypertension, diabetes, angina, cardiac rhythm disorders, arteritis, cardiac failure, myocardial infarction, hospitalization for stroke, asthma, Parkinson's disease, dyspnea, osteoporosis, thyroid diseases and/or 5 treatments prescribed and over.

Table 2: *Baseline plasma carotenoids, lipids and plasma carotenoid/lipids ratios, 3C Bordeaux and Montpellier samples, 1999-2018 (N=1,010)*

	Overall (n=1,010) Mean +/- SD
Plasma carotenoid (µmol/L)	
Total Carotenoids	1.99 +/- 1.01
Total Carotenes	1.33 +/- 0.82
- α-carotene	0.18 +/- 0.14
- β-carotene	0.71 +/- 0.56
- Lycopene	0.45 +/- 0.30
Total Xanthophylls	0.66 +/- 0.34
- Lutein	0.29 +/- 0.15
- Zeaxanthin	0.07 +/- 0.04
Lutein+Zeaxanthin	0.36 +/- 0.18
- β-cryptoxanthin	0.30 +/- 0.24
Plasma lipid (mmol/L)	
- Triglycerides (TG)	1.2 +/- 0.6
- Total Cholesterol (TC)	5.8 +/- 1.0
Plasma carotenoid /(TG+TC) ratio (µmol/mmol)	
Total Carotenoids	0.29 +/- 0.15
Total Carotenes	0.19 +/- 0.12
- α-carotene	0.03 +/- 0.02
- β-carotene	0.10 +/- 0.08
- Lycopene	0.06 +/- 0.04
Total Xanthophylls	0.10 +/- 0.05
- Lutein	0.04 +/- 0.02
- Zeaxanthin	0.01 +/- 0.01
Lutein+Zeaxanthin	0.05 +/- 0.03
- β-cryptoxanthin	0.04 +/- 0.03

SD=Standard Deviation; TG=Triglycerides; TC=Total Cholesterol

Table 3: *Plasma carotenoids and plasma carotenoid/lipids ratios and risk of depressive symptomatology (i.e. CES-D score≥16 and/or antidepressant treatment use), from the 3C Bordeaux and Montpellier samples, 1999-2018 (N=1,010)*

	OR [95% CI]*	***p-value****
Incident cases of DS during the follow-up = 436 (43.2 %)		
Plasma carotenoid (µmol/L)		
For +1 SD of the plasma level		
Total Carotenoids	0.85 [0.70;1.04]	*0.108*
Total Carotenes	0.93 [0.77;1.13]	*0.487*
α-carotene	1.03 [0.87;1.23]	*0.726*
β-carotene	0.88 [0.72;1.07]	*0.207*
Lycopene	0.95 [0.79;1.14]	*0.572*
Total Xanthophylls	0.86 [0.71;1.03]	*0.109*
Lutein	0.95 [0.79;1.14]	*0.580*
Zeaxanthin	**0.81 [0.67;0.99]**	***0.039***
Lutein+Zeaxanthin	*0.87 [0.72 ;1.06]*	*0.166*
β-cryptoxanthin	0.86 [0.71;1.03]	*0.109*
Plasma carotenoid / (TG+ TC) ratios (µmol/mmol)* **		
For +1 SD of the ratio		
Total Carotenoids	**0.78 [0.64 ;0.95]**	***0.015***
Total Carotenes	0.84 [0.69;1.03]	*0.088*
α-carotene	0.97 [0.80;1.17]	*0.712*
β-carotene	**0.79 [0.64;0.98]**	***0.034***
Lycopene	0.93 [0.77;1.12]	*0.439*
Total Xanthophylls	**0.78 [0.65;0.94]**	***0.009***
Lutein	0.85 [0.70;1.02]	*0.079*
Zeaxanthin	**0.79 [0.67;0.94]**	***0.008***
Lutein+Zeaxanthin	***0.80 [0.66;0.97]***	***0.023***
β-cryptoxanthin	0.84 [0.70;1.01]	*0.057*

OR=Odds ratio; CES-D=Center for Epidemiologic Studies-Depression ; CI=Confidence interval; SD=Standard Deviation; TG=Triglycerides; TC=Total cholesterol

** Random-effect logistic regression model with a random intercept and a random slope adjusted for age, sex, living condition, educational level, season of the blood drawing, smoking status, alcohol consumption, physical activity, body mass index, triglycerides, cholesterol total, multimorbidity and study center*

*** P-value of the log-likelihood ratio test*

****No longer adjusted for triglycerides and total cholesterol levels*

SUPPLEMENTARY MATERIALS

Table S1. Limit of quantification and detection of plasma carotenoid in blood sample

Table S2. Plasma carotenoids and plasma carotenoid/lipids ratios and risk of depressive symptomatology (i.e. CES-D score ≥ 16), from the 3C Bordeaux and Montpellier samples

Table S3. Plasma carotenoids and plasma carotenoid/lipids ratios and risk of depressive symptomatology identified by CES-D score ≥20 and/or antidepressant treatment use, from the 3C Bordeaux and Montpellier samples

Table S1. *Limit of quantification and detection of plasma carotenoid in blood sample, from 3C Bordeaux-Montpellier samples, 1999 (N=1,010)*

	n	LLQ Limit of Quantification	LOD Limit of Detection
α-carotene	*152*	0.056 µg/L	0.028 µg/L
β-carotene*	*0*	-	-
Lycopene	*14*	0.056 µg/L	0.028 µg/L
Lutein	*16*	0.007 µg/L	0.002 µg/L
Zeaxanthin*	*0*	-	-
β-cryptoxanthin*	*0*	-	-

**not concerned by undetected concentrations*

Table S2: *Plasma carotenoids and plasma carotenoid/lipids ratios and risk of depressive symptomatology (i.e. CES-D score ≥ 16), from the 3C Bordeaux and Montpellier samples, 1999-2018 (N=1,010)*

	OR [95% CI]*	***p-value****
Incident cases of DS during the follow-up = 339 (33.6 %)		
Plasma carotenoid (µg/L)		
For +1 SD of the plasma level		
Total Carotenoids	**0.81 [0.67;0.99]**	***0.043***
Total Carotenes	0.86 [0.70;1.05]	*0.132*
α-carotene	0.99 [0.82;1.19]	*0.918*
β-carotene	0.87 [0.71;1.06]	*0.174*
Lycopene	0.86 [0.71;1.04]	*0.130*
Total Xanthophylls	0.84 [0.69;1.02]	*0.072*
Lutein	0.94 [0.78;1.13]	*0.505*
Zeaxanthin	0.84 [0.69;1.02]	*0.075*
Lutein+Zeaxanthin	*0.91 [0.75;1.09]*	*0.308*
β-cryptoxanthin	**0.82 [0.68;0.99]**	***0.043***
Plasma carotenoid / (TG + TC) ratios (µg/mmol)***		
For +1 SD of the ratio		
Total Carotenoids	**0.76 [0.62 ;0.93]**	***0.007***
Total Carotenes	**0.80 [0.66 ;0.98]**	***0.033***
α-carotene	0.94 [0.78;1.14]	*0.518*
β-carotene	0.82 [0.66;1.01]	*0.056*
Lycopene	**0.82 [0.67;0.99]**	***0.042***
Total Xanthophylls	**0.78 [0.65;0.94]**	***0.009***
Lutein	0.87 [0.73;1.05]	*0.150*
Zeaxanthin	0.85 [0.72;1.01]	*0.066*
Lutein+Zeaxanthin	*0.86 [0.71;1.03]*	*0.108*
β-cryptoxanthin	**0.79 [0.66;0.96]**	***0.017***

OR=Odds ratio; CES-D=Center for Epidemiologic Studies-Depression ; CI=Confidence interval; SD=Standard Deviation; TG=Triglycerides; TC=Total cholesterol

** Random-effect logistic regression model with a random intercept and a random slope adjusted for age, sex, living condition, educational level, season of the blood drawing, smoking status, alcohol consumption, physical activity, body mass index, triglycerides, cholesterol total, multimorbidity and study center*

*** P-value of the log-likelihood ratio test*

****No longer adjusted for triglycerides and total cholesterol levels*

Table S3: *Plasma carotenoids and plasma carotenoid/lipids ratios and risk of depressive symptomatology identified by CES-D score ≥20 and/or antidepressant treatment use, from the 3C Bordeaux and Montpellier samples, 1999-2018 (N=1,010)*

	OR [95% CI]*	***p-value****
Incident cases of DS during the follow-up = 351 (34.8 %)		
Plasma carotenoid (µg/L)		
For +1 SD of the plasma level		
Total Carotenoids	0.84 [0.65;1.11]	*0.218*
Total Carotenes	0.90 [0.69;1.18]	*0.447*
α-carotene	0.99 [0.79;1.26]	*0.977*
β-carotene	0.82 [0.62;1.08]	*0.162*
Lycopene	0.99 [0.77;1.28]	*0.955*
Total Xanthophylls	0.84 [0.65;1.08]	*0.166*
Lutein	0.86 [0.66;1.11]	*0.243*
Zeaxanthin	0.77 [0.59;1.01]	*0.059*
Lutein+Zeaxanthin	*0.83 [0.63 ;1.08]*	*0.161*
β-cryptoxanthin	0.94 [0.74;1.19]	*0.597*
Plasma carotenoid / (TG + TC) ratios (µg/mmol)***		
For +1 SD of the ratio		
Total Carotenoids	0.78 [0.60 ;1.02]	*0.065*
Total Carotenes	0.78 [0.59 ;1.03]	*0.079*
α-carotene	0.90 [0.71 ;1.15]	*0.400*
β-carotene	0.76 [0.57;1.02]	*0.067*
Lycopene	0.97 [0.76;1.24]	*0.789*
Total Xanthophylls	0.82 [0.64;1.05]	*0.110*
Lutein	**0.76 [0.59;0.98]**	***0.037***
Zeaxanthin	**0.72 [0.57 ;0.91]**	***0.005***
Lutein+Zeaxanthin	*0.78 [0.60 ;1.01]*	*0.062*
β-cryptoxanthin	0.93 [0.73 ;1.18]	*0.564*

OR=Odds ratio; CES-D=Center for Epidemiologic Studies-Depression CI=Confidence interval; SD=Standard Deviation; TG=Triglycerides; TC=Total cholesterol

** Random-effect logistic regression model with a random intercept and a random slope adjusted for age, sex, living condition, educational level, season of the blood drawing, smoking status, alcohol consumption, physical activity, body mass index, triglycerides, cholesterol total, multimorbidity and study center*

*** P-value of the log-likelihood ratio test*

****No longer adjusted for triglycerides and total cholesterol levels*