# Carotenoid-rich dietary patterns during midlife and subsequent cognitive function

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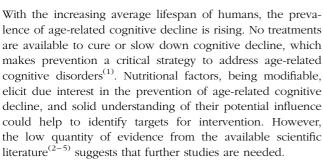
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## Abstract

Carotenoids may help to prevent the ageing of the brain. Previous findings regarding β-carotene alone are not consistent. In the present study, we evaluated the cross-time association between a carotenoid-rich dietary pattern (CDP) and subsequent cognitive performance using a sample of 2983 middle-aged adults participating in the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) study. Cognitive performance was assessed in 2007–9 using six neuropsychological tests, and a composite cognitive score was computed. The cognitive data were related to dietary data obtained by repeated 24 h dietary records (1994–6) and to measurements of baseline plasma concentrations of carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, *trans*-β-carotene and *cis*-β-carotene). DP were extracted using the reduced rank regression method for 381 participants and then extrapolated to the whole sample using plasma carotenoid concentrations as response variables. Associations between a CDP and cognitive function measured 13 years later were estimated with ANCOVA providing mean difference values and 95 % CI across the tertiles of CDP. A correlation between CDP and consumption of orange- and green-coloured fruits and vegetables, vegetable oils and soup was observed. CDP was found to be associated with a higher composite cognitive score (mean difference 1·04, 95 % CI 0·20, 1·87, *P* for trend 0·02), after adjustment for sociodemographic, lifestyle and health factors. Similar findings were obtained for scores obtained in the cued recall task, backward digit span task, trail making test and semantic fluency task (all *P* for trend <0·05). Further studies ought to confirm whether a diet providing sufficient quantity and variety of coloured fruits and vegetables may contribute to the preservation of cognitive function during ageing.

**Key words: Carotenoids: Dietary patterns: Carotene: Xanthophylls: Cognition: Ageing: Neuropsychological evaluation**

With the increasing average lifespan of humans, the prevalence of age-related cognitive decline is rising. No treatments are available to cure or slow down cognitive decline, which makes prevention a critical strategy to address age-related cognitive disorders[1]. Nutritional factors, being modifiable, elicit due interest in the prevention of age-related cognitive decline, and solid understanding of their potential influence could help to identify targets for intervention. However, the low quantity of evidence from the available scientific literature[2–5] suggests that further studies are needed.

Carotenoids are natural pigments present in plant-based foods and are well known for their role as efficient scavengers of reactive oxygen species and may thus help to protect the brain against oxidation occurring during the ageing process[6,7]. Indeed, the brain is especially prone to oxidative stress owing to its high content of long-chain PUFA that are extremely sensitive to peroxidation and to increased production of reactive oxygen species due to a high metabolic rate[6]. Reactive oxygen species are essential in signalling pathways; however, any increase in reactive oxygen species production may be detrimental to lipids, proteins and DNA (especially at the mitochondrial level) as a result of the increase in oxidative stress and dysfunction in signal cascades and/or apoptosis[8–10]. Carotenoids also exhibit anti-inflammatory properties probably through the modulation of the lipoxygenase enzyme and activation of the expression of genes involved in cell communication[11,12].

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Most of the available epidemiological literature on carotenoid-based prevention of brain ageing has been focused on β-carotene alone, in spite of other carotenoids being found to have interesting properties[7].

Cross-sectional and case–control studies have indeed reported associations between lower intake or plasma status of β-carotene and lower cognitive function[13–15] or higher risk of dementia[16–18]. Moreover, longitudinal studies have reported lower cognitive decline[19,20] or lower risk of Alzheimer's disease[21] among subjects with higher intake or plasma status of β-carotene. However, two cross-sectional studies on cognitive function[22,23] and five longitudinal studies on cognitive decline[24,25] and risk of dementia[26–28] have not reported such associations.

Besides, randomised controlled trials have not yet confirmed any potential benefits of β-carotene supplementation alone or in combination with other antioxidants regarding cognitive decline or dementia prevention[29–31], except for one such trial carried out among participants in the Physicians' Health Study II. In that study, short-term supplementation with a low dose of β-carotene was found to be not associated with cognitive function, while a beneficial impact of long-term supplementation was observed[32].

Prior research exploring the relationship between other carotenoids and cognitive ageing is scarce, with study designs and findings being inconsistent[14,18,22,24,33,34]. In a cross-sectional study, an association between cognitive impairment and low plasma lycopene and zeaxanthin concentrations has been reported[14].

Using data from the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) 2 study, the present study aimed to evaluate the association between dietary patterns (DP) that maximally account for the variation in plasma carotenoid concentrations and subsequent cognitive performance, employing the recently proposed reduced rank regression (RRR) statistical technique[35].

## Materials and methods

### Study population

The SU.VI.MAX study (1994–2002; n 12 741) was a randomised, double-blind, placebo-controlled, primary prevention trial evaluating the effect of daily low-dose antioxidant supplementation on the incidence of cancer and IHD[36,37]. At the end of the supplementation period (2002), a total of 6850 subjects agreed to participate in a post-supplementation follow-up study (SU.VI.MAX 2, 2007–9).

The SU.VI.MAX and SU.VI.MAX 2 studies were conducted according to the guidelines of the Declaration of Helsinki and were approved by the Ethics Committee for Studies with Human Subjects of Paris-Cochin Hospital (CCPPRB no. 706 and no. 2364, respectively) and the Comité National Informatique et Liberté (CNIL no. 334 641 and no. 907094, respectively). Written informed consent was obtained from all the participants. Clinical trial registration: clinicaltrial.gov (number NCT00272428).

### Measurement of carotenoid concentrations

Fasting blood samples were collected in evacuated tubes (Becton Dickinson) at baseline (1994–6). All biochemical measurements were carried out in a single laboratory. Blood samples were centrifuged immediately, frozen and stored at −80°C. Carotenoids were assessed in the same laboratory using a Biotek-Kontron HPLC system (UVK Lab), which consisted of a 525 dual pump, a 465 autosampler and a 540 diode array detector. The plasma concentrations of β-carotene were measured by Fluka (Sigma-France), and those of other carotenoids were measured by Hoffmann-La Roche (Hoffmann-La Roche). The liquid chromatography separation was carried out with an Alltech Adsorbosphere C18 column (150 × 4·5 mm inner diameter and 3 μm particle size; Templemars), which was thermostated at 28°C with a 402 column oven. Carotenoids were obtained after two extractions with a hexane–tetrahydrofuran mixture. For quantification, we used the method of Steghens et al.[38] with minor modifications. Specifically, we used a single (instead of two) 150 mm-long column, and we added 10 parts per million water in mobile phase A to improve the separation of retinol, lutein and zeaxanthin.

The limits of detection were calculated as 5-fold the maximum baseline noise in the region of the peaks. Thus, we found limits of detection of 0·02 μM for carotenoids. All the concentrations of retinol, lycopene and β-carotene were above the respective limits, and only 5 % of lutein, 8 % of zeaxanthin and 2 % of β-cryptoxanthin were below these limits.

### Dietary data assessment

During the SU.VI.MAX study, the subjects were asked to provide a 24 h dietary record every 2 months via computerised questionnaires. The participants were assisted by an instruction manual that included validated photographs of more than 250 generic foods shown in three main portion sizes[39]. A French food composition table was used to estimate nutrient intake[40].

### Cognitive assessment

During the SU.VI.MAX 2 study (2007–9), all the participants were invited to undergo a medical check-up. This included a clinical examination and a neuropsychological evaluation carried out by trained neuropsychologists. Episodic memory was evaluated with the RI-48 cued recall test (a list of forty-eight words belonging to twelve categories). The score was the number of words retrieved (maximum score of 48)[41]. Lexical–semantic memory was assessed by verbal fluency tasks including a semantic fluency task consisting of naming as many animals as possible and a phonemic fluency task consisting of citing words beginning with the letter P. The score was the number of correct words produced during a 2 min period for each task[42]. Short-term/working memory was assessed with the forward and backward digit spans. The subjects were asked to repeat two sequences of digits, forwards and backwards. The number of digits increased by one until the participants failed in two consecutive trials of

the same digit span. For each correct sequence repeated, one point was assigned, with a maximum score of 14 for a forward as well as a backward digit span[43]. Mental flexibility was assessed with the Delis–Kaplan trail making test (TMT) consisting of connecting numbers and letters alternating between the two series. The score was the time in seconds needed to complete a task[44], implying that a lower value indicated better performance.

## Covariates

Data pertaining to sex, date of birth, occupational category (unemployed, manual worker or blue- and white-collar worker), smoking status (never, former or current), physical activity (irregular, <1 h walking/d or ≥1 h walking/d), education (primary, secondary or post-secondary) and medication use were collected at baseline. In the SU.VI.MAX 2 study, medication use was self-reported.

Anthropometric and clinical measurements (including BMI and blood pressure) were obtained at baseline and at the end of follow-up as described previously[45]. Hypertension during follow-up was defined as blood pressure ≥140/90 mmHg at any follow-up examination or antihypertensive medication use.

Fasting blood glucose concentrations were measured using an enzymatic method (Advia 1650; Bayer Diagnostics), and diabetes during follow-up was defined as fasting blood glucose concentrations ≥7 mmol/l at any follow-up test or anti-diabetic medication use. Depressive symptoms were assessed at follow-up using the French version of the Center for Epidemiologic Studies Depression Scale, and the total score was used as a covariate[46]. Data pertaining to self-reported memory problems were collected at baseline. During follow-up, all the reported cardiovascular events were validated by an independent expert committee.

## Statistical analyses

For the present analyses, we selected subjects aged 45–60 years at baseline with available cognitive evaluation data (n 4447) and dietary data (i.e. ≥3 24 h dietary records over the first 2 years of follow-up; n 3362). Subjects with missing values for any of the covariates were excluded, leaving a sub-sample of 2983 participants.

In the present study, the 24 h dietary records provided during the first 2 years of follow-up were taken into account to compute the means of food and nutrient intakes. DP were extracted using the RRR technique described by Hoffman et al.[35]. The RRR method is used to derive coherent patterns from a number of predictor variables, thus maximising the explained variation in a pre-defined set of response variables, i.e. intermediate markers that are hypothesised to be related to the health outcome of interest. In the present analyses, responses chosen for the RRR method were plasma carotenoid concentrations available for a subsample of 381 participants.

Participants with and without data on plasma carotenoid concentrations were compared using the Kruskal–Wallis or $\chi^2$ test. We first carried out log transformation of plasma

carotenoid concentrations to improve normality. The RRR method can extract as many DP as there are response variables. In the present study, all the subsequent models pertain to the first DP, as it reflects the main part of the variation in plasma carotenoid concentrations. Next, an extrapolated DP score was computed for the entire sample. To test the stability and generalisability of the pattern, we applied the bootstrap method with 1000 replications using the sample with plasma carotenoid concentrations. Thus, we estimated the distribution of the Spearman correlation coefficients between these 1000 first RRR-extracted DP and the principal carotenoid-rich dietary pattern (CDP, described below) used in the present analyses. The mean Spearman correlation coefficient was 0·890 (95 % CI 0·889, 0·893). The consumption of a total of thirty different food groups was used as the predictor, which was energy adjusted using the residual method[47]. The grouping up of the various foods is described in Supplementary Table S1 (available online).

For the present analyses, we used the inverse of the TMT score, thus a higher score corresponded to a better result. The inverse TMT score was log-transformed to improve normality. Cognitive test scores were converted into $T$ scores (mean 50 (SD 10)). Thus, a 1-point difference in the test score corresponded to 1/10 of a SD difference. A composite cognitive score defined as the mean of the standardised test scores was rescaled to SD = 10.

Descriptive baseline characteristics are reported as means and standard deviations or percentages across tertiles of the extracted DP. The reported $P$ values refer to the Kruskal–Wallis or $\chi^2$ trend test, as appropriate. ANCOVA were used to estimate the cross-time associations between the tertiles of the retained baseline DP and the subsequent cognitive performance scores. $P$ values for linear contrast across the tertiles are reported. Mean differences in cognitive scores according to DP score modelled as continuous variables were also estimated using multivariable linear regression. In the initial model, the analyses were unadjusted. In the second set of models, the analyses were adjusted for follow-up time between baseline and cognitive evaluation (continuous variable), sex, supplementation group during the trial phase (active/placebo), education, baseline occupational status, age at cognitive evaluation (continuous variable), intervention group during the SU.VI.MAX trial phase (1994–2002), baseline energy intake (continuous variable), smoking, physical activity, number of available 24 h dietary records, baseline self-reported memory troubles, baseline BMI (continuous variable), depressive symptoms at follow-up (continuous variable), CVD incidence, hypertension and diabetes during follow-up. Further adjustment for the regular use of antioxidant supplements at cognitive evaluation did not have an impact on the estimations; thus, this covariable was not retained in the final model.

To test the robustness of the primary findings, three sets of supplementary analyses were carried out. First, for purposes of partly correcting for selection bias, additional analyses were carried out using inverse probability weighting[48,49]. The probability to be included in the present study was determined for each participant using data on baseline characteristics. The data were reanalysed using the inverse probability to be

included as the respective weight. Second, a simplified DP as described by Schulze *et al.*[50] was estimated and further tested with regard to subsequent cognitive function. For this purpose, food group consumption displaying a |correlation coefficient| > 0·2 within the DP was retained and standardised (mean 0 (SD 1)) before being summed up. Finally, analyses were carried out only for the subgroup receiving placebo during the trial phase.

Additionally, effect modifications by sex and supplementation group during the trial phase were tested.

Statistical tests were two sided (type 1 error < 0·05). All the analyses were carried out using SAS (release 9.2; SAS Institute, Inc.).

## Results

The preliminary analyses showed that participants with and without data on plasma carotenoid concentrations were similar in terms of sociodemographic factors, in particular, sex and education, cardiovascular risk factors (smoking, diabetes, hypertension and BMI) and food consumption (data not shown). However, the subsample with carotenoid status data was slightly older than the other group: 53·6 *v.* 51·8 years (P < 0·0001).

The retained DP factor, extracted using the RRR method with data from 381 participants with available plasma carotenoid status data, explained 11·66 % of the total variation in the response variables, i.e. plasma carotenoid concentrations, and about 6·46 % of the variation in food group consumption patterns (Table 1). This factor was positively correlated with all the carotenoids, with the highest correlations being evident for carotenes and β-cryptoxanthin. Hence, that factor was termed 'CDP'.

Correlations between the CDP score and the various food groups are presented in Table 2.

The CDP score was positively correlated with the consumption of green-coloured fruits and vegetables, vegetable oils, orange-coloured fruits and vegetables and soup and was negatively correlated with that of beer, cider and wine.

The mean follow-up duration was 13·6 (SD 0·5) years. The mean age of the population at the time of cognitive evaluation was 65·5 (SD 4·5) years. The characteristics of the participants

are given according to tertiles of the CDP score in Table 3. The CDP score was associated with a higher probability of being female and non-smoker, having more formal education and lower BMI, being a blue-collar worker, and being more likely to report the presence of depressive symptoms. It was also negatively associated with alcohol, lipid and protein intake and positively associated with carbohydrate intake.

No interaction between the CDP score and sex or supplementation group regarding cognitive performance was detected (all *P* values > 0·20).

The results of the analyses of the association between the CDP score and cognitive function are given in Table 4. In the unadjusted model, participants with a higher CDP score had higher composite cognitive scores as well as individual scores on the RI-48 cued recall task, backward digit span task, TMT and semantic fluency task. On the other hand, no association was observed for the forward digit span or phonemic fluency task. In the fully adjusted models, these relationships remained statistically significant, despite attenuation of the estimates in most cases.

The findings of the supplementary analyses (inverse probability weighting and the simplified DP) are presented in Supplementary Tables S2 and S3 (available online), respectively. After accounting for potential selection bias using inverse probability weighting, the estimates were slightly attenuated, but no association that was previously significant became non-significant or vice versa. In turn, modelling a simplified DP did not substantially modify the primary findings, except that the CDP and TMT performance were no longer associated. Finally, despite a loss of power, findings obtained for the placebo group were similar to those of the main analysis (data not shown).

## Discussion

In the present study, we used a statistical method termed RRR to extract a CDP correlated with the plasma status of various carotenoids. The DP extracted from our data was most strongly correlated with the plasma status of β-carotene, α-carotene, β-cryptoxanthin and lutein. This DP, estimated with midlife exposure data, was highly correlated with the consumption of green-coloured fruits and vegetables, vegetable oils, orange-coloured fruits and vegetables and soup. Furthermore, it was positively associated with the composite cognitive performance score assessed 13 years later, even after accounting for confounders such as sociodemographic factors, lifestyle characteristics and health status. More specifically, high CDP scores were related to better episodic memory, semantic fluency, working memory and executive functioning. The positive association between this DP and subsequent cognitive performance adds support to previous research reporting better cognitive status[13–15], lower cognitive decline[19,20] or lower probability[16–18] or risk of dementia[21] among participants with high β-carotene intake or biomarker status.

However, our findings cannot be directly compared with those of prior research investigating associations between a wide variety of carotenoids and cognitive outcomes. For example, in the EVA (Etude du Vieillissement Artériel) study, some carotenoids, i.e. lycopene and zeaxanthin but not

**Table 1.** Explained variation in the consumption of foods and in plasma carotenoid concentrations with the carotenoid-rich dietary pattern (*n* 381)

| | Carotenoid-rich pattern |
| --- | --- |
| Explained variation in food groups (%) | 6·46 |
| Explained variation in plasma carotenoid concentrations (%) | 11·66 |
| Pearson's correlation coefficients | |
| β-Carotene* | 0·50 |
| α-Carotene* | 0·44 |
| β-Cryptoxanthin* | 0·42 |
| Lutein* | 0·33 |
| Zeaxanthin* | 0·17 |
| Lycopene* | 0·14 |
| *Cis*-β-carotene* | 0·04 |

* Plasma concentrations.

**Table 2.** Food groups associated with carotenoid-rich dietary patterns (extracted from *n* 2983)

| | Pearson's correlation coefficient | Standardised scoring coefficient | Explained variation in score (%) |
|---|---|---|---|
| Green-coloured fruits and vegetables | 0·47 | 0·28 | 12·99 |
| Vegetable oils | 0·46 | 0·39 | 17·97 |
| Orange-coloured fruits and vegetables | 0·42 | 0·37 | 15·75 |
| Soup | 0·33 | 0·24 | 7·76 |
| Dairy products | 0·28 | 0·10 | 2·94 |
| Tea | 0·27 | 0·19 | 5·19 |
| Other fruits and vegetables | 0·25 | 0·003 | 0·07 |
| Legumes | 0·25 | 0·25 | 6·12 |
| Tomatoes | 0·25 | 0·10 | 2·60 |
| Fatty and/or sweet products | 0·20 | 0·27 | 5·27 |
| Red-coloured fruits and vegetables | 0·15 | 0·03 | 0·44 |
| Dried fruits | 0·12 | 0·03 | 0·32 |
| Salted products | 0·11 | 0·15 | 1·68 |
| Coffee | 0·08 | 0·17 | 1·38 |
| Nuts | 0·08 | − 0·03 | − 0·23 |
| Cheese | 0·05 | 0·14 | 0·69 |
| Sweet beverages | 0·02 | 0·01 | 0·01 |
| Starchy foods | 0·01 | 0·07 | 0·09 |
| Milk | 0·01 | 0·09 | 0·07 |
| Animal fat | − 0·01 | 0·11 | − 0·12 |
| Potatoes | − 0·02 | 0·10 | − 0·17 |
| Seafood and fish | − 0·01 | − 0·08 | 0·12 |
| Water | − 0·03 | − 0·13 | 0·37 |
| Snacks | − 0·09 | − 0·03 | 0·31 |
| Processed meat | − 0·13 | 0·06 | − 0·81 |
| Eggs | − 0·14 | − 0·13 | 1·85 |
| Alcoholic beverages | − 0·27 | − 0·08 | 2·15 |
| Meat | − 0·27 | − 0·17 | 4·48 |
| Beer and cider | − 0·34 | − 0·12 | 4·06 |
| Wine | − 0·49 | − 0·14 | 6·66 |

β-carotene, were found to be associated with specific cognitive tests mostly reflecting executive functioning. In the present study, the extracted CDP was not materially correlated with either lycopene or zeaxanthin, thus not allowing to draw a conclusion specifically with regard to these carotenoids. Other studies reporting results with regard to carotenoids did not find any association with cognitive function[22,24] or decline[24]. Besides, lower carotenoid status was observed among Alzheimer's disease cases compared with controls[18,34], but the case–control design of these studies prevented an inference about temporality or causality.

Lutein and zeaxanthin (xanthophylls, i.e. oxygenated carotenoids), which act as antioxidant and anti-inflammatory molecules, are preferentially accumulated in the macula of the retina and may be good candidates for eye/vision protection through various pathways[51]. They have also been posited to represent approximately 70 % of the carotenoids in the brain, and growing evidence favours a role of xanthophylls in the maintenance of cognitive function[33]. In the present study, lutein was found to be strongly associated with CDP, whereas zeaxanthin exhibited a weaker association; thus, the present study does not allow drawing conclusions regarding the role of xanthophylls. Carefully designed studies are needed to test whether serum xanthophylls, which have been found to be correlated with xanthophylls present in the brain[33], exhibit neuroprotective effects.

Generally, traditional reductive approaches used in nutritional epidemiology may not capture the synergy between foods and nutrients[35]. The RRR method yields a linear combination of food groups/nutrients maximising the proportion of the explained variation in intermediate response variables potentially implicated in the associations of interest. As such, the RRR method allows testing hypotheses regarding pathways of interest related to diet and diseases[52,53]. The RRR factor score is a combination of various food intakes influencing biomarker concentrations, but does not necessarily represent a combination of foods and drinks often consumed together. As with other dimension-reducing methods, the determination of the number of retained factors is often arbitrary. In the present study, we focused on the first extracted pattern that optimally explained the variation in carotenoid status. Indeed, the first pattern was positively correlated ($>0·20$) with most of the carotenoids and accounted for 11·66 % of the total variation, while other factors were much more weakly correlated with these biomarkers. In contrast, in the original study carried out by Hoffmann *et al.*[35], food intakes explained more than 90 % of the variation in nutrient intakes. This is not surprising, since nutrient intakes are directly estimated from food intakes. Besides, when biomarkers are introduced as response variables, as in the present study, the percentage of variation in response is much weaker as reported previously[54–56].

A major limitation of the present study was the unavailability of baseline cognitive performance measurements, preventing an inference of causality. No baseline differences in cognitive performance according to DP can be assumed. However, cognitive impairment at baseline leading to modified DP (and thus

**Table 3.** Baseline characteristics of the population across the tertiles of the reduced rank regression-extracted carotenoid-rich dietary pattern score (n 2983)*

(Mean values and standard deviations)

| Variable | Tertile 1 Mean | Tertile 1 SD | Tertile 2 Mean | Tertile 2 SD | Tertile 3 Mean | Tertile 3 SD | P† |
|---|---|---|---|---|---|---|---|
| Male (%) | 67 | | 50 | | 44 | | <0·0001 |
| Intervention group (%) | 52 | | 53 | | 55 | | 0·49 |
| BMI (kg/m$^2$) | 24·8 | 3·4 | 24·4 | 3·3 | 23·8 | 3·2 | <0·0001 |
| Age at cognitive evaluation (years) | 65·4 | 4·5 | 65·4 | 4·5 | 65·7 | 4·6 | 0·29 |
| CES-D score at cognitive evaluation | 8·3 | 7·2 | 8·7 | 7·5 | 9·1 | 7·6 | 0·05 |
| Number of 24 h dietary records | 10·0 | 3·2 | 10·3 | 3·0 | 10·1 | 3·2 | 0·10 |
| Energy intake (kJ/d)‡ | 8420 | 2345 | 8334 | 2222 | 9070 | 2354 | <0·0001 |
| Alcohol (g/d) | 32·5 | 25·8 | 16·9 | 15·9 | 12·0 | 12·7 | <0·0001 |
| Lipids‡ | 40·5 | 5·1 | 39·9 | 5·1 | 39·8 | 5·1 | 0·04 |
| Proteins‡ | 18·7 | 2·8 | 17·8 | 2·6 | 17·1 | 2·6 | <0·0001 |
| Carbohydrates‡ | 40·8 | 6·3 | 42·3 | 5·9 | 43·1 | 6·0 | <0·0001 |
| Plasma carotenoid concentration | | | | | | | |
| Lutein (μmol/l)§ | 0·24 | 0·09 | 0·30 | 0·13 | 0·33 | 0·15 | <0·0001 |
| Zeaxanthin (μmol/l)§ | 0·07 | 0·03 | 0·09 | 0·05 | 0·09 | 0·05 | 0·001 |
| β-Cryptoxanthin (μmol/l)§ | 0·20 | 0·17 | 0·31 | 0·22 | 0·39 | 0·27 | <0·0001 |
| Lycopene (μmol/l)§ | 0·59 | 0·53 | 0·58 | 0·47 | 0·62 | 0·42 | 0·07 |
| α-Carotene (μmol/l)§ | 0·17 | 0·18 | 0·23 | 0·17 | 0·30 | 0·19 | <0·0001 |
| β-Carotene (μmol/l)§ | 0·49 | 0·41 | 0·79 | 0·46 | 1·04 | 0·64 | <0·0001 |
| Cis-β-carorene(μmol/l)§ | 0·09 | 0·16 | 0·12 | 0·22 | 0·11 | 0·19 | 0·71 |
| Education (%) | | | | | | | 0·01 |
| Primary | 24 | | 19 | | 19 | | |
| Secondary | 26 | | 30 | | 26 | | |
| Post-secondary | 50 | | 51 | | 55 | | |
| Physical activity (%) | | | | | | | 0·49 |
| Irregular | 24 | | 23 | | 21 | | |
| < 1 h/d | 28 | | 30 | | 31 | | |
| ≥ 1 h/d | 48 | | 47 | | 47 | | |
| Smoking status (%) | | | | | | | <0·0001 |
| Non-smokers | 42 | | 53 | | 57 | | |
| Former smokers | 42 | | 39 | | 36 | | |
| Current smokers | 15 | | 8 | | 7 | | |
| Occupational status | | | | | | | |
| Unemployed | 7 | | 8 | | 8 | | 0·0002 |
| Manual | 7 | | 5 | | 5 | | |
| Blue collar | 49 | | 55 | | 58 | | |
| White collar | 36 | | 32 | | 28 | | |
| Self-reported memory problems (%) | 32 | | 36 | | 37 | | 0·02 |

CES-D, Center for Epidemiologic Studies Depression Scale.
* Except when otherwise specified.
† P values based on non-parametric Kruskal–Wallis test or Mantel–Haenszel $\chi^2$ trend test.
‡ Values are presented as the percentage of total daily energy intake (without alcohol).
§ Among n 381 participants.

leading to reverse causality) is unlikely, considering the relatively young age of the study population and the ability to follow the comprehensive study protocol (filling out many questionnaires). In addition, we extrapolated the CDP identified among a subsample to the entire sample. This may generate concerns about the generalisability of the DP and CDP data. However, we showed that the DP was relatively robust and that the subsample from which the DP was extracted was similar to the whole sample across many baseline and follow-up characteristics. Furthermore, some authors have suggested that the generalisability of RRR-extracted DP is possible as long as the populations of interest share similar profiles, in particular, with regard to dietary behaviour[57]. Next, caution is needed when generalising the present findings, as the participants were relatively healthy volunteers involved in a long-term nutritional study[58]. Another issue pertains to the additional potential bias due to non-response regarding

the dietary questionnaires and/or cognitive evaluation. However, the use of inverse probability weighting to correct for potential selection bias did not substantially modify the main findings. Finally, residual confounding cannot be excluded regardless of the extensive adjustment for confounders.

The present study also exhibits strengths and other original aspects including its large sample of community-dwelling subjects, its long follow-up period and the use of highly accurate dietary data reflecting midlife exposure. The availability of biomarker data for a subsample of the population allowed the calculation of DP. Next, DP extracted using the RRR procedure allowed analysing associations between food group consumption and cognitive function beyond the existing associations between food consumption practices, as would be estimated by principal component analysis. Indeed, the RRR method is original and versatile. The use of biomarkers as response variables in the RRR procedure allowed exploring

**Table 4.** Associations between the carotenoid-rich dietary pattern score (in tertiles (T) and as continuous variable) and cognitive performance

(Mean differences and 95 % confidence intervals)

| | Models | T1* Mean difference | T1* 95 % CI | T2* Mean difference | T2* 95 % CI | T3* Mean difference | T3* 95 % CI | P† | Continuous variable Mean difference | Continuous variable 95 % CI | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Composite cognitive scores | | | | | | | | | | | |
| | Model 1‡ | 0·00 | – | 1·19 | 0·32, 2·07 | 1·31 | 0·44, 2·19 | 0·003 | 0·66 | 0·34, 0·99 | <·0001 |
| | Model 2§ | 0·00 | – | 0·98 | 0·16, 1·79 | 1·04 | 0·20, 1·87 | 0·02 | 0·58 | 0·27, 0·90 | 0·0002 |
| RI-48 cued recall task | | | | | | | | | | | |
| | Model 1‡ | 0·00 | – | 1·53 | 0·66, 2·41 | 1·79 | 0·91, 2·66 | 0·0001 | 0·91 | 0·59, 1·23 | <0·0001 |
| | Model 2§ | 0·00 | – | 0·88 | 0·01, 1·75 | 0·90 | 0·01, 1·79 | 0·05 | 0·59 | 0·25, 0·92 | 0·001 |
| Forward digit span task | | | | | | | | | | | |
| | Model 1‡ | 0·00 | – | 0·04 | −0·84, 0·92 | −0·28 | −1·16, 0·60 | 0·53 | −0·08 | −0·40, 0·25 | 0·64 |
| | Model 2§ | 0·00 | – | 0·36 | −0·51, 1·23 | 0·14 | −0·75, 1·03 | 0·76 | 0·12 | −0·22, 0·45 | 0·50 |
| Backward digit span task | | | | | | | | | | | |
| | Model 1‡ | 0·00 | – | 1·05 | 0·17, 1·92 | 0·90 | 0·02, 1·78 | 0·05 | 0·34 | 0·01, 0·67 | 0·04 |
| | Model 2§ | 0·00 | – | 1·13 | 0·27, 2·00 | 1·00 | 0·12, 1·89 | 0·03 | 0·39 | 0·06, 0·72 | 0·02 |
| Trail making test | | | | | | | | | | | |
| | Model 1‡ | 0·00 | – | 0·70 | −0·18, 1·57 | 0·89 | 0·01, 1·76 | 0·05 | 0·40 | 0·07, 0·72 | 0·02 |
| | Model 2§ | 0·00 | – | 0·71 | −0·12, 1·54 | 1·09 | 0·25, 1·94 | 0·01 | 0·51 | 0·19, 0·82 | 0·002 |
| Semantic fluency task | | | | | | | | | | | |
| | Model 1‡ | 0·00 | – | 1·28 | 0·41, 2·16 | 1·22 | 0·34, 2·10 | 0·01 | 0·64 | 0·31, 0·96 | <0·0001 |
| | Model 2§ | 0·00 | – | 1·15 | 0·29, 2·01 | 1·00 | 0·12, 1·88 | 0·03 | 0·59 | 0·26, 0·91 | 0·001 |
| Phonemic fluency task | | | | | | | | | | | |
| | Model 1‡ | 0·00 | – | 0·01 | −0·87, 0·89 | 0·56 | −0·32, 1·44 | 0·21 | 0·35 | 0·02, 0·67 | 0·04 |
| | Model 2§ | 0·00 | – | −0·46 | −1·32, 0·39 | −0·14 | −1·02, 0·73 | 0·75 | 0·07 | −0·25, 0·40 | 0·66 |

* Values are mean differences and 95 % CI in cognitive test scores (ANCOVA) using the 1st (top) tertile of the carotenoid-rich dietary pattern score as reference.
† P for linear contrast.
‡ Model 1 is unadjusted.
§ Model 2 is adjusted for age, sex, education, follow-up time between baseline and cognitive evaluation, supplementation group during the trial phase, number of 24 h dietary records, energy intake, BMI, occupational status, tobacco use status, physical activity, reported memory problems at baseline, depressive symptoms concomitant with cognitive function assessment, and history of diabetes/hypertension/CVD.

possible mechanistic pathways through which food group consumption and even specific DP could act on a health outcome. Thus, the present study revealed the food groups whose consumption directly contributed to plasma carotenoid status. Finally, our findings are of major interest from a public health viewpoint, since the use of the RRR procedure allows formulating easily understandable diet-based public health messages. In the cognitive domain, such messages are of utmost importance, given that prevention is a cost-effective strategy and the prevention of dementia should be initiated in middle age when potential cognitive disorders are presymptomatic[1,59].

In conclusion, the present study adds new support with regard to the positive association between a CDP in midlife and subsequent cognitive function, especially in terms of executive functioning and episodic memory, which are cognitive domains particularly vulnerable during the pathological ageing of the brain. Upon confirmation in other settings, these findings may argue that sufficient quantity and variety of coloured fruits and vegetables in one's diet may help to maintain the health of the brain during ageing.

## Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114513003188

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