Iran Red Crescent Med J. 2018 November; 20(11):e64847. doi: 10.5812/ircmj.64847.

Published online 2018 October 31. **Research Article**

Dietary and Serum Level of Antioxidants in the Elderly with Mild Impaired and Normal Cognitive Function: A Case-Control Study

Hormoz Ayromlou[1], Parisa Pourvahed[2], Fatemeh Jahanjoo[3], Homayoun Dolatkhah[4], Seyyed Kazem Shakouri[3] and Neda Dolatkhah[3,*]

[1]Department of Neurology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Tabriz University of Medical Sciences, Tabriz, Iran
[3]Physical Medicine and Rehabilitation Research Center, Aging Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Department of Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

[*]*Corresponding author*: Assistant Professor, Physical Medicine and Rehabilitation Research Center, Faculty of Medicine, Tabriz University of Medical Sciences, Zip Code: 5163995479, Golgasht, Azadi Ave., Tabriz, Iran. Tel: +98-4133369128, Email: neda_dolatkhah@yahoo.com

Received 2017 December 07; **Revised** 2018 January 22; **Accepted** 2018 February 24.

Abstract

Background: Antioxidants are considered essential components in neurodegenerative disease management since they can protect cells from oxidative damage.

Objectives: The current study aimed at evaluating the intake and serum level of selected antioxidants in subjects possessing mild cognitive impairment (MCI) compared with a cognitively intact control.

Methods: The current prospective, case-control study was conducted in Tabriz from December 2016 to August 2017 on a total of 45 patients with MCI as a case group and another 45 healthy subjects matched by age and gender were recruited for the control group. After completing the mini-mental state examination (MMSE-12) questionnaire, the cases with MCI were identified using the modified Peterson standard. By a semi-quantitative food frequency questionnaire (FFQ), the common dietary intakes during the previous year were recorded and blood samples were collected.

Results: Of the 90 subjects in the current study, 61% were male and 39% female, with the mean age of 68.8 ± 5.5 years. There were no significant differences between the two groups with respect to dietary intakes of vitamin C, beta-carotene, and lycopene (P = 0.079, P = 0.413, and P = 0.455, respectively). The results of the studied parameters showed that serum beta-carotene and lycopene (P = 0.004 and P = 0.044, respectively) in healthy subjects were significantly higher than those of the elderly people with MCI. There was a significant correlation between Vitamin C dietary intake and serum level and cognitive scores in MMSE-12 (r = 0.231, P = 0.028 and r = 0.224, P = 0.033, respectively) and also between serum level of lycopene and cognitive scores(r = 0.388, P = 0.000).

Conclusions: The subjects with mild cognitive impairment had a significantly lower serum levels of lycopene beta-carotene antioxidants compared with healthy subjects, and there was a positive correlation between serum level of vitamin C and lycopene and also vitamin C dietary intake and scores in the MMSE-12 test.

Keywords: Antioxidants, Beta-Carotene, Diet, Intake, Lycopene, Mild Cognitive Impairment, Vitamin C

1. Background

Mild cognitive impairment (MCI) refers to the cognitive impairments that go beyond cognitive decline from a normal age, but not to the extent that it interferes significantly with daily activity (1). Although the six basic components of cognition (memory and learning, social function, language, mobility, attention, and performance) potentially can be involved (2, 3), the term "mild cognitive impairment" is synonymous with memory and cognitive changes characterized by age or normal aging (4).

The probability of cognitive decline along with other evidence of nerve degeneration is increasing with the aging population (some of them earlier than others and others more rapidly) (5). The incidence of MCI in people over 65 years old is 10% - 20% according to population studies (6-10). Diagnosis and early intervention in a mild cognitive disorder can delay or prevent the onset of dementia (11). Currently, no drug is approved by the Food and Drug Administration for the treatment of mild impairment (12).

Oxidative stress is an essential component in the pathology of the Alzheimer disease (AD), both in primary molecular changes and during its progression to the final stages (13). Oxidative stress induces mitochondrial anomalies that play an important role in degenerative diseases such as AD (14). Therefore, antioxidants are considered important components since they play a protective role in eliminating free oxygen radicals and preventing neuronal damage caused by these radicals (15). Although studies on the role of enzymes and antioxidant properties, the biological roles of vitamin C on the brain are only recently described, animal studies examined this biological link. Particularly, studies focused on marine guinea pigs, since their inability to biosynthesize vitamin C from glucose is similar to that of humans (16). In addition, it is proven that increasing the number of flavonoids inhibit the development of AD pathology and incite cognitive impairment in rodent models, thus suggesting that flavonoids have a potential therapeutic ability to treat dementia (17). Cross-sectional and longitudinal studies also show that the intake of more flavonoids from food can be accompanied by a better cognitive and evolutionary course (18, 19).

On the other hand, there is a significant correlation between the healthy dietary pattern and the lower risk of MCI or AD (20, 21). This conclusion is further established from the study on the Mediterranean diet, in which adherence to this diet is associated with a slower cognitive decline and a reduced risk of AD (22). It is also shown that the consumption of sufficient fruits, vegetables, and marine products may reduce the risk of MCI in the elderly (23). However, in a recent review of the relationship between vitamin C status and cognitive function in healthy people with cognitive impairment, the relationship between vitamin C concentration and cognitive function based on mini-mental state examination (MMSE) was not observed in people with cognitive impairment (16).

Many studies are conducted on the relationship between serum antioxidant levels and cognitive status, more on vitamins C and E, dementia samples and their possible role in preventing it (24-27), but studies on the role and effect of other carotenoids, especially in the case of MCI, are rare.

Recently, much attention is paid to lycopene and its potential protective properties in various models of central nervous system dysfunction. A nurses' health study (NHS) suggested that long-term high levels of lycopene from food sources were associated with a slower decrease in cognitive function both on a general and verbal scale (28).

The association of high beta-carotene serum levels with better comprehension skills among the healthy elderly population is also observed (29). Also, in patients with AD, serum levels of beta-carotene were low in some studies in comparison with healthy controls, which did not depend on individuals' age, age at the onset of AD symptoms, and individual scores on MMSE (30). However, in other studies, there was no inverse association between beta-carotene and flavonoid regimens with impaired or decreased cognitive function (31).

Generally, studies on oral intakes and serum levels of selected antioxidants, especially lycopene and beta-carotene, in MCI, are cognitively rare with respect to the healthy elderly, and studies on the healthy or demented elderly have conflicting results. It is often subject to restrictions (16).

2. Objectives

The current study aimed at evaluating the intake and serum level of selected antioxidants in subjects possessing MCI compared with a cognitively intact control.

3. Methods

3.1. Patients

The current prospective, case-control study was conducted on elderly people referred for paraclinical testing to the Neurology Department of Imam Reza state Hospital in Tabriz, Iran, from December 2016 to August 2017. Forty-five urban patients and 45 urban healthy control individuals were recruited in the study through non-random convince sampling method.

Patients were included if they had the following criteria: (1) Amnestic MCI diagnosis using the modified Peterson standard (Memory problems for a period of at least two weeks are not very severe symptoms and fulfill Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for dementia, cognitive function is 1.5 standard deviations under the soft-moderated age for at least one neurocytogenesis test, and daily life activities are basically preserved.) (32), which necessitates cognitive disorder that is inadequate to be dementia and commonly undamaged function, (2) age over 65 years, (3) non-acute psychosis, (4) lack of lethal disease or mental disorders (i.e., depression, schizophrenia, bipolar disorder, etc.), (5) no intake of any dietary supplementation recognized to interfere with nutrition condition or cognitive function three months before the study, (6) not living in a nursing home, and (7) capability to respond to queries. The exclusion criteria included the unwillingness of the elderly to participate in the study and other conditions such as daily calorie intake less than 800 or more than 3500 (33), intake of supplements and/or certain diets, using psychiatric and/or hypnotic drugs, presence of apparent malnutrition, mental illness, history of dementia in the family, history of brain surgery, brain injury, and smoking.

Subjects with MCI were categorized into amnestic or non-amnestic and single or multiple domains according to the cognitive domain(s) with deficiency, defined as 1.5 SD inferior than age proper reference standards (11). Diagnosis of amnestic MCI was primarily knowledgeable by California verbal learning test, 2nd edition (CVLT-II) short-form scores (the CVLT-II is an assessment speaking occasional memory with instant and 10-minute hindered recall scores (34)), and diagnosis of non-amnestic MCI was knowledgeable by scores on digit span, and category and verbal fluency tests. Digit span is an examination of consideration with forward and backward scores (35). Category and verbal fluency measure semantic recall, requiring the participant to say as many words as possible appropriate to a given class (for the WISE examination, "vegetables" and words that begin with the letter F) in one minute (36). If the criteria for MCI were encountered but the category of MCI could not be recognized, the subjects were classified as indeterminate MCI.

Of the 211 individuals examined for inclusion in the study, 86 patients were diagnosed with MCI. Six patients withdrew after further information. Two patients had depression. Five patients had an intake of antioxidant supplements during three months ago, and finally, eight patients were using psychiatric drugs. Forty-five out of the 65 remaining patients were randomly selected and entered into the study.

Simultaneously, a control group comprising subjects with no active medical history and no personal or family history of neuropsychiatric disorders were randomly assigned to a hospital by clinical interviews. The reason for these referrals to the hospital was musculoskeletal disease (n = 19), ear, throat, and nose problems (n = 10), digestive disease (n = 12), and general surgery (n = 4). They were functional in the normal range and did not have criteria for MCI and dementia.

3.2. Measurements

All participants were interviewed accompanied by their caregivers, and then all of them completed demographic and lifestyle information questionnaires through face-to-face interaction with a trained interviewer. The demographic and lifestyle questionnaire was designed to provide information on general characteristics of a person including the age, gender, education, marital status, use of alcohol, medical history, consumed drugs, dietary supplements, and lifestyle habits. This information was gained by asking the individual or his/her companion. The examined individuals were asked to report on the use of any supplements as part of their medications, including vitamins, fish oil, etc.

Measurement of height in a standing position without shoes to the nearest 0.1 cm using a non-stretched tape measure (Seca) and weight after overnight fasting, without shoes and wearing minimal clothing, to the nearest 0.1 kg by the use of a digital scale (Seca) was recorded by a nutrition expert. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

The cognitive status of the individuals was examined by a psychologist and MMSE (37). The translated version of this questionnaire was provided by the Iranian Research Centre of Ageing, the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran. The individuals fall into either of the categories of dementia (< 21), MCI (21 - 26) and cognitively healthy (> 26) based on points obtained in the test. To reject pseudo-dementia cases before completing the MMSE-12 questionnaire, individuals were examined by the 15-item geriatric depression scale (GDS-15) and were not included in the study if they scored 5 or higher. The validity of the Persian version of GDS-15, used to determine false dementia, was confirmed through a simultaneous narrative with the composite international diagnostic interview (CIDE) form, and its reliability was assessed by the Cronbach's alpha coefficient of 0.9 (38).

The common dietary intakes during the previous year were collected using a semi-quantitative food frequency questionnaire (FFQ) (39). In summary, the questionnaire contained 168 food items; the average of its food items was determined by the average of the serving size consumed by the Iranian population. To obtain the intake of each item of food, either the category was never or less than once a month, one to three times a month, once a week, two to four times a week, five to six times a week, or once a day, two to three times a day, four to five times a day, and six times or more per day, or three sizes: Too small to divide the share (half of the defined mean or less), medium (equal to the defined mean), and large (above the defined mean) (40). The reported frequencies were converted to daily intakes for any food based on the amount of home meal modules. Food and beverage coding, to assess the amount of energy and nutrients received, was determined using the Nutritionist IV software, adapted for Iranian foods. The reliability was assessed by the Cronbach's alpha coefficient of 0.87 (41).

3.3. Sample Preparation and Laboratory Evaluations

Blood samples (10 mL) were collected to measure serum vitamin C, alpha and beta-carotene, and lycopene at the laboratory of Imam Reza Hospital in Tabriz. All the necessary guidelines for blood sampling and prevention of hemolysis of blood during blood collection were observed. Ideal preparation and maintenance conditions including protection of samples from light, rapid serum separation, rapid acidification, and freezing at a temperature below -20°C were strictly adhered to in the collection of samples (42). The blood samples were quickly transferred to the centrifuge apparatus for serum separation and centrifuged at 3500 rpm for 10 to 15 minutes at 4°C. The serum samples were then frozen at -80°C until biochemical analysis.

Vitamin C level in the serum was measured using the colorimetric method introduced by McMurray and Gowenlock (43). Total lycopene and beta-carotene concentration were measured by high-performance liquid chromatography (HPLC) with ultraviolet detection (44).

3.4. Assessment of Physical Activity

The individuals were divided into three groups as per level of physical activity: Low physical activity (sedentary lifestyle and limited physical activity limited to cooking, sewing, computer work, etc.), moderate physical activity (such as cleaning, childcare and other tasks that require not too much body movement), and high physical activity (people accustomed to physical activities such as walking, running, cycling, and swimming regularly) (45).

3.5. Ethical Consideration

The study protocol was discussed and approved at the 228th session of the Ethics Committee of the Tabriz University of Medical Sciences (code number: IR.TBZMED.REC.1396.62). Before inclusion of participants in the study, the details of the study protocol were explained to them. Informed consents were obtained from all study participants, and they were excluded if they wished not to undergo the tests.

3.6. Sample Size

In details, the sample size was calculated with G-power software version 3.1.2 using the Pukak formula to compare differences between the groups. According to the effect size of 65% based on the study by Rinaldi et al. (46), a significance level of 0.05, and a power of 80%, approximately 40 subjects were estimated for each group. Assuming 10% drop-out rate, 45 subjects were required for each group. Finally, a case group of 45 patients with MCI and 45 healthy subjects were selected from the clients with a MMSE score of 26, matched by age and gender.

3.7. Statistical Analysis

The data were analyzed with SPSS version 16. The one-sample Kolmogorov-Smirnov test was performed to determine the frequency distribution of the studied variables. In the descriptive statistics section, frequency and percentage were used for qualitative variables and mean and standard deviation (SD) were used for quantitative variables. In the case of non-normal data, median and (25th percentile - 75th percentile) was used to measure central tendency and dispersion of data and also non-parametric statistical methods were used in analytical statistics.

In order to compare the quantitative demographic variables and the studied parameters between the two groups, independent samples *t*-test (the Mann-Whitney test if required) was applied. The Chi-square test was used to compare qualitative demographic variables (the exact test if required) between the two groups. The relationship between dietary intakes and serum levels of selected antioxidants with cognitive impairment score were evaluated using the Spearman correlation coefficient. All of the tests were two-way, and the significance level was considered as 0.05. Data entries and P-values were obtained via 1000 bootstrap and Monte Carlo samples.

4. Results

In the current study, 90 participants with the mean age of 68.84 ± 5.31 years and BMI of 26.65 ± 2.92 kg/m^2 were recruited. Of the 90 subjects in the study, 54.44% had higher education (college and university degrees), and 70.0% had physical illness; i.e., diabetes (5%), high blood pressure (44%), and dyslipidemia (21%). In terms of BMI, 35.55% were in normal status, 42.22% had overweight, and 22.22% had obesity (Class 1).

The descriptive information of the demographic variables studied in the study is presented in Tables 1 and 2. Table 1 shows the mean and SD of the quantitative demographic variables, including age, BMI, and sleep time per hour. Table 2 shows the frequency and percentage of each of the qualitative demographic variables. The results of the independent *t*-test for quantitative variables and Chi-square or the Fisher exact test for qualitative variables showed no statistically significant difference between the two groups based on demographic characteristics ($P > 0.05$).

The comparison of studied parameters showed no significant differences between the two groups with respect to dietary intakes of vitamin C, beta-carotene, and lycopene (P = 0.079, P = 0.413, and P = 0.455, respectively).

However, serum levels of beta-carotene and lycopene were significantly higher in the healthy subjects compared with those of the elderly subjects with MCI (P = 0.004 and P = 0. 044, respectively), there was no significant difference with respect to the serum level of vitamin C between the two studied groups (P = 0.280).

There was a positive and insignificant correlation between vitamin C intake and its serum level (r = 0.191, P = 0.072); therefore, this association was still positive and insignificant in each group ((r = 0.136, P = 0.373) for the case group and (r = 0.214, P = 0.158) for the control group).

There was a positive and significant correlation between vitamin C intake and MMSE scores (r = 0.592, $P < 0.001$); therefore, this association was still positive and significant in each group ((r = 0.905, $P < 0.001$) for the case group and (r = 0.762, $P < 0.001$) for the control group).

There was a positive significant correlation between serum vitamin C and MMSE scores (r = 0.224, P = 0.033). The relationship between the two groups was positively and significantly correlated with serum vitamin C and MMSE scores in the case group (r = 0.336, P = 0.024); however, this relationship was not significant in the control group (r = 0.141, P = 0.355).

There was no significant relationship between beta-carotene diet and its serum level (r = 0.008, P = 0.940),

Table 1. Quantitative Demographic Characteristics of the Study Participants[a]

Variable	Total (N = 90)	Case (N = 45)	Control (N = 45)	P Value[b]
Age, y	68.84 ± 5.31	68.13 ± 5.25	69.56 ± 5.34	0.713
Weight, kg	71.75 ± 10.48	70.10 ± 9.35	73.40 ± 11.38	0.503
Height, cm	163.81 ± 6.33	163.20 ± 6.05	164.42 ± 6.62	0.554
BMI, kg/m^2	26.65 ± 2.92	26.28 ± 2.74	27.03 ± 3.08	0.779
Sleep duration, h	7.15 ± 1.38	6.96 ± 1.36	7.36 ± 1.40	0.620

[a] All data are expressed as mean ± SD.
[b] Independent samples *t*-test.

and the relationship was not significant in the groups separately either ((r = 0.141, P = 0.356) in case group and (r = 0.010, P = 0.950) in the control group).

There was a positive and significant correlation between beta-carotene intakes and MMSE test scores (r = 0.145, P = 0.241), and was still positive and non-significant in each group ((r = 0.223, P = 0.141) in the case group and (r = 0.098, P = 0.522) in the control group).

No significant relationship was observed between serum beta-carotene and MMSE test scores, (r = 0.097, P = 0.363), and it was not significant in each group separately ((r = 0.216, P = 0.153) in the case group and (r = 0.021, P = 0.889) in the control group).

There was no significant relationship between dietary intake of lycopene and its serum level (r = 0.059, P = 0.581) ((r = 0.128, P = 0.401) in the case group and (r = 0.057, P = 0.710) in the control group).

There was a positive and insignificant correlation between consuming a lycopene diet and MMSE test scores (r = 0.178, P = 0.094) and between the two groups ((r = 0.157, P = 0.303) in the case group and (r = 0.100, P = 0.515) in the control group).

A positive and significant correlation was observed between serum lycopene levels and MMSE scores(r = 0.388, P < 0.001), and positive but insignificant between the groups ((r = 0.269, P = 0.074) in case group, and positive and significant (r = 0.347, P = 0.020) in the control group).

There was no significant difference in terms of consuming fruit between the two studied groups (P = 0.072). There was a positive and significant relationship between fruit intake and MMSE scores (r = 0.262, P = 0.012). In the two groups, this association was still positive, but not statistically significant ((r = 0.138, P = 0.367) in the case group and (r = 0.176, P = 0.248) in the control group).

Vegetables intake was significantly higher in the cases compared with controls (P = 0.048). There was a positive and significant correlation between consuming vegetables and MMSE test scores (r = 0.199, P = 0.059). Also there was no significant association in each group ((r = 0.008, P = 0.958) in the case group and (r = 0.161, P = 0.290) in the control group).

No significant correlation was observed between any of the serum levels and food intakes with BMI (Tables 3 and 4).

5. Discussion

Based on the authors‘ best knowledge, the current study was one of the few studies investigating dietary intakes and serum levels of selected antioxidants (vitamin C, beta-carotene, and lycopene) and their relationship with cognitive performance in the elderly people with MCI compared with the elderly with normal cognitive function. Based on the results of the current study, despite the fact that different levels of antioxidants were not consumed, serum levels of antioxidants (beta-carotene and lycopene) were significantly lower in patients with MCI compared with the elderly with normal cognitive function. There was a positive correlation between serum level of vitamin C and lycopene and also vitamin C dietary intakes and scores in the MMSE-12 test of the study participants.

According to the currently available knowledge, the positive impact of dietary intake of vitamins on better health status seems undeniable (47, 48). However, their impact on cognitive health is not so obvious. There is a huge interest among nutrition researchers regarding the dietary effects on cognitive function and delaying neurodegenerative disease in old age.

With the aging of societies, AD is the most common neurodevelopmental disorder (49). With recent advances in medical sciences, life expectancy in the developed countries increased, resulting in the over 65-year-old population being one of the most populated age groups in the world. The increased burden of these life-threatening conditions (50) and lack of disease-modifying drugs led studies towards developing preventive strategies.

Many epidemiological studies suggest that nutrition has the potential of a protective role in arresting the decline of cognitive function (51). Particularly, diets such as a Mediterranean diet rich in plant foods, namely fruits, vegetables, beans, and cereals are associated with a decreased risk of cognitive impairment and dementia (52).

Table 2. Qualitative Demographic Characteristics of the Study Participants[a]

Variable	Total (N = 90)	Case (N = 45)	Control (N = 45)	P Value
Gender				0.834[b]
Male	45	22 (48.9)	23 (51.1)	
Female	45	23 (51.1)	22 (48.9)	
BMI, kg/m^2				0.897[b]
Normal weight	32	16 (35.6)	16 (35.6)	
Overweight	38	18 (40.0)	18 (40.0)	
Obesity (class I)	20	11 (24.4)	11 (24.4)	
Marital status				0.569[c]
Single	4	1 (2.2)	3 (6.7)	
Married	63	31 (68.9)	32 (71.1)	
Other	23	13 (28.9)	10 (22.2)	
Occupational status				0.517[c]
Workless	43	22 (48.9)	21 (46.7)	
Employed	9	6 (13.3)	3 (6.7)	
Retired	39	17 (37.8)	21 (46.7)	
Educational status				0.684[b]
Literacy	2	2 (4.4)	0 (0.0)	
High school	39	17 (37.8)	22 (48.9)	
College level	38	20 (44.4)	18 (40.0)	
University level	11	6 (13.3)	5 (11.1)	
Physical illness				0.819[b]
Yes	63	31 (68.9)	32 (71.1)	
No	27	14 (31.1)	13 (28.9)	
Medical history				0.745[b]
Diabetes	5	2 (4.44)	3 (6.66)	
TIA/stroke	7	5 (11.11)	2 (4.44)	
MI	7	3 (6.66)	4 (8.88)	
Atrial fibrillation	9	6 (13.33)	3 (6.66)	
Hypertension	15	10 (22.22)	5 (11.11)	
Psychological illness				0.784[b]
Yes	16	7 (15.6)	9 (20.0)	
No	74	38 (84.4)	36 (80.0)	
Family history of cognitive disease				0.816[b]
Yes	16	9 (20.0)	7 (15.6)	
No	74	36 (80.0)	38 (84.4)	
Physical activity				> 0.999[b]
Inactive	74	37 (82.2)	37 (82.2)	
Moderate active	16	8 (17.8)	8 (17.8)	
Active	0	0 (0.0)	0 (0.0)	

[a] All values are expressed as No. (%).
[b] Chi-square test.
[c] The Fisher exact test.

Table 3. Comparison of the Dietary Intake Parameters Between the Two Groups[a]

Variable	Case (N = 45)	Control (N = 45)	P Value
Total energy intake, kCal/d	2189.00 (1979.00 - 238.50)	2164.00 (1846.50 - 23.02)	0.134[b]
Vitamin C intake, mg/d	93.58 (75.11 - 116.55)	106.66 (86.35 - 146.04)	0.079[b]
Beta-carotene intake, mg/d	945.28 (911.71 - 1074.52)	1029.65 (917.86 - 1180.33)	0.413[b]
Lycopene intake, μg/d	2884.87 (2072.58 - 4129.63)	2964.45 (1703.37 - 5990.40)	0.455[b]
Fruit, g/d	167.42 (122.30 - 191.44)	169.50 (103.40 - 218.06)	0.720[b]
Servings of fruit per day			0.826
0	1 (2.22)	2 (4.44)	
1 - 2	16 (35.55)	13 (28.88)	
3 - 4	28 (62.22)	28 (62.22)	
> 5	0 (0.00)	2 (4.44)	
Vegetable, g/d	261.55 (205.60 - 363.65)	318.64 (274.45 - 379.71)	0.09[b]
Servings of vegetables per day			0.006
< 2	1 (2.22)	0 (0.00)	
3 - 4	10 (22.22)	6 (13.33)	
> 5	34 (75.55)	39 (86.66)	

[a] Values are expressed as median (25th percentile - 75th percentile) or No. (%).
[b] The Mann- Whitney U test.

Table 4. Comparison of Serum Parameters Between the Two Groups[a]

Variable	Case (N = 45)	Control (N = 45)	P Value[b]
Serum vitamin C, mg/dL	14.73 (11.67 - 16.42)	15.88 (14.62 - 18.69)	0.280
Serum beta-carotene, μg/dL	1.58 (1.06 - 2.54)	2.04 (1.39 - 2.91)	0.004
Serum lycopene, μg/dL	1.35 (0.91 - 1.86)	1.50 (1.07 - 2.04)	0.044

[a] Values are expressed as median (25th percentile - 75th percentile).
[b] The Mann-Whitney U test.

These beneficial effects are partly attributable to nutritional compounds such as vitamin C, polyphenols, and carotenoids. However, the contribution of each nutrient to the protective effects of plant food against disorders and cognitive decline is still not fully understood (53).

Carotenoids are natural pigments produced by plants, algae, and photosynthetic bacteria. Based on the presence of oxygen, carotenoids are divided into two groups, carotene, and xanthophyll. The former does not have oxygen, and the latter has oxygen. Carotenoids owe their antioxidant activity to their dual conjugate bond. In addition, carotenoids affect cell cycle development, intercellular communication, growth factor, and the immune function (54).

Previous studies show the role of vitamin C in peripheral blood cells as an antioxidant biomarker in various diseases (55, 56). Based on the results of in vivo and in vitro studies, vitamin C also acts as a modulator of neuronal function in cholinergic and glutamatergic neurons (57), which have a strong association with the cognitive and pathological functioning of AD (58, 59). Vitamin C exists in fruit and vegetables, is often added as a preservative to food products, and is used as a dietary supplement as well (60).

Based on the results of the current study, vitamin C antioxidant dietary intake was lower in samples with MCI compared with controls without cognitive impairment; however, this difference was not statistically significant. A significant strong positive correlation was observed between vitamin C intakes and cognitive scores in MMSE-12 in both groups.

A study on 37 healthy adults in 2015 showed that consuming orange juice leads to the reduction of cognitive decline rate (61), which is attributed to high content of orange juice flavones since the association of flavonoids decreases the rate of cognitive decline (62, 63). But it is likely that the concentration of vitamin C in orange juice is involved in the observed effects, since other studies show that supplementation of elderly people with vitamins C and E can maintain cognitive function (64, 65).

In a study in the early 70's on people aged 65 years and above, vitamin C supplementation was recommended as the only predictive factor for cognitive ability (66) in assessing the association between vitamin C intake and cognitive function. However, in a sample of 5182 elderly subjects (aged 55 - 95 years) through a cross-sectional study, no significant relationship was observed between vitamins C and E intake and cognitive function (67).

Elders are often unable to prepare their meals and have problems with chewing; they may have poor and flawed food choices and do not include fruits and vegetables in their diet. Also, it should be noted that the assessment of food intake using a 24-hour dietary recall questionnaire, in people with cognitive impairment due to disrupted memory, is not very reliable.

Based on the results of the current study, the serum level of vitamin C in the elderly with MCI was lower than that of the elderly in the control group without cognitive impairment, although it was not significant statistically. Also, the results showed a weak significant positive correlation between serum levels of vitamin C and scores in MMSE test. This assessment separated the two groups with a significant positive correlation in the subject group with MCI, but not in the healthy controls, wherein they showed higher serum levels of vitamin C and higher MMSE scores.

Several studies demonstrated significantly lower concentration of vitamin C serum level in patients with AD when compared with healthy controls (68, 69).

Pearson et al. (26), in a cohort of 404 subjects, reported that lower levels of MCI were observed in individuals with the highest serum vitamin C concentrations. Compared with the dietary vitamin C, there was a strong correlation between the serum levels of vitamin C and cognitive impairment markers (Montreal cognitive assessment (MoCA) version 7.1).

However, in a number of other studies, in line with the current study results, there was no difference in serum vitamin C levels in patients with cognitive impairment and the Alzheimer dementia with those of their matched controls (27, 70-72).

In a cross-sectional study on the Maryland population in the United States, no correlation was observed between the serum levels of vitamin C and the cognitive performance scores measured with the MMSE (73). Furthermore, in a study (27) on patients with AD scored 27 - 28 in the MMSE test, in one of the public hospitals in Tokyo, there was a weak relationship between the level of vitamin C in peripheral blood flow and MMSE scores ($r = 0.17$).

The conflict in the results of previous studies suggested that in the patients with AD, the other pathological factors affected vitamin C level. It should be always considered that vitamin C is needed to neutralize free oxygen radicals and radical recycling of alpha-tocopherol. Therefore, the observed beneficial effects of vitamin E are often at least partially dependent on vitamin C levels, although this effect is not directly observed. All physiological and biochemical actions of vitamin C are due to their ability to donate electrons (as a reducing agent). At physiological concentrations, vitamin C (ascorbic acid) is a powerful antioxidant and a free radical cleanser in various tissue including the central nervous system (74). Oxidative stress is generally associated with aging, while old age is a major risk factor for AD. According to recent evidence, oxidative stress plays a major role in pre-AD phases, including MCI (75). Other basic mechanisms include modulating neuronal inflammation, inhibiting the amyloid peptide beta ($A\beta$) fibrillation, and chelation of iron, copper, and zinc (76). Furthermore, it should be noted that in these studies, only serum levels of vitamin C were investigated and vitamin C levels were not studied in lymphocytes and other blood components.

Regarding the use of carotenoids through diet, longitudinal studies generally failed to find evidence in favor of the protective effects of beta-carotene on the risk of dementia or AD, and the data collected in a recent meta-analysis from five cohort studies only yielded significant borderline results (17, 77).

Based on the results of the current study, serum levels of lycopene in patients with MCI were lower than that of the healthy controls. This difference was not significant in terms of dietary intake of this antioxidant. Although the lycopene was significantly correlated with MMSE-12 score in the control group, no significant correlation was observed between lycopene and MMSE-12 score in the case group. The effects of lycopene on the protection of cognitive function are shown in experimental studies on rats (78). In a study conducted to assess the protective effects of lycopene on learning and memory impairment and the potential mechanism for high-fat dietary rats (HFD), lycopene significantly reduced the learning and memory impairments and prevented the reduction of dendritic spine density (79). The results of case-control studies often show low levels of carotenoids in people with cognitive impairment compared with non-impaired controls. For example, Mecocci et al. (80), compared the serum levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and non-enzyme antioxidants in elderly patients with AD and healthy matched controls in terms of age and gender. Level of 8-OHdG was significantly higher than oxidative damage index in patients with AD, which was associated with a decrease in lycopene levels ($r = 0.560$, $P < 0.001$) in such patients. However, cross-sectional analyses had contradictory results (29, 81).

Several studies (82, 83) showed that lycopene inhibited the apoptotic mitochondrial pathway and free radical production of more oxidizing agents. Lycopene can inhibit neuronal inflammation too (84). In a cross-sectional study, Perrig et al., showed that high serum levels of beta-carotene were associated with better memory performance (reminding, recognition, and vocabulary) in 442 healthy subjects aged 65 - 94 years. Regarding the beta-carotene regimen, there was no relationship between oral intake and cognitive decline in the study by Morris et al. (85), while in subsequent studies low-level beta-carotene was associated with cognitive impairment measured with MMSE (67). Zaman et al. (86), in a case-control study, showed that serum levels of beta-carotene were significantly lower in patients with AD and multi-infarction dementia than in healthy controls (< 0.001 μM/L in AD, and < 0.13 - 0.30 μM/L in multivariate dementia compared to 0.31 - 13.5 μM/L in healthy controls).

In the current study, dietary intake of beta-carotene was not significantly different between the two groups; however, the serum level of beta-carotene was significantly higher in healthy samples than in elderly patients with MCI. The results of the Pearson correlation coefficient showed no significant and positive correlation between consumption and serum beta-carotene levels of cognitive scores in the MMSE-12 test in either of the groups.

In a number of studies, there was a greater emphasis on the type of foods to be used for nutrition, and not just on getting a specific micronutrient. For example, nutritional reminders with cognitive tests were studied and examined in a study on an over 60-year-old population. A strong correlation was observed between the ingestion of fruits and vegetables with cognitive function (87). Chen et al. (88), in their study on three dominant food patterns (vegetables, meat, and traditional) in the elderly population of China with cognitive function, found their effects applied to different components of cognitive function. According to the results of this study, the food pattern with moderate to high scores was associated with significant protection against logical memory loss (recall I: β = 0.16 - 0.18, odds ratio (OR) = 0.42 - 0.48; recall II: β = 0.17 - 0.21), while the score pattern of vegetables was associated with reduced performance (β = -0.22). The pattern of food intake of high meat products protected against attention decline (β = 0.20 - 0.22). The traditional Chinese food patterns protected against logic loss and memory (β = 0.20 - 0.22). However, there was no significant relationship between these food patterns and the global recognition.

The results of this study showed that the fruit and vegetable intake were not significantly different in subjects with MCI and controls without cognitive impairment. However, there was a positive correlation between intakes of fruits and MMSE score that was statistically significant (r = 0.262, P = 0.012).

In an animal study, the protective effects of spinach on neuronal function were more powerful than those of fruits (such as blackberries and blueberries) (89). Green vegetables are rich in vitamins, folate, flavonoids, and carotenoids. Tuberous vegetables are rich in protein, vitamin C, vitamin E, and beta-carotene (90-92).

The current study had limitations that should be considered in future studies. The observational nature in which the relationship is not causality was one of the most important limitations of the current study. While the current study focused on the comparison of diet and serum levels of vitamin C, beta-carotene, and lycopene, diet and serum levels of other nutrients can play a role in this difference. Moreover, the dietary questionnaires lack details on the dietary habits throughout the life of a person, and documentation of food intake is very difficult for the subjects. Food repellency and its accuracy are more and more endangered in elderly people with memory and cognitive function loss, and the shortage of dietary intake may be due to poor and inadequate dietary intake. It is very difficult to identify this problem in cross-sectional studies. On the other hand, the serum concentration of the examined antioxidants is related to the recent diet, rather than the long-term intake. The next limitation was the type of instrument used to evaluate the cognition. MMSE is an effective tool to measure cognition in patients with neurodegenerative disorders (25), but its sensitivity to detect cognitive difference is questionable in healthy people (93). Also, cognitive function is studied in general rather than in its components. The next limitation was the selection of healthy cases that should be considered in demographic studies.

Although, the current study was a single center and generalization of its results to the whole population is less reliable, the presence of control group and comparing data with normal healthy matched people was the strong point of the study.

5.1. Conclusions

In summary, the results of the current cross-sectional observational study suggested lower levels of serum lycopene and beta-carotene antioxidants in people with MCI compared with subjects without MCI despite the apparently similar dietary intakes. Also, there was a positive correlation between the serum levels of vitamin C and lycopene antioxidants and cognitive scores in the MMSE-12 test, although this relationship was not significant for dietary intakes of antioxidants except for vitamin C. These results can be the basis of new to investigate the role of antioxidants in protecting healthy cognitive function.

Acknowledgments

Authors are grateful to all participants that helped to conduct the current study. The authors would like to thank the staff of Physical Medicine and Rehabilitation Research

Center of Tabriz University of Medical Sciences, Tabriz, Iran for their assistance during the study.

Footnotes

Authors' Contribution: Hormoz Ayromlou, Parisa Pourvahed, and Neda Dolatkhah: Conducting the study and reporting data; Fatemeh Jahanjoo: Data collection, and drafting and reviewing the manuscript; Homayoun Dolatkhah and Seyyed Kazem Shakouri: Clinical examination, data collection, and drafting or reviewing the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding/Support: This study was financially supported by Physical Medicine and Rehabilitation Research Center of Tabriz University of Medical Sciences, Tabriz, Iran.

References

1. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Kokmen E, Tangelos EG. Aging, memory, and mild cognitive impairment. *Int Psychogeriatr*. 1997;**9 Suppl 1**:65–9. [PubMed: 9447429].
2. Sachdev PS, Blacker D, Blazer DG, Ganguli M, Jeste DV, Paulsen JS, et al. Classifying neurocognitive disorders: The DSM-5 approach. *Nat Rev Neurol*. 2014;**10**(11):634–42. doi: 10.1038/nrneurol.2014.181. [PubMed: 25266297].
3. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders (DSM-5®)*. 5th ed. American Psychiatric Publishing; 2013.
4. Sanford AM. Mild cognitive impairment. *Clin Geriatr Med*. 2017;**33**(3):325–37. doi: 10.1016/j.cger.2017.02.005. [PubMed: 28689566].
5. Noorbakhsh F, Overall CM, Power C. Deciphering complex mechanisms in neurodegenerative diseases: The advent of systems biology. *Trends Neurosci*. 2009;**32**(2):88–100. doi: 10.1016/j.tins.2008.10.003. [PubMed: 19135729].
6. Busse A, Hensel A, Guhne U, Angermeyer MC, Riedel-Heller SG. Mild cognitive impairment: Long-term course of four clinical subtypes. *Neurology*. 2006;**67**(12):2176–85. doi: 10.1212/01.wnl.0000249117.23318.e1. [PubMed: 17190940].
7. Di Carlo A, Lamassa M, Baldereschi M, Inzitari M, Scafato E, Farchi G, et al. CIND and MCI in the Italian elderly: Frequency, vascular risk factors, progression to dementia. *Neurology*. 2007;**68**(22):1909–16. doi: 10.1212/01.wnl.0000263132.99055.0d. [PubMed: 17536047].
8. Plassman BL, Langa KM, Fisher GG, Heeringa SG, Weir DR, Ofstedal MB, et al. Prevalence of cognitive impairment without dementia in the United States. *Ann Intern Med*. 2008;**148**(6):427–34. [PubMed: 18347351]. [PubMed Central: PMC2670458].
9. Manly JJ, Tang MX, Schupf N, Stern Y, Vonsattel JP, Mayeux R. Frequency and course of mild cognitive impairment in a multiethnic community. *Ann Neurol*. 2008;**63**(4):494–506. doi: 10.1002/ana.21326. [PubMed: 18300306]. [PubMed Central: PMC2375143].
10. Lopez OL, Jagust WJ, DeKosky ST, Becker JT, Fitzpatrick A, Dulberg C, et al. Prevalence and classification of mild cognitive impairment in the cardiovascular health study cognition study: Part 1. *Arch Neurol*. 2003;**60**(10):1385–9. doi: 10.1001/archneur.60.10.1385. [PubMed: 14568808].
11. Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, et al. Mild cognitive impairment. *Lancet*. 2006;**367**(9518):1262–70. doi: 10.1016/S0140-6736(06)68542-5. [PubMed: 16631882].
12. Petersen RC. Clinical practice. Mild cognitive impairment. *N Engl J Med*. 2011;**364**(23):2227–34. doi: 10.1056/NEJMcp0910237. [PubMed: 21651394].
13. Balmus IM, Strungaru SA, Ciobica A, Nicoara MN, Dobrin R, Plavan G, et al. Preliminary data on the interaction between some biometals and oxidative stress status in mild cognitive impairment and Alzheimer's disease patients. *Oxid Med Cell Longev*. 2017;**2017**:7156928. doi: 10.1155/2017/7156928. [PubMed: 28811866]. [PubMed Central: PMC5546061].
14. Raza H, John A. Streptozotocin-induced cytotoxicity, oxidative stress and mitochondrial dysfunction in human hepatoma HepG2 cells. *Int J Mol Sci*. 2012;**13**(5):5751–67. doi: 10.3390/ijms13055751. [PubMed: 22754329]. [PubMed Central: PMC3382802].
15. Samuel S, Kathirvel R, Jayavelu T, Chinnakkannu P. Protein oxidative damage in arsenic induced rat brain: Influence of DL-alpha-lipoic acid. *Toxicol Lett*. 2005;**155**(1):27–34. doi: 10.1016/j.toxlet.2004.08.001. [PubMed: 15585356].
16. Travica N, Ried K, Sali A, Scholey A, Hudson I, Pipingas A. Vitamin C status and cognitive function: A systematic review. *Nutrients*. 2017;**9**(9). doi: 10.3390/nu9090960. [PubMed: 28867798]. [PubMed Central: PMC5622720].
17. Crichton GE, Bryan J, Murphy KJ. Dietary antioxidants, cognitive function and dementia: A systematic review. *Plant Foods Hum Nutr*. 2013;**68**(3):279–92. doi: 10.1007/s11130-013-0370-0. [PubMed: 23881465].
18. Vauzour D. Effect of flavonoids on learning, memory and neurocognitive performance: Relevance and potential implications for Alzheimer's disease pathophysiology. *J Sci Food Agric*. 2014;**94**(6):1042–56. doi: 10.1002/jsfa.6473. [PubMed: 24338740].
19. Spencer JP. The impact of fruit flavonoids on memory and cognition. *Br J Nutr*. 2010;**104 Suppl 3**:S40–7. doi: 10.1017/S0007114510003934. [PubMed: 20955649].
20. Solfrizzi V, Panza F, Frisardi V, Seripa D, Logroscino G, Imbimbo BP, et al. Diet and Alzheimer's disease risk factors or prevention: The current evidence. *Expert Rev Neurother*. 2011;**11**(5):677–708. doi: 10.1586/ern.11.56. [PubMed: 21539488].
21. Cheung BH, Ho IC, Chan RS, Sea MM, Woo J. Current evidence on dietary pattern and cognitive function. *Adv Food Nutr Res*. 2014;**71**:137–63. doi: 10.1016/B978-0-12-800270-4.00004-3. [PubMed: 24484941].
22. Tangney CC, Kwasny MJ, Li H, Wilson RS, Evans DA, Morris MC. Adherence to a Mediterranean-type dietary pattern and cognitive decline in a community population. *Am J Clin Nutr*. 2011;**93**(3):601–7. doi: 10.3945/ajcn.110.007369. [PubMed: 21177796]. [PubMed Central: PMC3041601].
23. Zhao X, Yuan L, Feng L, Xi Y, Yu H, Ma W, et al. Association of dietary intake and lifestyle pattern with mild cognitive impairment in the elderly. *J Nutr Health Aging*. 2015;**19**(2):164–8. doi: 10.1007/s12603-014-0524-2. [PubMed: 25651441].
24. Lloret A, Badia MC, Mora NJ, Pallardo FV, Alonso MD, Vina J. Vitamin E paradox in Alzheimer's disease: It does not prevent loss of cognition and may even be detrimental. *J Alzheimers Dis*. 2009;**17**(1):143–9. doi: 10.3233/JAD-2009-1033. [PubMed: 19494439].
25. Riviere S, Birlouez-Aragon I, Nourhashemi F, Vellas B. Low plasma vitamin C in Alzheimer patients despite an adequate diet. *Int J Geriatr Psychiatry*. 1998;**13**(11):749–54. [PubMed: 9850871].
26. Pearson JF, Pullar JM, Wilson R, Spittlehouse JK, Vissers MCM, Skidmore PML, et al. Vitamin C status correlates with markers of metabolic and cognitive health in 50-year-olds: Findings of the CHALICE cohort study. *Nutrients*. 2017;**9**(8). doi: 10.3390/nu9080831. [PubMed: 28771190]. [PubMed Central: PMC5579624].
27. Ide K, Yamada H, Kawasaki Y, Yamanaka M, Kawakami N, Katsuyama Y, et al. Peripheral vitamin C levels in Alzheimer's disease: A cross-sectional study. *J Nutr Sci Vitaminol (Tokyo)*. 2016;**62**(6):432–6. doi: 10.3177/jnsv.62.432. [PubMed: 28202849].
28. Devore EE, Kang JH, Stampfer MJ, Grodstein F. The association of antioxidants and cognition in the nurses' health study. *Am J Epidemiol*. 2013;**177**(1):33–41. doi: 10.1093/aje/kws202. [PubMed: 23221724]. [PubMed Central: PMC3590037].
29. Johnson EJ, Vishwanathan R, Johnson MA, Hausman DB, Davey A, Scott TM, et al. Relationship between serum and brain carotenoids, alpha-tocopherol, and retinol concentrations and cognitive performance in the oldest old from the georgia centenarian study. *J Aging*

Res. 2013;**2013**:951786. doi: 10.1155/2013/951786. [PubMed: 23840953]. [PubMed Central: PMC3690640].
30. Jimenez-Jimenez FJ, Molina JA, de Bustos F, Orti-Pareja M, Benito-Leon J, Tallon-Barranco A, et al. Serum levels of beta-carotene, alpha-carotene and vitamin A in patients with Alzheimer's disease. *Eur J Neurol*. 1999;**6**(4):495–7. [PubMed: 10362906].
31. Kalmijn S, Feskens EJ, Launer LJ, Kromhout D. Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men. *Am J Epidemiol*. 1997;**145**(1):33–41. [PubMed: 8982020].
32. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. 2004;**256**(3):183–94. doi: 10.1111/j.1365-2796.2004.01388.x. [PubMed: 15324362].
33. Cahill LE, El-Sohemy A. Vitamin C transporter gene polymorphisms, dietary vitamin C and serum ascorbic acid. *J Nutrigenet Nutrigenomics*. 2009;**2**(6):292–301. doi: 10.1159/000314597. [PubMed: 20588054].
34. Delis DC, Kramer JH, Kaplan E, Ober BA. *California Verbal Learning Test – second edition. Adult version. Manual*. San Antonio, TX: Psychological Corporation; 2000.
35. Wechsler D. *Wechsler adult intelligence scale-III*. San Antonio, TX: Psychological Corporation; 1997.
36. Spreen O, Strauss E. *A compendium of neuropsychological tests: Administration, norms and commentary*. New York, NY: Oxford University Press; 1991.
37. Folstein MF, Folstein SE, McHugh PR. ["Mini-mental state". A practical method for grading the cognitive state of patients for the clinician]. *J Psychiatr Res*. 1975;**12**(3):189–98. Persian.
38. Malakouti K, Fathollahi P, Mirabzadeh A, Salavati M, Kahani SH. [Validation of geriatric depression scale (GDS-15) in Iran]. *Res Med*. 2006;**30**(4):361–9. Persian.
39. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the Tehran lipid and glucose study. *Public Health Nutr*. 2010;**13**(5):654–62. doi: 10.1017/S1368980009991698. [PubMed: 19807937].
40. Jalilpiran Y, Hezaveh E, Bahmanpour S, Faghih S. Healthy plant foods intake could protect against prostate cancer risk: A case-control study. *Asian Pac J Cancer Prev*. 2017;**18**(7):1905–12. doi: 10.22034/APJCP.2017.18.7.1905. [PubMed: 28749619]. [PubMed Central: PMC5648397].
41. Hilasaca-Mamani M, Barbosa Tde S, Fegadolli C, Castelo PM. Validity and reliability of the quality of masticatory function questionnaire applied in Brazilian adolescents. *Codas*. 2016;**28**(2):149–54. doi: 10.1590/2317-1782/20162015070. [PubMed: 27191878].
42. Michels AJ, Frei B. Myths, artifacts, and fatal flaws: Identifying limitations and opportunities in vitamin C research. *Nutrients*. 2013;**5**(12):5161–92. doi: 10.3390/nu5125161. [PubMed: 24352093]. [PubMed Central: PMC3875932].
43. Gowenlock AH. *Varley's practical clinical biochemistry*. 6th ed. London, UK: Heinemann Medical Books; 1988.
44. Kaplan LA, Miller JA, Stein EA. Simultaneous measurement of serum retinol, tocopherols, carotenes, and carotenoids by high performance liquid chromatography. *J Clin Lab Anal*. 1987;**1**(2):147–52. doi: 10.1002/jcla.1860010202.
45. Mahan LK, Escott-Stump S, Raymond JL, Krause MV. *Krause's food and the nutrition care process*. 13th ed. Elsevier/Saunders; 2012.
46. Rinaldi P, Polidori MC, Metastasio A, Mariani E, Mattioli P, Cherubini A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. *Neurobiol Aging*. 2003;**24**(7):915–9. [PubMed: 12928050].
47. Zhao LG, Shu XO, Li HL, Zhang W, Gao J, Sun JW, et al. Dietary antioxidant vitamins intake and mortality: A report from two cohort studies of Chinese adults in Shanghai. *J Epidemiol*. 2017;**27**(3):89–97. doi: 10.1016/j.je.2016.10.002. [PubMed: 28142039]. [PubMed Central: PMC5363781].
48. Kim K, Vance TM, Chun OK. Greater total antioxidant capacity from diet and supplements is associated with a less atherogenic blood profile in U.S. adults. *Nutrients*. 2016;**8**(1). doi: 10.3390/nu8010015. [PubMed: 26742057]. [PubMed Central: PMC4728629].
49. Chen W, Mao L, Xing H, Xu L, Fu X, Huang L, et al. Lycopene attenuates Abeta1-42 secretion and its toxicity in human cell and Caenorhabditis elegans models of Alzheimer disease. *Neurosci Lett*. 2015;**608**:28–33. doi: 10.1016/j.neulet.2015.10.009. [PubMed: 26453763].
50. Ientile L, De Pasquale R, Monacelli F, Odetti P, Traverso N, Cammarata S, et al. Survival rate in patients affected by dementia followed by memory clinics (UVA) in Italy. *J Alzheimers Dis*. 2013;**36**(2):303–9. doi: 10.3233/JAD-130002. [PubMed: 23609761].
51. Alles B, Samieri C, Feart C, Jutand MA, Laurin D, Barberger-Gateau P. Dietary patterns: A novel approach to examine the link between nutrition and cognitive function in older individuals. *Nutr Res Rev*. 2012;**25**(2):207–22. doi: 10.1017/S0954422412000133. [PubMed: 22874455].
52. Feart C, Samieri C, Alles B, Barberger-Gateau P. Potential benefits of adherence to the Mediterranean diet on cognitive health. *Proc Nutr Soc*. 2013;**72**(1):140–52. doi: 10.1017/S0029665112002959. [PubMed: 23228285].
53. Feart C, Letenneur L, Helmer C, Samieri C, Schalch W, Etheve S, et al. Plasma carotenoids are inversely associated with dementia risk in an elderly French cohort. *J Gerontol A Biol Sci Med Sci*. 2016;**71**(5):683–8. doi: 10.1093/gerona/glv135. [PubMed: 26286605].
54. Bendich A, Olson JA. Biological actions of carotenoids. *FASEB J*. 1989;**3**(8):1927–32. [PubMed: 2656356].
55. Yamada H, Yamada K, Waki M, Umegaki K. Lymphocyte and plasma vitamin C levels in type 2 diabetic patients with and without diabetes complications. *Diabetes Care*. 2004;**27**(10):2491–2. [PubMed: 15451922].
56. Ide K, Yamada H, Umegaki K, Mizuno K, Kawakami N, Hagiwara Y, et al. Lymphocyte vitamin C levels as potential biomarker for progression of Parkinson's disease. *Nutrition*. 2015;**31**(2):406–8. doi: 10.1016/j.nut.2014.08.001. [PubMed: 25592020].
57. Harrison FE, May JM. Vitamin C function in the brain: Vital role of the ascorbate transporter SVCT2. *Free Radic Biol Med*. 2009;**46**(6):719–30. doi: 10.1016/j.freeradbiomed.2008.12.018. [PubMed: 19162177]. [PubMed Central: PMC2649700].
58. Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. *Behav Brain Res*. 2011;**221**(2):555–63. doi: 10.1016/j.bbr.2010.11.058. [PubMed: 21145918].
59. Revett TJ, Baker GB, Jhamandas J, Kar S. Glutamate system, amyloid ss peptides and tau protein: Functional interrelationships and relevance to Alzheimer disease pathology. *J Psychiatry Neurosci*. 2013;**38**(1):6–23. doi: 10.1503/jpn.110190. [PubMed: 22894822]. [PubMed Central: PMC3529221].
60. Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am J Clin Nutr*. 1999;**69**(6):1086–107. doi: 10.1093/ajcn/69.6.1086. [PubMed: 10357726].
61. Kean RJ, Lamport DJ, Dodd GF, Freeman JE, Williams CM, Ellis JA, et al. Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: An 8-wk, randomized, double-blind, placebo-controlled trial in healthy older adults. *Am J Clin Nutr*. 2015;**101**(3):506–14. doi: 10.3945/ajcn.114.088518. [PubMed: 25733635].
62. Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P. Flavonoid intake and cognitive decline over a 10-year period. *Am J Epidemiol*. 2007;**165**(12):1364–71. doi: 10.1093/aje/kwm036. [PubMed: 17369607].
63. Touvier M, Druesne-Pecollo N, Kesse-Guyot E, Andreeva VA, Fezeu L, Galan P, et al. Dual association between polyphenol intake and breast cancer risk according to alcohol consumption level: A prospective cohort study. *Breast Cancer Res Treat*. 2013;**137**(1):225–36. doi: 10.1007/s10549-012-2323-y. [PubMed: 23132534].
64. Kesse-Guyot E, Fezeu L, Jeandel C, Ferry M, Andreeva V, Amieva H, et al. French adults' cognitive performance after daily supplementation with antioxidant vitamins and minerals at nutritional doses: A post hoc analysis of the supplementation in vitamins and mineral antioxidants (SU.VI.MAX) trial. *Am J Clin Nutr*. 2011;**94**(3):892–9. doi: 10.3945/ajcn.110.007815. [PubMed: 21775560].
65. Kang JH, Cook NR, Manson JE, Buring JE, Albert CM, Grodstein F. Vitamin E, vitamin C, beta carotene, and cognitive function among women with or at risk of cardiovascular disease: The women's antiox-

idant and cardiovascular study. *Circulation*. 2009;**119**(21):2772–80. doi: 10.1161/CIRCULATIONAHA.108.816900. [PubMed: 19451353]. [PubMed Central: PMC2752297].

66. Gale CR, Martyn CN, Cooper C. Cognitive impairment and mortality in a cohort of elderly people. *BMJ*. 1996;**312**(7031):608–11. [PubMed: 8595334]. [PubMed Central: PMC2350374].
67. Jama JW, Launer LJ, Witteman JC, den Breeijen JH, Breteler MM, Grobbee DE, et al. Dietary antioxidants and cognitive function in a population-based sample of older persons. The rotterdam study. *Am J Epidemiol*. 1996;**144**(3):275–80. [PubMed: 8686696].
68. Glaso M, Nordbo G, Diep L, Bohmer T. Reduced concentrations of several vitamins in normal weight patients with late-onset dementia of the Alzheimer type without vascular disease. *J Nutr Health Aging*. 2004;**8**(5):407–13. [PubMed: 15359361].
69. Polidori MC, Mecocci P. Plasma susceptibility to free radical-induced antioxidant consumption and lipid peroxidation is increased in very old subjects with Alzheimer disease. *J Alzheimer's Dis*. 2002;**4**(6):517–22. doi: 10.3233/jad-2002-4608.
70. Agbayewa MO, Bruce VM, Siemens V. Pyridoxine, ascorbic acid and thiamine in Alzheimer and comparison subjects. *Can J Psychiatry*. 1992;**37**(9):661–2. [PubMed: 1477827].
71. Paraskevas GP, Kapaki E, Libitaki G, Zournas C, Segditsa I, Papageorgiou C. Ascorbate in healthy subjects, amyotrophic lateral sclerosis and Alzheimer's disease. *Acta Neurol Scand*. 1997;**96**(2):88–90. [PubMed: 9272183].
72. Schippling S, Kontush A, Arlt S, Buhmann C, Sturenburg HJ, Mann U, et al. Increased lipoprotein oxidation in Alzheimer's disease. *Free Radic Biol Med*. 2000;**28**(3):351–60. [PubMed: 10699746].
73. Sato R, Helzlsouer KJ, Comstock GW, Hoffman SC, Norkus EP, Fried LP. A cross-sectional study of vitamin C and cognitive function in older adults: The differential effects of gender. *J Nutr Health Aging*. 2006;**10**(1):37–44. [PubMed: 16453056].
74. Grosso G, Bei R, Mistretta A, Marventano S, Calabrese G, Masuelli L, et al. Effects of vitamin C on health: A review of evidence. *Front Biosci (Landmark Ed)*. 2013;**18**:1017–29. [PubMed: 23747864].
75. Butterfield DA, Reed T, Newman SF, Sultana R. Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radic Biol Med*. 2007;**43**(5):658–77. doi: 10.1016/j.freeradbiomed.2007.05.037. [PubMed: 17664130]. [PubMed Central: PMC2031860].
76. Choudhry F, Howlett DR, Richardson JC, Francis PT, Williams RJ. Pro-oxidant diet enhances beta/gamma secretase-mediated APP processing in APP/PS1 transgenic mice. *Neurobiol Aging*. 2012;**33**(5):960–8. doi: 10.1016/j.neurobiolaging.2010.07.008. [PubMed: 20724034].
77. Li FJ, Shen L, Ji HF. Dietary intakes of vitamin E, vitamin C, and beta-carotene and risk of Alzheimer's disease: A meta-analysis. *J Alzheimers Dis*. 2012;**31**(2):253–8. doi: 10.3233/JAD-2012-120349. [PubMed: 22543848].
78. Kuhad A, Sethi R, Chopra K. Lycopene attenuates diabetes-associated cognitive decline in rats. *Life Sci*. 2008;**83**(3-4):128–34. doi: 10.1016/j.lfs.2008.05.013. [PubMed: 18585396].
79. Wang Z, Fan J, Wang J, Li Y, Xiao L, Duan D, et al. Protective effect of lycopene on high-fat diet-induced cognitive impairment in rats. *Neurosci Lett*. 2016;**627**:185–91. doi: 10.1016/j.neulet.2016.05.014. [PubMed: 27177726].
80. Mecocci P, Polidori MC, Cherubini A, Ingegni T, Mattioli P, Catani M, et al. Lymphocyte oxidative DNA damage and plasma antioxidants in Alzheimer disease. *Arch Neurol*. 2002;**59**(5):794–8. [PubMed: 12020262].
81. Akbaraly NT, Faure H, Gourlet V, Favier A, Berr C. Plasma carotenoid levels and cognitive performance in an elderly population: Results of the EVA study. *J Gerontol A Biol Sci Med Sci*. 2007;**62**(3):308–16. [PubMed: 17389729].
82. Prakash A, Kumar A. Implicating the role of lycopene in restoration of mitochondrial enzymes and BDNF levels in beta-amyloid induced Alzheimers disease. *Eur J Pharmacol*. 2014;**741**:104–11. doi: 10.1016/j.ejphar.2014.07.036. [PubMed: 25066110].
83. Qu M, Li L, Chen C, Li M, Pei L, Chu F, et al. Protective effects of lycopene against amyloid beta-induced neurotoxicity in cultured rat cortical neurons. *Neurosci Lett*. 2011;**505**(3):286–90. doi: 10.1016/j.neulet.2011.10.048. [PubMed: 22044877].
84. Stiernagle T. *Maintenance of C. elegans (February 11, 2006), WormBook, ed. The C. elegans research community, WormBook*. 2006.
85. Morris MC, Evans DA, Bienias JL, Tangney CC, Wilson RS. Vitamin E and cognitive decline in older persons. *Arch Neurol*. 2002;**59**(7):1125–32. [PubMed: 12117360].
86. Zaman Z, Roche S, Fielden P, Frost PG, Niriella DC, Cayley AC. Plasma concentrations of vitamins A and E and carotenoids in Alzheimer's disease. *Age Ageing*. 1992;**21**(2):91–4. [PubMed: 1575097].
87. Lee L, Kang SA, Lee HO, Lee BH, Park JS, Kim JH, et al. Relationships between dietary intake and cognitive function level in Korean elderly people. *Public Health*. 2001;**115**(2):133–8. doi: 10.1038/sj/ph/1900729. [PubMed: 11406779].
88. Chen YC, Jung CC, Chen JH, Chiou JM, Chen TF, Chen YF, et al. Association of dietary patterns with global and domain-specific cognitive decline in Chinese elderly. *J Am Geriatr Soc*. 2017;**65**(6):1159–67. doi: 10.1111/jgs.14741. [PubMed: 28092399].
89. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, et al. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci*. 1999;**19**(18):8114–21. [PubMed: 10479711].
90. Grodstein F, Kang JH, Glynn RJ, Cook NR, Gaziano JM. A randomized trial of beta carotene supplementation and cognitive function in men: The physicians' health study II. *Arch Intern Med*. 2007;**167**(20):2184–90. doi: 10.1001/archinte.167.20.2184. [PubMed: 17998490].
91. Jakobsen LH, Kondrup J, Zellner M, Tetens I, Roth E. Effect of a high protein meat diet on muscle and cognitive functions: A randomised controlled dietary intervention trial in healthy men. *Clin Nutr*. 2011;**30**(3):303–11. doi: 10.1016/j.clnu.2010.12.010. [PubMed: 21239090].
92. Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, et al. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. *Neurology*. 2000;**54**(6):1265–72. [PubMed: 10746596].
93. Wind AW, Schellevis FG, Van Staveren G, Scholten RP, Jonker C, Van Eijk JT. Limitations of the mini-mental state examination in diagnosing dementia in general practice. *Int J Geriatr Psychiatry*. 1997;**12**(1):101–8. [PubMed: 9050431].