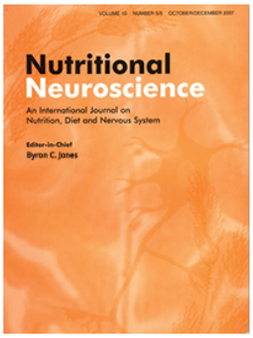

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Effects of daily almond consumption for six months on cognitive measures in healthy middle-aged to older adults: a randomized control trial

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ABSTRACT

Background: Age-related cognitive decline is a major public health issue. Almonds are rich in nutrients that benefit cognitive function.

Objective: To investigate the impact of almonds on cognition in elderly adults.

Design: In a six-month, single-blinded, randomized-controlled trial, the effects of an almond intervention on cognition in healthy, middle-aged/older adults (50–75 years) was tested. Subjects were assigned to one of three groups: 1.5 oz/d almond (n = 19), 3 oz/d almond (n = 24), or 3.5 oz/d snack (control, matched for macronutrients in 3.0 oz almonds, (n = 17). Serum analyses for tocopherols, oxidative status and inflammation, and cognition were assessed at baseline (M0), three (M3), and six (M6) months.

Results: At M6, serum alpha-tocopherol concentrations increased by 8% from M0 ($p < 0.05$) in the 3 oz almond group but did not increase in the other groups. Serum markers of inflammation and oxidative stress were not significantly different throughout the study among the groups. There was no difference in change over time in cognitive tests among the groups. However, there was a significant improvement in visuospatial working memory (p = 0.023), visual memory and learning (p = 0.017), and spatial planning and working memory ($p < 0.001$) in subjects receiving 3 oz/d almonds at M6, while the snack group showed no improvement.

Conclusions: Almonds did not significantly improve cognitive function in cognitively intact middle-aged/older adults over six months. However, a significant improvement at M6 in cognitive measures was observed with 3 oz/d almonds. While these results are encouraging, a study of longer duration in subjects at risk for age-related cognitive decline is warranted.

Trial registration: ClinicalTrials.gov identifier: NCT03093896.

KEYWORDS
Almonds; nuts; cognition; middle-aged to older adults; dementia

1. Introduction

Age-related cognitive impairment is a common disorder in elderly adults, ranging from mild cognitive impairment (MCI) to severe dementia, such as Alzheimer disease (AD) [1]. The prevalence of MCI in adults aged ≥65 years is approximately 10%–20% [1]. Although MCI does not interfere greatly with everyday activities, it is a risk factor for dementia and AD, which is currently the seventh leading cause of death in the United States [2,3].

Oxidative stress and inflammation have been recognized as mechanisms related to cognitive impairment in aging. The brain may be particularly susceptible to free radical attacks due to its relatively low antioxidant capacity, high polyunsaturated fatty acids (PUFA) concentrations, and high metabolic activity [3]. Given that increased oxidative stress and inflammation may lead to age-related cognitive deficits, interventions with nutrients that exhibit antioxidant and anti-inflammatory properties could postpone the development of cognitive impairment. Indeed, circulating levels of the lipophilic antioxidant, vitamin E, have been inversely associated with cognitive decline [4]. Moreover, observational and clinical studies reported that greater intake of dietary monounsaturated fatty acids (MUFA) is linked to better cognitive function [5,6]. A recent study also showed that a diet containing more than 30 g/d of fiber increased cognition performance in

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healthy adults aged 50–73 years [7]. Lastly, the concentration of plasma aminothiols, such as glutathione, cysteinylglycine and cysteine, were found to be associated with cognitive status in MCI or AD patients [8].

Almonds contain MUFAs, α-tocopherol, and fiber. Thus, it is possible that consuming almonds could delay age-related cognitive impairment. Despite previous studies showing an association between higher intake of mixed nuts and better cognitive performance [9–12], the impact of almond consumption on cognition capacity in individuals at an increased risk for cognitive decline has not been examined.

The primary objective of this study was to test the effects of a 6-month almond intervention on cognition in middle-aged to older adults who had no dementia. We hypothesized that subjects receiving almonds would have greater cognitive improvement (higher scores in each test and higher number of tests) than subjects receiving a macronutrient- and calorie-matched snack mix devoid of almonds (control group). A secondary objective was to investigate the effect of almond intake on serum antioxidants, anti-inflammatory cytokines, and lipids. We hypothesized that after 6 months these measures would be favorable in subjects receiving almonds as compared to the control group.

2. Subjects and methods

2.1. Subjects

Four hundred and twenty-three healthy, non-smoking men and post-menopausal women (50–75 y) were recruited and screened at the Human Nutrition Research Center on Aging (HNRCA) at Tufts University from March 2016 to January 2018 (Supplementary Figure 1). Sixty-eight subjects who met the eligibility criteria were enrolled, and 60 subjects completed the study by September 2018. All subjects underwent a screen that included a medical history, a physical examination, and a routine blood clinical chemistry profile. The eligibility criteria included: body mass index (BMI) 25–35 kg/m^2, Mini Mental State Examination score >24 [13], and Beck Depression Inventory <20 [14]. Individuals were ineligible to participate if there was any history of significant neurologic disorder, rheumatologic diseases, untreated hypertension, active cancers, endocrine disorders, pancreatic disease, gastrointestinal diseases that interfere with fat absorption, active bowel disease or resection, immune deficiency conditions, anemia, other hematologic disorders or any other major chronic illness that might interfere with the study outcomes. Other exclusion criteria included current use of antipsychotic, antimanic, anti-inflammatory (except for aspirin and non-steroidal anti-inflammatory drugs [NSAIDS], monoamine inhibitors, or dementia medications, drugs that interfere with metabolism of blood clotting, or with fat absorption), daily intake of proton pump inhibitors or H2 blockers. Moreover, MUFA intake >10% total calories, whole grain intake >1 serving/day, fruit and vegetable intake >5 servings/day, nuts >0.25 oz/day, unwillingness or inability to consume animal-based foods, nuts, coconuts; use of lutein, n-3 fatty acid, or choline supplements during the previous two months were grounds for exclusion. Additionally, individuals smoking or using nicotine patches or gum within the last 6 months and alcoholism (>2 drinks/day or 14 drinks/week) were excluded.

2.2. Experimental design

We conducted a single-blinded, controlled, randomized trial that tests the effects of supplementation with 1.5 or 3 oz (42 or 84 g, respectively) of almonds or 3.5 oz (100 g) of a snack mix containing cereal party mix, coconut, meat jerky, and butter per day on cognitive function in middle-aged to older adults older adults for six months. The nutrient content of the almonds and snack mix can be found in Table 1. A snack mix was chosen as the control group to approximate the calorie and macronutrient content of almonds, but not the nutrients of interest, e.g. MUFA, tocopherols, and fiber. Upon enrollment, 68 subjects were randomly assigned to one of the three dietary groups: supplementation with 1.5 oz of almonds, 3 oz of almonds, or 3.5 oz of snack mix using a randomization plan generator (http://www.randomization.com/). The subjects and study dietitian (HR) were aware of the dietary group assignment, while the study investigators were blinded to the randomization. The trial was conducted in the HNRCA at Tufts University in Boston. The protocol was approved by the Institutional Review Board of Tufts Medical Center and Tufts University Health Sciences. Informed consent was obtained from all subjects. This study used the

Table 1. Nutrient profiles of almonds and snack mix.

Nutrient	1.5 oz (42 g) Almonds	3.0 oz (84 g) Almonds	3.5 oz (100 g) Snack mix
Energy (kcal)	253	506	272
Protein (g)	9	18	9
Fat (g)	22	44	20
Carbohydrate (g)	9	18	17
Fiber (g)	5	10	3
Saturated fats (g)	1.7	3.4	13.0
Monounsaturated fat (g)	13.8	27.6	3.8
Polyunsaturated fat (g)	5.5	11.0	4.5
α-Tocopherol (mg)	10.1	20.2	1.5
γ-Tocopherol (mg)	0.3	0.6	1.8
Magnesium (mg)	119	238	21

CONSORT reporting guidelines [15]. This study was registered at ClinicalTrials.gov (Clinical Trial Registry #NCT03093896).

2.3. Study protocol

The subjects visited the HNRCA a total of 11 times (one screening visit, four visits to pick-up a monthly supply of study food, three visits to pick-up pre-study day meals and three visits for study outcome measures). Pre-study day meals were composed of breakfast, lunch, dinner, and snacks and considered a menu low in polyphenols, MUFA, and vitamin E. Breakfast included a plain white-flour bagel, white cream cheese, 4 ounces of low-fat milk (if requested), and habitual coffee or tea consumption (to avoid caffeine withdrawal symptoms; ≤18 ounces of coffee or tea). Lunch included chicken slices on white bread with low fat mayonnaise, diet ginger ale, and a slice of angel food cake. Snacks (for late afternoon and evening) included white cheddar cheese on saltine crackers, and diet ginger ale. Dinner included macaroni and white cheese with cubed chicken breast, parmesan cheese, diet ginger ale, and a slice of angel food cake. If preferred, subjects could have regular ginger ale in place of diet ginger ale. At the beginning of the study subjects were given instructions on how to incorporate the supplemented food and offset these calories by substitution of other foods in order to maintain their body weights throughout the trial. Subjects were otherwise instructed to maintain their usual dietary habits. At baseline (M0) and study months three (M3) and six (M6) subjects visited the HNRCA and provided an overnight fasting blood sample and urine. Fasting blood samples were processed within 1 h (15 min, 1000×g, 4°C) and plasma or serum samples were stored at −70°C, until analyzed. During those visits, measures of cognitive function and dietary interviews were performed. The web-based diet history questionnaire II (DHQ II) developed by the Risk Factor Monitoring and Methods Branch of the National Cancer Institute, was administered by the study dietitian. The Diet*Calc Analysis Program (Version 1.5.0., National Cancer Institute), was used to interpret the DHQ II data to provide nutrient and food group intake estimates. A study dietitian (HR) assessed compliance with the study protocol. Monthly compliance was monitored using calendars given at each study visit. Monthly phone calls were made for an assessment of compliance and wellness.

2.4. Cognitive measures

The subjects underwent computerized cognitive assessment (CANTAB, Cambridge Cognition Ltd., Cambridge, UK) designed to test several cognitive domains, including memory, processing speed, and attention [16]. The CANTAB has been extensively evaluated for reliability and validity and has been used in studies of dietary supplementation and cognition in older adults [17–19], as well as age-related cognitive decline [20–24]. Description of cognitive tests and measures performed in this study can be found in Supplementary Table 1. Tests were administered in the same order as shown in Supplementary Table 1. Before each testing session at all time points, subjects also underwent a very brief, standardized training session provided by CANTAB to ensure they understood the instructions.

2.5. Serum analysis for tocopherols, aminothiol and biomarkers of oxidative status and inflammation

Serum α- and γ- tocopherols were measured by a reversed phase HPLC (Waters HPLC Empower® Network system with the 717plus Wisp, 515 pump, Waters Corporation, Milford, MA) procedure after the extraction of the vitamins into a suitable solvent according to the protocol of Bieri et al. [25], with intra- and inter-assay CVs of 4.5% and 5.5% respectively. The *ex vivo* resistance of serum low density lipoprotein (LDL) resistance against Cu^{2+}-induced oxidation, a biomarker of oxidative stress, was analyzed by spectrophotometric assay at an absorbance of 234 nm as previously described [26]. Markers of inflammation- C-reactive protein (CRP), interleukin (IL)-6, IL-12 and soluble intercellular adhesion molecule-1 (ICAM-1) - were measured in serum by electrochemiluminescence detection validated sandwich immunoassays using a MULTI-ARRAY technology (V-PLEX Human Cytokine Assays, Meso Scale Diagnostics, Rockville, MD) on the Meso Scale Discovery SECTOR Imager 2400. Markers of oxidative stress - plasma and red blood cell (RBC) superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione (GSH) - were measured using enzymatic assays as specified in procedural documentation (Cayman Chemical Company, Ann Arbor, MI). RBC GSH LOD was 1.0 μM, and samples with a concentration lower than LOD were imputed with 0.5 μM.

2.6. Lipoprotein analysis

Total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides in plasma were measured on a clinical chemistry analyzer (AU480 Clinical Chemistry Analyzer, Beckman Coulter, Inc., Brea CA) as specified in the manufacturer's

procedural documentation with the intra- and inter-assay CVs less than 2.4% and 3.8%, respectively.

2.7. Sample size calculation and statistical analyses

Given the novelty of this work, a sample size calculation was not possible. We proposed a sample size of 20/group. This is based on data from a study with a similar study design evaluating the effect of consuming 1 avocado/d (13 g/d MUFA) or chickpeas/potatoes (<1 g/d MUFA) for 6 months on cognitive function in 40 middle to older adults (>50 yrs) [19]. Data are expressed as mean ± SD. Paired Student's t-test was performed to compare all cognitive and serum measures between M3 and M0, and between M6 and M0. To determine the effect of group, time, and the group x time interaction on any cognitive or serum measure, repeated measure ANOVA was performed. Log transformation was applied to serum tocopherols, IL-6, CRP, and sICAM-1 to normalize the data and satisfy the assumption of normal distribution for statistical tests. One-way ANOVA and Fisher's Exact Test were performed to detect any differences at baseline among the three dietary groups and to assess the effectiveness of randomization. All statistical tests were performed in R version 3.5.1, and statistical significance was set at $\alpha = 0.05$.

3. Results

3.1. Subject characteristics

Among 68 healthy subjects who were enrolled, 8 subjects (12%) did not complete the study due to the following reasons: drop-out or lost to follow-up ($n = 4$), non-compliance with study protocol ($n = 3$), situational exclusion ($n = 1$). No adverse events were reported. Dropout rates were not statistically different among groups. Of the 60 subjects who have completed the study, 19 were in the 1.5 oz almond group, 24 were in the 3 oz almond group, and 17 were in the snack mix group. Subjects were on average 61.5 ± 6.3 years old and 45% were women. Forty subjects (67%) were Caucasians, 11 subjects (18%) were African Americans, and 9 subjects (15%) were other races. The average BMI was 29.0 ± 2.7 kg/m^2, and 68% graduated from a 4-year college or graduate school. As shown in Table 2, there were no statistical differences among the three intervention groups in age, sex, race, and highest education level at randomization. No significant change in BMI occurred during the study (Supplementary Table 2). Nutrient intakes at all visits are reported in Supplementary Table 3. Among groups, the intake of nutrients of interest was not significantly different throughout the study.

Table 2. Subject characteristics ($n = 60$).

Characteristics	1.5 oz Almonds ($n = 19$)	3 oz Almonds ($n = 24$)	3.5 oz Snack mix ($n = 17$)	*P* value[a]
Age (years), mean ± SD	61.6 ± 6.3	60.4 ± 6.8	63.0 ± 5.6	0.44
Sex, *n* (%)				0.90
Female	9 (47%)	10 (42%)	8 (47%)	
Male	10 (53%)	14 (58%)	9 (53%)	
Race				0.66
Caucasian	14 (74%)	16 (67%)	10 (59%)	
African American	4 (21%)	4 (17%)	3 (18%)	
Others	1 (5%)	4 (17%)	4 (24%)	
BMI (kg/m^2), mean ± SD	28.9 ± 2.9	28.9 ± 2.8	29.2 ± 2.6	0.93
Highest education level				0.98
High school or less	3 (16%)	4 (17%)	3 (18%)	
Some college	3 (16%)	4 (17%)	2 (12%)	
Four year college	8 (42%)	8 (33%)	8 (47%)	
Graduate school	5 (26%)	8 (33%)	4 (24%)	

[a]Comparisons among the three groups were performed using ANOVA for age and BMI, and Fisher's exact test for sex, race, and education.

3.2. Cognitive measures

At baseline, there was no significant difference in the 16 cognitive outcomes among the three dietary groups (Table 3). Repeated measure ANOVA tests revealed that the effect of time was significant for MOT ($p_{time} = 0.002$), SSP Reverse ($p_{time} = 0.036$), PAL (FAMS, $p_{time} = 0.009$; TEA, $p_{time} < 0.001$), RVP (A', $p_{time} < 0.001$; PFA, $p_{time} = 0.031$), OTS (PSFC, $p_{time} < 0.001$) outcomes, suggesting there is an improvement in cognitive performance after repeated exposure to these tests over time, independent of dietary intervention. No treatment effect was observed ($p_{group} > 0.05$ for all cognitive measures). The interaction between dietary intervention and time was marginally significant for SSP Reverse (SL, $p_{group\ x\ time} = 0.052$) and PAL (TEA, $p_{group\ x\ time} = 0.061$).

Results from paired t-tests were also shown in Table 3. Only subjects receiving 3 oz of almonds daily had a significant improvement at M6 in visuospatial working memory (SSP Reverse, Spatial Span Reverse) ($p = 0.023$), the first attempt memory score (FAMS, $p = 0.017$) in visual memory and learning (PAL, Paired Associate Learning), and the problems solved on first choice (PSFC, $p < 0.001$) in spatial planning and working memory (OTS, One Touch Stockings of Cambridge). Adjustment for multiple comparisons across 18 cognitive measures were performed with false discovery rate, and only PSFC remained significant (adjusted $p = 0.013$). Subjects in the snack mix groups showed no improvement in these measures at M6 (although a

Table 3. Cognitive measures (mean ± SD) at baseline (M0), three months (M3), and six months (M6) in healthy adults consuming almonds (1.5 or 3.0 oz/d) or a snack mix (3.5 oz/d).

	1.5 oz Almonds ($n = 19$)					3 oz Almonds ($n = 24$)					3.5 oz Snack mix ($n = 17$)				
Measure [a]	M0	M3	M6	P_{M3-M0}	P_{M6-M0}	M0	M3	M6	P_{M3-M0}	P_{M6-M0}	M0	M3	M6	P_{M3-M0}	P_{M6-M0}
MOT															
ML	976 ± 268	857 ± 197	850 ± 203	0.051	0.046*	1108 ± 626	946 ± 268	912 ± 249	0.133	0.071	1022 ± 279	936 ± 174	918 ± 225	0.303	0.261
RTI															
FMDMT	318 ± 75	311 ± 91	323 ± 86	0.502	0.615	347 ± 149	324 ± 74	319 ± 65	0.433	0.271	338 ± 73	360 ± 73	346 ± 68	0.338	0.717
FMDRT	400 ± 40	400 ± 31	397 ± 41	0.966	0.770	406 ± 49	415 ± 64	421 ± 55	0.268	0.072	401 ± 51	420 ± 34	419 ± 43	0.052	0.051
DMS															
PCS	97 ± 7	95 ± 13	98 ± 6	0.494	0.331	98 ± 6	97 ± 8	97 ± 8	0.162	0.426	94 ± 12	95 ± 9	94 ± 9	0.668	1
PC0	87 ± 14	88 ± 15	86 ± 15	0.834	0.804	83 ± 18	83 ± 17	78 ± 18	1	0.328	89 ± 12	75 ± 23	85 ± 19	0.035*	0.299
PC4	78 ± 23	75 ± 17	79 ± 23	0.615	0.893	83 ± 15	80 ± 17	81 ± 15	0.575	0.732	85 ± 15	89 ± 17	81 ± 19	0.431	0.484
PC12	79 ± 22	78 ± 22	83 ± 20	0.871	0.520	83 ± 16	77 ± 19	84 ± 20	0.258	0.714	75 ± 22	72 ± 20	80 ± 14	0.484	0.482
PCAD	81 ± 11	80 ± 11	83 ± 12	0.737	0.686	83 ± 11	80 ± 10	81 ± 12	0.341	0.626	83 ± 10	79 ± 13	82 ± 12	0.221	0.691
PEGE	0.17 ± 0.19	0.19 ± 0.20	0.08 ± 0.14	0.731	0.091	0.09 ± 0.16	0.11 ± 0.17	0.10 ± 0.16	0.681	0.963	0.09 ± 0.21	0.13 ± 0.17	0.05 ± 0.11	0.299	0.490
SSPF															
SL	5.7 ± 0.8	6.1 ± 1.1	5.8 ± 0.8	0.250	0.695	5.8 ± 1.5	6.1 ± 1.5	6.2 ± 1.4	0.207	0.175	5.7 ± 0.7	5.6 ± 1.3	5.8 ± 1.2	0.668	0.608
SSPR															
SL	5.6 ± 1.0	5.0 ± 0.7	5.5 ± 0.7	0.023*	0.772	5.6 ± 1.7	6.0 ± 1.9	6.0 ± 2.0	0.106	0.023*	5.5 ± 1.3	5.3 ± 1.4	5.7 ± 1.2	0.387	0.565
PAL															
FAMS	10.1 ± 3.9	12.1 ± 4.0	10.9 ± 5.1	0.019*	0.472	11.2 ± 3.9	11.9 ± 5.0	13.1 ± 4.4	0.347	0.017*	9.0 ± 4.2	9.2 ± 4.4	11.4 ± 4.5	0.815	0.059
TEA	23.6 ± 14.6	18.4 ± 12.9	22.8 ± 17.1	0.045*	0.770	21.4 ± 15.0	19.2 ± 17.2	13.8 ± 13.0	0.284	0.004**	29.7 ± 16.8	23.5 ± 14.8	20.2 ± 15.5	0.092	0.030*
RVP															
A′	0.92 ± 0.05	0.93 ± 0.04	0.94 ± 0.05	0.387	0.008**	0.91 ± 0.06	0.92 ± 0.07	0.93 ± 0.05	0.245	0.014*	0.89 ± 0.05	0.90 ± 0.05	0.92 ± 0.05	0.351	0.002**
MDL	532 ± 103	543 ± 94	513 ± 89	0.566	0.342	527 ± 95	522 ± 124	511 ± 112	0.786	0.449	529 ± 92	527 ± 98	489 ± 68	0.681	0.151
PFA	0.65 ± 0.96	0.41 ± 0.37	0.51 ± 0.71	0.341	0.380	3.61 ± 11.1	1.56 ± 3.36	2.22 ± 5.61	0.226	0.323	3.53 ± 6.70	1.30 ± 2.36	0.64 ± 0.71	0.162	0.101
OTS															
MDLFC	144 ± 65	136 ± 46	148 ± 77	0.546	0.837	159 ± 113	195 ± 159	167 ± 105	0.261	0.632	159 ± 83	160 ± 81	152 ± 82	0.980	0.601
PSFC	8.8 ± 3.7	8.7 ± 3.2	9.9 ± 3.6	0.878	0.109	8.5 ± 3.2	8.6 ± 2.6	10.4 ± 2.9	0.734	<0.001***	7.8 ± 3.9	8.9 ± 3.1	8.4 ± 2.8	0.086	0.440

Abbreviations: MOT: Motor Screening Task, ML: Mean Latency, RTI: Reaction Time, FMDMT: Five-Choice Median Movement Time, FMDRT: Five-Choice Median Reaction Time, DMS: Delayed Matching to Sample, PCS: Percent Correct Simultaneous, PC0: Percent Correct 0 s Delay, PC4: Percent Correct 4 s Delay, PC12: Percent Correct 0 s Delay, PCAD: Percent Correct All Delays, PEGE: Probability of Error Given Error, SSPF: Spatial Span Forward, SL: Span Length, SSPR: Spatial Span Reverse, PAL: Paired Associate Learning, FAMS: First Attempt Memory Score, TEA: Total Errors Adjusted, RVP: Rapid Visual Information Processing, A′: A Prime, MDL: Median Response Latency, PFA: Probability of False Alarm, OTS: One Touch Stockings of Cambridge, MDLFC: Median Latency to First Choice, PSFC: Problems Solved on First Choice.

[a]Units are as follows: ML, FMDMT, FMDRT, MDL in milliseconds; PCS, PC0, PC4, PC12, PCAD, PFA in %; SL in boxes; FAMS, TEA in times; MDLFC in seconds; PSFC in trials; PEGE and A′ have no unit.

* $p<0.05$, ** $p < 0.01$, *** $p < 0.001$.

trend was observed for PAL [FAMS, $p = 0.059$]). As for subjects who received 1.5 oz of almonds daily, the only improvement observed was at M3 for SSP Reverse (SL, $p = 0.023$) and PAL (FAMS, $p = 0.017$), but they did not remain significant at M6.

Improvement in sustained attention (RVP, Rapid Visual Information Processing) as measured by the A' metric, occurred at M6 regardless of which intervention group they were in (1.5 oz almond, $p = 0.008$; 3 oz almond, $p = 0.014$; snack mix, $p = 0.002$, paired t-test, Table 3), but only the snack mix group remained statistically significant after the adjustment for multiple comparisons (adjusted $p = 0.048$). Similarly, total errors adjusted (TEA) of the PAL, a measure of visual memory and learning, was improved among the 3 oz almond group ($p = 0.004$) and the snack mix group ($p = 0.030$) at M6, and among the 1.5 oz almond group at M3 ($p = 0.045$) but not M6. Only the 3 oz almond group remained statistically significant after the adjustment for multiple comparisons (adjusted $p = 0.039$). Subjects in the 1.5 oz almond group also had an improvement in the Motor Screening Task (MOT), which is a general assessment of sensorimotor function and comprehension, at M3 ($p = 0.046$) but not at M6.

These results suggest that changes in any cognitive measure over time did not statistically differ among three dietary groups, and that the independent effect of almonds on cognitive performance was relatively small as compared to the effect of repeated exposure to the tests.

3.3. Serum tocopherols

Baseline serum tocopherols were not significantly different among groups ($p > 0.05$). In the 3 oz almond group, serum α-tocopherol concentrations increased from M0 (1251 ± 381 μg/dL) at M3 (1356 ± 313 μg/dL, $p = 0.004$) and M6 (1346 ± 325 μg/dL, $p = 0.019$), and serum γ-tocopherol concentrations decreased from M0 (148 ± 74 μg/dL) at M3 (123 ± 84 μg/dL, $p = 0.004$) and was borderline significantly lower at M6 (128 ± 77 μg/dL, $p = 0.054$) (Table 4). Serum α- and γ-tocopherols did not significantly change over time in the other two groups. Repeated measure ANOVA also demonstrated the group x time effect is significant for γ-tocopherol ($p_{\text{group x time}} = 0.026$) but not α-tocopherol ($p_{\text{group x time}} > 0.10$), indicating that changes in serum γ-tocopherol concentrations over time in the 3 oz almond group were significantly different than the other two groups. It should be noted that serum γ-tocopherol concentrations in the 3 oz almond group were significantly higher than those in 1.5 oz almond group at M0 (148 ± 74 vs 99 ± 49 μg/dL, Bonferroni adjusted $p = 0.041$).

Given that changes were observed with serum tocopherols at M3 and M6, as well as SSP Reverse (SL), PAL (FAMS), and OTS (PSFC) only in the 3 oz almond group, additional analyses were performed to evaluate the relationship between changes in serum tocopherols and changes in cognitive measures from M0 to either M3 or M6 in this group. No significant correlations were observed at either time intervals.

3.4. Serum biomarkers of inflammation and oxidative status

Serum IL-6, CRP, and sICAM-1 concentrations were not significantly different at M0 among the three groups (Table 4). They also remained unchanged at M3 and M6 in all groups. Concentrations of serum IL-12 were below the LOD in 66% of all samples and thus excluded from all analyses.

Similarly, measures of oxidative status (Table 4) were not significantly different at baseline. Red blood cell (RBC) glutathione peroxidase (GPX) activities significantly increased at M6 in the 1.5 oz Almonds group (1.96 ± 0.68 μmol·min^{-1}·mL^{-1} at M0, 2.24 ± 0.64 μmol·min^{-1}·mL^{-1} at M6, $p = 0.039$), but this increase was not statistically different from the non-significant changes in the other two groups. No significant changes were observed for other oxidative status measures at M3 or M6 from M0 within each group, and comparisons among the three groups yielded no significant difference.

3.5. Serum cholesterol and triglyceride

Serum total cholesterol, VLDL-C, LDL-C, and HDL-C, and total triglyceride concentrations were comparable among the three groups at baseline. As shown in Table 4, total serum cholesterol significantly increased at M3 (224 ± 30 mg/dL, $p = 0.048$) and M6 (225 ± 29 mg/dL, $p = 0.043$) from M0 (214 ± 38 mg/dL) in the snack mix group but not in the other two almond groups, and the group x time interaction approached statistical significance ($p_{\text{group x time}} = 0.072$). Serum VLDL-C, LDL-C, and HDL-C and triglyceride concentrations remained unchanged in all groups at M3 and M6. The non-HDL-C:HDL-C as well as the triglyceride:HDL-C ratios were also constant in all groups at M3 and M6.

4. Discussion

The study found encouraging improvement in cognitive performance among middle-aged to older adults who consumed 3 oz of almonds daily over a six-month

Table 4. Serum, plasma, or red blood cell measures (mean ± SD) of retinol, tocopherols, biomarkers of inflammation and oxidative status, and lipids at baseline (M0), three months (M3), and six months (M6) in healthy adults consuming almonds (1.5 or 3.0 oz/d) or a snack mix (3.5 oz/d).

	1.5 oz Almonds ($n = 19$)					3 oz Almonds ($n = 24$)					3.5 oz Snack mix ($n = 17$)				
Measure [a]	M0	M3	M6	P_{M3-M0}	P_{M6-M0}	M0	M3	M6	P_{M3-M0}	P_{M6-M0}	M0	M3	M6	P_{M3-M0}	P_{M6-M0}
α-Tocopherol	1.35 ± 0.52	1.41 ± 0.61	1.35 ± 0.42	0.257	0.505	1.25 ± 0.38	1.36 ± 0.31	1.35 ± 0.33	0.004**	0.019*	1.27 ± 0.4211	1.28 ± 0.28	1.31 ± 0.37	0.568	0.180
γ-Tocopherol	99 ± 49	89 ± 54	97 ± 57	0.196	0.916	148 ± 74	123 ± 84	128 ± 77	0.004**	0.054	4 ± 55	128 ± 59	121 ± 50	0.153	0.310
IL-6	0.57 ± 0.31	0.59 ± 0.30	0.59 ± 0.52	0.511	0.667	0.88 ± 0.87	0.74 ± 0.70	0.84 ± 0.79	0.349	0.777	0.70 ± 0.60	0.75 ± 0.82	1.06 ± 0.90	0.996	0.082
CRP	1.71 ± 1.11	1.50 ± 0.97	2.59 ± 4.00	0.624	0.781	4.13 ± 6.34	3.18 ± 3.88	8.45 ± 23.13	0.946	0.249	3.38 ± 5.74	2.60 ± 3.77	3.57 ± 4.93	0.434	0.792
sICAM-1	320 ± 47	315 ± 54	342 ± 105	0.485	0.404	360 ± 94	339 ± 92	381 ± 91	0.341	0.083	345 ± 81	330 ± 62	333 ± 69	0.328	0.592
Plas-SOD	2.16 ± 0.65	2.04 ± 0.57	2.16 ± 0.57	0.351	0.993	1.90 ± 0.35	1.93 ± 0.49	2.00 ± 0.43	0.659	0.235	1.86 ± 0.63	1.77 ± 0.38	2.02 ± 0.51	0.541	0.297
RBC-SOD	146 ± 21	146 ± 16	136 ± 20	0.939	0.152	140 ± 20	140 ± 19	137 ± 16	0.754	0.557	137 ± 21	137 ± 22	135 ± 20	0.967	0.773
Plas-GR	19.3 ± 9.4	16.4 ± 6.0	15.7 ± 8.9	0.321	0.170	18.5 ± 9.3	19.0 ± 7.6	17.8 ± 7.7	0.756	0.723	15.7 ± 9.4	18.3 ± 9.1	21.1 ± 10.0	0.377	0.114
RBC-GR	178 ± 81	211 ± 109	161 ± 71	0.124	0.662	206 ± 179	175 ± 51	179 ± 59	0.405	0.508	151 ± 72	182 ± 66	162 ± 102	0.287	0.632
RBC-GPX	1.96 ± 0.68	2.03 ± 0.70	2.24 ± 0.64	0.654	0.039*	1.96 ± 0.84	1.91 ± 0.59	1.90 ± 0.66	0.535	0.567	2.05 ± 0.67	2.09 ± 0.94	2.05 ± 0.67	0.724	0.992
RBC-GSH	45.1 ± 43.6	40.4 ± 39.7	51.3 ± 47.6	0.670	0.703	48.3 ± 46.7	64.9 ± 56.4	61.1 ± 64.6	0.099	0.205	79.5 ± 43.3	69.9 ± 63.2	58.2 ± 61.5	0.473	0.153
Total-C	218 ± 47	215 ± 45	210 ± 40	0.435	0.100	222 ± 37	218 ± 46	217 ± 38	0.368	0.351	214 ± 38	224 ± 30	225 ± 29	0.048*	0.043*
VLDL-C	24 ± 13	24 ± 14	24 ± 11	1	0.920	35 ± 27	34 ± 24	31 ± 20	0.565	0.302	27 ± 12	25 ± 9	31 ± 15	0.357	0.083
LDL-C	126 ± 35	122 ± 31	119 ± 32	0.323	0.054	123 ± 34	120 ± 32	123 ± 27	0.607	0.963	126 ± 37	136 ± 25	133 ± 28	0.112	0.145
HDL-C	67 ± 15	69 ± 17	66 ± 13	0.276	0.584	64 ± 22	64 ± 23	63 ± 18	0.712	0.260	60 ± 13	62 ± 16	60 ± 14	0.109	0.845
TG	122 ± 64	121 ± 68	122 ± 56	0.909	0.979	177 ± 138	170 ± 122	158 ± 101	0.570	0.306	137 ± 61	128 ± 46	156 ± 77	0.384	0.079

IL-6: interleukin-6, CRP: c-reactive protein, sICAM-1: soluble intercellular adhesion molecule-1, Plas: Plasma, SOD: superoxide dismutase, RBC: red blood cell, GR: glutathione reductase, GPX: glutathione peroxidase, GSH: reduced glutathione, Total-C: total cholesterol, VLDL-C: very low density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride.

[a]All measures are in serum if not indicated otherwise. Units are as follows: retinol and tocopherols in μg/dL; IL-6 in pg/mL; CRP in mg/L; sICAM-1 in ng/mL; Plas-SOD, RBC-SOD in U/mL; Plas-GR, RBC-GR in $nmol \cdot min^{-1} \cdot mL^{-1}$; RBC-GPX in $\mu mol\ min^{-1}\ mL^{-1}$; RBC-GSH in μM; cholesterols and TG in mg/dL.

* $p < 0.05$, ** $p < 0.01$.

period. Subjects in the 3 oz almond group, but not the control group, had improved memory and executive function as measured by improved performance on SSP Reverse (SL), PAL (FAMS), and OTS (PSFC) at the end of the study. However, changes in any cognitive measure over time did not statistically differ among three dietary groups. Therefore, the independent effect of almonds on cognitive performance is may be small compared to the effect from repeated exposure to cognitive tests. Indeed, almonds are rich in nutrients such as MUFA, α-tocopherol, and fibers [27], that are known to exhibit biological roles such as potent antioxidants and anti-inflammatory-agents [28–31]. However, we were not able to observe any significant changes in the markers of systemic inflammation and oxidative stress, despite detecting a significant increase in serum concentrations of lipophilic antioxidant α-tocopherol in the 3 oz almond group. In our subject population, the measures were within a healthy normal range at the start of the study, which may explain an inability to detect significant changes in these markers. Our findings are consistent with previous studies reporting an increase in antioxidant concentrations without observing changes in oxidative stress or total antioxidant capacity in plasma of adequately nourished adults [32].

While our findings suggest increased serum α-tocopherol to be a good marker of compliance to the intervention, the increase was not associated with the improved performance in any cognitive tests. Still, the significance of α-tocopherol on a brain function should not be neglected. Brain is enriched in PUFAs, which exert anti-inflammatory and pro-resolving activities [33]. Chronic low-grade inflammation, which is characteristic for the aging population, has been found to have detrimental effects on normal function of many tissues, including nervous tissue [34]. One of the major functions of α-tocopherol is being a protector of the integrity of PUFA, and thereby cell membrane morphology and function, indicating an importance of maintaining adequate α-tocopherol levels in a brain [35]. Nevertheless, a recently published review paper reported increased all-cause mortality risk with antioxidant supplementation, which also contained supraphysiological doses of vitamin E [36]. These findings are in agreement with previous recommendations against vitamin E supplementation in cardiovascular disease and cancer [36,37] *(Supplemental Vitamins and Minerals for CVD Prevention and Treatment, 2018*) and emphasize a significance of achieving daily micronutrients recommendations through a healthy diet, including those for vitamin E. Possibly, other nutrients and bioactives found in almonds, but not addressed in this study, may play an important role in the cognition process through other unknown biological pathways. Emerging data suggest that polar lipids, such as phospholipids and sphingolipids that are found in almonds [38,39], exhibit many key roles important for proper brain function, from maintaining synaptic function and plasticity to signal transduction and anti-inflammatory function [40,41]. Therefore, identifying nutritional intervention with anti-inflammatory properties could serve as an important mean to prevent or postpone age-related cognitive decline.

It has been shown that feeding almonds to healthy rats increased brain acetylcholine levels and enhanced memory function [42]. Acetylcholine synthesis is dependent on choline [43], whose levels are found in the amount of 52 mg/100 g almonds (or 44.2 mg/3 oz almonds) [27]. Administration of 3 oz of almonds also provides 72% and 85% of the recommended dietary allowance (RDA) of riboflavin for men and women, respectively [27,44]. Riboflavin functions in many cognitive processes, such as synthesis of monoamine neurotransmitters through the folate-dependent pathway. Riboflavin also exhibits antioxidant properties, which are known to be neuroprotective [45]. Additionally, polyphenol intakes are associated with reduced cognitive decline in some studies [46], and a number of phenolic compounds in almonds have been identified and demonstrated to prevent the formation of thiobarbituric acid reactive substances (TBARS) in brain cells and lipid peroxidation in biomembranes [47,48]. Also, the observed improvement in cognition may be due to a collective effort of all almond nutrients. Further studies simultaneously evaluating different micronutrients, phytochemicals in almonds, and their synergistic effect are warranted.

Findings from our study support previously reported associations of nut consumption, including almonds, and better cognitive performance in older adults [10–13]. However, the cognitive improvement observed in the 3 oz almond group was not statistically different from the other two groups. A longer duration in dietary intake may be required to yield larger cognitive effects. While a greater adherence to healthy dietary patterns is associated with lower risk of cognitive impairment [49–51], an intervention with a single food, such as mixed berries or avocados, may have small yet significant effects on cognitive changes [19,52], as we observed in this study. Alternatively, stronger cognitive effects from an almond intervention could possibly be observed in participants who had poorer health or nutrition status or were at higher risk of cognitive impairment. We did not observe significant changes in serum measures of oxidative status and inflammation

in this study. However, it was previously reported that consumption of ~2.6 oz of almonds per day for one month reduced oxidized LDL and lipid peroxidation in older hyperlipidemic subjects [53]. Consuming 2 oz almonds per day for 2 weeks reduced serum inflammatory markers IL-6 and CRP in subjects with type 2 diabetes mellitus [54]. Moreover, given that higher education is consistently associated with lower risk of cognitive decline [55,56], and that 68% of our participants graduated from a 4-year college or graduate school, our study population were possibly at lower risk of cognitive decline than the general population.

Interestingly, we observed an improvement in cognitive capacity in all three groups, independent of dietary intervention, after repeated exposure to the tests over time (known as the practice effect) [57]. Our findings are consistent with previous studies reporting improvement in cognitive measures with repeated exposure to the cognitive tests in the control group [19]. It may be possible to minimize the practice effect with a more extensive training session before each testing. However, it is likely that the effect of almonds in this present study was under the influence of the practice effect. Consequently, only when the effect of the treatment is sufficiently large that the group x time effect is statistically significant. While the observed improvement in cognitive function was not significantly different among all groups, when each group was analyzed independently, a significant improvement in some cognitive tests, such as SSP Reverse, PAL, and OTS, was observed only in subjects receiving 3 oz at M6 vs. M0. These results suggest that the almond intervention may have an effect on cognitive function.

This study is the first trial with the primary aim to assess the effect of almond consumption on changes of cognition over a 6-month period. Strengths of the present study include the randomized control design, use of a well-validated dietary questionnaire, comprehensive evaluation and diagnostic protocol at baseline and each examination, and the use of a broad battery of standardized cognitive tests for a more-detailed characterization of cognitive function. However, this study was not without limitations. First, our study had a relatively small sample size, which may affect the statistical power of the analyses, including cognitive function and serum measurements of oxidative stress and inflammation biomarkers. Second, 6 months may not be long enough to observe cognitive improvements resulting from almond administration, which is independent of the practice effect. Indeed, in the PREDIMED-NAVARRA study the beneficial effect of a Mediterranean diet supplemented with either olive oil or mixed nuts on cognitive function was observed after 6.5 y of nutritional intervention [6]. Third, the ceiling effect was observed in some cognitive tests, such as the percent correct 4 (PC4) and 12 s delay (PC12) in the Delayed Matching to Sample (DMS) where 37% and 35% of participants, respectively, received the maximum scores at baseline. Therefore, these tests may not be sensitive to gauge cognitive improvement in this study population. Additionally, OTS has a relatively poor accuracy since participants responded to a prompt by only providing one final number without demonstrating a reasoning process.

Findings from this study are impactful. Although there was no significant difference in cognition improvement among the three groups at M6, only the 3 oz almond group showed significant improvement in measures of executive function, visual memory, and learning ability at M6 compared to M0. RCTs of longer duration and larger sample size are warranted to provide added evidence of beneficial effects of almond consumption on cognitive function in the middle-aged to older adults.

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References

[1] Langa KM, Levine DA. The diagnosis and management of mild cognitive impairment: a clinical review. JAMA. 2014;312:2551–61.

[2] Roberts RO, Knopman DS, Mielke MM, Cha RH, Pankratz VS, Christianson TJH, et al. Higher risk of progression to dementia in mild cognitive impairment cases who revert to normal. Neurology. 2014;82:317–25.

[3] Johnson EJ. A possible role for lutein and zeaxanthin in cognitive function in the elderly. Am J Clin Nutr. 2012;96:1161S–5S.
[4] Mangialasche F, Solomon A, Kåreholt I, Hooshmand B, Cecchetti R, Fratiglioni L, et al. Serum levels of vitamin E forms and risk of cognitive impairment in a Finnish cohort of older adults. Exp Gerontol. 2013;48:1428–35.
[5] Naqvi AZ, Harty B, Mukamal KJ, Stoddard AM, Vitolins M, Dunn JE. Monounsaturated, trans, and saturated fatty acids and cognitive decline in women. J Am Geriatr Soc. 2011;59:837–43.
[6] Martínez-Lapiscina EH, Clavero P, Toledo E, San Julián B, Sanchez-Tainta A, Corella D, et al. Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. J Nutr Health Aging. 2013;17:544–52.
[7] Nilsson A, Tovar J, Johansson M, Radeborg K, Björck I. A diet based on multiple functional concepts improves cognitive performance in healthy subjects. Nutr Metab. 2013;10:49.
[8] Hernanz A, De la Fuente M, Navarro M, Frank A. Plasma aminothiol compounds, but not serum tumor necrosis factor receptor II and soluble receptor for advanced glycation end products, are related to the cognitive impairment in Alzheimer's disease and mild cognitive impairment patients. Neuroimmunomodulation. 2007;14:163–7.
[9] O'Brien J, Okereke O, Devore E, Rosner B, Breteler M, Grodstein F. Long-term intake of nuts in relation to cognitive function in older women. J Nutr Health Aging. 2014;18:496–502.
[10] Valls-Pedret C, Lamuela-Raventós RM, Medina-Remón A, Quintana M, Corella D, Pintó X, et al. Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. J Alzheimers Dis. 2012;29:773–82.
[11] Nooyens ACJ, Bueno-de-Mesquita HB, van Boxtel MPJ, van Gelder BM, Verhagen H, Verschuren WMM. Fruit and vegetable intake and cognitive decline in middle-aged men and women: the Doetinchem Cohort study. Br J Nutr. 2011;106:752–61.
[12] Nurk E, Refsum H, Drevon CA, Tell GS, Nygaard HA, Engedal K, Smith AD. Cognitive performance among the elderly in relation to the intake of plant foods. The Hordaland health study. Br J Nutr. 2010;104:1190–201.
[13] 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. Pergamon. 1975;12:189–98.
[14] Beck AT, Steer RA, Carbin MG. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. Clin Psychol Rev. 1988;8:77–100.
[15] Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Br Med J. 2010;340:c332.
[16] Robbins TW, James M, Owen AM, Sahakian BJ, McInnes L, Rabbitt P. Cambridge neuropsychological test automated battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. Dementia. 1994;5:266–81.
[17] Wade AT, Davis CR, Dyer KA, Hodgson JM, Woodman RJ, Keage HAD, Murphy KJ. A Mediterranean diet with fresh, lean pork improves processing speed and mood: cognitive findings from the MedPork randomised controlled trial. Nutrients [Internet]. 2019;11. Available from: http://dx.doi.org/10.3390/nu11071521
[18] Furlong ON, Parr HJ, Hodge SJ, Slevin MM, Simpson EE, McSorley EM, McCormack JM, Magee PJ. Consumption of a soy drink has no effect on cognitive function but may alleviate vasomotor symptoms in post-menopausal women; a randomised trial. Eur J Nutr [Internet]. 2019; Available from: http://dx.doi.org/10.1007/s00394-019-01942-5
[19] Scott TM, Rasmussen HM, Chen O, Johnson EJ. Avocado consumption increases macular pigment density in older adults: a randomized, controlled trial. Nutrients [Internet]. 2017;9. Available from: http://dx.doi.org/10.3390/nu9090919
[20] Simpson EE, Maylor EA, McConville C, Stewart-Knox B, Meunier N, Andriollo-Sanchez M, et al. Mood and cognition in healthy older European adults: the Zenith study. BMC Psychol. 2014;2(11).
[21] Polcher A, Frommann I, Koppara A, Wolfsgruber S, Jessen F, Wagner M. Face-name associative recognition deficits in subjective cognitive decline and mild cognitive impairment. J Alzheimers Dis. 2017;56:1185–96.
[22] Fowler KS, Saling MM, Conway EL, Semple JM, Louis WJ. Paired associate performance in the early detection of DAT. J Int Neuropsychol Soc. 2002;8:58–71.
[23] Soares FC, de Oliveira TCG, de Macedo LDD, Tomás AM, Picanço-Diniz DLW, Bento-Torres J, et al. CANTAB object recognition and language tests to detect aging cognitive decline: an exploratory comparative study. Clin Interv Aging. 2015;10:37–48.
[24] Lee A, Archer J, Wong CKY, Chen S-HA, Qiu A. Age-related decline in associative learning in healthy Chinese adults. PLoS One. 2013;8:e80648.
[25] Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr. 1979;32:2143–9.
[26] McKay DL, Chen C-YO, Zampariello CA, Blumberg JB. Flavonoids and phenolic acids from cranberry juice are bioavailable and bioactive in healthy older adults. Food Chem. 2015;168:233–40.
[27] US Department of Agriculture, Agriculture Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28 [Internet]. 2015 [cited 2019 Sep]. Available from: https://ndb.nal.usda.gov/ndb/
[28] Rocha DM, Bressan J, Hermsdorff HH. The role of dietary fatty acid intake in inflammatory gene expression: a critical review. Sao Paulo Med J. 2017;135:157–68.
[29] Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. Free Radic Biol Med. 2007;43:4–15.
[30] Nielsen FH. Magnesium deficiency and increased inflammation: current perspectives. J Inflamm Res. 2018;11:25–34.
[31] Andoh A, Bamba T, Sasaki M. Physiological and anti-inflammatory roles of dietary fiber and butyrate in intestinal functions. JPEN J Parenter Enteral Nutr. 1999;23:S70–S73.

[32] Li L, Chen C-YO, Aldini G, Johnson EJ, Rasmussen H, Yoshida Y, et al. Supplementation with lutein or lutein plus green tea extracts does not change oxidative stress in adequately nourished older adults. J Nutr Biochem. 2010;21:544–9.
[33] Laye S, Nadjar A, Joffre C, Bazinet RP. Anti-inflammatory effects of omega-3 fatty acids in the brain: physiological mechanisms and relevance to pharmacology. Pharmacol Rev. 2018;70(1):12–38.
[34] Calder PC, Bosco N, Bourdet-Sicard R, Capuron L, Delzenne N, Dore J, et al. Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition. Ageing Res Rev. 2017;40:95–119.
[35] Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. Free Radic Biol Med. 2007;43(1):4–15.
[36] Jenkins DJA, Spence JD, Giovannucci EL, Kim YI, Josse R, Vieth R, et al. Supplemental vitamins and minerals for CVD prevention and treatment. J Am Coll Cardiol. 2018;71(22):2570–2584.
[37] Klein EA, Thompson Jr IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the Selenium and vitamin E Cancer Prevention trial (SELECT). JAMA. 2011;306(14):1549–56.
[38] Fallico B, Ballistreri G, Areana E, Tokusoglu O. Nut bioactives: phytochemicals and lipid-based components of almonds, hazelnuts, peanuts, pistachios, and walnuts. In: Tokucoglu O, Hall C, editors. Fruits and cereal bioactives: sources, chemistry, and applications, 1st ed.; Boca Raton, FL: CRC Press, 2011. p. 185–212.
[39] Malisiova F, Hatziantoniou S, Dimas K, Kletstas D, Demetzos C. Liposomal formulations from phospholipids of Greek almond oil properties and biological activity. Z Naturforsch C J Biosci. 2004;59(5-6):330–4.
[40] Zheng L, Fleith M, Giuffrida F, O'Neill BV, Schneider N. Dietary polar lipids and cognitive development: a narrative review. Adv Nutr. 2019;10(6):1163–1176.
[41] Perez-Galvez A, Jaren-Galan M, Garrido-Fernandez J, Visitacion Calvo M, Visioli F, Fontecha J. Activities, bioavailability, and metabolism of lipids from structural membranes and oils: promising research on mild cognitive impairment. Pharmacol Res. 2018;134:299–304.
[42] Batool Z, Sadir S, Liaquat L, Tabassum S, Madiha S, Rafiq S, et al. Repeated administration of almonds increases brain acetylcholine levels and enhances memory function in healthy rats while attenuates memory deficits in animal model of amnesia. Brain Res Bull. 2016;120:63–74.
[43] Taylor P, Brown JH. Synthesis, storage and release of acetylcholine. Philadelphia: Lippincott-Raven; 1999.
[44] Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, its Panel on Folate, Vitamins OB, Choline. Riboflavin. National Academies Press (US); 1998.
[45] Kennedy DO. B vitamins and the brain: mechanisms, dose and efficacy–a review. Nutrients. 2016;8(2). doi:10.3390/nu8020068.
[46] Colizzi C. The protective effects of polyphenols on Alzheimer's disease: A systematic review. Alzheimers Dement (NY). 2019;5:184–196.
[47] Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA. Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. Food Chem Toxicol. 2008;46:2230–5.
[48] Sang S, Lapsley K, Jeong W-S, Lachance PA, Ho C-T, Rosen RT. Antioxidative phenolic compounds isolated from almond skins (Prunus amygdalus Batsch). J Agric Food Chem. 2002;50:2459–63.
[49] Mattei J, Bigornia SJ, Sotos-Prieto M, Scott T, Gao X, Tucker KL. The mediterranean diet and 2-year change in cognitive function by status of type 2 diabetes and glycemic control. Diabetes Care. 2019;42:1372–9.
[50] Wright RS, Waldstein SR, Kuczmarski MF, Pohlig RT, Gerassimakis CS, Gaynor B, et al. Diet quality and cognitive function in an urban sample: findings from the healthy aging in neighborhoods of diversity across the life Span (HANDLS) study. Public Health Nutr. 2017;20:92–101.
[51] Wengreen HJ, Neilson C, Munger R, Corcoran C. Diet quality is associated with better cognitive test performance among aging men and women. J Nutr. 2009;139:1944–9.
[52] Nilsson A, Salo I, Plaza M, Björck I. Effects of a mixed berry beverage on cognitive functions and cardiometabolic risk markers; A randomized cross-over study in healthy older adults. PLoS One. 2017;12:e0188173.
[53] Jenkins DJA, Kendall CWC, Marchie A, Josse AR, Nguyen TH, Faulkner DA, et al. Almonds reduce biomarkers of lipid peroxidation in older hyperlipidemic subjects. J Nutr. 2008;138:908–13.
[54] Liu J-F, Liu Y-H, Chen C-M, Chang W-H, Chen C-YO. The effect of almonds on inflammation and oxidative stress in Chinese patients with type 2 diabetes mellitus: a randomized crossover controlled feeding trial. Eur J Nutr. 2013;52:927–35.
[55] Wilson RS, Hebert LE, Scherr PA, Barnes LL, de Leon CF M, Evans DA. Educational attainment and cognitive decline in old age. Neurology. 2009;72:460–5.
[56] Zahodne LB, Stern Y, Manly JJ. Differing effects of education on cognitive decline in diverse elders with low versus high educational attainment. Neuropsychology. 2015;29:649–57.
[57] Goldberg TE, Harvey PD, Wesnes KA, Snyder PJ, Schneider LS. Practice effects due to serial cognitive assessment: implications for preclinical Alzheimer's disease randomized controlled trials. Alzheimers Dement. 2015;1:103–11.