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A Lower Ratio of Omega-6 to Omega-3 Fatty Acids Predicts Better Hippocampus-Dependent Spatial Memory and Cognitive Status in Older Adults

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Objective: Evidence from several cross-sectional studies indicates that an increase in omega-6 to omega-3 fatty acids (FAs) may negatively affect cognition in old age. The hippocampus is among the first neural structures affected by age and atrophy in this brain region is associated with cognitive decline. Therefore, we hypothesized that a lower omega-6:3 FA ratio would predict better hippocampus-dependent spatial memory, and a higher general cognitive status. ***Method:*** Fifty-two healthy older adults completed a Food Frequency Questionnaire, the Montreal Cognitive Assessment test (MoCA; a test of global cognition) and virtual navigation tasks that assess navigational strategies and spatial memory. ***Results:*** In this cross-sectional study, a lower ratio of omega-6 to omega-3 FA intake strongly predicted more accurate hippocampus-dependent spatial memory and faster learning on our virtual navigation tasks, as well as higher cognitive status overall. ***Conclusions:*** These results may help elucidate why certain dietary patterns with a lower omega-6:3 FA ratio, like the Mediterranean diet, are associated with reduced risk of cognitive decline.

General Scientific Summary
Numerous studies in the literature have demonstrated the importance of dietary elements in healthy cognitive aging. Results in the current paper show that a lower omega-6 to omega-3 ratio in the diet significantly correlated to spatial memory associated to the hippocampus, a structure of the brain involved in healthy cognition. These results can help explain why certain dietary patterns, like the Mediterranean diet which includes a lower omega-6:3 ratio, are effective at delaying the onset of Alzheimer's disease.

Keywords: Omega-6:3 ratio, navigation, hippocampus, cognitive mapping, older adults

A low omega-6 to omega-3 fatty acid (FA) ratio in the diet has been shown to reduce the risk of Alzheimer's disease (AD) and cognitive decline in older adults (Loef & Walach, 2013; Samieri et al., 2008; Vercambre, Boutron-Ruault, Ritchie, Clavel-Chapelon, & Berr, 2009). However, to date, no studies have assessed the relationship between the omega-6:3 FA ratio and cognitive mea-

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sures that depend on the hippocampus, one of the first areas of the brain affected in AD (Braak & Braak, 1991), such as spatial memory (O'Keefe & Nadel, 1978). Understanding the relationship between the omega-6:3 ratio and hippocampal function may create new avenues for AD prevention.

The relative amount of omega-6 to omega-3 FA could modulate neuronal processes and disease progression through both their individual functions and their interaction. Indeed, omega-6 FAs can modulate the amount of omega-3 FAs available in the body (Taha et al., 2014) as linoleic acid (18:2n-6) and alpha-linolenic (18:3n-3) acid compete for the same desaturase enzymes (Nakamura & Nara, 2004). Thus, their metabolisms are closely linked and competitive (Nakamura & Nara, 2004; Yehuda, Rabinovitz, & Mostofsky, 1999). The eicosanoid derivatives of omega-3 and omega-6 FA are anti- and proinflammatory, respectively, and function distinctly (Nakamura & Nara, 2004). Indeed, docosahexaenoic acid (DHA; 22:6n-3), an essential FA that is created when alpha-linolenic acid elongates, has essential functions in the brain (Salem, Litman, Kim, & Gawrisch, 2001; Yehuda, 2003). Further, omega-3 FA reduces LDL insertion in atherosclerotic plaques in arterial tissue, and thus can affect health overall (Deckelbaum & Torrejon, 2012). Thus, the balance of each of these essential fatty acids may impact various health outcomes.

The ratio of omega-6:3 FA in the Western diet increased by 77% from 1909–1999 mostly due to the increased consumption of processed foods and grain-fed animals (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011) which, in turn, may have an impact on health and cognition. This increase is not present in other dietary patterns, like the Mediterranean diet, that are associated with a reduced prevalence of cardiovascular disease, cancer, diabetes, and AD (Otaegui-Arrazola, Amiano, Elbusto, Urdaneta, & Martinez-Lage, 2014; Simopoulos, 2011; Sofi, Cesari, Abbate, Gensini, & Casini, 2008; Sofi, Macchi, Abbate, Gensini, & Casini, 2014). A lower omega-6:3 FA ratio is associated with a reduced risk of cognitive decline (Vercambre et al., 2009) while lower scores on a test of general cognition are associated with a higher omega-6:3 FA ratio in blood plasma (Cherubini et al., 2007) or as measured by a food frequency questionnaire (Gonzalez, Huerta, Fernandez, Patterson, & Lasheras, 2010). Further, individuals with mild cognitive impairment show a higher neuronal content of arachidonic acid (AA; 20:4n-6), a derivative of linoleic acid, and lower content of DHA (Milte et al., 2011). The relationship between the ratio of omega-6:3 FA and hippocampal-dependent spatial memory remains to be investigated.

Spatial memory abilities are closely linked with global cognition in older adults; they rely on the hippocampus and decline with age (Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005). During navigation, individuals can use the hippocampus-based spatial memory system to build a cognitive map of the environment (O'Keefe & Nadel, 1978) or use the caudate nucleus of the striatum to navigate in a stimulus-response manner (Packard, Hirsh, & White, 1989). Use of the spatial memory system and the stimulus-response system are associated with increased fMRI activity and gray matter in the hippocampus and caudate nucleus, respectively (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003). To date, no studies have looked at the relationship between the dietary ratio of omega-6:3 FA and spatial memory in healthy older adults. Therefore, we investigated the relationship between hippocampal-dependent spatial memory, cognition, and the dietary ratio of omega-6:3 FA in a group of healthy older adults free of cognitive decline. We hypothesized that individuals whose diets contained a lower ratio of omega-6:3 FA would have better spatial memory, perform better on our virtual navigation tasks and perform better on a measure of global cognition.

Subjects and Method

Subjects

A total of 52 healthy older adults between the ages of 60 to 75 years of age (33 women, 19 men, mean age: 65.71 ± 0.62, mean education: 16.48 ± 0.47 years) completed this study and were recruited from the greater Montreal area as part of a larger study at the Douglas Mental Health University Institute in Verdun, Quebec. Participants were recruited through newspaper advertisements for an aging and memory study. Participants completed screening and testing individually in an interview room in either French or English, depending on their first or most proficient language. All participants were right-handed, and had normal or corrected vision. Participants were free of neurological or psychiatric disorders, drug and alcohol abuse, hypertension, high cholesterol, and diabetes, as assessed by a prescreening questionnaire. Participants underwent neuropsychological assessments to exclude individuals with mild cognitive impairment, dementia, and/or depression. Informed consent was obtained according to the guidelines put forth by the local ethics committee. The study protocol was approved by the Research Ethics Board at the Douglas Mental Health University Institute.

Method

Wayfinding task. A subset of participants (20 women, 14 men, mean age: 65.94 ± .74, mean education: 16.24 ± .65 years) completed a wayfinding task to assess their spatial memory abilities. The wayfinding task was created using the Unreal Editor program (Unreal Tournament, 2003; Epic Games, Raleigh, NC) and has been described previously (Dahmani, Ledoux, Boyer, & Bohbot, 2012; Etchamendy & Bohbot, 2007). The town was modeled after a virtual town by Hartley, Maguire, Spiers, and Burgess (2003). Briefly, the virtual town is made up of streets, alleys, buildings, and eight landmarks that include a convenience store, a pool, a park, a bank, among others (see Figure 1). Participants explored the town for 20 min to learn the locations of the landmarks. An experimenter ensured that they passed by each landmark at least twice and that they traveled through every passageway in the town. When the participant passed by each landmark the experimenter notified them to ensure that they attended to it. The landmarks were situated in such a way that the participants were unable to see others from their locations. This layout ensured that participants formed a spatial representation of the environment as they could not form "stimulus-response" type associations between the landmarks.

After the 20-min exploration, participants were administered probe trials. They completed six probe trials where the experimenter instructed them to navigate between two landmarks taking the shortest route possible. For each probe trial, participants were placed in front of a predetermined starting location (e.g., the pool)

Figure 1. Wayfinding task. In order from top left to bottom right: an aerial view of the virtual town's layout with the eight landmarks indicated with red dots; a bird's-eye-view of the virtual town; view of streets from a participant's perspective; an example of a landmark, in this case, a Future Shop. See the online article for the color version of this figure.

and asked to take the shortest route to a predetermined target location (e.g., the park). The landmarks were paired together only once and the same landmark was never used twice as a starting point or end point. The participants had 5 min to complete each probe trial (i.e., find the target landmark); if they did not succeed the experimenter stopped the trial and moved to the next one. Participants' accuracy was recorded based on whether they found the target location with the 5 min and computed as a percentage out of a score on six (i.e., if a participant reached four of the six landmarks they had 67% accuracy). These probe trials helped assess participants' spatial acquisition of the town's layout as participants who acquired a cognitive representation of the space would find significantly more target location. In sum, in the wayfinding task, the measure of learning was the participants' percent accuracy on the six probe trials. This variable was used as an index of their ability to use a spatial memory to complete the task.

The 4 on 8 Virtual Maze. All participants completed the 4 on 8 Virtual Maze (4/8 VM). The 4/8 VM is a virtual navigation task modeled after the rodent eight-arm radial maze. It was created using the Unreal Editor program (Unreal Tournament, 2003; Epic Games, Raleigh, NC) and has been described previously (Bohbot, Iaria, & Petrides, 2004; Bohbot et al., 2007; Iaria et al., 2003). The task consists of a minimum of five trials, each with two parts. In each part of the task, participants are placed in the center platform surrounded by eight radiating pathways, where each of them leads to a pit. In the first part of each trial, barriers block four pathways, while the other four pathways are accessible. The four accessible pathways each have an object (a golden statue) located at the bottom of their pits. Participants start each trial in the center of the radial maze and are always facing the same direction. In the first part of each trial, participants are instructed to go into all of the accessible pathways and pick up the objects located in the pit. They are told to remember which pathways they have visited because in the second part of each trial, all of the pathways are accessible (there are no more barriers), and they must avoid the pathways that they had previously visited in Part 1 in order to retrieve the objects. The goal in Part 2 of each trial was therefore to avoid entering a pathway that they visited in Part 1 and also reentering a pathway that they already visited. The radial maze is located in an environment enriched with landmarks, such as a tree, a rock, and a mountain chain (Figure 2A). Participants solve this task by spontaneously using one of two navigation strategies: the spatial strategy or the response strategy. A spatial strategy entails remembering the location of objects in relation to landmarks, for example, one might remember the location of an object relative to the tree and the mountain. A response strategy involves numbering arms from a specific starting position to remember the location of objects. Both strategies can be used equally to solve the task and one is not more advantageous than the other.

Participants reach criterion and move on to the probe trial when they solve Part 2 of a trial without error and complete a minimum of three trials. If participants do not reach criterion within the first three trials, participants can complete a maximum of five extra trials until they reach criterion. Trials to criterion was used as a cognitive measure of interest.

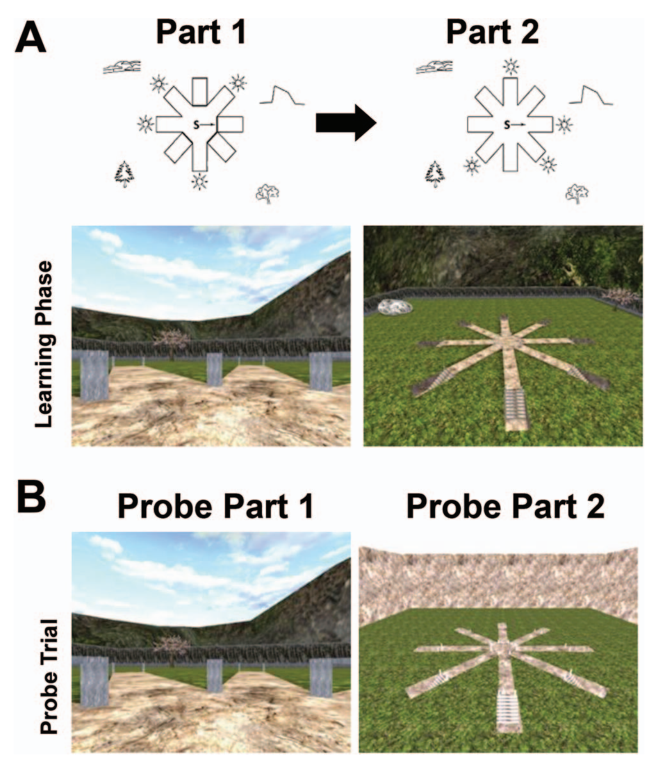

Figure 2. 4 on 8 Virtual Maze. (A) Example of a learning trial, in the first part of the trial half of the arms are blocked by barriers, and half are accessible. The participant must remember where they have been. In Part 2 of the trial all of the paths are accessible and the participant must avoid the pathways they previously visited. (B) Probe trial. Part 1 of the probe trial is the same as Part 1 of the learning trials. In Part 2 of the probe trial the environment is not visible. See the online article for the color version of this figure.

Probe trial. After reaching criterion, participants are administered the probe trial that consists of two parts. The first part of the probe trial is identical to the first part of all of the other trials with the objects located in the same pathways; the participants find themselves in the radial maze surrounded by landmarks and a landscape, and they are instructed to enter the pathways that are open, and to remember where they have been for the next part of the trial. In Part 2 of the probe trial, all external landmarks are removed and the surrounding environment appears uniform (Figure 2B); therefore, the environment contains no distinguishable features. As in Part 2 of the other trials, all of the pathways are accessible. Participants are instructed that the objects are still located in the pathways that were previously blocked by barriers. They are then told to retrieve all of the objects by avoiding the pathways that they previously visited in Part 1. Participants who rely on external landmarks to remember which pathways they have visited, that is, use the spatial strategy, make more errors during the probe trial because their reference points for navigation are removed, whereas participants who use a response strategy generally do not make errors because they relied more on a counting strategy that does not depend as much on external landmarks. Therefore, this section of the task helps distinguish participants who use a spatial strategy from those who use a response strategy.

At the end of the task participants were debriefed and gave a verbal report. The experimenter asked how they remembered the objects' locations. Verbal responses were recorded and transcribed. Two experimenters independently rated each verbal report as a spatial strategy or response strategy. In the case of disagreement, a third rater assessed the verbal report and the majority rating was chosen as the strategy.

Dietary assessment. Dietary intakes were assessed using the semiquantitative 78-item Food Frequency Questionnaire (FFQ) developed and validated in both French and English among adults by Shatenstein, Nadon, Godin, and Ferland (2005) which captures participants' usual food intake over the previous year. Specifically, the FFQ was validated using multiple nonconsecutive food records known for face validity for specific macro and micronutrients including polyunsaturated fatty acids (Shatenstein et al., 2005). The FFQ queries frequency and relative portion size of foods commonly found in the Canadian diet. Quantity is estimated as smaller than, equal to, or larger than portion size photos located on the facing page. The FFQ also contains a set of 23 questions that acquire additional dietary habit information, as well as physical activity, anthropomorphic, lifestyle, and demographic information. FFQ data were analyzed using customized software in Microsoft Access (Microsoft, Redmond, WA). Nutrient values were computed from a database used for the FFQ, based on the Canadian Nutrient File (Canada, 1982). The analysis is based on an algorithm that computes energy intakes, macro- and micronutrient values from the foods reported, and the frequencies and portion sizes selected by the respondent. For this study, the dietary variables of interest generated by the FFQ included total daily omega-3 intake and total daily omega-6 intake in grams/d as generated by the FFQ data analysis. These values were then used to calculate the omega-6:3 ratio for each participant.

To minimize the prevalence of self-reporting error, a set of established criteria was used to assess FFQ data plausibility (Shatenstein & Jabbour, 2009). Questionnaires were excluded if they contained one or more blank page, energy intakes that were less than 800 kcal or more than 4,000 kcal, or if >10% of food items were missing responses for frequency and portion size. SPSS (Version 16, SPSS Inc., Chicago, IL) was used for the plausibility analysis after the data was exported to Microsoft Excel. The plausibility analysis generated a score ranging from 1 to 5 with 1 being the most plausible and 5 leading to a firm recommendation to eliminate the FFQ from analysis; a score of 4 indicates poor plausibility and suggests eliminating the FFQ unless the respondent provides clarification. As previously stated, the dietary variables of interest generated by the FFQ included total daily omega-3 intake and total daily omega-6 intake. These values were used to calculate the omega-6:3 ratio for each participant.

Montreal cognitive assessment. Participants were screened for mild cognitive impairment and dementia using the French and English versions of the Montreal Cognitive Assessment Test (MoCA). The MoCA assesses several cognitive domains including short- and long-term recall, visuospatial abilities, executive function, attention, concentration, working memory, orientation, and language. Participants were excluded if they scored below 26 out of 30, the standard cutoff score to assess for mild cognitive impairment (Nasreddine et al., 2005). We used the MoCA score as a measure of general cognitive status.

Neuropsychological assessment. Participants underwent a battery of neuropsychological tasks to assess specific cognitive domains. The Rey Auditory Verbal Learning Task (RAVLT; Rey, 1964) assessed participants' verbal memory while the Rey-Osterrieth Complex Figure (ROCF; Osterrieth, 1944) assessed participants' visuospatial memory. Participants' IQ was measured with the Test of Non-Verbal Intelligence (TONI-III; Brown, Sherbenou, & Johnson, 1997).

Statistical analyses. All data were analyzed using SPSS version 20 (IBM Corp., Armonk, NY). Linear Regression Models were used to assess the dietary variables of interests' (ratio of omega 6:3, omega 3 and omega 6 independently) ability to predict measures of cognition for each task. Because of the limited sample size, we controlled only for age and education to include an appropriate number of predictors for each model (rule of thumb; 1 predictor for every 10 N).

All predictor variables were assessed for normality, independence, linearity, and equality of error variance both visually and statistically. Histograms and box plots revealed an extreme outlier in the ratio score and another for the omega-3 score, each from separate participants. Both data points were removed since they were greater than three standard deviations from the mean. We detected no other abnormalities in those two participants' food frequency questionnaire scores and thus the rest of the data were included in analysis. Skewness measures ($<\pm 1$) and histograms indicated the following variables were skewed: 4/8 VM trials to criterion, total omega-6, and total omega-3. Thus, we chose to bootstrap (bias-corrected accelerated 95% confidence intervals) our statistical models to account for deviations from parametric assumptions.

Group differences based on sex and strategy were assessed with independent samples t tests to determine if these characteristics (sex and strategy on the 4/8 VM) could influence our cognitive variables (MoCA score, 4/8 VM trials to criterion, and accuracy on the wayfinding task). We conducted a preliminary bivariate correlation analysis to assess the relationship between our primary dietary predictor variables of interest (omega-6:3 ratio, omega-3 intake and omega-6 intake) and the cognitive variables to justify inclusion in a linear regression model.

We used a linear regression model for accuracy on the wayfinding task and included the omega 6:3 ratio, age, and education as predictors to ensure model accuracy because of the limited sample size for this task ($N = 34$; i.e., rule of thumb, 1 predictor for every 10 N). Bootstrapped bias-corrected accelerated (BCa) 95% CIs were used to assess statistical significance.

For the 4/8 VM cognitive variable, a linear regression analysis using bootstrapped BCa 95% CIs was used. The original model contained all dietary variables, age, and education as predictors; however, we discovered high variance inflation factors (VIF > 10) for total omega-3 and total omega-6 intake due to their significant correlation in the model. Thus, we decided to run two separate regression models for the cognitive variable, including total omega-3 intake and total omega-6 intake separately.

Results

Subjects

All 52 participants completed the study, however, six participants were excluded from the analysis due to implausible responses on the FFQ (plausibility score >3). Therefore, a total of 46 participants were included in our analysis (28 women, 18 men, mean age: 65.91 ± 0.66). Three of the participants were smokers but indicated they did not smoke more than 10 cigarettes per day. Twenty-three of the participants reported taking vitamin/mineral supplements regularly, however, the type was not reported. Independent samples t tests indicated that men and women consumed comparable amounts of all dietary variables of interest (independent samples t test: bootstrapped BCa 95% CIs all passing 0) and performed comparably on wayfinding accuracy, the number trials needed to reach criterion, average latency, and average learning errors on the 4/8 VM, and the MoCA score (independent samples t test: bootstrapped BCa 95% CIs all passing 0). Thus participant sex was not included in the linear regression models. To ensure that participants' consumed ratio of omega-6:3 was not related to physiological factors that could affect health overall, we assessed whether amount of physical activity and BMI were associated with the omega-6:3 ratio. We found no association between both factors and the omega-6:3 ratio (Pearson correlation: BMI: $p > .05$, bootstrapped BCa 95% CI [−0.09, 0.50]; physical activity: $p > .05$, bootstrapped BCa 95% CI [−0.18, 0.19]). Demographic, anthropometric information, and FFQ data are presented in Table 1.

Wayfinding Task

Older adults on average found 76.76 ± 21.06% of the target location on the wayfinding task. Individuals who consumed a lower omega-6:3 ratio were more able to find the location of landmarks in the virtual town, that is, were better able to locate the local pool, post office, and so forth. Initial bivariate correlation analysis indicated that the ratio negatively correlated with wayfinding accuracy (bivariate correlations: $r = -0.34$, $p < .05$, bootstrapped BCa 95% CI [−0.56, −0.11]) while total omega-6 and omega-3 intake did not (bootstrapped BCa 95% CIs all passing zero).

Linear regression analysis indicated that a lower ratio score predicted better accuracy on the wayfinding task, that is, better spatial memory ($p < .01$, bootstrapped BCa 95% CI [−5.43, −.331]) (Figure 3A). Specifically, for every one standard deviation decrease in the ratio score participants' accuracy on the wayfinding task increased by 0.26 standard deviations. The results of this regression model are found in Table 2. This

Table 1
Participant Demographics and Performance Data[1]

	Men ($N = 18$)	Women ($N = 28$)	All participants ($N = 46$)
Demographic variables			
Age (y)	66.17 ± 1.08	65.75 ± .845	65.91 ± .659
IQ	104.0 ± 2.65	102.1 ± 2.41	102.8 ± 1.79
Education (y)	16.67 ± .953	16.02 ± .596	16.34 ± .516
BMI	27.00 ± .705	25.24 ± .944	25.93 ± .644
Performance variables[1]			
MoCA	27.39 ± .363	28.00 ± .262	27.76 ± .216
4/8 VM TTC	2.88 ± .290	2.36 ± .314	2.56 ± .223
Wayfinding % accuracy	78.6 ± 6.40[2]	75.5 ± 4.32[3]	76.8 ± 3.61[4]
Daily dietary intake as per the Food Frequency Questionnaire[1]			
Energy (kcal)	2009.9 ± 106.6	1861.9 ± .85.43	1919.8 ± 66.78
Total lipid (g)	76.74 ± 5.88	76.44 ± 4.98	76.56 ± 3.77
Omega-6 PUFA (g)	11.54 ± 1.15	13.16 ± 1.14	12.53 ± .828
Omega-3 PUFA (g)[5]	1.19 ± .108	1.49 ± .131	1.37 ± .092
Omega-6:3 ratio[5]	9.69 ± .297	8.57 ± .417	9.02 ± .287

Note. IQ = Intelligence Quotient; 4/8 VM TTC = 4 on 8 virtual maze trials to criterion; MoCA = Montreal Cognitive Assessment.
[1] Mean ± SEM for all variables. Group means for men and women were not significantly different from another. [2] $N = 20$. [3] $N = 14$. [4] $N = 34$, a subset of participants completed the Wayfinding task. [5] In these cases one outlier was excluded, therefore $N = 27$ women or $N = 45$ for all participants.

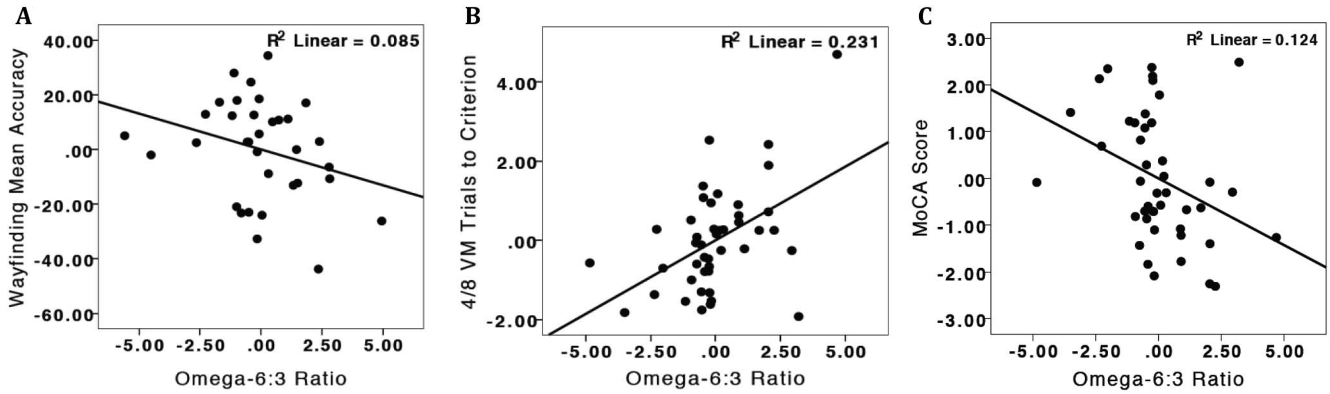

Figure 3. Omega-6:3 ratio predicts wayfinding accuracy, learning of the 4/8 VM, and MoCA score. A lower ratio score predicted spatial memory on the wayfinding task, faster learning on the 4/8 Virtual Maze, and higher scores of general cognition. Graphs are partial regression plots showing the significant relationship between the ratio of omega-6:3 and cognitive variables while controlling for all other covariates in the models (Note: regression model that contained omega-3 as a predictor used to generate plots for MoCA and 4/8 VM TTC). (A) Relationship between accuracy on the wayfinding and omega-6:3 ratio. (B) Relationship between trials to criterion on the 4/8 VM and omega-6:3 ratio. (C) Relationship between MoCA score and omega-6:3 ratio. Both variables in all plots are residuals.

regression model accounted for 31% of the total variance of accuracy on the wayfinding task ($F_{(3,30)} = 4.46$, $p = .01$).

Learning on the 4/8 VM

All participants learned the 4/8 VM within eight trials. The mean number of trials required to reach criterion was 2.56 ± 1.51 trials.

A lower ratio score was associated with fewer trials to reach criterion on the 4/8 VM. Initial bivariate correlations indicated that both the ratio score and omega-3 correlated with trials to criterion (Ratio score: $r = .51$, $p < .001$, bootstrapped BCa 95% CI [0.16, 0.72]; omega-3: $r = -0.29$, $p > .05$, bootstrapped BCa 95% CI [−0.45, −.04]). As such, with higher omega-3 intake, fewer trials were needed to reach criterion on the 4/8 VM. There was no association between omega-6 intake and trials to criterion on the 4/8 VM (bootstrapped BCa 95% CIs all passing zero).

Linear regression analysis indicated that the ratio of omega-6:3 significantly predicted the number of trials needed to reach criterion on the 4/8 VM in both models (omega-6 model: $p < .05$, bootstrapped BCa 95% CI [−.497, −.205], omega-3 model: $p < .05$, bootstrapped BCa 95% CI [.030, .688]; Figure 3B). Specifically, in the model that contained omega-6, for every one standard deviation increase in the ratio score, the number of trials needed to reach criterion, that is, the rate at which participants learned the task, increased by 0.5 standard deviations when all other predictors were held constant. The model that included total omega-3 intake as a predictor variable accounted for 43% of the total variance, $F(4, 39) = 7.27$, $p < .001$. The model that included total omega-6 intake as a predictor accounted for 43% of the total variance of trials to criterion on the 4/8 VM, $F(4, 40) = 7.60$, $p < .001$). In this model, education also predicted trials to criterion but to a lesser degree than the ratio score ($p < .05$, bootstrapped BCa 95% CI [−.240, −.066]; see Table 2).

Verbal reports indicated that 47.8% of participants used a spatial memory strategy while the remaining 52.2% of participants used a stimulus-response strategy to solve the task. We found no difference in predictor variables between strategy groups (bootstrapped BCa 95% CIs all passing 0).

Montreal Cognitive Assessment

A lower omega-6:3 ratio was associated with a higher overall cognitive status. Initial bivariate correlation indicated that the ratio score was significantly associated with MoCA score ($r = -0.43$, $p < .01$, bootstrapped BCa 95% CI [−0.14, −0.64]), while total omega-6 and omega-3 intakes independently were not associated with MoCA score (bootstrapped BCa 95% CIs all passing 0).

Linear regression analysis indicated that the ratio score was the only predictor variable to significantly predict MoCA score in both models (omega-6 model: $p < .01$, bootstrapped BCa 95% CI [−.497, −.205], omega-3 model: $p < .05$, bootstrapped BCa 95% CI [−.603, −.044]) (Figure 3C). Thus, a lower ratio predicted a higher MoCA score. Specifically, using the model that includes omega-6, for every one standard deviation increase in the ratio score the MoCA score decreased by 0.42 standard deviations when all other predictors in the model were held constant. When total omega-3 intake was included in the analysis, the model uniquely explained 23% of the total variance of MoCA score ($F(4, 39) = 2.89$, $p < .05$; see Table 2). When total omega-6 intake was included in the analysis, the model uniquely explained 27% of the total variance of MoCA score ($F(4, 40) = 3.72$, $p < .05$).

Neuropsychological Tests

We found no associations between the three dietary variables of interest (omega-6:3 ratio, total omega-3 intake and total omega-6 intake) and total and delayed recall on the RAVLT, delayed recall on the ROCF, and nonverbal IQ (bootstrapped BCa 95% CIs all passing zero).

Table 2
Linear Regression Analyses Predicting Cognitive Performance With The Omega-6:3 Ratio, Omega-3, Omega-6, Age, and Education

	β	95% CI
Regression Model That Predicts Accuracy on the Wayfinding Task (N = 34)[1]		
Ratio score	$-.26^{*}$	[−5.43, −.331]
Education	$.39^{\dagger}$	[.123, 4.11]
Age	−.28	[−3.19, .982]
Regression model that predicts trials to criterion on the 4/8 VM score with total omega-3 intake (N = 45)[2]		
Ratio score	$.45^{\dagger}$	[.030, .688]
Education	$-.38^{*}$	[−.277, −.068]
Total omega-3 intake	$-.22^{*}$	[−1.27, .286]
Age	.11	[−.035, .126]
Regression model that predicts trials to criterion on the 4/8 VM score with total omega-6 intake (N = 45)[3]		
Ratio score	$.50^{\dagger}$	[.115, .702]
Education	$-.34^{\dagger}$	[−.240, −.066]
Total omega-6 intake	−.23	[−.151, .023]
Age	.17	[−.015, .132]
Regression model that predicts Montreal Cognitive Assessment score with total omega-3 intake (N = 45)[4]		
Ratio score	$-.35^{\dagger}$	[−.603, −.044]
Education	.26	[−.026, .271]
Total omega-3 intake	.14	[−.337, 1.42]
Age	−.07	[−.120, .066]
Regression model that predicts Montreal Cognitive Assessment score with total omega-6 intake (N = 45)[5]		
Ratio score	$-.42^{*}$	[−.497, −.205]
Education	$.26^{\dagger}$	[−.008, .244]
Total omega-6 intake	.14	[−.038, .161]
Age	−.08	[−.117, .059]

Note. 4/8 VM = 4 on 8 virtual maze.
Linear regression models were used to assess the dietary variables of interests' (ratio of omega 6:3, omega 3, and omega 6 independently) ability to predict measures of cognition for each task. Bootstrapped bias-corrected accelerated 95% confidence intervals were used to assess statistical significance. Beta coefficients for each independent variable are listed in the middle column. The beta coefficients demonstrate the direction and amount of change in the cognitive variables for every one standard deviation increase in significant predictor variables when the other predictor variables in the model are held constant. R^2 values for each model are located below. Each model contains the ratio of omega-6:3 (called ratio score) and are where our main results are drawn from. For the wayfinding task, the omega-6:3 ratio, age, and education were included in the model as the initial bivariate correlation analysis indicated that omega-3 and omega-6 intake were not associated with performance on this task. We ran two models each for the 4/8 VM task and the Montreal Cognitive Assessment (MoCA) score; one model containing omega 6 and one model containing omega 3 since including them both in the same model caused a variance inflation factor >10. All models included age and education as control variables. Results from this table show that in all models, the ratio score was the strongest predictor of performance on the virtual navigation tasks and the MoCA score.
[1] R^2 = .31 (p = .01). [2] R^2 = .43 (p = .001). [3] R^2 = .43 (p = .001). [4] R^2 = .23 (p = .05). [5] R^2 = .27 (p = .05).
† $p < .05$. * $p < .01$.

Discussion

This study was the first to examine the relationship between the ratio of omega-6:3 FA and hippocampus-dependent spatial memory and navigation in healthy older adults. In line with our hypothesis, we found that the ratio of omega-6:3 FA was strongly associated with participants' ability to form a spatial representation of a virtual town and their ability to learn a virtual navigation task. Further, the omega-6:3 FA ratio was associated with overall cognitive status. Specifically, as the ratio of omega-6:3 FAs *lowered,* participants performed more accurately on the wayfinding task when they searched for target landmarks. Thus, participants who consumed a lower ratio of omega-6:3 FAs were better able to form a spatial representation of the environment. On the 4/8 VM, a lower ratio score, which may have been the result of greater omega-3 FA intake in this case, was associated with faster learning of the task. These results were robust even in the presence of age and education as covariates. Therefore, these data indicate that the ratio of omega-6:3 FAs may impact spatial memory and cognition in older adults. Here, for the first time, we show that the ratio of omega-6:3 FAs that individuals consume predicts spatial memory ability in a population of healthy older adults. Indeed, the present population was free of diabetes, hypertension, high cholesterol, history of cancer, and psychiatric and neurological disorders suggesting that the present results were independent of participants' health status. Further, we found that the omega 6:3 ratio was independent of potentially health-related physiological characteristics such as BMI. Thus, the omega-6:3 ratio may be independently related to spatial memory and cognition in this population. Because the hippocampus is one of the first neural structures to decline in both structure (Raz, Rodrigue, Head, Kennedy, & Acker, 2004) and function with age (Bohbot et al., 2012; Lupien et al., 1998), the present results may help elucidate the mechanisms by which a decreased omega-6:3 FA ratio is associated with a decreased risk of cognitive decline (Barberger-Gateau et al., 2007; Heude, Ducimetiere, & Berr, 2003; Samieri et al., 2008).

The hippocampus is crucially involved in spatial processing (O'Keefe & Nadel, 1978) in both rodents (McDonald & White, 1994) and humans (O'Keefe & Nadel, 1978). Because here, we show that the relative amount of omega-6 FA to omega-3 FA in the diet predicts spatial memory in older adults, the relative amounts of each of these FAs may modulate each other's ability to affect neural structures like the hippocampus. Specifically, both of these FAs compete for the same desaturase enzymes, thus they competitively affect the synthesis of the other's downstream mediators which have differential pro- (omega-6) and anti-inflammatory roles (omega-3) in the body (Nakamura & Nara, 2004). This interaction underlines the importance of assessing the relative amount of each of these FAs that individuals consume, however, currently, the majority of studies that assess FAs' effects on the brain tend to assess omega-3 FA intake alone. Indeed, in vitro, DHA facilitates long-term potentiation, the synaptic basis for learning and memory (Bliss & Collingridge, 1993), in the hippocampus (Fujita, Ikegaya, Nishikawa, Nishiyama, & Matsuki, 2001) and animal models indicate that DHA plays a role in synaptic transmission, function, neurogenesis, and has neuroprotective properties (Yehuda, 2012; Yehuda et al., 1999; Yehuda, Rabinovitz, Mostofsky, Huberman, & Sredni, 1997). Further, arachidonic acid (AA; 20:4n-6) is also involved in long-term potentiation in the hippocampus (Carta et al., 2014; Fujita et al., 2001) despite its potential involvement with the pathogenesis of some forms of cognitive decline in humans (Cole, Ma, & Frautschy, 2010). In animals, high amounts of cortical omega-6 FA concentrations impair spatial memory in both transgenic Alzheimer's and wild type mice, irrespective of the level of omega-3 FA supplementation (Arendash et al., 2007). Thus, these studies indicate that omega-3 and omega-6 FAs seem to affect the hippocampus distinctly, with high amounts of omega-6 FA being detrimental to hippocampal processing. This statement is consistent with a rodent study showing that rats fed a diet with a lower omega-6:3 FA ratio had faster latencies and better spatial memory on the Morris Water Maze than rats fed a high ratio diet (Hajjar et al., 2012). Thus, this literature taken with the present results suggest that differences in the relative amount of omega-6 to omega-3 FAs may affect hippocampal-based spatial memory. In the current study, a ratio range of 7.8–8.74 was found for participants who performed the best on the cognitive measures. Thus, our findings support evidence that suggests certain dietary patterns that contain a lower omega-6:3 ratio are associated with reduced risk of cognitive decline (Simopoulos, 2011). Therefore, these dietary patterns may affect cognition in old age through omega-6 and omega-3 FA's ability to affect each other's bioavailability (Taha et al., 2014) and their differential effects on the hippocampus.

We also demonstrated that the ratio of omega-6:3 FAs in the diet is associated with learning and memory and performance on navigation tasks in general. The 4/8 VM is unique because individuals can solve it using one of two different navigation strategies, the spatial strategy or the response strategy, with older adults being less likely to spontaneously use the spatial strategy (Bohbot et al., 2012; Etchamendy, Konishi, Pike, Marighetto, & Bohbot, 2012). Here, we found that both individuals who used a spatial strategy and those who used a response strategy consumed similar ratios of omega-6:3 FAs. These results indicate that although the dietary omega-6:3 FA ratio affects spatial learning and memory, the effect is general and it is not specific to navigational strategies dependent on the hippocampus versus caudate nucleus. Interestingly, our results are in line with investigations in animals that indicate the ratio of omega-6:3 FAs can affect cognitive functioning on several types of tasks. Indeed, dietary DHA supplementation in mice (used to lower the omega-6:3 FA ratio) increased neural membrane DHA, and improved learning in a footshock avoidance test compared with mice fed a standard diet (Petursdottir, Farr, Morley, Banks, & Skuladottir, 2008).

Although no studies to date have assessed the relationship between the omega-6:3 FA ratio and spatial memory in humans, several observational investigations have looked at the relationship between the omega-6:3 FA ratio and dementia. Heude, Ducimetiere, and Berr (2003) found that a higher omega-3:6 FA ratio (or in other words a lower omega-6:3 FA ratio) in erythrocyte membranes conferred a lower risk of cognitive decline in a large sample of older adults. In another study, individuals who consumed a higher ratio, conferred by a higher intake of omega-6 FAs and a lower amount of omega-3 FAs, showed an increased incidence of dementia after 4 years (Barberger-Gateau et al., 2007) and a high plasma omega-6:3 FA ratio was associated with an increased risk of dementia (Samieri et al., 2008). Given the present results, and the evidence that indicates the omega-6:3 FA ratio may reduce the risk of dementia, the relative amount of both omega-6 and omega-3 FAs may be important dietary mediators of cognition in old age.

In the present population, a lower omega-6:3 FA ratio was associated with a higher cognitive status. Indeed, several other investigations also observed this finding using other measures of global cognition (Cherubini et al., 2007; Gonzalez et al., 2010). Presently, it is unclear how changing the amount of omega-3 or omega-6 FAs in the diet may affect cognition in older adults since results from trials supplementing older adults with omega-3 FAs are generally inconclusive. For example, in three large randomized controlled trials of older adults free of cognitive decline, the placebo and the omega-3 FA supplement groups scored comparably on the MMSE and on other cognitive measures after the study periods that reached up to 40 months (Dangour et al., 2010; Geleijnse, Giltay, & Kromhout, 2012; van de Rest et al., 2008). It is possible that other uncontrolled dietary factors, such as dietary omega-6 FA, could modulate the availability of omega-3 FA in the brain. Indeed, one study found that older adults who consumed a higher omega-3:6 FA ratio (which confers a lower omega-6:3 FA ratio) had better spatial working memory (Pase et al., 2015). Taken together with the present results, relative proportion of omega-6 and omega-3 FAs in the diet may impact spatial memory, learning, and general cognitive function while the absolute amount of omega-3 or omega-6 FAs may have no effect on these measures. Thus, investigations that fail to assess the relative amount of each of these FAs in the diet may be more likely to see weak or no effects of an intervention in older adults free of cognitive decline. More trials that consider the interaction of these nutrients in the body are required to understand the relationship between dietary FAs and cognition throughout aging.

In the current study, although we found an association between omega-6:3 ratio and performance on the MoCA and spatial memory tasks, no association was found with IQ and performance on the RAVLT and ROCF. Past studies have shown that our spatial memory tasks are more sensitive to the integrity of the hippocampus compared to standard neuropsychological tests such as the RAVLT and ROCF. In Konishi and Bohbot (2013), gray matter in the hippocampus in older adults significantly correlated with our spatial memory measure but did not correlate with performance on the RAVLT and ROCF. The reason for this may be that although the RAVLT and ROCF assess memory, brain areas other than the hippocampus may contribute to these functions. As such, these measures may not be as sensitive to hippocampal structure and function and in turn omega-6:3 ratio. In addition to hippocampal function, studies have shown that omega-6:3 ratio is also associated with global cognition (Cherubini et al., 2007; Gonzalez et al., 2010). This may explain why omega-6:3 ratio also correlated with MoCA scores in the current study but not the RAVLT and ROCF. The MoCA was specifically designed to be sensitive to cognitive impairment and assesses various cognitive domains. There are several components to this test beyond visual and verbal memory. This test also assesses executive function, attention, concentration, working memory, orientation, and language, which may be sensitive to areas of the brain other than the hippocampus, such as the prefrontal cortex. Indeed, a study in rodents demonstrated that omega-3 PUFA deficiency leads to hyperactivity in the hypothalamic—pituitary—adrenal (HPA) axis, which in turn resulted in atrophy in the prefrontal cortex (Larrieu et al., 2014). Therefore, overall omega-6:3 ratio may be sensitive to variability in the hippocampus and prefrontal cortex; however, this hypothesis requires further investigation.

The present study includes several limitations. The FFQ is based on self-report which includes controls for implausible responses through its plausibility analysis (Shatenstein, Amre, Jabbour, & Feguery, 2010). Furthermore, participants' erythrocyte ratio of omega-6:3 FAs was not measured and thus physiological concentrations of these two FAs could not be included in our analysis. Based on the sample size, the current analyses were limited in the number of covariates that could be included. There are other factors that may be associated with the ratio of omega-6:3 FAs such as socioeconomic status, IQ, BMI, physical fitness, and other health factors that should be taken into consideration with larger sample size analyses. Further, despite being included as a covariate in the statistical analysis, the high average level of education in the present population may affect the generalizability of the results. On the other hand, studies in rodents support the current results (Hajjar et al., 2012; Petursdottir et al., 2008). The current study lays an important ground, showing for the first time, the relationship between the relative amount of omega-6 and omega-3 FAs in the diet and spatial cognition in old age.

In conclusion, we found that older adults who consumed a lower dietary omega-6:3 FA ratio had better spatial memory on a virtual wayfinding task. Further, older adults who consumed a lower omega-6:3 FA ratio learned a virtual navigation task faster than those who consumed a higher ratio. Participants with a lower omega-6:3 FA ratio had a higher overall cognitive status. These results provide evidence of a plausible link between diet and cognitive function with aging, and highlight the importance of the balance of dietary FAs on spatial memory abilities and learning. Therefore, a dietary pattern that promotes a lower omega-6:3 FA ratio combined with intact spatial memory abilities may support healthy cognition in old age and may be a key feature for clinical trials that aim to reduce conversion rates to Alzheimer's disease.

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