Impact of a diet rich in n-3 fatty acids on Alzheimer's disease

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Background
Alzheimer's disease (AD) is a progressive neurodegenerative disease. The potential effect of nutrition on development of AD has become a topic of increasing scientific and public interest. High intakes of saturated and *trans*-unsaturated (hydrogenated) fats were positively associated with increased risk for AD, whereas intakes of polyunsaturated and monounsaturated fats were protective against cognitive decline in the elderly. Would foods rich in these fatty acids delay cognitive decline in elderly people who are vulnerable to AD?

Objectives
The aim of this study was to measure the concentration of plasma fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in patients with AD and study the relationship between foods rich in these fatty acids and severity of cognitive decline.

Methods
A total of 62 individuals were screened for cognitive decline using the mini-mental status examination test and were diagnosed with AD using the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed., diagnostic criteria. Data on nutrition were obtained and blood samples were withdrawn to determine the plasma levels of the fatty acids EPA and DHA.

Results
Patients with late-onset AD have significantly higher intake of food and food supplements containing both fatty acids.

Conclusion
High intake of food and food supplements rich in EPA and DHA fatty acids may delay the onset of AD.

Keywords:
Alzheimer, diet, n-3 fatty acids

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects millions in the aging population worldwide and will affect millions more in the next 20 years. Over 90% of all cases are sporadic, with genetics playing a minor role in the etiology of AD. Therefore, it is crucial to investigate environmental factors and diet as primary risk factors in pathology of AD (Dosunmu *et al.*, 2007). At present, it is estimated that 25% of the population older than 85 years have significant cognitive impairment. The global prevalence of cognitive impairment and dementia, including AD, is expected to rise significantly in proportion to increased life expectancy. Therefore, various attempts have been made to identify means of either delaying the onset of cognitive impairment or improving memory function in patients affected by AD. The importance of dietary fatty acids, in particular ω-3-based fatty acids, has gained significant momentum (Itua and Naderali, 2010). Cognitive impairment can be influenced by a number of factors. The potential effect of nutrition has become a topic of increasing scientific and public interest. In particular, there are arguments that nutrients (food and/or supplements) such as vitamins, trace minerals, and lipids can affect the risk of cognitive decline and dementia, especially in frail elderly people at risk for deficiencies. High intake of saturated and *trans*-unsaturated (hydrogenated) fats was positively associated with increased risk for AD, whereas intake of polyunsaturated and monounsaturated fats was protective against cognitive decline in the elderly, as cited in previous studies (Cole *et al.*, 2009). Many epidemiological studies have reported that reduced intake or levels of ω-3 fatty acids or reduced fish consumption is associated with increased risk for age-related cognitive decline or dementia such as AD (Huang, 2010). In multiple epidemiological studies, increased dietary consumption or blood levels of docosahexaenoic acid (DHA) appear protective against AD and other dementias (Gillette-Guyonnet *et al.*, 2007).

The major dietary sources of DHA and eicosapentaenoic acid (EPA) are fish and shellfish, from both salt water and fresh water. DHA can also be synthesized in the body

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from the n-3 fatty acid linolenic acid (18:3), which is present in some vegetable oils and some nuts and seeds. However, this synthetic step is relatively inefficient. DHA is 22 carbon atoms long and has six double bonds in the n-3 configuration. It is the most prominent fatty acid in the brain, retina, and spermatozoa and is necessary for vision, cognition, and sperm motility. DHA is especially abundant in the neurons and synaptosomes of the cerebral cortex, in which it occupies the second position of the membrane phospholipid backbone (Connor, 2000; Connor *et al.*, 2007).

DHA is broadly neuroprotective through multiple mechanisms that include neuroprotective DHA metabolites, reduced arachidonic acid metabolites, and increased trophic factors or downstream trophic signal transduction. DHA is also protective against several risk factors for dementia including head trauma, diabetes, and cardiovascular disease. In addition, DHA is specifically protective against AD (Huang, 2010).

On the basis of epidemiological and basic research data, expert panels have emphasized on the need for clinical trials with ω-3 fatty acids, especially DHA, for the prevention or treatment of age-related cognitive decline. Nowadays, clinical trials are underway to prevent and treat AD. Results to date suggest that DHA may be more effective if consumed from early stages of life or if used in conjunction with antioxidants (Cole *et al.*, 2009).

Recent years have witnessed the discovery of a new class of inflammation-dampening and resolution-promoting n-3 polyunsaturated fatty acid (PUFA)-derived lipid mediators called resolvins and protectins. Chemically, these compounds are hydroxylated derivatives of the parent n-3 PUFA EPA in the case of the E-resolvins and DHA in the case of D-resolvins and protectin D1. Although a relatively large number of these compounds have been identified and characterized until now, with differences in the position of the hydroxyl groups and chirality of the different carbon atoms, all compounds share common precursor metabolites: 17-hydroperoxydocosahexaenoic acid [17-H(p)DHA] for the DHA-derived compounds and 18-hydroperoxyeicosapentaenoic acid [18-H(p)EPE] for the EPA-derived compounds (Weylandt *et al.*, 2012).

These metabolites are associated with signal transduction processes involved in downregulation of oxidative stress, neuroinflammation, and apoptosis. EPA-derived E-series resolvins (RvE_1 and RvE_2), DHA-derived D-series resolvins (RvD_1 and RvD_2), and neuroprotectins have potent anti-inflammatory, proresolution, and antioxidant properties. They not only retard excessive inflammation but also promote resolution by enhancing clearance of apoptotic cells and debris from inflamed brain tissue and vasculature, leading to tissue homeostasis. These actions may underlie the beneficial effects of EPA and DHA on neurotraumatic and neurodegenerative diseases and may lead to normal human health (Farooqui, 2012).

With this background, a logical question is: Could consumption of foods rich in these fatty acids delay the decline in cognitive function that might occur in an elderly population, and can it delay the onset of AD? Therefore, in this study, our objectives were to measure the concentration of plasma fatty acids DHA and EPA in elderly patients suffering from AD and to compare early-onset AD with late-onset AD with regard to consumption of food and food supplements rich in the fatty acids DHA and EPA.

Methods

Study design

This study was a cross-sectional comparative study.

Tools

(1) Complete psychogeriatric assessment.
(2) Cognitive screening.
(3) Psychiatric diagnosis.
(4) Laboratory investigations comprising measurement of plasma levels of the fatty acids DHA and EPA.
(5) Measurement of food intake using 24 h recall and food frequency methods concentrating on foods containing the fatty acids DHA and EPA.
(6) Data analysis.

Subjects

This study was carried out at the Geriatric Psychiatry Clinic of the Institute of Psychiatry, Ain Shams University, from January 2008 to May 2009. A total of 62 patients, belonging to either sex, with AD fulfilling the following inclusion criteria were selected.

(1) Age above 60 years.
(2) With a mild or moderate stage of AD according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed., criteria.

Exclusion criteria

(1) Age below 60 years.
(2) History of medical or other neuropsychiatric disorders or of drug intake, which may be the cause of or explanation for his/her cognitive complaint.
(3) Patients with a disturbed level of consciousness or a late stage of AD.

Study proper

Participants included in the study were attending the geriatric psychiatry clinic for follow-up and counseling. Caregivers accompanying the patients were invited to participate in the study, and those who approved gave written informed consent on behalf of the patients.

Comprehensive psychogeriatric assessment of personal and sociodemographic data, medical history, and history of drug intake by the patient was carried out using the semistructured clinical interview sheet of the Institute of Psychiatry, Faculty of Medicine, Ain Shams University. Complete physical examination was carried out to detect

chronic conditions and exclude patients fulfilling the previously mentioned exclusion criteria.

Cognitive screening was performed using the mini-mental status examination (MMSE) test, which is a brief 30-point questionnaire used to screen for cognitive impairment (Folstein *et al.*, 1975; Arabic version by El Okl, 2002). MMSE is commonly used in medicine to screen for dementia. It samples various functions including arithmetic, memory, and orientation. A score greater than or equal to 25 points is effectively normal. Scores below 25 points can indicate severe ($\leq$9 points), moderate (10–20 points), or mild (21–24 points) illness. Low to very low scores correlate closely with the presence of dementia.

Patients with AD were diagnosed using the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed., Axis II Disorders (First *et al.*, 1996). The development of multiple cognitive deficits manifested as impairment of both memory and one or more other cognitive functions represents a decline from previous functioning and causes significant impairment in social or occupational functioning, with a gradual onset and progressive course and not due to other central nervous system, systemic, or substance-induced conditions.

Staging of dementia was carried out using the Clinical Dementia Rating Scale (CDRS) (Morris, 1993). The CDRS is a dementia staging instrument used to rate cognitive function on five levels of impairment, from none to maximal (rated as 0, 0.5, 1, 2, or 3), in each of the six following domains: memory, orientation, judgment and problem solving, functioning in community affairs, home and hobbies, and personal care (personal care does not have a 0.5 impairment level). Only impairment caused by cognitive dysfunction is rated. A score of 0 on CDRS indicates no dementia and scores of 0.5, 1, 2, and 3 represent very mild, mild, moderate, and severe dementia, respectively.

Assessment of plasma fatty acids

Fasting blood samples were collected into heparinized evacuated tubes and centrifuged at 1000*g* for 10 min. The method adopted by the Association of Official Analytical Chemists (AOAC, 2000) was followed for lipid extraction from the samples; chloroform and methanol (2 : 1 v/v) were used. A set of standard fatty acids of 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, and 22:0 with a stated purity of 99% determined using gas liquid chromatography (GLC) was purchased from Nu-Check Prep (Elysian, Minnesota, USA). The purity of each fatty acid methyl ester was checked using GLC, and it gave one peak. The method described by Farag *et al.* (1986) was applied for determination of fatty acids using GLC. The methyl esters of fatty acids obtained from oils of samples and standard materials were analyzed using a Pye Unicam Series 304 gas chromatograph (gas liquid chromatography TRACE GC Ultra-Thermo TR-FAME; ThermoFisher Scientific Inc., USA) equipped with a dual flame ionization detector and a dual-channel recorder. The separation of fatty acid methyl esters was carried out using a coiled glass column (1.5 m $\times$ 4 mm) packed with diatomite (100–120 mesh) and coated with 10% polyethylene glycol adipate. The column oven temperature was programmed at 8°C/min from 70 to 190°C, then isothermally at 190°C for 25 min, with nitrogen at 30 ml/min. The unsaponifiable fatty acids were also fractionated on a coiled glass column (2.8 m $\times$ 4 mm) packed with diatomite (100–120 mesh) and coated with 3% OV-17. The oven temperature was programmed at 10°C/min from 70 to 270°C, then isothermally at 270°C for 25 min, with a nitrogen flow rate of 30 ml/min. The detector and injector temperatures and hydrogen and air flow rates were generally 300°C, 280°C, 33 ml/min, and 330 ml/min, respectively. Peak identification was carried out by comparing the retention time (RT) of each compound with those of standard materials. The linear relationship between the log of the retention times of the standard hydrocarbons and the number of carbon atoms in these compounds was used to characterize the unavailable authentic hydrocarbons. Peak area was measured using a computing integrator (PU 4810; Philips, UK). The results of each fatty acid were expressed as a percentage of total fatty acids.

Dietary history

Food intake was studied using a questionnaire planned by the dieticians of NNI, based on 24-h recall and food frequency methods, concentrating on fish consumption and consumption of foods rich in n-3 fatty acids.

Twenty-four-hour recall method: in this method each caregiver was asked to recall the exact food and beverages consumed by the patient in the previous 24-h period. Quantities of food and beverages consumed were recorded in household measures and grams.

The obtained information included eating events in sequence, beginning with the first eating event of the day, specifying each event whether major or minor and recording food items in each event.

Dietary pattern 'Food Frequency Questionnaire': this method was used to obtain qualitative descriptive information on the usual food and beverage consumption pattern per day and per week (Dehghan *et al.*, 2012). The dietary assessment was completed at the clinic after written informed consent was obtained. During the interview, few of the caregivers had some knowledge about the importance of including fish in the diet of AD patients to improve their cognitive function.

For further comparison between early-onset and late-onset AD, patients were further divided into two groups according to age: one group included patients up to 65 years in age (early-onset AD) and the other included patients older than 65 years (late-onset AD).

Statistical analysis

Analysis of the data was carried out using SPSS version 10 (SPSS Inc., Chicago, Illinois, USA).

Table 1 Clinical Dementia Rating Scale in relation to age group in Alzheimer's patients

	Age group			
CDRS	>65 years [*N* (%)]	≤65 years [*N* (%)]	Total [*N* (%)]	*P*
Mild	14 (43.8%)	14 (46.7%)	28 (45.2%)	
Moderate	18 (56.3%)	16 (53.3%)	34 (54.8%)	0.818
Grand total	32 (100%)	30 (100%)	62 (100%)	

CDRS, Clinical Dementia Rating Scale.

(1) Quantitative variables were presented as means and SD. Means were compared using unpaired Student's *t*-test and analysis of variance.
(2) Relationship between variables was identified by linear regression analysis.
(3) Qualitative data was presented in the form of proportions and percentages.
(4) The χ^2-square test was used as a significance test.
(5) The nonparametric test (Mann–Whitney *U*-test) was used for data not fulfilling the normal distribution.

Results

Sociodemographic data

This study was carried out on 62 patients with AD. The distribution of patients according to age and sex was as follows: 48.4% of the patients were up to 65 years in age and 51.6% were older than 65 years; 41.9% of the patients were men and 58.1% were women.

Severity of cognitive function

MMSE showed lower scores (8.5 ± 0.9) in the age group of greater than 65 years compared with the scores (10.4 ± 1.2) in the age group of up to 65 years, but this difference was insignificant ($P = 0.235$). Severity of the disease was determined using CDRS, and it was found that 46.7% of the patients who were 65 years of age or younger showed mild degree of the disease, whereas 53.3% of patients in the same age group showed moderate manifestations. Among the patients who were 65 years or younger, 43.8% had a mild case of the disease compared with 56.3% who showed moderate manifestations. Statistically, this difference in the severity of the disease between the two age groups was insignificant ($P = 0.818$; Table 1).

Food consumption and fatty acids

Using the 24-h recall method and a food frequency questionnaire for both groups, we studied the frequency of consumption of food rich in the fatty acids EPA and DHA. In the questionnaire, the frequency was determined to be either less than three times per week or more than three times per week (Table 2).

Table 2 shows that there are more patients older than 65 years (late-onset disease) who consumed food rich in EPA and DHA more than 3 times per week compared with those 65 years or younger. This increase in consumption among the late-onset disease group was statistically significant for certain types of foods such as vegetable oil and canned sardines (*P*-value 0.003 and 0.017, respectively). Consumption of some food items such as herrings, sardines, and fruits showed a tendency toward significance.

Table 2 Distribution of patients according to age group by frequency of consumption of food rich in fatty acids (eicosapentaenoic acid and docosahexaenoic acid)

	N (%)		
Food item	>65 years		≤65 years
Herrings	32 (61.5%)		20 (38.5%)
P		0.068	
Sardines	34 (58.1%)		25 (41.6%)
P		0.091	
Canned sardines	26 (56.5%)		20 (43.5%)
P		0.017	
Canned tuna	36 (60%)		24 (40%)
P		0.725	
Vegetable oil	28 (60.9%)		18 (39.1%)
P		0.003	
Peanuts	36 (60%)		24 (40%)
P		0.662	
Green salad	34 (56.6%)		26 (43.3%)
P		0.937	
Cooked vegetables	36 (60%)		24 (40%)
P		1.0	
Fruits	33 (55.3%)		27 (45%)
P		0.077	
Sunflower oil	33 (54.8%)		27 (45.2%)
P		0.597	

Table 3 shows that 10 patients older than 65 years consumed food supplements rich in the fatty acids EPA and DHA, with a frequency greater than that observed in patients 65 years or younger; this increase in the frequency of intake was statistically significant ($P = 0.008$).

When risk assessment was carried out between frequency of intake of food items rich in EPA and DHA and severity of the disease, the probability of developing mild AD in patients consuming fruits less than three times per week was found to be three times greater and this finding was significant only with respect to fruits. The lack of participants with moderate manifestations of AD among the low-consumption group was the cause for the nonconformity of risk assessment between them.

Plasma fatty acid concentration

The mean level of the plasma fatty acid EPA in patients 65 years old or younger (early-onset disease) was insignificantly higher than that in patients older than 65 years (late-onset disease; $P = 0.232$). In contrast, the mean level of the plasma fatty acid DHA was insignificantly

Table 3 Distribution of patients according to age group by frequency of consuming vitamin supplements rich in fatty acids (eicosapentaenoic acid and docosahexaenoic acid)

			Frequency of supplement intake			
Age group	Number of patients	Nonsupplemented	Once/day	Twice/day	Three times/day	*P*-value
>65 years	32	22	2	2	6	0.018
≤65 years	30	24	6	0	0	

Table 4 Plasma fatty acid concentration in Alzheimer's disease patients according to age group

Plasma fatty acids	Patients>65 years	Patients≤65 years	*P*-value
EPA	*n*=32 0.152±0.040	*n*=30 0.230±0.052	0.232
DHA	*n*=32 1.234±0.137	*n*=30 1.005±0.110	0.201

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Table 5 Plasma fatty acid concentration in Alzheimer's disease patients according to severity of disease

Plasma fatty acids	Mild disease	Moderate disease	*P*-value
EPA	*n*=28 0.062±0.034	*n*=34 0.295±0.072	0.004
DHA	*n*=28 1.301±0.168	*n*=34 0.977±0.183	0.368

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

lower in patients 65 years or younger than in patients older than 65 years ($P = 0.201$; Table 4).

According to severity of the disease, the concentration of the plasma fatty acid EPA was significantly higher (0.295 ± 0.034) in patients showing moderate manifestations compared with those having a mild degree of disease (0.062 ± 0.034; $P = 0.004$). Concentration of the plasma fatty acid DHA was insignificantly higher (1.301 ± 0.168) among patients with mild disease (Table 5).

Dietary recall information was correlated with blood levels of EPA and DHA. Plasma fatty acid concentrations were higher among groups consuming food items rich in EPA and DHA more than three times per week. This finding was significant for EPA fatty acid in correlation to sunflower oil, green salad, and vegetable oil (Table 6).

Discussion

The aim of this study was to answer the question of whether consumption of food and food supplements rich in EPA and DHA fatty acids retards the decline in cognitive function that might occur in an elderly population and delays the onset of AD.

Fatty acid measurement

Fatty acids that are largely of exogenous origin – that is, n-3, n-6, *trans*, and odd-numbered fatty acids – could provide the best quantitative estimate of their intakes (Baylin and Campos, 2006). These fatty acids can be measured in various blood fractions and tissues, for example, plasma or serum, erythrocytes, and adipose tissue (Baylin and Campos, 2006). One well-conducted controlled dietary trial clearly showed that serum n-3 fatty acid concentrations responded more quickly to recent dietary supplementation with fish oil than did erythrocyte n-3 fatty acid concentrations (Katan *et al.*, 1997).

In a Norwegian population in whom intake of n-3 fatty acids of marine origin was high, total serum reflected a long-term intake as strong that in adipose tissue (Andersen *et al.*, 1999). In another study, the n-3 fatty acid concentration in erythrocytes was even better correlated with intake than was the n-3 fatty acid concentration in adipose tissue (Godley *et al.*, 1996). Nondietary factors, such as absorption, metabolism, and genetic and lifestyle determinants, can affect fatty acid concentrations in human tissues (Willett, 1998). In addition to the differences in fatty acid composition in blood fractions (plasma and erythrocytes) and dietary fat, considerable differences were observed between these two blood fractions. These differences may have been due to the endogenous synthesis of some fatty acids, the different physiological functions of certain fatty acids in different blood fractions, or different roles of these fractions as vehicles for fatty acid transport (Willett, 1998). However, because measurement errors of the biomarkers and the food frequency questionnaires are independent, the methodology for measuring DHA and EPA was not our concern. We used the methodology commonly used in our laboratories, which is measuring the concentration of DHA and EPA in plasma.

Fatty acid level and food consumption

Although comparing patients with early-onset AD with those with late-onset AD, as regards the mean level of plasma fatty acids DHA and EPA, in relation to the onset or severity of AD did not reveal significance, baseline diet history and past food habits of the patients showed that intake of food items rich in the fatty acids EPA and DHA was significantly higher among patients of the age group of greater than 65 years (Table 2). Further, this increase in consumption was statistically significant for certain food items such as vegetable oil and canned sardines. Consumption of some food items such as herrings, sardines, and fruits showed a tendency toward significance.

Table 6 Nonparametric test (Mann–Whitney) to correlate frequency of consumption of food rich in fatty acids (eicosapentaenoic acid and docosahexaenoic acid) with plasma fatty acid concentrations

Food item	Plasma fatty acids	Median (25th–75th percentiles)	Min–Max	*Z*-value	Two-tailed significance
Sunflower oil	EPA	0.147 (0.0–0.304)	0.0–0.586	−2.159	0.031[a]
	DHA	0.996 (0.798–1.448)	0.0–2.061	−0.417	0.677
Fruits	EPA	0.0 (0.0–0.230)	0.0–0.842	−1.064	0.287
	DHA	0.973 (0.802–1.461)	0.0–2.318	−0.077	0.939
Cooked vegetables	EPA	0.0 (0.0–0.234)	0.0–0.586	−1.520	0.128
	DHA	0.955 (0.798–1.436)	0.0–2.318	−0.627	0.531
Green salad	EPA	0.0 (0.0–0.251)	0.0–0.842	−1.658	0.097[b]
	DHA	0.948 (0.734–1.411)	0.0–2.318	0.645	0.519
Peanuts	EPA	0.0 (0.0–0.260)	0.0–0.586	−0.423	0.672
	DHA	0.996 (0.811–1.411)	0.0–2.158	−1.041	0.298
Vegetable oil	EPA	0.0 (0.0–0.304)	0.0–0.586	−3.876	0.000[a]
	DHA	1.028 (0.945–1.461)	0.0–1.981	−0.430	0.667
Canned tuna	EPA	0.0 (0.0–0.242)	0.0–0.571	−1.577	0.115
	DHA	0.996 (0.802–1.461)	0.0–2.158	−0.035	0.972
Canned sardines	EPA	0.0 (0.0–0.206)	0.0–0.484	−1.117	0.264
	DHA	0.996 (0.635–1.461)	0.0–1.981	−1.313	0.189
Sardines	EPA	0.0 (0.0–0.242)	0.0–0.842	−0.882	0.378
	DHA	0.952 (0.675–1.410)	0.0–1.981	−1.031	0.303
Herrings	EPA	0.0 (0.0–0.211)	0.0–0.484	−0.817	0.414
	DHA	0.973 (0.710–1.461)	0.0–1.981	−1.136	0.256

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a]Significant.
[b]Borderline significance.

Our findings may suggest a possible role of the n-3 fatty acids EPA and DHA in delaying the onset of AD. These results were consistent with those of Boukje *et al.* (2007), who studied the association between the intake of n-3 fatty acids EPA and DHA from fish and other food and food supplements and subsequent 5-year cognitive decline; they reported that elderly men who consumed an average of 400 mg of EPA and DHA per day had lesser (by 1.1 points) cognitive decline than those who consumed 20 mg of EPA and DHA per day. In the study by Boukje and colleagues, fish intake was the main source of EPA and DHA, and it is therefore recommended that the intake of these fatty acids be increased.

Dietary sources of eicosapentaenoic acid and docosahexaenoic acid

Our results showed that a large amount of EPA and DHA ingested came from food items other than fish, for example, vegetable oil and fruits in addition to food supplements; therefore, their consumption can also contribute to higher intake of EPA and DHA. Before 2000, only few studies had investigated the relationship between fish consumption and the intake of n-3 fatty acids and cognitive decline. Meanwhile, the findings of our study were consistent with those of four other large prospective studies, as reported by Larrieu *et al.* (2004); these studies found that higher intakes of fish and DHA from diet sources were associated with a lower risk for dementia and AD. These studies had an average follow-up period of 2–3 years for dietary assessment and dementia diagnosis.

In contrast, results of the current study differ from results of the Zutphen Elderly Study conducted earlier, in which no clear inverse association between fish consumption and 3-year cognitive decline could be shown (Kalmijn *et al.*, 1997). Although fish is the major source of EPA and DHA (71%), these fatty acids are also present in other foods, such as meat (20%), eggs (6%), and plant foods [such as leek and cereal-based products (3%)] (Meyer *et al.*, 2003). In the past few years, several foods enriched with n-3 fatty acids have been included in the studies because of the suggested positive effect of these n-3 fatty acids on health. Therefore, information on the EPA and DHA content of all foods in the diet is now required when associations between these fatty acids and cognitive functioning are studied.

Possible causes for different results in this study and other studies

In previous studies, the use of fish as a main source of EPA and DHA in food may explain some of the differences. Therefore, our results were inconsistent with those of the study by Elizabeth *et al.* (2009), in which 5395 participants aged 55 years were free of dementia and reported dietary information at baseline. The study evaluated dementia and AD risk across categories of typical fish intake (none, low, and high) and fish type consumed (none, lean, and fatty). Across tertiles of ω-3 PUFA intake, specifically EPA and DHA, during an average follow-up period of 9.6 years, dementia developed in 465 participants (365 with a diagnosis of AD). Total fish intake was unrelated to dementia risk; therefore, the study concluded that these dietary factors did not appear to be associated with long-term dementia risk. The reason for these different results may be related to the type of the study, as our study depended on the diet history and previous food habits of the patients. At the same time, the study by Elizabeth *et al.* (2009) assessed the risk for development of dementia in healthy elderly, whereas our study measured frequency, type, and level of consumption of DHA and EPA in patients who already suffered from AD. Moreover, the method of assessment of dietary intake and plasma level may be another cause for

the differences between the studies. Multiple epidemiological studies examining fish consumption and tissue DHA levels are cited to support the proposition that increased DHA intake might protect the brain from the devastating effects of dementia. However, epidemiological studies cannot tease apart an isolated contribution of DHA as the determining beneficial factor in fish and seafood (Umhau, 2011).

The baseline diet habits of the patients obtained through diet history taking can be more accurate than plasma levels of both fatty acids because earlier diet may be most relevant to dementia development as lifestyle factors many years before clinical disease are thought to best predict later dementia risk (Launer, 2005). On the basis of the epidemiological and basic research data, expert panels have emphasized on the need for clinical trials with ω-3 fatty acids, especially DHA, for the prevention or treatment of age-related cognitive decline, with a focus on the most prevalent cause, AD. Clinical trials are underway to prevent and treat AD. Results to date suggest that DHA may be more effective if consumed earlier in life or used in conjunction with antioxidants (Cole *et al.*, 2009).

Vedin *et al.* (2012) suggested that dietary fish oil rich in n-3 fatty acids, for example, DHA and EPA, regulates inflammatory reactions by various mechanisms, such as gene activation. They concluded in their study that 6 months of dietary n-3 fatty acid supplementation affected expression of genes that might influence the inflammatory processes and could be of significance for AD.

In our study, the mean level of plasma EPA was insignificantly higher in patients who were 65 years or younger (early-onset disease); whereas plasma DHA was insignificantly lower in the same age group. Meanwhile, plasma EPA was significantly higher in patients showing moderate manifestations, whereas plasma DHA was insignificantly higher among patients with mild disease. These findings were inconsistent with those of other studies. Samieri *et al.* (2011) estimated the association between plasma levels of EPA and DHA and subsequent cognitive decline over 7 years, taking into account the apolipoprotein E (ApoE-ϵ4; the main genetic risk factor for AD) status and depressive symptoms, in a prospective population-based cohort. Participants ($\geq$65 years, $n = 1228$, nondemented at baseline) were evaluated at least once over three follow-up visits using four cognitive tests. Plasma EPA was associated with a slower decline in the Benton Visual Retention Test performances in ApoE-ϵ4 carriers and in subjects with high depressive symptoms at baseline. Plasma DHA was associated with slower decline in Benton Visual Retention Test performances in ApoE-ϵ4 carriers only.

In another study carried by Tan *et al.*, 2012, DHA and EPA levels in dementia-free Framingham Study participants ($n = 1575$; 854 women, age 67 ± 9 years) were related to performance on cognitive tests and to volumetric brain MRI, with serial adjustments for age, sex, and education (model A, primary model); ApoE-ϵ4 and plasma homocysteine (model B); physical activity and BMI (model C), and for traditional vascular risk factors (model D). Participants with a lower DHA and ω-3 index (RBC DHA + EPA) levels had lower scores on tests of visual memory, executive function, and abstract thinking in model A, the results remaining significant in all models. Lower RBC DHA levels are associated with smaller brain volumes and a 'vascular' pattern of cognitive impairment even in individuals free from clinical dementia.

Strengths and limitations

This study is one of the few studies conducted in Egypt and developing countries. Baseline dietary assessment may overcome the limitations of some previous studies. In this study, we used multiple valid tools as well as the dietary pattern 'Food Frequency Questionnaire' for estimation of plasma levels of DHA and EPA. This method was used to obtain qualitative descriptive information on the usual food and beverage consumption pattern per day and per week (Dehghan *et al.*, 2011).

A possible confounder in our study was the lack of control for other protective or risk factors for AD, such as antioxidants. However, ours was a cross-sectional descriptive study focusing only on one possible protective and risk factor for AD in a group of patients already suffering from AD; therefore, a long-term study that estimates this factor is recommended.

There was a great difficulty in the recruitment of a large number of AD patients fulfilling the inclusion and exclusion criteria of this study. In addition, the high cost of performing the blood analysis was another confounder, as the study was not funded by any advocacy. Therefore, it is our recommendation to perform the study on a larger scale in the form of a field research including AD patients and healthy individuals of the same age group, with sponsorship from a governmental institute.

Conclusion

Although there was no statistically significant difference in the plasma level of fatty acids between the two groups (early-onset and late-onset AD patients), patients with late-onset AD showed significantly higher intake of food and food supplements containing both fatty acids. Our findings may support the hypothesis that high intake of food and food supplements rich in EPA and DHA fatty acids may delay the onset of AD. Although there were only few statistically significant results in this study, we can partially prove from the increased consumption of food rich in DHA and EPA by late-onset AD patients that consumption of these fatty acids may delay the onset of AD. Lack of laboratory evidence as regards levels of DHA and EPA may be partially related to the fact that we studied a group of patients who already developed AD. This study was a very small step in the search for a possible available and safe treatment for AD. However, further research is required to verify the role of n-3 fatty acids as a protective agent against cognitive decline.

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Conflicts of interest

There are no conflicts of interest.

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