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Disturbed choline plasmalogen and phospholipid fatty acid concentrations in Alzheimer disease prefrontal cortex

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Abstract

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by brain deposition of senile (neuritic) plaques containing β-amyloid, neurofibrillary tangles, synaptic loss, neuroinflammation, and overexpression of arachidonic acid (AA, 20:4n-6) metabolizing enzymes. Lipid concentration changes have been reported in different brain regions, but often partially and/or as a percent of the total concentration. In this study, we measured absolute concentrations (per gram wet weight) of a wide range of lipids in postmortem prefrontal cortex (Brodmann area 9) from 10 AD patients and 9 controls. Mean total brain lipid, phospholipid, cholesterol and triglyceride concentrations did not differ significantly between AD and controls. There was a significant 73% decrease in plasmalogen choline, but no difference in other measured phospholipids. Fatty acid concentrations in total phospholipid did not differ from control. However, docosahexaenoic acid (DHA, 22:6n-3) was reduced in ethanolamine glycerophospholipid and choline glycerophospholipid, but increased in phosphatidylinositol. AA was reduced in choline glycerophospholipid, but increased in phosphatidylinositol, while docosatetraenoic acid (22:4n-6), an AA elongation product, was reduced in total brain lipid, cholesteryl ester and triglyceride. These lipid changes may contribute to membrane instability and synaptic loss in AD, and reflect neuroinflammation and excitotoxicity.

Keywords

Alzheimer; brain; arachidonic; docosahexaenoic; phospholipid; plasmalogen; cholesteryl ester; postmortem; lipid

Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by dementia and accumulation in brain of neurofibrillary tangles, senile (neuritic) plaques containing β-amyloid and activated microglia, as well as by synaptic loss [1]. Increased brain cytokine levels and increased expression of cytosolic phospholipase A_2 ($cPLA_2$), secretory $sPLA_2$ and cyclooxygenase (COX)-2 in postmortem AD brain are consistent with neuroinflammation and upregulated arachidonic acid (AA, 20:4n-6) metabolism [2–8], as is increased brain AA uptake in patients, measured with positron emission tomography (PET) [9].

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Conflicts of Interest: None

Disturbances of other fatty acids and lipids also have been reported in AD. For example, the n-3 polyunsaturated fatty acid (PUFA), docosahexaenoic acid (DHA, 22:6n-3), was reported reduced in postmortem brain and plasma of AD patients [10–12, 13. Additional studies identified abnormal fatty acid or phospholipid concentrations in the AD brain {Brown, 1989 #30, 14–22]. In many of these, however, the reported lipid profile was incomplete and/or fatty acid concentrations were measured as percent of total fatty acid concentration, rather than per gram tissue wet weight (wt), protein or phosphorus. In such cases, a statistically significant change in the percent concentration of one fatty acid may actually reflect a change in the opposite direction of others. Studies also were performed on different brain regions and on tissue obtained under different criteria, making comparisons difficult.

To examine AD brain lipids more thoroughly, and to relate changes if any to pathological mechanisms, in this paper we determined a relatively complete lipid concentration profile (measured per gram brain wet weight) in prefrontal association cortex (Brodmann area 9) of brains obtained under strict criteria from confirmed AD patients and age-matched controls. Prefrontal cortex was chosen because abnormalities in glucose metabolism, blood flow and AA metabolism have been reported in this region in patients, and characteristic AD pathology is found there on post-mortem [9, 23–31].

Materials and Methods

Postmortem brain samples

This study was approved by the Institutional Review Board of the McLean Hospital (Belmont, MA, USA) and by the NIH Office of Human Subjects Research (Protocol No. #4380). It was performed on frozen prefrontal cortex (Brodmann area 9) from 10 pathologically confirmed AD patients and 9 age-matched controls. Age, gender, postmortem interval (PMI), disease staging according to a Global Dementia scale [32], reported cause of death, medications taken at the time of death, and ApoE genotype are given in Table 1. Brain tissue was provided by the Harvard Brain Tissue Resource Center http://www.brainbank.mclean.org/, McLean Hospital (Belmont, MA, USA) under PHS grant R24MH068855 awarded to one of us (J. S. Rao). ApoE genotyping was performed on the samples (see below), and their pH was measured by the method of Harrison et al. [33].

Materials

Di-heptadecanoate phosphatidylcholine (di-17:0 PC), free heptadecanoic acid (17:0), and thin layer chromatography (TLC) standards for cholesterol, triglycerides, and cholesteryl esters were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fatty acid methyl ester (FAME) standards for gas chromatography (GC) were purchased from NuChek Prep (Elysian, MN, USA). Identification standards for individual phospholipids were purchased from Avanti® Polar Lipids (Alabaster, AL, USA). 6-p-Toluidine-2-naphthalene sulfonic acid was purchased from Acros Organics (Fairlawn, NJ, USA). Other chemicals and reagents were purchased from Sigma-Aldrich or Fisher Scientific.

ApoE genotyping

ApoE genotyping was performed on brain tissue using an ApoE4/Pan-ApoE4 ELISA kit (MBL International, Woburn, MA, USA), according to the manufacturer's instruction. Tissue was homogenized with 3 vol of homogenization buffer (10 mM HEPES, pH 7.5 containing 1 mM EDTA, 0.34 μM sucrose and protease inhibitor cocktail (Roche, Indianapolis, IN, USA)) using a glass homogenizer. The homogenized sample was centrifuged at 100,000 g for 1 h at 4 °C, and the supernatants were kept at −80 °C until used for ELISA. Protein concentration was analyzed by the Bradford assay [34]. 150 μl of control, AD or standard protein samples were incubated in a precoated ApoE polyclonal 96-

well plate. The bound protein-antibody complex was detected by adding peroxidase conjugated anti-ApoE4 monoclonal antibody or a peroxidase conjugated anti-ApoE polyclonal antibody. Color was developed by adding peroxidase substrate to the antibody complex, and measured with a spectrophotometer. The concentration of ApoE4 or Pan-ApoE was calibrated from a standard curve based on reference standards. The ELISA kit can measure the difference among ApoE4 homozygotes (E4/E4), heterozygotes (E2/E4, E3/E4), and non-ApoE4 zygotes (E2/E2, E2/E3, E3/E3), but cannot distinguish between ApoE4-containing heterozygotes (E2/E4, E3/E4). Results are read as (E4/E4), (E2/E4, E3/E4) or (E2/E2, E2/E3, E3/E3).

Chemical analyses

Brain lipid extraction and separation of lipid classes—We employed lipid analytical methods that have been validated for both human and microwaved rat brain [35, 36]. Because we wished to measure concentrations of "stable" lipids (e.g., phospholipids, triacylglycerol, cholesteryl ester), of esterified fatty acids in stable lipids, and of unesterified fatty acids in the same tissue sample, we did not add fatty acid or phospholipid standards to the tissue before processing. Instead, as described below, we first used the Folch method to extract > 99% of brain lipid [37], then used TLC and specific solvent systems to separate individual lipid classes [38]. Less than 5% of lipid is lost during TLC (Igarashi, M., unpublished observations). We visualized individual lipid bands on the gel and verified the identify of the bands by comparing them to bands from authentic standards. We added di-17:0 PC and 17:0 as internal standards to the phospholipids and unesterified fatty acids, respectively, and then prepared fatty acid methyl esters (FAMEs) from the hydrolyzed and unesterified fatty acids before measuring concentrations by GC. We used a phosphorous assay to measure concentrations of phospholipids.

More explicitly, total lipids were extracted from frozen brain by the Folch method [37]. The sample (~0.2 g) was homogenized in 5 ml methanol with a homogenizer (Model T25, IKA® Works, NC, USA) for 1 min, 5 ml methanol and 20 ml chloroform were added to the homogenate, and then the mixture was mixed vigorously for 1 min. After 7.5 ml of 0.5 M KCl was added, the sample was mixed vigorously for 1 min and centrifuged for 10 min at 1,000 rpm. The bottom chloroform phase was transferred to a tube, and 10 ml chloroform was added to the upper aqueous phase, which then was mixed and centrifuged. The second chloroform phase was combined with the first chloroform phase and dried under a nitrogen stream. The residue was dissolved in chloroform/methanol (2:1, v/v) as the total lipid extract, and kept at −80 °C until used.

The total lipid extract was separated into phospholipid and neutral lipid subclasses using TLC on silica gel-60 plates (EM Separation Technologies, Gibbstown, NJ, USA). Phospholipid classes were separated using chloroform:methanol:glacial acetic acid:water (60:50:1:4, by vol) [38]. This separates EtnGpl, phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), and choline glycerophospholipid (ChoGpl). Neutral lipid subclasses (cholesteryl esters, triacylglycerol, unesterified fatty acids, cholesterol, and total phospholipid) were separated using heptane:diethylether:glacial acetic acid (60:40:3, by vol) [38]. Authentic standards of phospholipids, triacylglycerol, cholesterol, cholesteryl ester and unesterified fatty acids were run on the plates to identify the lipids. The plates were sprayed with 0.03% 6-p-Toluidine-2-naphthalene sulfonic acid in 50 mM Tris-HCl buffer (pH 7.4) (w/v), and the lipid bands were visualized under ultraviolet light. The bands were scraped, and silica gel was directly used to prepare fatty acid methyl esters (FAMEs) and to determine phosphorus concentration.

Brain fatty acids—Fatty acid concentrations (nmol/g brain wet wt) were determined by a modification of the method of Makrides et al. [39]. Unesterified and esterified fatty acids, which had been separated by TLC as described above, were methylated with 1% H_2SO_4-methanol for 3 h at 70 °C [40]. To analyze fatty acid concentrations in total lipids, total lipid extracts were used directly for methylation. Before methylation, an internal standard (di-17:0 PC for phospholipids or 17:0 for unesterified fatty acids) was added to each tube. The prepared FAME samples were analyzed using a GC (6890N, Agilent Technologies, Palo Alto, CA, USA) equipped with an SP™-2330 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Supelco, Bellefonte, PA, USA) and a flame ionization detector. Concentrations were calculated by proportional comparison of peak areas of samples to the area of the 17:0 internal standard.

Brain phospholipids—Total and individual phospholipid concentrations were quantified using a phosphorus assay [41]. An adequate amount of total lipids scraped from the silica gel after TLC was added to a tube and digested in 0.5 ml water and 0.65 ml perchloric acid (70%) at 180°C for 1 h. After the samples had cooled to room temperature, 0.5 ml ascorbic acid (10%, w/v), 0.5 ml ammonium molybdate (2.5%, w/v), and 3.0 ml water were added. The mixture was boiled for 5 min to develop color. Absorbance was read at 797 nm. Phospholipid concentrations were determined using standard curves as reported [42].

Brain plasmalogens—Concentrations of the plasmalogens, plasmenylethanolamine (PlsEtn) and plasmenylcholine (PlsCho), were determined by the iodine uptake method [43]. Phospholipid classes in total lipid extracts were separated by TLC, as described above. The EtnGpl and ChoGpl bands were scraped and added to a tube containing a mixture of methanol (0.5 ml) and iodine reagent (0.5 ml) (6.0×10^{-4} N iodine in 3% aqueous KI). The mixture was mixed vigorously, then left at room temperature for 20 min. Absorbencies of the samples and of iodide (control) were measured at 355 nm after adding 4.0 ml 95% ethanol. Plasmalogen concentrations were calculated with absorbance coefficients: plasmalogen μmol/tube = 10^{-6} x [(absorbance of iodine control - absorbance of sample)/ 27500].

Brain cholesterol—Total cholesterol was determined with a commercial kit, according to the manufacturer's instructions (BioVision Research, Mountain View, CA). The total lipid extract was dried in a SpeedVac, and the residue was dissolved in 0.1% Triton X-100. The concentration was expressed in μmol/g brain wet weight.

Statistical analysis

We screened for outliers and tested assumptions of normality and homogeneity of variance with the Shapiro-Wilk and Levene tests, respectively, using SPSS 12.0 (SPSS Inc, Chicago, IL, USA). A Mann-Whitney U test was applied if the normality assumption was violated and an Aspin-Welch test was used if there was significant heterogeneity of variance. An unpaired Student's t-test was applied if statistical assumptions were met. The alpha level was set at 0.05 for all statistical tests.

Results

As illustrated in Table 1, AD patients differed from controls with regard to the drugs that they were taking at the time of death (e.g., mood stabilizers carbamazepine and valproate; acetylcholinesterase inhibitors donepezil and rivastigmine; atypical antipsychotics sertraline, alprazolam, quetiapine and risperidone; escitalopram for depression or anxiety; pantoprazole for esophagitis). The AD patients were in Stages V – VI on the Global Deterioration Scale (Range I to VII), with Stage VI representing moderate-severe dementia [32]. Four of the 10

AD patients were E4/E4 homozygous and 5 were E2/E4 or E3/E4 heterozygous; one could not be classified. Control subjects were E2/E4 or E3/E4 heterozygous. As summarized in Table 2, mean age, PMI, and tissue pH did not differ significantly between AD and control cortex.

Mean absolute concentrations (nmol/g brain wet wt) of total phospholipid, individual phospholipids (EtnGpl, ChoGpl, PtdIns and PtdSer), and total cholesterol did not differ significantly between AD and control prefrontal cortex (Table 3). There also was no significant difference in the mean PlsEtn concentration between the groups. However, the mean PlsCho concentration was decreased significantly by 73% ($p < 0.001$), from 4061 to 1111 nmol/mg brain wet wt, in AD compared to control cortex.

Table 4 summarizes mean esterified fatty acid concentrations in stable brain lipids of AD and control prefrontal cortex, as well as mean unesterified fatty acid concentrations (the latter are overestimates, as they are released on death [44]). Of the n-6 PUFAs in control brain, the concentration of AA was highest and that of docosatetraenoic (adrenic) acid (DTA, 22:4n-6), an AA elongation product, second highest in stable lipids. The DTA concentration was reduced significantly (by 28%) in total lipids, as well as in cholesteryl ester, triglyceride and unesterified fatty acids of the AD brain. There was no significant difference of any other fatty acid, including AA and DHA, within total lipids between the two groups, and no significant difference in the concentration of any fatty acid in total phospholipid. Fatty acid concentrations in cholesteryl ester, triglyceride and unesterified compartments showed a number of changes in AD compared with control brain.

Differences were evident in esterified fatty acid concentrations in individual brain phospholipids between AD and control brain, suggesting remodeling (Table 5). In control brain, highest concentrations of DHA were in EtnGpl and PtdSer, of DTA (22:4n-6) in EtnGpl and PtdSer, and of AA in EtnGpl and ChoGpl. The mean DHA concentration was decreased significantly in EtnGpl (by 22%) and in ChoGpl (by 22%) of the AD cortex, but was increased in PtdIns (by 89%). 22:5n-3 (docosapentaenoic acid, DPAn-3) was increased in EtnGpl but decreased in ChoGpl and PtdSer. The mean AA concentration was decreased in ChoGpl (by 25%) of the AD brain, but increased in PtdIns (by 38%). 22:5n-6 (DPAn-6), an AA elongation product, was decreased in EtnGpl (by 29%) and PtdSer (by 31%). Stearic acid (18:0) was decreased in EtnGpl (by 25%) and ChoGpl (by 30%), palmitic acid (16:0) was increased in ChoGpl (by 20%), and monounsaturated 18:1n-7 was decreased in ChoGpl. Total n-6 PUFAs were increased in PtdIns (by 40%), while the total n-3 PUFA concentration was decreased in EtnGpl (by 23%) and ChoGpl (by 12%), it was increased in PtdIns (by 89%) of the AD compared with control cortex. AA/DHA and n-6/n-3 ratios did not differ significantly between groups.

There was no significant concentration difference for any stable lipid or fatty acid between AD patients classified as homozygous for ApoE4 and those classified as heterozygous or having no ApoE4 (data not shown).

Discussion

The present study demonstrates a statistically significant 73% decrease in PlsCho in postmortem prefrontal cortex from AD patients compared with controls, and significant reductions in the concentration of 22:4n-6 (DTA) in total lipid, cholesteryl ester and triglyceride. Esterified AA and DHA did not show any significant concentration difference in total phospholipid or in total lipid between AD and control cortex, but did show group differences in individual phospholipids. The AA concentration was reduced in ChoGpl but increased in PtdIns, while the DHA concentrations was reduced in EtnGpl and ChoGpl but

increased in PtdIns. The AA elongation product, 22:5n-6 (DPAn-6), was decreased in EtnGpl and PtdSer. The total esterified n-6 PUFA concentration was increased in PtdIns (by 40%) of the AD cortex, while the total n-3 PUFA concentration was decreased in EtnGpl (by 23%) and ChoGpl (by 12%) but increased in PtdIns (by 89%). AA/DHA and n-6/n-3 ratios did not differ significantly between groups. Of esterified unsaturated or monounsaturated fatty acids, stearic acid (18:0) was decreased in EtnGpl and ChoGpl, palmitic acid (16:0) was increased in ChoGpl, and 18:1n-7 was decreased in ChoGpl. Significant differences in fatty acid concentrations also were evident in cholesteryl ester.

While we did not find a significant change in phospholipid concentrations in the AD brain except for the reduced PlsCho, other studies have reported decreased PtdCho and PtdEtn concentrations [19] and lower total phospholipid in the frontal cortex [45]. A lower PtdEtn concentration and higher PtdSer concentration also were reported in [46], but one study found no change in phospholipid composition [47]. The different results could have been due to differences in methodology, regions analyzed, tissue source, tissue quality, sample size, dementia severity, diet, drug history and other factors.

In the human brain, plasmenyl-type ether phospholipids (plasmalogens), which have 1-*O*-alk-1'-enyl (plasmenyl) linkage rather than an ester linkage at the *sn*-1 position of the glycerol backbone, compose approximately 23% of total phospholipids [48]. Plasmalogens are synthesized in peroxisomes and microsomes, then are transferred to myelin or synaptic membranes [49–51]. Thus, the 73% reduced PlsCho in the AD brain may be related to changes in myelin in AD [52] and to synaptic loss and dysfunction [29, 53, 54].

The decreased PlsCho also may contribute to the reduced lipid critical temperature reported in AD association cortex, leading to membrane instability and increased membrane phospholipid turnover [14, 15, 19, 55]. The reduced critical temperature has been ascribed to a reduced PlsEtn concentration [15], but the present study suggests a more critical role for reduced PlsCho. Total brain plasmalogen was reduced in cortical but not cerebellar gray matter in one study [16], and serum PlsEtn was reduced in AD patients in another [17].

Several studies have suggested a role for reduced DHA in AD pathophysiology. A reduced plasma DHA concentration was noted in patients [10, 56], and dietary n-3 PUFA deficiency increased risk for AD in multiple studies [56–60]. However, dietary n-3 PUFA supplementation has yet to prove useful in patients [12, 61], although it was beneficial in transgenic animal models of AD, reversing synaptic loss in some of them [61–63].

We did not find a significant reduction in the total DHA concentration in the AD prefrontal cortex, in agreement with other studies [64–66]. This suggests that the patients were not n-3 PUFA deprived, which in animal studies leads to a reduced total brain DHA concentration [67]. However, total DHA was reported reduced in AD neocortex in some studies [20, 68], whereas others reported a decrease in the hippocampus but not neocortex [11, 69].

In one of these studies, the DHA concentration also was unaltered in the parietal and temporal cortices from AD patients, and the AA concentration was in the temporal cortex, that of stearic acid (18:0) in frontal and temporal cortices [66]. In that study, fatty acid concentrations were unrelated to ApoE genotype (as in the present paper), age, gender, or PMI.

The altered fatty acid profiles in individual phospholipids that we found suggest increased remodeling, associated with breakdown and resynthesis of phospholipid, or with accelerated deacylation and reacylation [70–72]. Excess remodeling may have been caused by energy insufficiency, β-amyloid accumulation, or neuroinflammation and excitotoxicity associated with increased expression of $cPLA_2$ and $sPLA_2$ [2–7, 72–77]. Consistent with the changes in

these enzymes, which hydrolyze AA from membrane phospholipid [7], brain AA metabolism is elevated in AD patients [9, 78].

AA and DHA are concentrated in synaptic membrane phospholipids [27, 28]. Thus, the significant decreases in AA and DHA concentrations in ChoGpl in the AD cortex, the reduced concentration of 22:5n-6 (DPAn-6) in EtnGpl and PtdSer, and the lower DHA concentration in EtnGpl, may be related to synaptic loss in this disorder [1, 29, 53, 54].

We did not find any significant relation between ApoE genotype and brain cholesterol or other lipid concentrations in this study, possibly because of the small number of samples and the presence of E4 in the heterozygous group, but nevertheless in agreement with a prior report on a large number of subjects [66]. Furthermore, mean values of PMI and tissue pH did not differ between the AD and control cortex. In this regard, a PMI of 18 h compared with a shorter PMI did not alter brain phospholipid composition in rats [79].

Our control phospholipid and fatty acid concentrations agree with published human brain concentrations measured per gram wet weight, protein or DNA, in which internal standards were employed for the fatty acids [11, 19, 35, 80]. The fatty acid concentrations in cholesteryl ester also agree with reported values [81], as do the plasmalogen concentrations [82].

Like the AD brain, the postmortem brain from bipolar disorder patients shows neuroinflammation. excitotoxicity and elevated $cPLA_2$ and $sPLA_2$ expression [83–85]. Using the lipid analytical methods of this paper, we found no statistically significant difference between bipolar and control frontal cortex in mean concentrations (per gram wet weight) of total lipid, phospholipid, individual phospholipids, or cholesterol, or of esterified AA or DHA within individual phospholipids [35], which was confirmed in another study [86]. Thus, the significant concentration changes in the AD cortex in the present paper appear specific to AD, and indicate more severe lipid metabolic changes in AD than in bipolar disorder [83, 87].

In summary, postmortem prefrontal cortex (Brodmann area 9) from AD compared with control subjects showed multiple statistically significant differences in fatty acid concentrations measured per gram wet weight, particularly of AA and DHA, within phospholipids, as well as a 73% reduced PlsCho concentration. Overall AA or DHA concentrations did not differ significantly between groups, arguing against a diet-related n-3 or n-6 PUFA disturbance. The changes likely reflect and/or contribute to AD pathology, particularly synaptic loss and a reduced membrane lipid critical temperature, associated with neuroinflammation and excitotoxicity.

Abbreviations

AA	arachidonic acid
AD	Alzheimer disease
ApoE	apolipoprotein E
COX	cyclooxygenase
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DTA	docosatetraenoic (adrenic) acid
FAME	fatty acid methyl ester

GC	gas chromatography
PET	positron emission tomography
cPLA$_2$	cytosolic phospholipase A$_2$
iPLA$_2$	calcium-independent phospholipase A$_2$
sPLA$_2$	secretory phospholipase A$_2$
PMI	postmortem interval
PUFA	polyunsaturated fatty acid
EtnGpl	ethanolamine glycerophospholipid
PtdCho	phosphatidylcholine
PtdIns	phosphatidylinositol
PtdSer	phosphatidylserine
ChoGpl	choline glycerophospholipid
PlsEtn	plasmenylethanolamine
PlsCho	plasmenylcholine
sn	stereospecifically numbered
TLC	thin layer chromatography

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Table 1

Characteristics of control subjects and Alzheimer disease patients

Group	Age (yr)	Sex	PMI (hr)	Cause of death	Medications	Disease Stage	Genotype
Control	60	F	21	Cardiopulmonary attack	Aspirin		ApoE2/4/ ApoE2/3
Control	80	M	15	Myocardial infarction	Furosemide		ApoE2/4 ApoE2/3
Control	79	F	19	Cardiopulmonary attack	Isosorbide		ApoE2/4 ApoE2/3
Control	72	M	22	Cardiopulmonary attack	Atorvastatin		ApoE2/4 ApoE2/3
Control	64	M	15	Acute myocardial infarction	Vitamins		ApoE2/4 ApoE2/3
Control	74	M	18	Cardiac arrest	Atenolol, Digoxin		ApoE2/4 ApoE2/3
Control	61	M	22	Myocardial infarction	Vitamins		ApoE2/4 ApoE2/3
Control	78	F	17	Myocardial infarction	Atorvastatin		ApoE2/4 ApoE2/3
Control	63	M	15	Heart failure	Furosemide		ApoE2/4 ApoE2/3
AD	76	F	25	Stroke	Sertraline, Carbamazepine, Alendronate, Aspirin	V	ApoE4/4
AD	83	M	17	Stroke	Donepezil, Valproate, Tamsulosin, Escitalopram	V	ApoE4/4
AD	72	M	24	AD	Atenolol, Donepezil, Valproate, Alprazolam	VI	ApoE4/4
AD	78	F	17	AD	Fexofenadine, Donepezil, Aspirin	VI	ApoE4/4
AD	75	M	15	Atherosclerosis	Gabapentin, Furosemide, Quetiapine, Escitalopram	VI	ApoE2/4 ApoE2/3
AD	66	M	23	Broken hip, AD	Rivastigmine. Donepezil	V	ApoE2/4 ApoE2/3

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Group	Age (yr)	Sex	PMI (hr)	Cause of death	Medications	Disease Stage	Genotype
AD	63	M	18	Pneumonia	Carbamazepine, Sertraline, Aspirin, Quetiapine	VI	ApoE2/4 ApoE2/3
AD	71	M	16	End of AD	Atorvastatin, Donepezil, Pantoprazole, Verapamil, Vitamin E, Risperidone	VI	ApoE2/4 ApoE2/3
AD	61	F	21	End of AD	Pantoprazole, Verapamil, Vitamin E, Risperidone	VI	ApoE2/4 ApoE2/3
AD	61	M	17	Pneumonia	Valproate Quetiapine, Olanzapine	STAGE VI	Not available

PMI, postmortem interval; Not available, sample volume was not enough for this experiment; Staging according to Reisberg et al. [32]

Table 2

Mean parameters for control and Alzheimer disease subjects

	Control	Alzheimer disease
Age, years	70.1 ± 8.1	70.6 ± 7.6
Post-mortem interval, hours	18.2 ± 2.9	19.3 ± 3.6
pH	6.7 ± 0.2	6.8 ± 0.2

Mean ± SD, n = 9 for controls, 10 for AD patients

Student's *t*-test was performed for all results. No mean differed significantly between groups.

Table 3

Phospholipid, total cholesterol and triglyceride concentrations in postmortem prefrontal cortex from Alzheimer Disease and control subjects

Lipid	Control	Alzheimer Disease
	nmol/g brain wet weight	
Total phospholipids	52425±17230	50545±12544
Ethanolamine glycerophospholipid	15691±4266	12966±3634
Choline glycerophospholipid	15542±4103	13065±1854 *b*
Phosphatidylserine	6379±2274	6336±2500 *a*
Phosphatidylinositol	†2764±646	2696±820
Total cholesterol	28561±9124	24186±10716 *a*
Total triglyceride	1252±740	‡869±386
Plasmalogens		
Plasmenylethanolamine	10521±3808	8478±4398 *a*
Plasmenylcholine	4061±3438	1111±637***, *a*

Mean ± SD, n = 9 for controls (†n = 8), 10 for AD (‡n = 9) patients

*** $p < 0.001$

a Mann-Whitney test was performed;

b Aspin-Welch test was performed. All other results were obtained by Student's t-test.

Table 4

Esterified and unesterified fatty acid concentrations in post mortem cortex from Alzheimer disease and control subjects

Fatty acid	*Total lipids*		*Total phospholipid*		*Cholesteryl ester*		*Triglyceride*		*Unesterified fatty acid*	
	Control	**AD**	**Control**	**AD**	**Control**	**AD**	**Control**	**AD**	**Control**	**AD**
	nmol/g brain wet weight									
16:0	17443±4071	15423±2131	16512±3066	15088±2083	21.6±16.3	††34.6±12.4*, *a*	18.2±8.8	††4.2±3.4**, *a*	429±93	322±82*
16:1n-7	581±183	612±292 *b*	476±203	523±209	1.3±0.6	††2.7±2.5 *b*	2.1±1.6	††0.3±0.2*, *b*	28±9	16±8**
18:0	24521±6292	20301±4836	23101±5617	18478±4069	23.1±9.4	††26.7±8.4	17.1±5.5	††8.4±3.8**	654±156	527±140
18:1n-9	16036±5612	14616±6615 *a*	14654±6777	11827±4874 *a*	8.1±10.3	††24.0±16.8**, *a*	21.7±12.9	††2.5±1.3**, *b*	186±63	119±49*
18:1n-7	6716±2109	5785±2610	6405±2510	6969±3183 *a*	5.7±8.5	††20.6±15.6**, *a*	10.1±6.5	††2.5±2.1**, *b*	238±147	73±81*
18:2n-6	735±210	807±189	577±107	663±119	2.7±2.2	††9.7±9.4 *b*	7.7±3.7	††2.0±1.4**, *b*	46±15	35±16
20:3n-6	704±231	610±191	643±165	529±106	3.1±2.3	††2.2±1.0	1.4±1.6	††0.3±0.2 *a*	10±6	7±5
20:4n-6 (AA)	6965±1606	5708±968	5358±1161	4671±494 *b*	11.6±15.4	††15.5±8.4*a*	6.7±2.2	††4.0±3.6*a*	373±89	218±108**
20:5n-3	158±98	156±115 *a*	208±200	184±180 *a*	0.8±0.4	††1.0±0.5*a*	0.9±0.9	††0.3±0.2*a*	3.3±2	2.6±1.4
22:4n-6	4087±1182	2996±938*	3929±1622	3061±1013	20.2±8.3	††11.7±7.2*	16.1±12.5	†4.1±1.7*, *b*	135±172	43±21**, *a*
22:5n-6	1019±346	792±242	958±367	774±185	1.5±0.8	††1.5±1.0 *a*	1.3±1.2	†0.5±0.3 *a*	17±6	9±3**, *b*
22:5n-3	310±119	261±61	205±70	222±45 *b*	0.4±0.2	††0.5±0.4*a*	0.2±0.1	††0.1±0.1*a*	5.1±2.6	3.8±2.4
22:6n-3 (DHA)	10333±2695	9077±1883	7244±1903	6626±1038	1.8±2.9	††4.7±3.8*, *a*	1.5±0.6	††0.9±0.7*	111±30	66±34**
Total	89510±20520	77145±18938	80170±19849	69614±14938	102±68	††155±68*, *a*	105.0±41.9	††29.6±12.7***,*b*	2236±531	1516±514**
Total n-6	13412±3231	10914±2153	11365±3151	9698±1644	39.0±22.9	††40.6±17.2*a*	33.3±17.5	††10.4±5.1**, *b*	581±226	312±142**
Total n-3	10801±2652	9494±1984	7657±1980	7032±988	3.0±3.1	††6.2±4.0*, *a*	2.6±1.2	††1.3±0.7*	120±31	72±37**
Total saturated	41964±10295	35725±6741	39613±8467	33565±5931	44.7±24.6	††61.3±18.1*, *a*	35.5±11.5	††12.6±7.1***	1083±240	850±221*
Total monounsaturated	23332±7709	21012±9256	21535±9311	19319±8045 *a*	15.1±19.1	††47.3±34.1**, *a*	33.8±17.2	††5.3±3.5**, *b*	452±138	282±133*
n-6/n-3 ratio	1.25±0.13	1.16±0.14	1.51±0.32	1.40±0.31	16.8±6.3	††8.4±4.4**	13.4±6.0	††8.8±3.0	1.4±0.5	1.4±0.4
AA/DHA ratio	0.67±0.05	0.64±0.06	0.76±0.13	0.72±0.13	10.5±5.9	††5.0±2.6*	4.9±2.2	††4.7±2.2	0.9±0.3	1.1±0.3

Mean ± SD, n = 9 for controls, n = 10 (†n=7 ††n = 8) for AD patients

* $p<0.05$,

** $p < 0.01$,

*** $p < 0.001$

[a] Mann-Whitney test was performed;

[b] Aspin-Welch test was performed. All other results were obtained by Student's t-test.

Table 5

Fatty acid concentrations in individual glycerophospholipids in prefrontal cortex from Alzheimer disease and control subjects

Fatty acid	***EtnGpl***		***ChoGpl***		***PtdIns***		***PtdSer***	
	Control	**AD**	**Control**	**AD**	**Control**	**AD (n = 9)**	**Control**	**AD**
	nmol/g brain wet weight							
16:0	1520±383	1506±478	10113±1989	12046±1701*	558±259	††466±172	327±302	505±236 ^{a}
16:1n-7	135±68	162±68	145±77	291±93**	19±15	††16±8^{a}	13.7±8.5	20.1±19.3 ^{a}
18:0	6702±1730	5052±1228*, ^{a}	5032±1173	3523±871**	1829±679	††1752±440	7495±3792	6660±2100
18:1n-9	4009±2278	3502±2470^{a}	8401±2225	7829±2213	335±189	††336±152	2451±2319	2984±1938^{a}
18:1n-7	1289±569	2499±1902 ^{a}	2212±391	1506±195**, ^{a}	142±81	††204±65	553±569	462±288^{a}
18:2n-6	212±53	201±62	312±54	445±233^{a}	57±49	††74±46^{a}	67±52	93±68^{a}
20:3n-6	275±84	222±83	248±79	255±179^{a}	58±45	††58±35	120±101	101±29^{a}
20:4n-6 (AA)	3233±573	2744±670	1380±262	1036±189**	636±234	††878±160*	290±235	275±74^{a}
20:5n-3	141±129	35±24**, ^{a}	†22±11	††24±17	9.1±6.4	¥10.5±7.6	111±124	17.1±13.6**, ^{a}
22:4n-6	3400±1020	2591±1187	254±67	185±84	63±38	¥94±46	736±439	508±226^{a}
22:5n-6	601±207	427±111*	78±28	74±68^{a}	13.5±7.1	††15.0±10.7	372±139	258±67*, ^{b}
22:5n-3	21±12	111±71**, ^{a}	28±7	††12±13**	‡4.8±1.3	‡4.9±2.3	47±33	18±17*, ^{a}
22:6n-3 (DHA)	5113±1212	4007±786*	599±134	469±88*	64±28	††121±42**	2649±1607	1951±358 ^{b}
Total	26650±4987	23061±7193	28820±5300	27693±5216	3799±1383	††4017±972	15234±7732	13853±4327 ^{b}
Total n-6	7720±1770	6186±1915	2272±421	1995±676^{a}	828±287	††1109±214*	1586±900	1235±338 ^{b}
Total n-3	5275±1162	4153±801*	645±141	502±93*	76±31	††133±45**	2807±1621	1986±355 ^{b}
Total saturated	8223±1712	6558±1500*	15146±2985	15569±2542	2386±909	††2219±574	7822±3987	7165±2140
Total monounsaturated	5433±2890	6163±4377	10758±2604	9627±2397	495±271	††556±220	3019±2625	3466±2191^{a}
Ratio of n-6/n-3	1.5±0.4	1.5±0.5	3.6±0.5	3.9±0.8 ^{a}	11.3±2.1	††9.0±2.6	0.58±0.16	0.64±0.20 ^{a}
AA/DHA	0.7±0.2	0.7±0.2	2.3±0.3	2.2±0.3	10.4±2.2	††8.0±2.8	0.11±0.05	0.14±0.04

Mean ± SD, n = 9 for controls(‡=6), 10 for AD patient (‡=6, †=7, ¥=8, ††=9,)

* $p<0.05$

** $p<0.01$

[a] Mann-Whitney test was performed

[b] Aspin-Welch test was performed. All other results were obtained by Student's t-test.