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Original Article

Lipid Profile in Human Frontal Cortex Is Sustained Throughout Healthy Adult Life Span to Decay at Advanced Ages

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Abstract

Fatty acids are key components in the structural diversity of lipids and play a strategic role in the functional properties of lipids which determine the structural and functional integrity of neural cell membranes, the generation of lipid signaling mediators, and the chemical reactivity of acyl chains. The present study analyzes the profile of lipid fatty acid composition of membranes of human frontal cortex area 8 in individuals ranging from 40 to 90 years old. Different components involved in polyunsaturated fatty acid biosynthesis pathways, as well as adaptive defense mechanisms involved in the lipid-mediated modulation of inflammation, are also assessed. Our results show that the lipid profile in human frontal cortex is basically preserved through the adult life span to decay at advanced ages, which is accompanied by an adaptive proactive anti-inflammatory response possibly geared to ensuring cell survival and function.

Keywords: Brain aging, Desaturases, Cytochrome p450 epoxygenase, Peroxisomal β-oxidation, Inflammatory factors

During the physiological aging process, human brain suffers from structural and physiological changes which become the substrate of age-associated cognitive decline (1–6). Among these, genomic and proteomic neuronal changes involved in energy metabolism, cytoskeleton, proteostasis, neurotransmission, O_2/CO_2 and heme metabolism, synaptic plasticity, vesicular transport, mitochondrial function, and stress-adaptive response have the greatest impact (2–4,6–10).

Human neurons are functional throughout the adult life span but the mechanisms that guarantee survival and preserve function, and protect them against harmful conditions, are poorly understood. Some recent evidence suggests adaptive response of stress resistance mechanisms based on the sustained expression of REST (6,7), and modifications in the expression of Akt and mTOR are substantial players (6) in maintaining neural cell survival and function.

Lipids have played a cardinal role in the evolution of the brain (11,12). Besides their quantitative importance (13), lipids of the nervous system show great structural and functional diversity (5,14). This diversity is linked to an intense dynamic cellular activity

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involved in lipid biosynthesis, remodeling, turnover, specific organization at the membrane level, and synthesis of lipid mediators which represents an energy investment of around 25% of total brain energy demand (15). All lipid classes are represented in the nervous system, which expresses the structural and functional complexity of the system (14,16). Fatty acids are primary components in the structural diversity of lipids and they play a key role in the functional properties of the integrity of cell membranes, generation of lipid signaling mediators, and chemical reactivity of the acyl chains (5).

Changes in phospholipid composition are documented in human brain aging (17–22) and in several age-associated neurodegenerative diseases such as Alzheimer disease (5). However, little is known about changes occurring throughout the life span.

The present study analyzes the membrane lipid fatty acid compositional profile in human frontal cortex area 8 in individuals ranging from 40 to 90 years old. Components of polyunsaturated fatty acid (PUFA) biosynthesis and adaptive defense mechanisms involved in lipid-mediated modulation of inflammation are also determined. The selection of frontal cortex is based on the fact that this region developed recently during primate evolution and is implicated in cognitive function (23,24). Our results demonstrate that the lipid profile in human frontal cortex is maintained throughout the adult life span to decay at advanced ages; this maintenance is accompanied by an adaptive proactive anti-inflammatory response geared to ensuring cell survival and function.

Methods

Chemicals

Unless otherwise specified, all reagents were from Sigma-Aldrich, and were of the highest purity available.

Human Samples

Brain samples were obtained from the Institute of Neuropathology Brain Bank, a branch of the HUB-ICO-IDIBELL Biobank, following the guidelines of Spanish legislation and the approval of the local ethics committee, and in accordance with recently published criteria for sample quality (25).

The neuropathological study was carried out in every case as previously described (26). At autopsy, one hemisphere was fixed in 4% buffered formalin for about three weeks while the other hemisphere was cut in coronal sections 1 cm thick; selected samples of the brain were dissected and kept in labeled plastic bags, immediately frozen on dry ice, and stored at −80°C until use. The neuropathological study was carried out on formalin-fixed, paraffin-embedded samples of 26 brain regions (6). Dewaxed sections, 5 μm thick, were stained with hematoxylin and eosin, and Kluver Barrera, or processed for immunohistochemistry to β-amyloid, phosphorylated tau, α-synuclein, ubiquitin, p62, TDP43, glial fibrillary protein, and microglia markers.

Selected cases showed no lesions on neuropathological examination, excepting neurofibrillary tangle pathology at stages I–II of Braak and β-amyloid Phase 1 of Thal in some old individuals, a feature which was consistent with normal aging. Cases with AD-related pathology, α-synuclein deposits, TDP43 proteinopathy, vascular diseases (other than mild small blood vessel disease), inflammatory and autoimmune diseases of the nervous system, and decompensated systemic metabolic diseases (such as hepatic insufficiency, kidney failure, and metabolic syndrome) and hypoxia were excluded. Following initial screening, the present series include 58 cases: 30 males and 28 females, with an age ranging from 43 to 86 years. The postmortem delay ranged from 2 hours to 19 hours 30 minutes. Supplementary Table S1 summarizes cases examined in the present series. The grey matter of frozen samples from frontal cortex area 8 was dissected and used for biochemical studies.

Fatty Acid Profile

Fatty acids from human frontal cortex homogenates were analyzed as methyl ester derivatives (FAMEs) by gas chromatography as previously described (27,28). Separation was performed with a DBWAX capillary column (30 m × 0.25 mm × 0.20 μm) in a GC System 7890A with a Series Injector 7683B and a FID detector (Agilent Technologies, Barcelona, Spain). Identification of fatty acid methyl esters was made by comparison with authentic standards (Larodan Fine Chemicals, Malmö, Sweden). Results are expressed as mol%.

The following fatty acyl indices were also calculated: saturated fatty acids; unsaturated fatty acids; monounsaturated fatty acids; PUFA from n-3 and n-6 series (PUFAn-3 and PUFAn-6); and average chain length = $([\Sigma\%Total_{14} \times 14] + [\Sigma\%Total_{16} \times 16] + [\Sigma\%Total_{18} \times 18] + [\Sigma\%Total_{20} \times 20] + [\Sigma\% Total_{22} \times 22] + [\Sigma\% Total_{24} \times 24])/100$. The density of double bonds in the membrane was calculated with the Double Bond Index = ([1 × Σmol% monoenoic] + [2 × Σmol% dienoic] + [3 × Σmol% trienoic] + [4 × Σmol% tetraenoic] + [5 × Σmol% pentaenoic] + [6 × Σmol% hexaenoic]). Membrane susceptibility to peroxidation was calculated with the Peroxidizability Index (PI) = ([0.025 × Σmol% monoenoic] + [1 × Σmol% dienoic] + [2 × Σmol% trienoic] + (4 × Σmol% tetraenoic] + [6 × Σmol% pentaenoic] + [8 × Σmol% hexaenoic]).

Elongase and desaturase activities were estimated from specific product/substrate ratios: Δ9(n-7) = 16:1n-9/16:0; Δ9(n-9) = 18:1n-9/18:0; Δ5 (n-6) = 20:4n-6/20:3n-6; $\Delta6(n\text{-}3)^1$ = 18:4n-3/18:3n-3; $\Delta6(n\text{-}3)^2$ = 24:6n-3/24:5n-3; Elovl3(n-9) = 20:1n-9/18:1n-9; Elovl6 = 18:0/16:0; Elovl1-3-7a = 20:0/18:0; Elovl1-3-7b = 22:0/20:0; Elovl1-3-7c = 24:0/22:0; Elovl 5(n-6) = 20:2n-6/18:2n-6; Elovl2-5 (n-6) = 22:4n-6/20:4n-6; Elovl 2-5(n-3) = 22:5n-3/20:5n-3, and Elovl 2(n-3)= 24:5n-3/22:5n-3. Finally, peroxisomal β-oxidation was estimated according to the ratio 22:6n-3/24:6n-3.

Western Blotting

The protein expression related with (i) PUFA biosynthesis pathway, (ii) peroxisomal β-oxidation pathway, and (iii) neuroinflammatory markers was estimated using western blot analyses in samples from frontal cortex. Samples (50 mg) were homogenized in a buffer containing 180 mM KCl, 5 mM 3-(*N*-morpholino)propanesulfonic acid, 2 mM ethylenediaminetetraacetic acid, 1 mM diethylenetriaminepentaacetic acid, 1 μM butylated hydroxyltoluene, protease inhibitor mix (80-6501-23, Amersham Biosciences), and phosphatase inhibitors (Na_3VO_4 1 mM, NaF 1 mM). After a brief centrifugation (1,000 rpm at 4°C for 3 minutes) to pellet cellular debris, protein concentrations were measured in the supernatants using the Bradford method (Bio-Rad Protein Assay 500-0006).

Proteins were separated with one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis, and for immunodetection proteins were transferred using a Mini Trans-Blot Transfer Cell (Bio Rad) to polyvinylidene difluoride membranes (Immobilon-P Millipore, Bedford, MA) as previously described (6). The primary specific antibodies used were: anti-FADS1 (Fatty Acid Desaturase 1, ref. ab124363-Abcam, dilution: 1:300), anti-FADS2 (Fatty Acid Desaturase 2, ref. ab72189-Abcam, dilution: 1:500), anti-ELOVL2 (Elongation of very-long-chain fatty acids 2, ref. NBP1-80719-Novus,

dilution: 1:300), anti-DBP-1 (D-Bifunctional protein, ref. NBP1-85296-Novus, dilution: 1:300), anti-ACAA1 (Acetyl-CoA acyltransferase 1, ref. ab84635-Abcam, dilution: 1:1,000), anti-SCP2 (Sterol Carrier Protein 2, ref. ab82382-Abcam, dilution: 1:200), anti-5-LOX (Lipoxigenase-5, ref. ab169755-Abcam, dilution: 1:1,000), anti-COX-2 (Cyclooxygenase-2, ref. ab52237-Abcam, dilution: 1:300), anti-NF-kBp65 (Nuclear Factor-KB-RelA, ref. ab32536-Abcam, dilution: 1:1,000), anti-15-LOX (Lipoxygenase-15, ref. ab119774-Abcam, dilution: 1:500), anti-CYP450-2J2 (Cytochrome P450 2J2/Epoxygenase, ref. ab139160-Abcam, dilution: 1:500), anti-Actin (Sigma-a5441, dilution 1:5,000), and anti-Tubulin (ab7291-Abcam, dilution 1:5,000).

Each membrane was washed and incubated with the appropriate secondary antibodies: ECL Anti-mouse IgG, horseradish peroxidase linked whole antibody-NA93IV GE Healthcare (1:30,000) and ImmunoPure Goat Anti-rabbit IgG peroxidase conjugated-31460 Pierce Biotechnology (1:100,000) as previously described (6).

Bands were visualized using an enhanced chemiluminescence HRP substrate (Millipore, MA). Signal quantification and recording was performed with ChemiDoc equipment (Bio-Rad Laboratories, Inc., Barcelona, Spain). The amounts of the determined proteins were specifically calculated from the ratio of their densitometry values in reference to the densitometry values of actin or tubulin.

RNA Purification, Retrotranscription Reaction, and TaqMan PCR

The purification of RNA from human frontal cortex (n = 3–4 samples per decade) was carried out with RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) following the protocol provided by the manufacturer, following DNase digest to avoid extraction and later amplification of genomic DNA. Quality of isolated RNA was first measured with Bioanalyzer Assay (Agilent, Santa Clara, CA). The concentration of each sample was obtained from A260 measurements with Nanodrop 2000 (Thermo Scientific, Wilmington, DE). RNA integrity (RIN) was tested using the Agilent 2100 BioAnalyzer (Agilent Technologies, Palo Alto, CA). RIN values were higher than 6 for all samples used.

The retrotranscriptase reaction was carried out using a High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA) following the protocol provided by the supplier. Parallel reactions for an RNA sample were run in the absence of MultiScribe Reverse Transcriptase to assess the degree of contaminating genomic DNA.

TaqMan quantitative RT-PCR assays for each gene were performed in triplicate on cDNA samples obtained from the retrotranscription reaction using the ABI Prism 7900 Sequence Detection system (Applied Biosystems, Life Technologies, Waltham, MA). For each 10 μL TaqMan reaction, 4.5 μL cDNA was mixed with 0.5 μL 20× TaqMan Gene Expression Assays and 5 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems). The reactions were carried out using the following parameters: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Finally, all TaqMan PCR data were captured using the Sequence Detection Software (SDS version 1.9; Applied Biosystems). The identification numbers and names of all TaqMan probes used are shown in Supplementary Table S2. Samples were analyzed with the double-delta cycle threshold (ΔΔCT) method. The ΔCT values represent normalized target gene levels with respect to the internal control (ACTB). The ΔΔCT values were calculated as the ΔCT of each test sample minus the mean ΔCT of the calibrator samples for each target gene. The fold change was determined using the equation $2^{(-\Delta\Delta CT)}$.

Statistics

To model the relationships between frontal cortex age and fatty acid indexes, both generalized additive model (GAM) and Jointpoint regression were used as previously described (6). These two analyses were made using the R statistical package 2.10 in conjunction with the "mgcv" and "segmented" library.

Comparisons between groups were made with one-way analysis of variance. The minimum level of statistical significance was set at p less than .05 in all the analyses. These statistical analyses were performed using the SPSS software (SPSS, Chicago, IL).

Results

In the present study, we evaluated the temporal trajectories over the adult life span in frontal cortex grey matter fatty acid composition using gas chromatography in subjects aged 40–90 years (n = 58). Our prediction was that increasing age would be associated with significant progressive and specific changes in the fatty acid profile and fatty acid indexes. Age-related changes in fatty acid indexes are

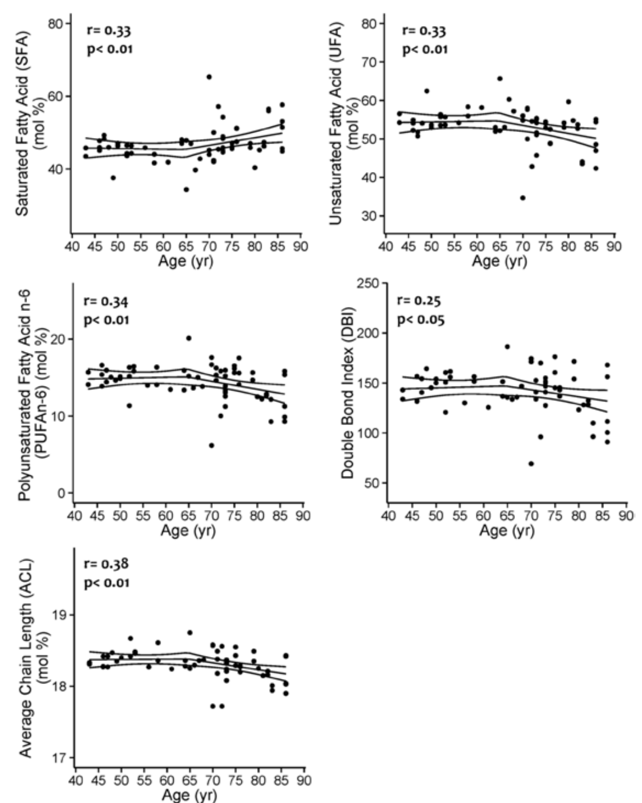

Figure 1. Relationship between age and different fatty acid indexes in human frontal cortex. Graphics show the trend for each variable with age, analyzed by generalized additive model (GAM). Significance of the GAM analysis is: for saturated fatty acids (SFA), R^2(adj) = 0.112, p < .01, Deviance explained = 1.34%; for unsaturated fatty acids (UFA), R^2(adj) = 0.112, p < .01, Deviance explained = 1.34%; for Polyunsaturated Fatty Acids series n-6 (PUFAn-6), R^2 (adj) = 0.116, p < .01, Deviance explained = 13.8%; for Double Bond Index (DBI), R^2(adj) = 0.063, p < .05, Deviance explained = 8.39%; and for Average Chain Length (ACL), R^2(adj) = 0.146, p < .01, Deviance explained = 16.9%.

shown in Figure 1. Our results demonstrate the existence of a significant relationship between frontal cortex fatty acid indexes and age analyzed by generalized additive model. In particular, this relationship shows an increase with age in the saturated fatty acid content (R^2[adj] = 0.112, $p < .01$, Deviance explained: 1.34%), and decrease with age in the unsaturated fatty acid content (R^2[adj] = 0.112, $p < .01$, Deviance explained: 1.34%), basically due to the decrease with age in the PUFAn-6 content (R^2[adj] = 0.116, $p < .01$, Deviance explained: 13.8%) which, in turn, leads to a decrease with age in the Double Bond Index (R^2[adj] = 0.063, $p < .05$, Deviance explained: 8.39%). A decrease with age in the average chain length was also observed (R^2[adj] = 0.146, $p < .01$, Deviance explained = 16.9%). In contrast, no changes associated with age in the monounsaturated fatty acid content (R^2[adj] = 0.041, $p = .287$, Deviance explained: 7.85%), PUFA content (R^2[adj] = 0.027, $p = .0861$, Deviance explained: 7.89%), PUFAn-3 content (R^2[adj] = −0.0269, $p = .375$, Deviance explained: 0.729%), or PI (R^2[adj] = 0.0136, $p = .153$, Deviance explained: 6.16%) were observed (data not shown). Potential interference of the variables of gender and postmortem time in the temporal trajectories of the different fatty acid indexes was ruled out after applying the corresponding generalized additive model analysis (see Supplementary Figure S1).

To shed light upon a possible juncture at which brain aging begins, we applied a jointpoint regression model to determine the breakpoint where the temporal trajectories for fatty acid indexes showed a significant tipping point followed by a more pronounced increase/decrease in fatty acid indexes. Our analysis indicates that the breakpoint is at 50 years old for average chain length (52.16 ± 3.60 years old, R^2[adj] = 0.153), at 60 years old for saturated fatty acids and unsaturated fatty acids (59.57 ± 7.58 years old, R^2[adj] = 0.103, for both), and at 75 years old for PUFAn-6 (75.90 ± 4.53 years old, R^2[adj] = 0.128), with no significant breakpoints for the rest of the fatty acid indexes.

Table 1 shows changes in frontal cortex of individual fatty acids with age, and with subjects grouped by decades. Of particular relevance is the significant increase in the saturated fatty acid palmitic acid (16:0) content in the decade following age 70, and the significant decay in the polyunsaturated fatty acids arachidonic acid (AA, 20:4n-6), adrenic acid (22:4n-6), docosapentaenoic acid (DPA, 22:5n-6), and docosahexaenoic acid (DHA, 22:6n-3) in the decade following age 80, a decade in which more significant changes are verified, in contrast to the previous decades during which the fatty acid profile is sustained without significant changes. The observed changes at advanced ages could potentially be ascribed to defects in the PUFA biosynthesis pathways in accordance with the ratios estimating desaturase and elongase activity, which suggests an alteration in delta-6-desaturase (FADS2) and ELOVL2 activity, as well as in peroxisomal β-oxidation (Supplementary Tables S3, S4, and S5).

Because the most significant changes observed in the fatty acid profile are found at advanced ages and affect polyunsaturated fatty acids, we investigated protein expression related to PUFA biosynthesis. In particular, we focused our attention on the enzymes participating in PUFA biosynthesis FADS1, FADS2, and ELOVL2, as well as the enzymes DBP-1, ACAA1, and SCP2, belonging to peroxisomal β-oxidation. Our results showed a significant decrease with age in the content of FADS2 and ELOVL2, with this trajectory especially evident at advanced ages (Figure 2A). These changes are likely post-transcriptional because no changes were observed in the mRNA content with age (Figure 2B). In contrast, the expression of FADS1 and proteins of peroxisomal β-oxidation did not show any differences with age (Figure 2A and C).

Table 1. Membrane Lipid Fatty Acid Compositional Profile of the Human Frontal Cortex in Individuals Ranging from 40 to 90 Years Old

Fatty Acid	Decade 40–49 (years), *n* = 6	Decade 50–59 (years), *n* = 10	Decade 60–69 (years), *n* = 9	Decade 70–79 (years) *n* = 22	Decade 80–89 (years) *n* = 11
14:0	1.48 ± 0.17	1.21 ± 0.07	1.53 ± 0.13[b*]	1.19 ± 0.07[a*,c**]	1.31 ± 0.06
16:0	21.19 ± 0.66	20.27 ± 0.74	20.15 ± 0.85	25.32 ± 0.65[a***,b***,c***]	24.17 ± 0.71[a**,b***,c**]
16:1n-7	1.47 ± 0.13	1.45 ± 0.10	1.85 ± 0.17[a*,b*]	1.47 ± 0.08[a*,b*,c***]	1.24 ± 0.08[c***]
18:0	21.43 ± 0.97	21.27 ± 0.61	20.30 ± 1.47	20.11 ± 0.59	22.45 ± 1.08[d*]
18:1n-9	26.56 ± 1.46	26.35 ± 1.04	27.82 ± 1.30	23.87 ± 0.66[c**]	27.46 ± 1.25[d**]
18:2n-6	1.11 ± 0.10	1.16 ± 0.10	1.21 ± 0.13	1.16 ± 0.06	1.04 ± 0.07
18:3n-3	0.20 ± 0.05	0.19 ± 0.04	0.32 ± 0.15	0.25 ± 0.02	0.25 ± 0.04
18:4n-3	0.37 ± 0.20	0.20 ± 0.05	0.44 ± 0.24	0.36 ± 0.05	0.41 ± 0.06
20:0	0.37 ± 0.07	0.74 ± 0.42	0.42 ± 0.12	0.49 ± 0.03	0.46 ± 0.04
20:1n-9	1.36 ± 0.17	1.41 ± 0.17	1.81 ± 0.18[a*]	1.13 ± 0.07[c***]	1.43 ± 0.19
20:2n-6	0.43 ± 0.12	0.31 ± 0.05	0.51 ± 0.19	0.36 ± 0.02	0.38 ± 0.02
20:3n-6	1.18 ± 0.26	1.04 ± 0.05	1.54 ± 0.46	1.21 ± 0.08	1.09 ± 0.15
20:4n-6	6.92 ± 0.43	6.73 ± 0.38	6.26 ± 0.32	6.55 ± 0.30	5.37 ± 0.32[a**,b**,d**]
20:5n-3	0.33 ± 0.12	0.25 ± 0.04	0.38 ± 0.18	0.34 ± 0.04	0.51 ± 0.27
22:0	0.38 ± 0.10	0.73 ± 0.43	0.46 ± 0.13	0.42 ± 0.02	0.40 ± 0.04
22:4n-6	4.27 ± 0.33	4.64 ± 0.20	4.40 ± 0.31	4.26 ± 0.23	3.51 ± 0.26[b**,c*,d*]
22:5n-6	1.19 ± 0.24	1.08 ± 0.12	1.16 ± 0.30	0.98 ± 0.07	0.72 ± 0.08[a*]
22:5n-3	0.58 ± 0.18	0.53 ± 0.05	0.60 ± 0.22	0.61 ± 0.04	0.44 ± 0.04
22:6n-3	8.07 ± 0.95	9.15 ± 0.67	7.42 ± 0.60	9.28 ± 0.63	6.40 ± 0.67[b*,d**]
24:0	0.59 ± 0.09	0.78 ± 0.18	0.71 ± 0.14	0.65 ± 0.05	0.69 ± 0.09
24:5n-3	0.29 ± 0.06	0.28 ± 0.02	0.40 ± 0.09[b*]	0.15 ± 0.01[a**,b**,c***]	0.14 ± 0.02[a**,b**,c***]
24:6n-3	0.24 ± 0.05	0.24 ± 0.04	0.31 ± 0.08	0.15 ± 0.02[c**]	0.11 ± 0.02[a*,b*,c***]

Note: Values are means ± SEM from 6 to 22 different cases and are expressed as mol%.

[a]Significantly different from 40 to 49 group. [b]Significantly different from 50 to 59 group. [c]Significantly different from 60 to 69 group. [d]Significantly different from 70 to 79 group.

*$p < .05$, **$p < .01$, ***$p < .001$.

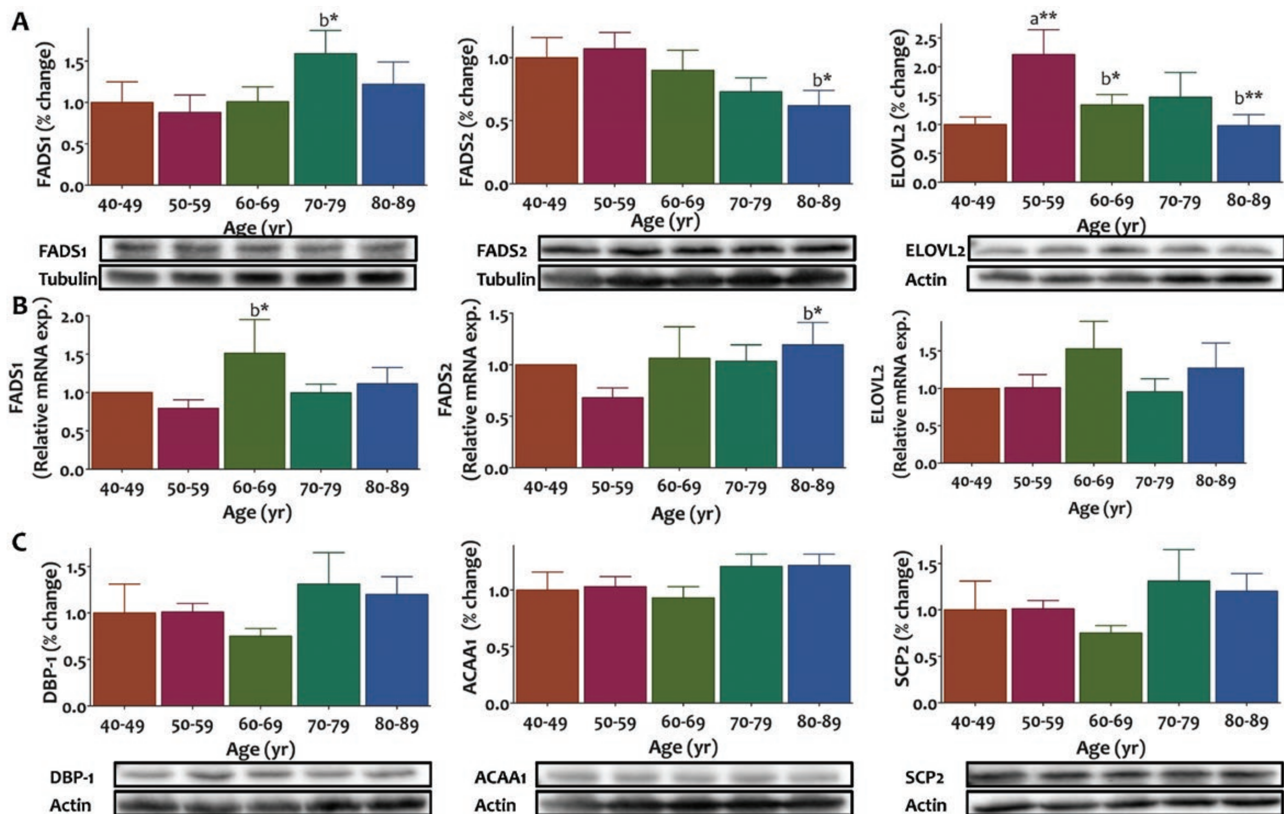

Figure 2. Representative immunoblots showing the relative levels of protein involved in PUFA biosynthesis (**A**) and peroxisomal β-oxidation (**C**). The mRNA relative expression of FADS1, FADS2, and ELOVL2 is also illustrated (**B**). Values are expressed as mean ± SEM from five different frontal cortex human samples normalized for tubulin or actin protein levels as a tissue marker. [a]Significantly different from 40 to 49 group, [b]significantly different from 50 to 59 group, [c]significantly different from 60 to 69 group, [d]significantly different from 70 to 79 group. $*p < .05$, $**p < .01$.

Our findings suggest that the membrane lipid fatty acid profile in human prefrontal cortex is sustained over the adult life span only to decay at advanced age, and that this decay could be ascribed to particular defects in PUFA biosynthesis. However, other mechanisms may also be considered. Thus, the decrease in particular polyunsaturated fatty acids observed at advanced ages might be associated with an increase in the consumption of them to generate lipid signaling mediator or second messengers to ensure neuronal survival and function. To this end, we investigated the content of pro-inflammatory factors lipoxigenase-5 (5-LOX), cyclooxygenase-2 (COX2), and NF-kB, as well as anti-inflammatory factors lipoxygenase-15 (15-LOX) and cytochrome P450 epoxygenase (CYP450 2J2). Our results showed a significant progressive increase in the protein expression of CYP450 2J2 with age (Figure 3B) which was consistent with the increase (albeit without significant) in its mRNA content (Figure 3C), as well as sustained protein content of 15-LOX despite decreased mRNA levels. These showed a significant decline in the decades following age 70 probably due to an increased translation rate (Figure 3B and C). In contrast, the expression of none of the proinflammatory factors showed any difference with age (Figure 3A).

Discussion

The physiological aging process favors the induction of changes at all levels of the biological organization which are balanced by homeostatic adaptive response mechanisms geared to preserving the composition and function within physiological limits (29). Cell membranes are not an exception; consequently, the longer the optimal membrane lipid composition is maintained, the better cell survival and function.

The studies by Burger & Seidel (30) and Rouser & Yamamoto (31) were the first evidence that the lipid profile of the human brain changes with aging. Burger & Seidel (30) found that the amount of total lipids increased during the first two decades of life and then began to decrease. Rouser & Yamamoto (31) also demonstrated a curvilinear regression of human brain lipid levels with age; however, as pointed out by Ledesma et al. (32), in both studies the use of whole brains to analyze lipids resulted in variations in lipid content that precluded an evaluation of separate changes in the grey and white matter.

Later on, several studies of different areas of human brain confirmed the occurrence of age-related lipid changes. Overall, changes are in line with the assumption of the progressive and deleterious character of the aging process, as the contents of most lipids in the human brain decrease after the age of 50. Thus, phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) brain levels decrease very slowly with age, with less than 10% loss in the period between the ages of 40 and 100 years old (33). Another study described 10%–20% loss of phospholipids in different brain regions (gray matter, white matter, nucleus caudate, hippocampus, pons, cerebellum, medulla oblongata) only in individuals aged from 89 to 92 when compared with individuals aged from 33 to 36, whereas phospholipid composition remained unchanged (19). Phospholipid decrease starts slowly at the age of 20 and becomes

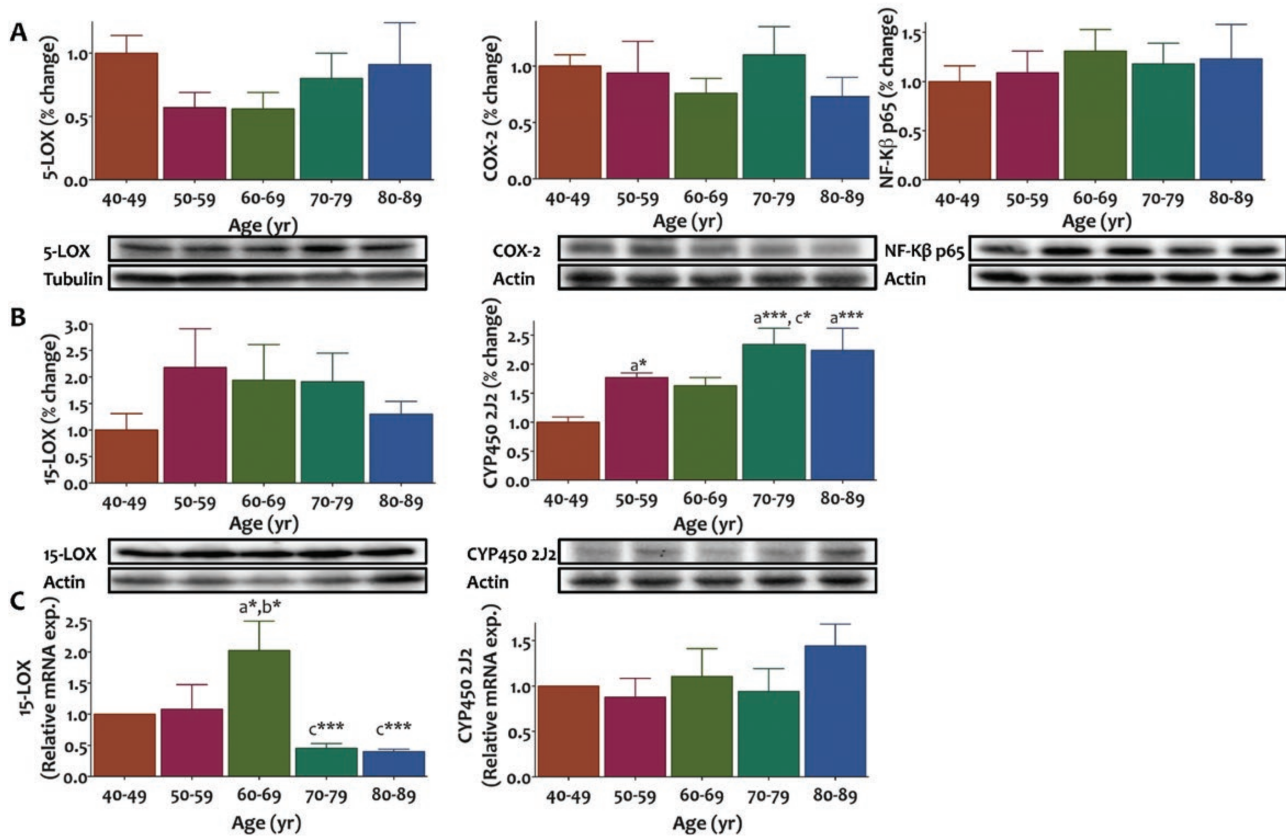

Figure 3. Representative immunoblots showing the relative levels of protein involved in proinflammatory (**A**) and anti-inflammatory pathways (**B**). The mRNA relative expression for the anti-inflammatory factors lipoxygensase-15 and cytochrome P450 epoxygenase is also illustrated (**C**). Values are expressed as mean ± SEM from five different human brain samples normalized for tubulin or actin protein levels. [a]significantly different from 40 to 49 group, [b]significantly different from 50 to 59 group, [c]significantly different from 60 to 69 group, [d]significantly different from 70 to 79 group. $^*p < .05$, $^{***}p < .001$.

more pronounced by the age of 80, with no significant differences between male and female (34,35). More recent studies focused on the mitochondrial and microsomal lipidome of frontal cortex and hippocampus of subjects from 20 to 100 years old (21,22), showing that minor fractions of phospholipids containing adrenic and arachidonic acid specifically decrease along adult life in both regions, whereas particular phospholipids containing docosahexanoic acid increase during the same period. These data, however, must be approached with care because fractionation methods from frozen human tissue do not prevent contamination of membrane fractions. Additionally, no changes are observed in the mole percentage of lipid classes and fatty acid composition of lipid rafts from normal human frontal cortex throughout the human life span (24–85 years) (36).

Only two recent studies have focused on the human frontal cortex fatty acid profile, analyzing subjects aged 20–80-year old (37,38). Our results, together with the findings of these two previous studies, suggest the following three basic points: (i) maintenance or minor changes with age in the saturated and monounsaturated fatty acid content, (ii) decrease in the polyunsaturated fatty acid series n-6 content with age, particularly affecting arachidonic (20:4n-6) and adrenic acid (22:4n-6), and (iii) maintenance of docosahexaenoic acid (22:6n-3) content during aging with eventual reduction at a very advanced age.

Maintenance of saturated-monounsaturated fatty acid and the highly polyunsaturated fatty acid DHA content with aging can be interpreted as a sign of stability. Saturated, monounsaturated, and docosahexaenoic acid are key fatty acids determining the geometric properties of lipids, and affecting functional properties such as exocytosis and membrane domain formation (14). However, changes in the average chain length and double bond index with aging can influence the geometric properties of lipids and functional properties. Yet the impact of these changes in neural cell membrane physiology during aging remains to be explored.

In contrast to these observations, PUFAn-6, and particularly the 20:4n-6 and 22:4n-6 fatty acid content, has an effect as a substrate for lipid mediators. Thus, in addition to the defects in PUFA biosynthesis pathway observed in our work, the decrease in these fatty acids may possibly be ascribed to increased consumption by enzymes involved in anti-inflammatory pathways which synthesize a diversity of compounds with neuroprotective properties to ensure cell survival and functioning during normal aging.

The present observations may have implications in neuronal physiology and, by extension, brain function. Thus, the described changes in PUFA content (particularly 20:4n6 and 22:6n3), average chain length, and double bond index—the major factors determining the geometric properties of membrane lipids (14)—have key consequences on functional properties of neurons such as synaptic vesicle exocytosis, ion channel regulation, membrane domain formation, G-protein signaling or survival pathways (14,39), mechanisms underlying memory and learning processes and affected during aging (32).

Available evidence suggests that an adequate dietary intake of PUFAs (n3 and/or n6) could prevent or slow down cognitive decline

and attenuate the physiological changes of the brain that are associated with aging (40–45). However, the outcomes from these studies are not conclusive, and more experimental data are needed to determine PUFA's dose, type, duration, targets of action in brain, impacted cognitive traits, involved mechanisms, or responsive populations. In a similar way, another nutritional intervention such as caloric restriction seems to improve synaptic plasticity and activation of neuroprotective pathways in the brain (at least in animal studies) but again fail to offer conclusive outcomes when applied to human studies, basically due to the scarcity of works in this research line (46–48).

Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None reported.

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