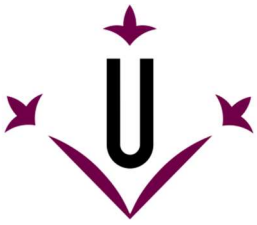

Redox lipidomics to better understand brain aging and function

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Review

Abbreviations

AA, arachidonic acid

ACO2, aconitate hydratase

ACTB, beta-actin

ALE, advanced lipoxidation endproduct

ATP5F1A, ATP synthase subunit alpha

ATP5F1B, ATP synthase subunit beta

BASP1, brain acid soluble protein 1

BLVRB, NADPH-flavin reductase

CA1, carbonic anhydrase 1

CEL, carboxyethyl-lysine

ChoGpl, glycerophosphocholines

CKB, creatine kinase B-type

CML, carboxymethyl-lysine

CMRg, cerebral metabolic rate of glucose

COX, cyclooxigenases

CR, caloric restriction

CRYAB, alpha-crystallin B chain (heat shock protein B5)

DHA, docosahexaenoic acid

DLD, dihydrolipoyl dehydrogenase

DPYSL2, dihydropyrimidinase-related protein 2

ENO1, alpha-enolase

ENO2, gamma-enolase

EtbGpl, glycerophosphoethanolamines

ETC, electron transport chain

GAPDH, glyceraldehyde-3-phosphate dehydrogenase

GFAP, glial fibrillary acidic protein

GL, glycerolipids

GLUD1, glutamate dehydrogenase 1

GLUL, glutamine synthetase

GNB1, guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta1

GOT1, aspartate aminotransferase

GP, glycerophospholipids

GPx4, phospholipid hydroperoxide glutathione peroxidase

GST, glutathione-S-transferase

HBA1, hemoglobin subunit alpha

HNE, 4-hydroxy-2-nonenal

HSPD1, heat shock protein 60KDa

IF, intermittent fasting

IMS, imaging mass spectrometry

InoGpl, glycerophosphoinositols

LOX, lipoxigenases

MDA, malondialdehyde

MetR, methionine restriction

MRI, magnetic resonance image

MS, mass spectrometry

MUFA, monounsaturated fatty acids

NEFL, neurofilament light polypeptide

NEFM, neurofilament medium polypeptide

NKT, neuroketals

Nrf2, nuclear factor erythroid 2-related factor

ONE, 4-oxo-2-nonenal

OxPhos, oxidative phosphorylation

PARK7, protein/nucleic acid deglycase DJ-1

PC, phosphatidylcholine

PE, phosphatidylethanolamine

PEBP1, phosphatidylethanolamine-binding protein 1

PET, positron emission tomography

PFC, prefrontal cortex

PGAM1, phosphoglycerate mutase 1

PI, peroxidizability index

PIGpl, phosphatidylinositols

PKM, pyruvate kinase

PR, protein restriction

PS, phosphatidylserine

PUFA, polyunsaturated fatty acids

RCS, reactive carbonyl species

REST, RE1-silencing transcription factor

ROS, reactive oxygen species

SerGpl, glycerophosphoserines

SFA, saturated fatty acids

SM, sphingomyelins

SP, sphingolipids

SYN1, synapsin-1

TCA, tricarboxylic acid cycle

TUBA1B, tubulin alpha 1B chain

TUBB, tubulin beta chain

TPPP, tubulin polymerization-promoting protein

UCHL1, ubiquitin carbosyl-terminal hydrolase L1

UCP, uncoupling protein

UFA, unsaturated fatty acids

UQCRC1, ubiquinol-cytochrome c reductase complex core protein 1

VIM, vimentin

YWHAG, 14-3-3 protein gamma

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Highlights

1. Human prefrontal cortex (PFC) has unique and specific lipidome that undergoes progressive changes with aging

2. PFC protein damage is selective and affects specific biological processes

3. Human PFC lipid oxidation and lipoxidation-derived protein damage increase with aging

4. Human PFC lipid oxidation and lipoxidation-derived protein damage processes may have a role in the age- associated PFC cognitive decline.

Abstract

Human prefrontal cortex (PFC) is a recently evolutionary emerged brain region involved in cognitive functions. Human cognitive abilities decline during aging. Yet the molecular mechanisms that sustain the preservation or deterioration of neurons and PFC functions are unknown. In this review, we focus on the role of lipids in human PFC aging. As the evolution of brain lipid concentrations is particularly accelerated in the human PFC, conferring a specific lipid profile, a brief approach to the lipidome of PFC was consider along with the relationship between lipids and lipoxidative damage, and the role of lipids in human PFC aging. In addition, the specific targets of lipoxidative damage in human PFC, the affected biological processes, and their potential role in the cognitive decline associated with aging are discussed. Finally, interventions designed to modify this process are considered. We propose that the dysfunction of key biological processes due to selective protein lipoxidation damage may have a role the cognitive decline of PFC during aging.

1. Introduction

One critical trait of human evolution is the rapid expansion of brain size and the emergence of unique and complex cognitive capabilities. These changes have demanded metabolic adaptations, in particular with respect to increased energy supplies [1]. Thus, humans allocate about 20% of their total energy to the brain, compared with 12% for apes and 5% for other mammalian species [2]. This higher metabolic activity, supported by increased expression of genes related to neuronal functions and energy metabolism [3, 4], as well as the increased density of glia cells relative to neurons [5, 6], shows its greatest demand in human prefrontal cortex (PFC) [5, 7]. Furthermore, PFC has been shown to undergo greater expansion at the gross anatomy level in human evolutionary lineage than other brain regions [8]; it is the brain region that has emerged and evolved most recently during primate evolution. PFC is involved in complex cognitive functions including reasoning, planning, social behavior, and general intelligence [9-11].

Cognitive abilities decline during aging. Yet brain aging does not affect all neurons identically. Non-invasive techniques such as structural brain imaging and functional magnetic resonance imaging (MRI) are being widely used and combined with post-mortem analyses of aging human brain to identify the most age-sensitive regions of the brain. Volumetric brain measurements as well as positron emission tomography (PET) measures of whole brain and regional cerebral metabolic rates of glucose (CMRg) reinforce the idea that aging affects some brain regions more than others. Thus, in addition to the volume changes in, particularly, the association cortex during aging, it has been shown that the PFC shows the greatest and most consistent decrements in CMRg as compared with all other regions of the aging brain [12-15]. In this line, cognitive functions that rely on PFC, such as learning, memory, and executive functions, show considerable age-related decline. Consequently, it is plausible to postulate that the decline in both energy metabolism and cognition are closely linked.

These changes in metabolic activity in PFC during aging have been associated with different, and non-exclusive, molecular mechanisms and biological processes comprising altered neuronal expression of proteins involved in the metabolic state of neurons and glial cells, altered activation of synapses, and the disruptive effects of abnormal neuronal excitability. But, in any case, the evidence clearly suggests that neuronal loss during aging

is very modest, whereas a decrease in the number of synapses appears to be a more prominent trait of brain aging. Indeed, most of the functional decline associated with physiological aging is caused by relatively subtle changes, such as loss of dendrites, reductions in spine density, altered spine morphology, changes in the molecular profile of synapses, and a general loss of synaptic plasticity, all of which is paralleled by declining cognitive function and elevated risk of neurodegenerative disease [16-22]. Nevertheless, the molecular mechanisms behind aging-related phenotypic changes are only scarcely understood.

Lipids have allowed and lent support to the brain's evolution toward complexity [23]. In addition to their quantitative relevance (50% of the dry matter [24]), brain lipids show great structural and functional diversity, with most lipid categories represented in cells as expressions of the different needs and functions ascribed to them which are related to the generation of membranes, cell signaling, and energy storage [23,25]. By regulating the chemical and physical properties of membranes, lipids direct vesicle fusion and fission processes, ion flux, and lateral diffusion of membrane proteins, they also create the microdomains necessary for optimal cellular communication, among other functions [26] [27, 28]. However, despite growing evidence of the critical role of lipids in brain function, little is known about the participation of lipids in PFC aging. In this review, we used data from targeted and untargeted mass spectrometry (MS)-based lipidomics and redox lipidomics to characterize the lipidome composition of human PFC and its association with the cognitive decline linked to the aging process.

2. Lipidomics of human prefrontal cortex

Recent progress in mass spectrometry (MS)-based lipidomics has provided experimental opportunities for the large-scale quantitative profiling of thousands of individual lipid molecular species in human brain, providing novel insights into the mechanisms and evolution of brain functionality. Thus, current data demonstrate the existence of lipidomic signatures which are tissue-specific [29, 30] and brain region-dependent [29, 31, 32], with the lipid profile of the brain seen to be markedly different from that of the other tissues (verified in mammals, non-human primates, and humans) [29]. This supports the

idea that lipids play specific roles in brain function [25]. Interestingly, the largest lipidome divergence between the brain and other tissues is found in humans, who show the greatest cognitive complexity. Furthermore, lipidome divergence follows the genetic divergence among animal species, supporting the idea that concentration levels of lipids enriched in brain evolved at a much more rapid pace than the concentration levels of lipids characteristic of non-neural tissues [29]. As stated by Khaitovich and coworkers [29], these features suggest a link between brain lipidome evolution and the evolution of brain functionality.

Importantly, the greater acceleration of lipidome changes in the human evolutionary lineage is specific to brain enriched lipids in PFC, the region commonly associated with human-specific brain functions. Lipids showing human PFC-specific concentration changes belong to three lipid categories: glycerolipids (GL), glycerophospholipids (GP), and sphingolipids (SP); these in turn are represented in very specific lipid subclasses, namely: 1-(1Z-alkenyl), 2-acylglycerophosphoethanolamines (plasmalogens of phosphatidylethanolamines), diacylglycerols, N-acylsphinganines (dihydroceramides), and N-acylsphingosines (ceramides) [29].

This specific lipid pattern present in human PFC seems to also be projected and reflected at the cellular, subcellular, and molecular levels. Thus, for example, at the cellular level, by using imaging mass spectrometry (IMS) [33, 34] it is possible to generate density maps for several lipid species, mainly glycerophosphocholines (ChoGpl), glycerophosphoethanolamines (EtnGpl), and sphingomyelins (SM), offering important information about the lipidome of the human brain and, in particular, the human PFC. The IMS images show that the diverse lipid molecular species found in human PFC exhibit a different and specific distribution, and even allow us to distinguish among the six different layers of the PFC based on the slight differences in lipid density throughout the cortex [33] (see **Figure 1**).

A recent study has used a lipidomic approach to analyze the lipid profile at the mitochondrial and microsomal levels in grey matter of human PFC [35]. The results show that, independently of the subcellular fraction and with a very similar distribution, the three major phospholipid classes are ChoGpl, EtnGpl, and glycerophosphoserines

(SerGpl). Overall, ChoGpls comprise around 55% of total phospholipids, and the main molecular species are PC(16:0/18:1), PC(16:0/16:0), and PC(18:0/18:1). EtnGpls represent about 30% of total phospholipids, with the major molecular species being PE(18:0/22:6), PE(18:0/20:4), PE(18:0/22:4), and PE(16:0/22:6). Finally, SerGpls represent 15% of total phospholipids, with the major molecular species being PS(18:0/22:6) and PS(18:0/18:1). Other minor phospholipids such us phosphatidylinositols (PIGpl) and cardiolipin were not analyzed.

At the membrane level, lipid rafts are microdomains which provide a highly-saturated liquid-ordered microenvironment that promotes protein-lipid and protein-protein interactions [36, 37]. Lipid rafts comprise a highly dynamic clustering of proteins and lipids playing a central role in signal transduction and intercellular communication. Lipid rafts from human PFC are characterized by their high level of cholesterol and sphingolipids (SM, cerebrosides, and sulfatides), and lower content in GP (particularly EtnGpl and SerGpl, both aminophospholipids, and to a lesser degree ChoGpl and PIGpl) compared to the non-rafts membranes [38] (see **Table 1**).

Fatty acids are prime components of the structural diversity of lipids of neurons and glial cells. Indeed, with different permutations of head group and fatty acids, up to 10,000 theoretical lipid molecular species could be generated [39]. Fatty acids determine the functional properties of lipids in PFC which can be ascribed to their roles in the structural and functional integrity of membranes, the generation of lipid signaling mediators, and the chemical reactivity of the acyl chains [23]. In human PFC, the fatty acid profile is characterized by an average chain length of 18 carbon atoms, and a relative distribution between saturated (SFA) and unsaturated (UFA) fatty acids of about 40:60 [32]. Among SFA, 16:0 and 18:0 are the most abundant. The most abundant UFA are the monounsaturated (MUFA) 18:1n-9, and the polyunsaturated fatty acids (PUFAs) 20:4n-6 (arachidonic acid, AA), and 22:6n-3 (docosahexaenoic acid, DHA) [32, 40, 41] (see **Table 2**). The content of these PUFAs in PFC is the highest across human brain regions [32], is supported at transcriptional and protein expression levels, and can probably be ascribed to inherent neuronal activity [32]. The specific finding that DHA (22:6n-3) is the main PUFA present in brain regions is in line with previous findings pointing up the very high level of DHA in the whole brain [24]. Interestingly, lipid rafts from human PFC are

characterized by their high level of SFA, as well as by reduced amounts of PUFA compared to the non-rafts membranes [38].

The high concentration of PUFAs in neuronal and glial cell membrane GP in PFC not only makes them prime targets for lipoperoxidative damage but also enables them to participate in long free radical chain reactions. Considering that the peroxidizability index (PI) combines the membrane fatty acid composition with the relative susceptibility of individual fatty acids to peroxidation [42], the available evidence indicates that PFC shows the highest value of PI in the human brain and, consequently, helps explain the greater susceptibility of the membrane bilayer to lipid peroxidation [32]. Considering that lipid rafts from PFC show enrichment in SFA, it is proposed that this vulnerability particularly affects non-raft territories of the cell membrane. However, a greater PI is not necessarily associated with a higher degree of molecular damage. In fact, quite the reverse is the case. Thus, the higher PI of the human PFC compared to other human brain regions is associated with a lower steady-state level of lipoxidation-derived protein damage [32, 43]. This dissociation is probably due to the lower mitochondrial stress present in PFC (based on the levels of the mitochondrial stress marker succinyl-cysteine (SC)) and the relevant presence of neuronal adaptive response mediated by the antioxidant response-signaling pathway Nrf2 (Nuclear factor erythroid 2-related factor), as well as by the stress resistance adaptive response mediated by the transcriptional factor REST (RE1-silencing transcription factor, also known as neuron-restrictive silencer factor (NRSF)) [32, 44].

Globally, human PFC has a specific lipidome that significantly differs from those of other human brain regions, tissues, and animal species. Inside the PFC, this lipidome varies in the cell layers of the grey matter, the subcellular level (mitochondria and microsomes), and even the membrane levels (non-raft vs lipid raft domains). Furthermore, PFC presents a specific fatty acid profile characterized by the presence of a high content in PUFAs, conferring to PFC a high vulnerability to oxidative conditions, which in turn probably demands the presence of adaptive responses to ensure neuronal survival during adult lifespan.

3. The meaning of lipid oxidation and lipoxidation-derived protein adducts

Polyunsaturated fatty acids (PUFAs) are abundant constituents preferentially located at the sn-2 position in glycerophospholipids (to pointing up plasmalogen-PE) within cellular membranes. The fact that lipids constitute a major portion of the plasma, and mitochondrial and endoplasmic membranes, establishes the presence of substantial concentrations of PUFAs within membranous structures. It is known that PUFA residues of lipids (and particularly GP) are very sensitive to oxidation, and this sensitivity increases as a function of the number of double bonds per fatty acid molecule [45, 46]. In this context, PFC is the human brain region with the highest content in PUFAs which also determines that it has the highest content in double bond and peroxidation indexes [32] (see **Table 2**).

PUFAs are vulnerable to diverse oxidation reactions and radical reactions, both of which result in the formation of so-called electrophilic lipid products. Certain oxidation reactions involving PUFAs are enzymatically mediated by families of enzymes including the lipoxygenases (LOX), cyclooxygenases (COX), and cytochrome P450 [47]. In contrast to the highly regulated PUFA oxidation mediated by enzymes, the electrophilic lipid products generated by free radical-mediated lipid oxidation are part of the lipid peroxidation process (for a review see [48, 49]). Lipid peroxidation occurs in three major phases, comprised of an initiation event, chain propagation, and termination (for more details, see [50, 51]). Of note, a single initiation reaction is postulated to result in 200 to 400 propagation cycles, rapidly amplifying free radical damage in highly oxidizing PUFA-rich environments. The resulting electrophilic lipid products are reactive aldehydes characterized by the common group R-CHO consisting of a carbonyl center bonded to hydrogen and an R group arising predominantly as a consequence of oxidative damage within the cellular microenvironment. These reactive carbonyl species (RCS) have signaling properties, but can also be cytotoxic [23, 42]. It is estimated that over a hundred different reactive species can be generated, each with a wide range of reactivity, size, and specificity. The most reactive RCS are α,β-unsaturated aldehydes [4-hydroxy-2-nonenal (HNE) and acrolein], di-aldehydes [malondialdehyde (MDA) and glyoxal], and keto-aldehydes [4-oxo-2-nonenal (ONE) and isoketals] [50, 51]. Special mention must be made of neuroketals (NKT) which are keto-aldehydes formed by the non-enzymatic oxidation of the 22:6n-3 through the neuroprostane pathway [52]. 2-Hydroxyheptanal, 4-

hydroxyhexenal, cyclopentenone prostaglandins, levuglandins, and oxidized phospholipids are other significant aldehydic products of lipid peroxidation of PUFAs.

As mentioned above, lipid rich membranes provide an optimal environment for producing a large abundance of these cytotoxic compounds. For instance, microsomal and mitochondrial intra-membrane concentrations of these lipid peroxidation-derived electrophilic compounds have been estimated to accumulate to as many as 1-10 mM *in vivo* [50]. The regulatory function and/or cytotoxicity of these aldehydes depend on abundance, reactivity, and half-life. Half-life varies significantly, with compounds such as ONE and HNE exhibiting half-lives of about 1 second and 2 minutes, respectively. So compared with reactive oxygen species (ROS), RCSs have a much longer half-life. This property along with the non-charged structure of aldehydes allows them to transiently modify distant proteins, lipid membranes (particularly aminophospholipids), and nucleic acids [42]. These properties may contribute to a localized bias for molecular adduction by RCSs, particularly those with short half-life, as cellular components located within the vicinity of lipid peroxidation targets remain more susceptible to generated RCS. By contrast, long-lived RCSs can be more destructive because they may have a more pronounced global impact by altering a broader array of molecular targets due to their transient nature [42, 49].

RCS react with nucleophilic groups in macromolecules (lipoxidation reactions) such as proteins, nucleic acids, and aminophospholipids, resulting in their chemical and non-enzymatic modification (see **Figure 2**). The result is the formation of a diversity of adducts and intra- and inter-molecular cross-links collectively called Advanced Lipoxidation Endproducts (ALEs) [53]. Thus, by reacting with nucleophilic sites in proteins (basically to cysteine (Cys), lysine (Lys), arginine (Arg), and histidine (His) residues), RCSs generate ALE adducts such as MDA-Lys, HNE-Lys, NKT-lys, FDP-Lys, carboxymethyl-lysine (CML), and S-carboxymethyl-cysteine, as well as the cross-links glyoxal-lys dimer (GOLD) and lys-MDA-lys, among others [53-55]. Several of these compounds have been detected, characterized, and located (by mass spectrometry, redox proteomics, and immunohistochemistry) in the human PFC. RCSs can also react with the exocyclic amino groups of nucleosides to form alkylated products. Guanine is the most vulnerable and commonly modified DNA base because of its high nucleophilicity, while MDA-

deoxyguanosine (M1dG) is the most common adduct [56]. Finally, the amino group of aminophospholipids can also react with RCS, leading to the formation of adducts such as MDA-PtdEtn and carboxymethyl-PE [57]. To the best of our knowledge, no data are currently available about lipoxidation-derived damage to DNA and aminophospholipids in human brain.

The molecular consequences of ALE formation in proteins mostly include deleterious structural and functional changes [42]. Thus, ALE formation induces alterations in physico-chemical properties (e.g., conformation, charge, hydrophobicity, and solubility), formation of intra- and inter-molecular protein crosslinks and aggregates, and loss of enzymatic activity, among others; yet it also includes protein activation when the ALE formation is in proteins particularly designed to sense and respond to cellular RCS production. In other words, protein activation seems to be associated with specific protein targets designed to detect RCS which will act as signaling products (see next paragraph). In a deleterious way, ALE formation on nucleic acids induces DNA damage and mutagenesis, and alterations in physico-chemical and biological properties of the lipid bilayer [56, 57]. In this context, a relevant recent study demonstrated that MDA causes neuronal mitochondrial dysfunction by directly promoting ROS generation and modifying mitochondrial proteins [58].

In contrast to their cytotoxic effects, RCSs can also work by sending regulatory signals activating specific protein targets which act as adaptive responses designed to decrease lipoxidative damage and improve antioxidant defenses. Among these mechanisms involved in the modulation of oxidative damage by RCS are i) the modification and activation of uncoupling proteins (UCPs) by the RCS hydroxynonenal and the subsequent decrease in mitochondrial ROS production [59], and ii) the activation of the antioxidant response signaling pathway Nrf2 that includes, among others, the expression of enzymes such as glutathione-S-transferase (GST), specifically designed to detoxify reactive carbonyl compounds, and GPx4 (phospholipid hydroperoxide glutathione peroxidase), designed to restore reduced states of membrane fatty acids from phospholipids to ensure membrane lipid homeostasis [60-62]. The existence of both mechanisms at the brain level and the relevant content of Nrf2 in PFC suggest a particular adaptive response in the face of the

presence of a lipid microenvironment showing the higher unsaturation degree of human brain [32].

4. Redox lipidomics and adductomics in human prefrontal cortex aging

Aging induces changes at all levels of the biological organization which are offset by allostatic adaptive response mechanisms geared to preserving the composition and function within homeostatic limits. The cell membrane is not an exception [63], and, consequently, the longer the optimal membrane lipid composition is maintained, the better the cell survival and function. These adaptive mechanisms seem to be particularly effective in human PFC since cognitive functions are relatively well-preserved during normal aging. Among these mechanisms, the preservation of the lipid membrane composition seems to be a key component.

The first evidence that the membrane lipid composition of the human PFC changes with aging emerged in 1990s. The work of Svennerholm and coworkers [64], analyzing the membrane lipid composition of human PFC in 118 subjects from 20 to 100 years old, found that phospholipids and cholesterol decreased slightly from 20 years of age, whereas gangliosides differed from the other lipids, showing an almost constant concentration between 20 and 70 years of age. Later, a study focused on the mitochondrial and microsomal lipidome of PFC of subjects from 20 to 100 years old [35] showed that minor lipid molecular species of ChoGpl, EtnGpl, and SerGpl and containing adrenic acid (22:4n-6) and AA specifically decreased along adult life in the PFC, whereas particular ChoGpl, EtnGpl, and SerGpl containing DHA increased or remained the same during the same period. Finally, in the most recent work [65], the comprehensive assessment of lipid concentration changes in the gray matter of the human PFC over the adult lifespan (from 30 to 99 years of age) demonstrated that aging affects 14% of lipidome (682 from 5024 lipid species showed significant concentration differences) with changes starting predominantly at 50-55 years of age, and with differences existing between females and males. The lipid functional pathways analyses revealed that the biosynthesis of UFA, the glycerolipid metabolism, and the cannabinoid signaling pathways were the most affected by aging.

Lipidomic changes with aging have also been described in lipid raft from human PFC. Thus, Diaz and coworkers [38], using lipid rafts isolated from PFC of healthy subjects aged 24 to 85, demonstrated that that human cortical lipid rafts are modified by aging in a gender-dependent way. The main changes were in levels of plasmalogens, PUFAs (especially AA and DHA), total polar lipids (mainly InoGpl, SM, sulfatides, and cerebrosides), and total neutral lipids (particularly cholesterol and sterol esters). These findings may provide molecular support for the modifications in protein-protein dynamics and protein clustering in lipid raft underlying alterations in synaptic plasticity, memory retention, and learning during aging [38]. Further studies are, however, needed to consolidate these new ideas.

An additional research line is to be found in studies focused on analysis of the fatty acid compositional profile of human PFC [35, 66, 67] in healthy subjects ranging from 20 to 80 years old. The findings of these studies point to: i) a general sustained and preserved fatty acid profile throughout the adult lifespan in PFC; ii) the maintenance or minor changes with age in the SFA and MUFA content; iii) the decrease in the PUFA content from series n-6 with age, particularly affecting 20:4n-6 and 22:4n-6; and iv) the maintenance or minor increase in 22:6n-3 content during aging, with eventual reduction at a very advanced age. Sustained SFA, MUFA, and DHA content with age could be interpreted as an adaptive response to aging to preserve neurons and cerebral function by helping to maintain the geometric properties of lipids and, consequently, functional properties such as exocytosis and membrane domain formation [25]. The decreases in PUFAn-6, and particularly the 20:4n-6 and 22:4n-6 fatty acid content, could have biological effects in that they are substrates for lipid mediators. Thus, one explanation for this finding lies in the reported decline of the PUFA biosynthesis pathway [67]; however, there is also increased consumption by enzymes involved in anti-inflammatory pathways which synthetize a diversity of compounds with neuroprotective properties to ensure cell survival and functioning during normal aging [67]. For this reason, it is proposed that during normal human brain aging, the lipid profile is particularly resistant to changes with age as a result of strict control designed to ensure neuronal survival and function.

Although the membrane lipid composition is largely maintained to guarantee cell survival and function, there is a continuous physiological lipoperoxidative attack on the

membrane, and the membrane becomes a source of carbonyl compounds with the ability to damage other cellular components. In this line, a recent initial study analyzing selected specific protein damage markers with mass spectrometry in PFC from healthy humans ranging in age from 43 to 86 years old was published [68]. The results demonstrated that there is an increase in the steady-state level of oxidative and lipoxidative protein damage in human PFC over the adult lifespan, with a breakpoint at 60 years of age (the detected and quantified markers for protein oxidation were glutamic semialdehyde and aminoadipic semialdehyde, and for protein lipoxidation carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL)). In two additional recent studies, changes with age in the protein damage markers derived from the RCS neuroketals (NKT) and malondialdehyde (MDA) in human PFC in two groups of individuals, middle-aged and old-aged, were analyzed. Steady-state levels of these markers were also analyzed as continuous variables in function of age. The levels of NKT- and MDA-protein adducts were significantly increased in old-aged human PFC when comparing the two groups, and they remained significantly increased considering age as a continuous variable [43, 69].

Consequently, it is suggested that progressive lipoxidation-derived molecular damage is a conserved, central mechanism of age-related functional decline in human PFC. Indeed, several alterations occur in the human PFC with age including increased levels of cytokines and of mediators of the innate inflammatory response [70], increased cytotoxic oligomeric species [69], regression of dendritic arbors and dendritic spines [71], neurotransmitter deficits [72], and reduction in the volume of the gray and white matter [73].

5. Lipoxidation-derived protein damage targets in human prefrontal cortex

Although the steady-state levels of ALEs in proteins increase in human PFC during aging, such evidence does not elucidate the specific mechanisms that cause losses in particular cellular/tissue functions. Is there a selective pattern of lipoxidative protein damage? If there is, what proteins are the targets? Recent proteomic studies performed in human PFC demonstrate that there is only a subset of lipoxidatively-modified proteins, that the degree of lipoxidative damage increases with age, and that in all the identified proteins increased lipoxidation is not due to a higher content of the corresponding protein but

rather to increased flux of protein damage [69, 74]. Importantly, this small but significant pool of lipoxidized protein will probably increase over time because the ALEs detected are limited to those derived from MDA and NKTs. Consequently, further studies are needed to develop a more detailed view.

The identification of specific lipoxidation-modified proteins in the PFC of adult and aged subjects provides an overview of the selective cellular functions that are affected during the aging process. The proteins listed in **Table 3** represent the biological processes affected by RCS-related protein damage. If we consider these molecular pathways, we can verify that energy metabolism (with 34.2% of modified proteins, n=12), cytoskeleton (22.8% of modified proteins, n=8), and neurotransmission (including neuronal communication, synaptic plasticity, and other processes) (with 20% of modified proteins, n=7) are the main affected cellular functions. Other significant molecular pathways involved, but with a lesser number of modified proteins, are proteostasis (11.4%, n=4 modified proteins), O_2/CO_2/heme metabolism (8.5%, n=3), and signal transduction (2.8%, n=1) (see **Figure 2**).

There is extensive evidence that **energy metabolism** is particularly affected during PFC aging [14, 19, 75]. The reported observations give some clues about the molecular substrates of energy failure with PFC aging after the identification of key proteins as targets of lipoxidative damage including proteins of glycolysis such as enolases (ENO1, ENO2), GAPDH, PGAM1, and PKM; proteins of the tricarboxylic acid (TCA) cycle ACO2, and GLUD1; protein subunits of the mitochondrial electron transport chain complexes DLD and UQCRC1; and different protein subunits of mitochondrial ATP synthase or complex V (ATP5F1A, and ATP5F1B) responsible for oxidative phosphorylation. All of these proteins are components of coupled processes which are necessary to fulfill the ATP requirements of neurons and glial cells. Neuronal activity is highly dependent on these processes since, under normal conditions, glucose is the exclusive energy substrate for the brain [76]. In addition, CKB, being a key player in the 'phosphocreatine circuit' for cellular energy homeostasis, is in charge of rapid ATP production from phosphocreatine reservoirs in response to acute increased energy demands in neurons [77]. Thus, CKB provides neurons with a reservoir and also an alternative source of ATP to glycolysis, TCA cycle, and respiration.

Cytoskeletal proteins are also lipoxidized in the second major biological process with a great number of modified proteins. The protein targets are beta-Actin, glial fibrillary acidic protein, neurofilament light polypeptide, neurofilament medium polypeptide, tubulin alpha 1B chain, tubulin beta chain, tubulin polymerization-promoting protein (TPPP), and vimentin. Proteins belonging to the main components of cytoskeleton seem to be affected: microfilaments, intermediate filaments, and microtubules. These findings are in line with previous findings showing that neurofilaments are the main targets of RCS adduction in mouse nervous system [78, 79]. Consequently, general neuronal and glial processes linked to cytoskeleton can become dysfunctional as a consequence of ALE formation in their protein components, but additional functions more specifically linked to neurons which require cytoskeleton integrity can also be affected secondarily to protein lipoxidation [18, 19]. Some of these functions are vesicle trafficking, maintenance of the neuronal caliber, axon guidance, synaptogenesis, and synaptic plasticity.

The third major biological process that is affected during aging based on the number of lipoxidized proteins is **neurotransmission**. The affected proteins are aspartate aminotransferase (GOT1), brain acid soluble protein (BASP1), dihydropyrimidinase Like 2 (DPYSL2), glutamate-ammonia ligase (GLUL), phosphatidylethanolamine-binding protein (PEBP1), 14–3–3 protein gamma (YWHAG), and synapsin 1 (SYN1). These proteins are involved in several functions such as neurotransmitter metabolism, axon growth and guidance, growth cone collapse and cell migration, neuron differentiation, synaptic transmission and plasticity, synaptic vesicle trafficking, signaling pathways, and regulation transcriptional activity, among others. These damaged proteins together with those linked to the high-energy demands make synapses especially vulnerable to oxidative stress damage. Interestingly, these biological processes potentially affected by the modified proteins are in line with the concept that most of the functional decline associated with normal PFC aging is caused by relatively subtle changes such as those in the molecular profile of synapses, altered spine morphologies, reductions in spine densities, and loss of dendrites [14] [18].

Four damaged proteins are involved in **proteostasis**. These proteins are alpha-crystallin B chain (heat shock protein B5) (CRYAB), heat shock protein 60KDa (HSPD1), protein/nucleic acid deglycase DJ-1 (PARK7), and ubiquitin carboxyl-terminal hydrolase L1 (UCHL1).

Whereas HSPD1 participates in mitochondrial protein import and macromolecular assembly, folding of proteins, and apoptotic process, among other potential functions, the rest of the proteins (UCHL1, CRYAB, and PARK7) play a relevant role in oxidative stress homeostasis. The case of HSPD1 may be particularly important since decreased content of mitochondrial ETC complexes during aging has been described, without any explanation [68]. So, it is proposed that defects in HSPD1 activity could induce a decreased import of nuclear subunits to the mitochondria with the subsequent deficiencies in complex formation which, in turn, affect energy production. As for the other proteins, UCHL1 participates in processing of ubiquitin precursors and ubiquitinated proteins for proteasomal degradation [80], and CRYAB shows chaperone-like activity and prevents aggregation of proteins under stress conditions [81]. PARK7 is a protein and nucleotide deglycase that catalyzes the degradation of adducts formed between amino groups of proteins or nucleotides and RCS acting as a protein repair system [82-85]. Furthermore, it plays an important role in cell protection against oxidative stress and cell death, acting as an oxidative stress sensor and redox-sensitive chaperone and protease, and it is also involved in neuroprotective mechanisms linked to mitochondrial uncoupling proteins, L-type channels, and inflammatory responses. So, the modified chaperones are implied in maintaining neuronal oxidative stress homeostasis. Assuming that non-enzymatic modifications are mostly linked to the loss of function, it may be proposed that the inactivation of the modified chaperones favors PFC aging by promoting cellular oxidative stress.

Three proteins related to **heme metabolism and oxygen and carbon dioxide regulation**, biliverdin reductase B (BLVRB), carbonic anhydrase (CA), and hemoglobin A1 (HbA1), are also lipoxidatively damaged. CA participates in the conversion of carbon dioxide into bicarbonate and participates in the transport of carbon dioxide out of the tissues. BLVDR regulates the final step in heme metabolism, but it also regulates glucose metabolism and has neuroprotective effects [86]. HbA1 is a component of hemoglobin, the role of which in the nervous system is still poorly understood. Hemoglobin has been found in neurons where it probably plays a role in oxygen transport or in the regulation of cytosolic neuronal oxygen [87]. Lipoxidative damage to these three proteins tags cell oxygen and

carbon dioxide regulation as a target of putative cellular respiratory dysfunction in the aged PFC.

Structural characterization of the modified proteins in the healthy adult human PFC with PredictProtein software analysis (https://www.predictprotein.org/) reveals some shared specific traits which may explain this specificity, rendering proteins more susceptible to oxidative damage [69]. Thus, i) the predominant structures of lipoxidized proteins are alpha helix and loops, ii) most proteins (excepting cytoskeletal proteins) are globular and form soluble coiled-shaped molecules with hydrophobic groups at the core and exposed hydrophilic groups to the medium, and iii) among the most recurrent amino acids encountered in the exposed regions is lysine, in addition to glutamic acid and aspartic acid, a frequent target of lipoxidation on proteins. Another factor that may be important in the selectivity of targeted proteins is the cellular location. Localization of proteins may make them more vulnerable as a result of their environmental conditions. Thus, several lipoxidized proteins are located in the mitochondria, the principal source of damaging free radicals; other proteins are located in membranes belonging to different subcellular compartments or even plasma membrane, which show a high content in PUFAs. In contrast, yet other damaged proteins, located in the cytoskeleton and the growth cones, are involved in neurotransmission, or present in synaptic vesicles. It is worth stressing that axons and synaptic terminals have high bioenergetic demands met by continuous mitochondrial activity and recruitment, and rapid energy transduction. In addition, axons are radial structures with a small diameter in comparison to the cell body, which heightens the probability of stochastic interactions between free radicals and membrane lipids, thus propitiating lipid peroxidation and further non-enzymatic modification of proteins.

Overall, these findings show that i) the PFC in healthy adults contains lipoxidized proteins, ii) the ALE-modified proteins increase in aged subjects, and iii) the selectivity of molecular damage is associated with specific structural traits and spatial location.

6. Advanced lipoxidation end-products (ALEs) and soluble oligomers

Aberrant protein structures are able to generate soluble oligomers with a high cytotoxic potential [88]. For instance, NKT-modified proteins generate aberrant structures with

cross-linking and aggregation [52]. For this reason, global levels of NKT-protein adducts and the presence of oligomers were analyzed in a recent work [69]. A positive significant relation between NKT-modified proteins and soluble oligomer levels in human PFC was found, suggesting that modifications of proteins parallels soluble oligomer formation. Furthermore, levels of oligomers and NKT increase with age in the PFC. These findings are complementary to previous observations showing increased expression levels of soluble oligomers in the PFC when comparing middle-aged individuals lacking sAD (Alzheimer disease)-related pathology with older cases with sporadic AD-related pathology stages I-II. Thus, increased levels of soluble oligomers are indeed a characteristic feature of the aging human brain coincidental with the first stages of AD-related pathology [70]. Importantly, increased levels of oligomeric species parallel regulation of brain cytokines and mediators of the immune response in old age [70]. Whether these coincidences have a cause-effect relationship needs further study using appropriate *in vitro* and *in vivo* models. Studies in post-mortem brains serve to demonstrate that such changes do really exist in the human PFC and that they deserve to be investigated deeper.

7. Interventions against PFC aging

The identification of interventions that delay PFC aging and can be easily implemented in humans is especially important because, to date, no pharmacological treatments able to maintain brain function and/or reduce cognitive decline, the risk of dementia, and neurodegenerative pathologies have been discovered. In recent decades there have been major advances in our understanding of the biology of aging, along with the development of nutritional interventions that delay aging including calorie restriction (CR), intermittent fasting (IF), protein restriction (PR), methionine restriction (MetR), and chemicals that influence pathways linking nutrition and aging processes [89, 90].

Growing evidence supports the view that CR/IF/PR/MetR may be helpful in preventing age-related changes in the brain associated with cognitive dysfunction [89, 91-93]. CR improves cognitive function, including learning and memory, in old rodents and humans [94-97], as well as improving the plasticity and recovery of the brain [98, 99], possibly mediated by its effects on cell-mitochondrial metabolism [91], nutrient-sensing pathways [89], and specific brain-related mechanisms [100].

One of the most relevant mechanisms proposed to explain this protective effect involves oxidative stress. In this line, a number of studies have shown that CR, as well as PR and MetR, may act at the mitochondrial level, reprogramming and improving mitochondrial metabolism and thereby decreasing free radical production [91, 101, 102]. In parallel to the improvement in mitochondrial free radical generation, there is a reduction in the steady-state levels of oxidative damage to proteins, lipids, and DNA in animals subjected to CR, IF, PR, and MetR, in whole brain as well as in brain cortex [32, 103-110]. Importantly, the set of brain proteins affected in normal aged rats is protected from oxidative damage by CR in association with improvement in cognitive functions [111].

With regards to lipids, several studies have demonstrated that these interventions (CR, PR, and MetR) have an impact on brain lipid profiles, inducing changes toward a lipid profile that is more resistant to lipid peroxidation which, in turn, leads to a significantly lower lipoxidation-derived protein damage level and, consequently, lower loss of function [32, 105, 106, 108, 111]. These interventions can also influence brain lipidome. Thus, it has been found that 80% MetR in mice induces marked changes in the brain, spinal cord, and liver lipidomes [108]. Interestingly, at least 50% of the lipids changed are common in the brain and spinal cord but not in the liver, suggesting a nervous system-specific lipidomic profile for MetR. The differentially expressed lipids basically include specific phospholipid species, which could reflect adaptive membrane responses, and sphingolipids, which could lead to changes in ceramide signaling pathways. In addition, specific oxidation products derived from cholesterol, ChoGpl, and EtnGpl are significantly decreased in brain, spinal cord, and liver from MetR mice. These results demonstrate the importance of adaptive responses of membrane lipids leading to increased stress resistance in brain.

Overall, these data provide a valuable insight into the mechanism of dietary restriction (CR, IF, PR, MetR) on oxidative stress reduction and functional improvements in the aged brain.

8. Healthy aging brain as prevention for neurodegenerative diseases

Various morphological, biochemical, metabolic and circulatory alterations, which are translated into functional changes, occur in brain aging. The degree of these functional

changes will in turn determine whether or not neurodegenerative diseases take place. When damage exceeds certain limits age- associated cognitive deterioration occurs. Indeed, it has been shown that the physiopathological processes associated to the disease begin more than 20 years before the clinical onset of dementia [112]. This provides a window of opportunity for finding interventions which can slow or even prevent the progress of the disease. Genetics, along with the lifestyle, nutrition, environmental factors, etc. can prevent the appearance of these diseases that occur with cognitive impairment [113]. This is of the utmost importance, as in many occasions there is no treatment for these diseases once the symptoms have debuted. As stated by our group in [114] "Only prevention makes sense", i.e. maintaining a healthy aging brain is the best way to prevent neurodegenerative diseases. In this regard, there have been some attempts to intervene early in neurodegenerative diseases. The FINGER study is a randomised controlled trial with a multidomain intervention consisting of a 2 years intervention including diet, exercise, cognitive training and vascular risk monitoring, which demonstrated that this multidomain approach could improve or maintain cognitive functioning in at-risk elderly people [115]. Two years later, a 24-month multicentre trial in which prodromal Alzheimer's patients received a specific multinutrient showed that they had better results on the cognitive-functional measurements and less brain atrophy as assessed by magnetic resonance imaging (MRI) [116].

The Lancet Commission stated in 2017: that we should "be ambitious about prevention" [117]. "Active treatment of hypertension in middle aged (45–65 years) and older people (aged older than 65 years) without dementia to reduce dementia incidence. Interventions for other risk factors including more childhood education, exercise, maintaining social engagement, reducing smoking, and management of hearing loss, depression, diabetes, and obesity might have the potential to delay or prevent a third of dementia cases". Therefore, maintaining a healthy brain throughout all life by healthy lifestyle habits seems to be one of the best ways to prevent neurodegenerative diseases.

9. Conclusions

Overall, the available evidence provides robust information about changes in lipidome and increased steady-state levels of protein damaged by lipoxidation reactions with PFC

aging. The set of damaged proteins compromise vital cell functions such as energy metabolism, cytoskeleton, neurotransmission, proteostasis, and oxygen/carbon dioxide/heme homeostasis. The affected biological processes may be considered putative collaborative factors contributing to the cognitive decline associated with PFC aging. Different nutritional interventions can have an impact on these mechanisms, improving brain function and delaying changes associated with PFC aging. Since molecular damage is already identified in normal middle-aged individuals and increases physiologically in the elderly, it seems reasonable to act upon the identified free radical-producing targets and lipid metabolism in the appropriate middle-age window.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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Figure Legends

Figure 1. Imaging mass spectrometry (IMS) of a human brain section, built by integrating selected lipids. The six layers of the gray matter and the white matter (WM) are labeled. GPCho, glycerophosphocholine; SPM, sphingomyelin; GPEtn, glycerophosphoethanolamine. Modified from [33].

Figure 2. Lipid peroxidation in human prefrontal cortex aging. Increased levels of lipid peroxidation induce formation of reactive carbonyl species-protein adducts which triggers the impairment of energy metabolism, cytoskeleton, neurotransmission, proteostasis, and oxygen/carbon dioxide/heme metabolism. The progressive dysfunction of these biological processes probably underlies the cognitive decline of PFC associated with the aging process.

Table 1. Lipid class comparison of lipid rafts and non-raft membranes from gray matter of prefrontal cortex from healthy subjects.

	Nonrafts	Lipid rafts
Sphingomyelin	1.97 ± 0.86	11.43 ± 1.44
Phoshatidylcholine (PC)	9.86 ± 0.87	5.35 ± 0.49
Phosphatidylserine (PS)	10.85 ± 1.08	6.82 ± 0.50
Phosphatidylinositol (PI)	3.67 ± 0.59	2.16 ± 0.17
Phosphatidylglycerol (PG)	2.71 ± 0.58	0.70 ± 0.10
Phosphatidylethanolamine (PE)	28.72 ± 1.82	20.97 ± 0.57
Sulfatides	0.00 ± 0.00	10.56 ± 0.68
Cerebrosides	0.00 ± 0.00	5.10 ± 0.79
Cholesterol	27.68 ± 1.98	33.04 ± 1.18
Free fatty acids	10.68 ± 0.92	2.17 ± 0.28
Sterol esters	0.00 ± 0.00	1.68 ± 0.60
Total neutral lipids	42.23 ± 2.66	36.90 ± 1.41
Total polar lipids	57.77 ± 2.66	63.09 ± 1.41
Phospholipid/cholesterol	2.15 ± 0.18	1.11 ± 0.06

Results are expressed as mole % and represent mean ± SEM. Data from: [38]

Table 2. Fatty acid profiles of healthy adult human prefrontal cortex.

Fatty Acid	Taha et al., 2013	Hamazaki et al., 2016	Naudi et al., 2017
PFC area	BA10	BA8	BA8
Age (years)	49.7 years	47.9 years	40-49 years
14:0	nd	0.4	1.3
16:0	19.4	21.8	20.01
16:1n-7	0.5	0.5	1.5
18:0	23.6	25.1	20.4
18:1n-9/n-7	23.5	19.6	24.6
18:2n-6	1.0	0.7	1.2
18:3n-3	nd	nd	0.2
18:4n-3	nd	nd	0.4
20:0	nd	0.2	0.5
20:1n-9	nd	0.6	1.7
20:2n-6	nd	0.1	0.4
20:3n-6	1.0	0.9	1.1
20:4n-6	8.9	8.6	6.9
20:5n-3	0.1	nd	0.4
22:0	nd	0.1	0.5
22:1n-9	nd	0.06	nd
22:4n-6	6.5	5.1	4.8
22:5n-6	1.5	nd	1.04
22:5n-3	0.5	0.3	0.7
22:6n-3	13.5	14.5	10.4
24:0	nd	0.2	0.9
24:1n-9	nd	0.9	nd
24:5n-3	nd	nd	0.2
24:6n-3	nd	nd	0.1
Double bond index	182.1	169.9	160.0
Peroxidation Index	185.8	176.6	153.3

Values are expressed as mol %. Nd, not detected, identified, or quantified.

Data were obtained and modified from [32, 40, 41]

Table 3. Lipoxidized proteins identified by redox proteomics in healthy adult/aged human prefrontal cortex.

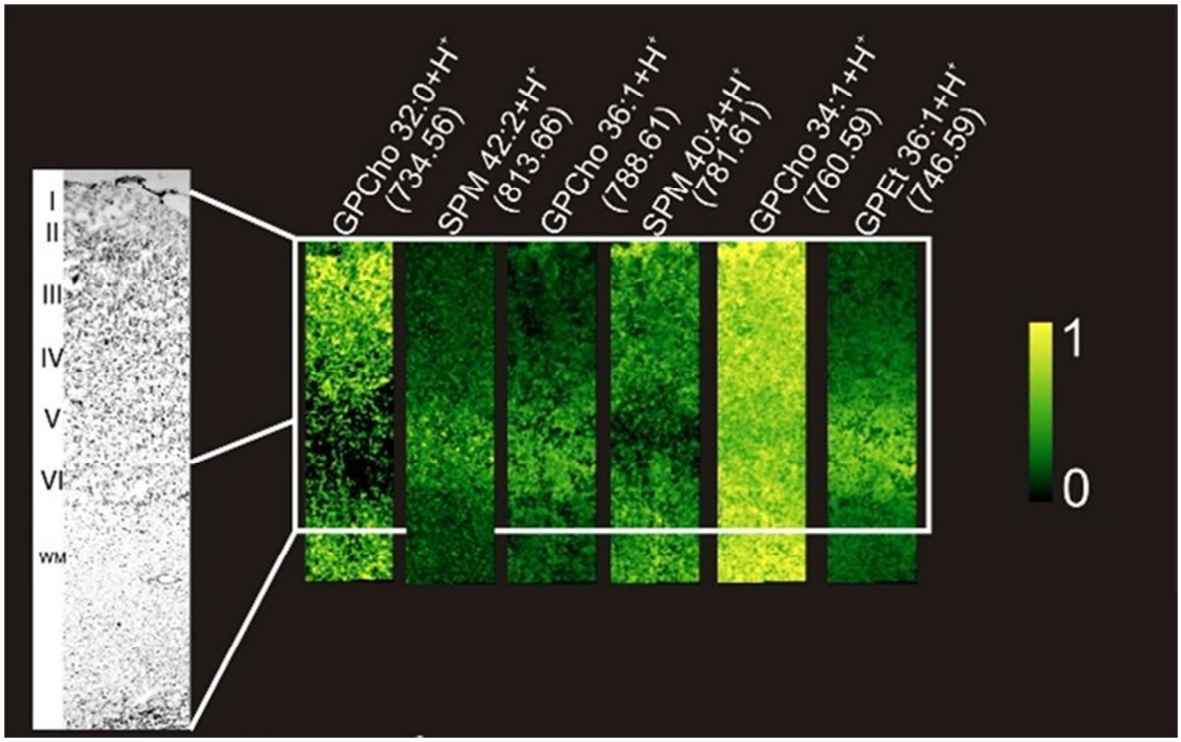
ID (entry human)	Protein	Gene	Main location	Protein modification by	Biological process
P06733	alpha-Enolase	ENO1	Cytosol, cell membrane, nucleus	MDA, NKT	Energy metabolism (glycolysis)
P09104	gamma-Enolase	ENO2	Cytosol, cell membrane	MDA	Energy metabolism (glycolysis)
P04406	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	Cytosol, cytoskeleton, nucleus	NKT	Energy metabolism (glycolysis)
P18669	Phosphoglycerate mutase 1	PGAM1	Cytosol, EVE, nucleus	NKT	Energy metabolism (glycolysis)
P14618	Pyruvate kinase	PKM	Cytosol, nucleus	NKT	Energy metabolism (glycolysis)
Q99798	Aconitate hydratase	ACO2	Mitochondrion	NKT	Energy metabolism (TCA cycle)
P00367	Glutamate dehydrogenase 1	GLUD1	Mitochondrion	MDA	Energy metabolism (TCA cycle)
P09622	Dihydrolipoyl dehydrogenase	DLD	Mitochondrion, nucleus	NKT	Energy metabolism (ETC)
P31930	Ubiquinol-cytochrome c reductase complex core protein 1	UQCRC1	Mitochondrion	MDA	Energy metabolism (ETC)
P25705	ATP synthase subunit alpha	ATP5F1A	Mitochondrion	NKT	Energy metabolism (OxPhos)
P06576	ATP synthase subunit beta	ATP5F1B	Mitochondrion	MDA	Energy metabolism (OxPhos)
P12277	Creatine kinase B-type	CKB	Cytosol	MDA, NKT	Energy metabolism (energy transduction)
P17174	Aspartate aminotransferase	GOT1	Cytosol	NKT	Neurotransmission
P80723	Brain acid soluble protein 1	BASP1	Cell membrane, growth cone	NKT	Neurotransmission
Q16555	Dihydropyrimidinase-related protein 2	DPYSL2	Cytosol, cytoskeleton, membrane	MDA, NKT	Neurotransmission
P15104	Glutamine synthetase	GLUL	Cytosol, mitochondrion	MDA	Neurotransmission
P30086	Phosphatidylethanolamine-binding protein 1	PEBP1	Cytosol	NKT	Neurotransmission
P61981	14-3-3 protein gamma	YWHAG	Cytosol	NKT	Neurotransmission
P17600	Synapsin-1	SYN1	Golgi apparatus, synaptic vesicle	NKT	Neurotransmission
P60709	beta-Actin	ACTB	Cytosol (cytoskeleton)	MDA	Cytoskeleton
P14136	Glial fibrillary acidic protein	GFAP	Cytosol (cytoskeleton)	MDA, NKT	Cytoskeleton
P07196	Neurofilament light polypeptide	NEFL	Cytosol (cytoskeleton)	MDA, NKT	Cytoskeleton
P07197	Neurofilament medium polypeptide	NEFM	Cytosol (cytoskeleton)	NKT	Cytoskeleton
P68363	Tubulin alpha 1B chain	TUBA1B	Cytosol (cytoskeleton)	MDA	Cytoskeleton
P07437	Tubulin beta chain	TUBB	Cytosol (cytoskeleton)	MDA	Cytoskeleton
O94811	Tubulin polymerization-promoting protein	TPPP	Cytosol (cytoskeleton), nucleus	NKT	Cytoskeleton
P08670	Vimentin	VIM	Cytosol (cytoskeleton), nucleus	MDA	Cytoskeleton
P02511	alpha-Crystallin B chain (Heat Shock Protein B5)	CRYAB	Cytosol, nucleus	NKT	Proteostasis
P10809	Heat shock protein 60KDa	HSPD1	Mitochondrion	MDA, NKT	Proteostasis
Q99497	Protein/nucleic acid deglycase DJ-1	PARK7	Cytosol, nucleus, mitochondrion, cell membrane	NKT	Proteostasis
P09936	Ubiquitin carboxyl-terminal hydrolase L1	UCHL1	Cytosol, endoplasmic reticulum	NKT	Proteostasis
P00915	Carbonic anhydrase 1	CA1	Cytosol	NKT	O_2/CO_2/heme metabolism

P69905	Hemoglobin subunit alpha	HBA1	Cytosol, EVE	NKT	O_2/CO_2/heme metabolism
P30043	NADPH-Flavin reductase	BLVRB	Cytosol	NKT	O_2/CO_2/heme metabolism
B1AKQ8	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta1	GNB1	Not described	MDA	Signal transduction

Gene, main location, and biological process are based on what was reported in the UniProt database (http://www.uniprot.org/). Abbreviations: EVE, extracellular vesicular exosome; TCA cycle, tricarboxylic acid cycle; ETC, electron transport chain; OxPhos, oxidative phosphorylation; ALEs, advanced lipoxidation end-products; NKT, neuroketals; MDA, Malondialdehyde.

Sources: [69, 74].

Figure 1.


GPCho 32:0+H+ (734.56)
SPM 42:2+H+ (813.66)
GPCho 36:1+H+ (788.61)
SPM 40:4+H+ (781.61)
GPCho 34:1+H+ (760.59)
GPEt 36:1+H+ (746.59)
I
II
III
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1
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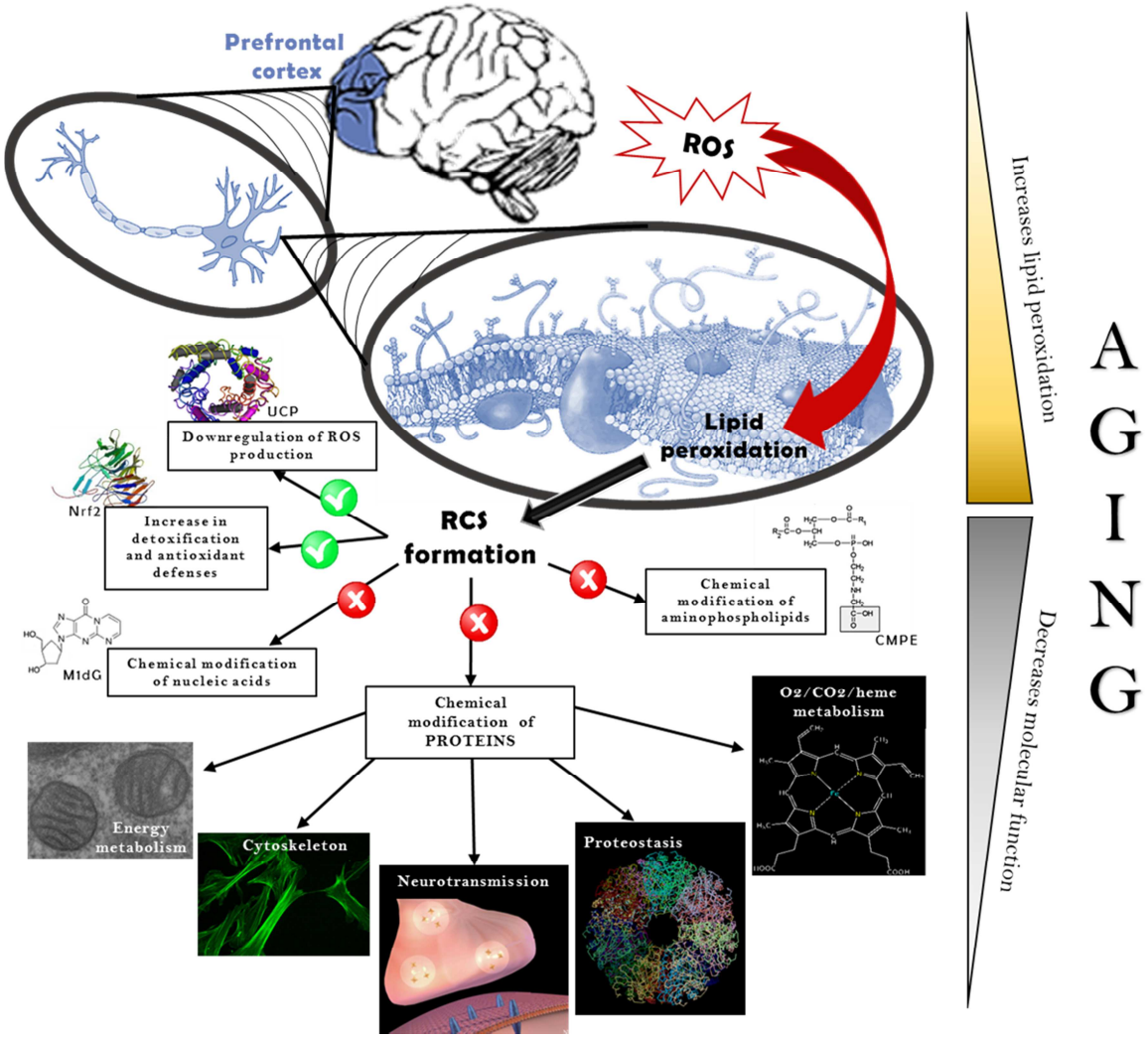
Figure 2.


Prefrontal cortex
ROS
Lipid peroxidation
UCP
Downregulation of ROS production
Nrf2
Increase in detoxification and antioxidant defenses
RCS formation
Chemical modification of aminophospholipids
CMPE
M1dG
Chemical modification of nucleic acids
Chemical modification of PROTEINS
O2/CO2/heme metabolism
Energy metabolism
Cytoskeleton
Neurotransmission
Proteostasis
Increases lipid peroxidation
Decreases molecular function
AGING