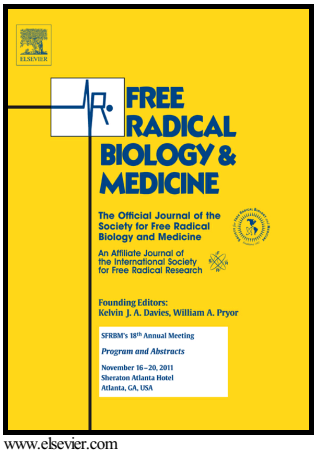

Sixty years old is the breakpoint of human frontal cortex aging

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Sixty years old is the breakpoint of human frontal cortex aging

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Abstract

Human brain aging is the physiological process which underlies as cause of cognitive decline in the elderly and the main risk factor for neurodegenerative diseases such as Alzheimer's disease. Human neurons are functional throughout a healthy adult lifespan, yet the mechanisms that maintain function and protect against neurodegenerative processes during aging are unknown. Here we show that protein oxidative and glycoxidative damage significantly increases during

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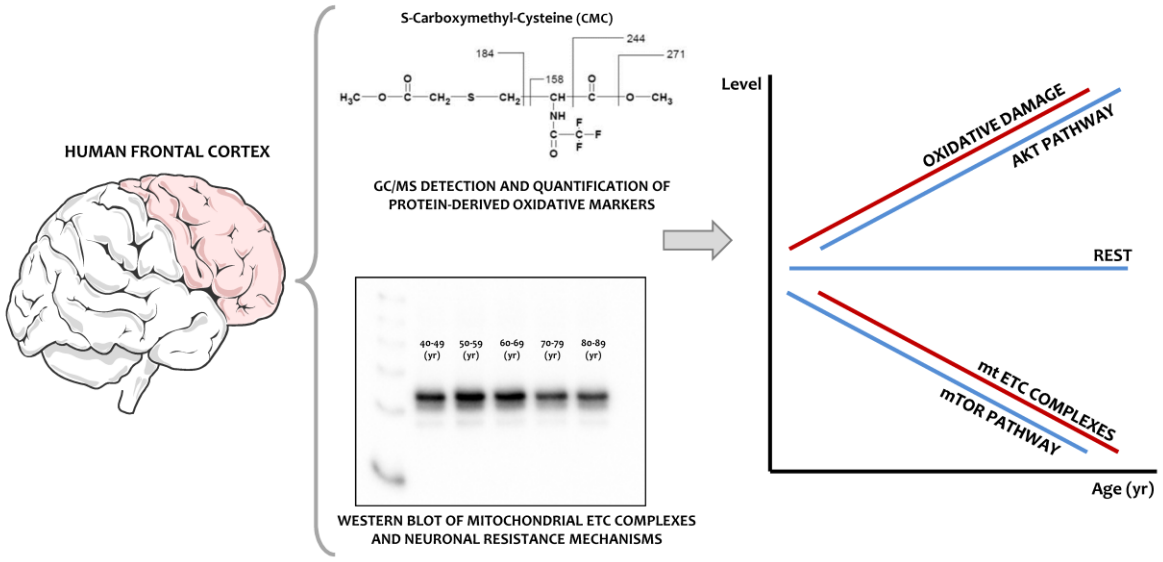
human brain aging, with a breakpoint at 60 years old. This trajectory is coincident with a decrease in the content of the mitochondrial respiratory chain complex I to IV. We suggest that the deterioration in oxidative stress homeostasis during aging induces an adaptive response of stress resistance mechanisms based on the sustained expression of REST, and increased or decreased expression of Akt and mTOR, respectively, over the adult lifespan in order to preserve cell neural survival and function.



Keywords: cell survival pathways, mechanistic target of rapamycin (mTOR), mitochondria respiratory chain, mitochondrial stress, protein oxidation, repressor element 1-silencing transcription factor (REST)

Introduction

Human brain aging is a process characterized by structural and physiological changes at all levels of the biological organization leading to cognitive functional losses [1–4]. Indeed cognitive decline is becoming a health challenge of the present century. This challenge is consequence of the increase in life expectancy of the population, origin of the increase in the prevalence of

cognitive decline and dementia, mostly in the form of Alzheimer's disease (AD). Thus, current data indicate that eighty-one percent of people who have AD are age 75 or older in the United States [5]. So, the main cause and risk factor for cognitive decline and AD in the elderly is age itself. Functional genetic analysis has identified signaling pathways and phenotypes that influence aging in model organisms and brain aging in mammals. Among them, of particular note are changes in genes involved in both mitochondrial and synaptic function [2,3,6–13].

Human neurons, however, are functional throughout an entire healthy adult lifespan, in contrast to AD pathology or other neurodegenerative diseases, but the mechanisms that maintain function and protect against neurodegeneration during aging are unknown. Available evidence suggest that oxidative stress is a conserved mechanism of age-related functional decline [13,14], but surprisingly little is known about the changes occurring in oxidative damage in the human brain over the adult lifespan. Furthermore, the methods used in these studies did not allow measurement of specific markers and their trajectories during aging, and any changes at this level are yet to be determined.

In the present work, the use of mass spectrometry technologies allowed us to determine a specific non-enzymatic oxidative protein damage profile of the human frontal cortex in individuals ranging from 40 to 90 years old. The choice of frontal cortex is based on the fact that it is a brain region that emerged recently during primate evolution and it is implicated in cognitive function including reasoning, planning, social behavior, and general intelligence. In addition to the protein damage marker analysis, we also measured the content of mitochondrial complexes of the electron transport chain, as well as different factors associated with stress resistance and cell survival such as the repressor element 1-silencing transcription factor REST, a regulator of neuroprotective stress response, and the serine/threonine kinase AKT, a member of the AKT signal transduction pathway that promotes cell survival, and the master regulator that senses cell nutrient and energy status, the mechanistic target of rapamycin mTOR.

Experimental Procedures

Chemicals

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

Human samples

Brain samples were obtained from the Institute of Neuropathology Brain Bank following the guidelines of the local ethics committee, and in accordance with recently published criteria of sample quality [15]. The selection of cases examined in the present study corresponded to a consecutive series of donations having in common (i) lack of neurological symptoms and signs, (ii) lack of known hepatic or renal function impairment, and (iii) lack of evidence of prolonged agonal state.

The neuropathological study was performed in every case as previously describe [16]. Briefly, 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 labelled 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 the frontal, primary motor, primary sensory, parietal, temporal superior, temporal inferior, anterior cingulated, anterior insular, and primary and associative visual cortices; entorhinal cortex and hippocampus; caudate, putamen and globus pallidus; medial and posterior thalamus; subthalamus; Meynert nucleus; amygdala; midbrain (two levels), pons and medulla oblongata; and cerebellar cortex and dentate nucleus. De-waxed sections, 5 mm thick, were stained with haematoxylin and eosin, and Klüver Barrera, or processed for immunohistochemistry to β-amyloid, phosphorylated tau, α-synuclein, ubiquitin, p62, TDP43, glial fibrillary protein, and microglia markers.

Selected cases did not show lesions on neuropathological examination including any kind of β-amyloid, tau, hypoxic, or vascular pathology. Following initial screening, the present series included 41 cases: 22 men and 19 women, with an age ranging from 43 to 86 years, and with post-mortem delay ranging from 2h to 19h 30 min (Table 1). Frozen samples of frontal cortex area 8 were used for biochemical studies.

Protein oxidation, glycoxidation, and succination

Markers of protein oxidation (the protein carbonyls glutamic [GSA] and aminoadipic [AASA] semialdehydes), glycoxidation (carboxyethyl-lysine [CEL], carboxymethyl-lysine [CML], and carboxymethyl-cysteine [CMC]), and succination (S-(2-succinyl) cysteine [2SC]), were determined as trifluoroacetic acid methyl ester (TFAME) derivatives in acid hydrolyzed delipidated and reduced tissue protein samples by gas chromatography/mass spectrometry (GC/MS) [17] using an HP6890 Series II gas chromatograph (Agilent, Barcelona, Spain) with an MSD5973A Series detector and a 7683 Series automatic injector, a HP-5MS column (30-m x 0.25-mm x 0.25-μm), and the described temperature program [17]. Quantification was performed by internal and external standardization using standard curves constructed from mixtures of deuterated and non-deuterated standards. Analyses were carried out with selected ion-monitoring GC/MS (SIM-GC/MS). The ions used were: lysine and [$^{2}H_{8}$]lysine, m/z 180 and 187, respectively; 5-hydroxy-2-aminovaleric acid and [$^{2}H_{5}$]5-hydroxy-2-aminovaleric acid (stable derivatives of GSA), m/z 280 and 285, respectively; 6-hydroxy-2-aminocaproic acid and [$^{2}H_{4}$]6-hydroxy-2-aminocaproic acid (stable derivatives of AASA), m/z 294 and 298, respectively; CEL and [$^{2}H_{4}$]CEL, m/z 379 and 383, respectively; CML and [$^{2}H_{2}$]CML, m/z 392 and 394, respectively; CMC and [$^{13}C_{3}$-^{15}N]CMC, m/z 271 and 273, respectively; and 2-SC and [$^{2}H_{2}$]SC, m/z 284 and 286. The amounts of product were expressed as μmoles of GSA, AASA, CEL, CML, CMC, or SC per mole of lysine.

Western blotting

The amounts of a) the mitochondrial respiratory chain complex I to IV, b) the complex I regulatory factors AIF (apoptosis-inducing factor), c) the repressor element 1-silencing

transcription factor REST, and d) the serine/threonine kinase AKT, a member of the AKT signal transduction pathway that promotes cell survival, and the master regulator that senses cell nutrient and energy status, the mechanistic target of rapamycin mTOR, were estimated using western blot analyses in samples from brain tissue as previously describe [18]. Briefly, brain samples (50mg) were homogenized in a buffer containing 180 mM KCl, 5 mM MOPS, 2 mM EDTA, 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 1mM). After a brief centrifugation (1000 rpm at 4°C for 3 min) to pellet cellular debris, protein concentrations were measured in the supernatants using the Bradford method (Bio-Rad Protein Assay 500-0006). Proteins were separated by one-dimensional SDS-PAGE. Samples were mixed with sample buffer (62.5 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 20% 2-β-mercaptoethanol and 0.02% bromophenol blue) and heated at 95°C for 5 min. Proteins (20 μg for respiratory chain complexes and AIF, and 35 μg for the others) were subjected to electrophoresis on 10% SDS-polyacrylamide minigels. For immunodetection, proteins were transferred using a Mini Trans-Blot Transfer Cell (Bio Rad) in a buffer containing 25 mM TRIS, 192 mM glycine, and 20% methanol, to polyvinylidene difluoride (PVDF) membranes (Immobilon-P Millipore, Bedford, MA). The membranes were immersed in blocking solution (0.2% I-Block Tropix AI300 or 0.5% BSA Sigma-Aldrich A4503, 0.1% Tween in TBS) at room temperature for 1 hour. After blocking, the membrane was washed two times using 0.05% TBS-T buffer. Afterwards, the membrane was incubated in primary solution using specific antibodies: anti-29kDa (NDUFS3) subunit of complex I (1:1000, ref. 459130, Invitrogen), anti-70kDa subunit (Flavoprotein) of complex II (1:500, ref. 459200, Invitrogen), anti-48kDa (CORE 2) subunit of complex III (1:1000, ref. 456220, Invitrogen), anti-57kDa (COXI subunit) of complex IV (1:1000, ref. 459600, Invitrogen), anti-AIF (1:1000, ref. A7549, Sigma), anti-porin (1:1000, ref. A31855, Molecular Probes), anti-actin (1:5000, ref. A5411, Sigma), anti-REST (1:1000, ref. ab21635, Abcam), anti-phospho-Akt (1:1000, ref. 9271, Invitrogen) and anti-Akt (1:1000, ref. 44-

609G, Invitrogen), and anti-phospho-mTOR and anti-mTOR (1:1000 in both cases, ref. 2971s and 2972-Cell Signaling Technology, respectively).

The primary antibody was incubated at room temperature for 1 hour for mtComplexes and AIF, and at 4°C for 16 h for the other factors. The membrane was washed three times in 0.05% TBS-T buffer and incubated at room temperature for 1 hour with the appropriate secondary antibodies [ECL Anti-mouse IgG, horseradish Peroxidase linked whole antibody-NA93IV GE Healthcare (1:30000) and ImmunoPure Goat Anti-Rabbit IgG peroxidase conjugated-31460 Pierce Biotechnology (1:100000)]. After five washes with 0.05% TBS-T buffer, bands were visualized using an enhanced chemiluminescence HRP substrate (Millipore, MA, USA). Signal quantification and recording was performed with ChemiDoc equipment (Bio-Rad Laboratories, Inc., Barcelona, Spain). The amounts of the determined factors were specifically calculated from the ratio of their densitometry values in reference to the densitometry values of their own porin content. The amount of REST was specifically calculated from the ratio of their densitometry values in reference to the actin content. Ratios of phospho-mTOR to total-mTOR, and phospho-AKT to total-AKT, were also calculated.

RNA purification, retrotranscription reaction, and TaqMan PCR

RNA purification, retrotranscription reaction and taqman PCR were performed as previously described [19]. Briefly, 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, after 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 (see Table 1).

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 10µL TaqMan reaction, 4.5µL cDNA was mixed with 0.5µL 20x TaqMan Gene Expression Assays and 5µL of 2x TaqMan Universal PCR Master Mix (Applied Biosystems). The reactions were carried out using the following parameters: 50°C for 2min, 95°C for 10 min, and 40 cycles of 95°C for 15 seconds and 60°C for 1min. 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 Supporting Information Table S1. 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 $2^{(-\Delta\Delta CT)}$.

Immunohistochemistry

Tissue immunohistochemistry was made as previously described [19]. Briefly, Tissue samples were embedded in paraffin, and 4-µm thick coronal sections were obtained with a sliding microtome. Endogenous peroxidases were blocked by incubation in 10% methanol–1% H2O2 solution for 15 minutes, followed by 3% normal horse serum solution, and then incubated at 4°C overnight with one of the primary antibodies indicated-that is, anti-mtComplex 1 (ref. 459130, Invitrogen), anti-REST (ref. ab21635, Abcam), anti-Akt (ref. 44-609G, Invitrogen), and anti-mTOR (ref. 2972-Cell Signaling Technology). Sections were subsequently rinsed and incubated with biotinylated secondary antibody (Dako, Glostrup, Denmark), followed by EnVision+

system peroxidase (Dako) and, finally, the chromogen diaminobenzidine and H_2O_2. Some sections were incubated without the primary antibodies; no immunostaining was detected in these sections. Sections were lightly counterstained with hematoxylin. After staining, the sections were dehydrated and coverslipped for microscopic assessment. The mounting medium used for image acquisition was DPX. Images were acquired with an Olympus BX51 microscope (UPlan FL N 20 × /0.50 Ph1) (Olympus Corporation, Tokyo, Japan) connected to an Olympus DP71 camera and Cell^B software (Olympus Corporation).

Statistics

To model the relationships between brain age and protein oxidative damage biomarkers were used, both generalized additive model (GAM) and joinpoint regression. GAM [20] is an extension of the Generalized Linear Model (GLM) where the assumption of linear dependency between the response variable and the covariates is relaxed. GAMs provide more flexibility than GLMs to capture non-linear relationships by using smooth functions like piecewise polynomials that join at points called knots. After the GAM analysis, in order to search for a potential break point in the age variable in relation to each independent variable (protein oxidative damage biomarkers) a joinpoint regression was applied. Joinpoint regression is a statistical modeling technique that explains the relationship between two variables by means of a segmented linear regression constrained so as to be continuous everywhere, in particular in those places where the slope of the regression function changes. These two analyses were done using the libraries "mgcv" and "segmented" of the R statistical software v.2.10.

Regression equations were obtained using nonlinear regression analyses with the curve estimation method of the SPSS/PC software for Windows (SPSS, Chicago, IL). Comparisons between groups were analyzed with one way ANOVA. These statistical analyses were performed using the SPSS software (SPSS, Chicago, IL, USA). The level of statistical significance was set at $p<0.05$ in all the analyses.

Results

Comparative studies of gene expression and evolution suggest that neurons from human neocortex are characterized by high levels of energy metabolism [21,22]. Furthermore, available evidence suggest that progressive oxidative damage, mitochondrial dysfunction, and defects of energy metabolism are mechanisms of age-related functional decline of the human brain [13]. We applied this idea to a key region of the adult human brain, the frontal cortex, to evaluate temporal trajectories of specific protein damage markers using a mass spectrometry technique, as direct markers of protein oxidation. Our results demonstrate the existence of a significant relationship between frontal cortex specific protein damage markers and age analysed by generalized additive model (GAM). In particular, this relationship shows an increase with age for the protein oxidation-derived markers GSA and AASA, and the glycoxidation-derived markers CEL and CML (p-values of the age smooth term were $p < 0.00001$ for GSA and AASA, $p=0.0003$ for CEL, and $p=0.012$ for CML) (Figure 1).

Protein cysteine residues can suffer post-translational modifications by oxidation, S-glutathionylation, S-nitrosylation, glycoxidation, and succination reactions, all of which modify protein structure and function in response to a changing intracellular redox environment [23]. In particular, succination is a nonenzymatic chemical modification of cysteine in proteins by the Krebs cycle metabolite, fumarate, yielding 2-SC. 2-SC serves as a biomarker of mitochondrial stress [24]. In contrast to oxidative and glyoxidative markers, no changes associated with age for the protein non-enzymatic modification of the cysteine residues CMC and the cysteine-derived succination marker SC were detected. (p-values of the age smooth term were $p=0.114$ for CMC, and $p=0.155$ for 2-SC) (Figure 1).

Reinforcing our findings, the potential interference of the variables gender and post-mortem time in the temporal trajectories of the different protein damage markers was ruled out after applying the corresponding GAM analysis (see Supporting Information).

To shed light upon a possible juncture when brain aging begins, we applied a jointpoint regression model to determine the breakpoint where the temporal trajectories for oxidative and glycoxidative markers show a significant inflection point followed with a more pronounced increase in protein damage. Our analysis suggests that the breakpoint is located around 60 years old (GSA: 62.64 ± 6.79 years old, R^2(adj)=0.5459; AASA: 63.77 ± 5.64 years old, R^2(adj)=0.538; CEL: 63.60 ± 20.44 years old, R^2(adj)=0.2868).

Because studies of brain aging has demonstrated a progressive decline in mitochondrial gene expression in different animal species such as rats, rhesus macaques, and humans [8,9,25], and mitochondrial function is involved in the aging process [26], we investigated the content of mitochondrial electron transport chain complexes during human brain aging. Our results showed a significant decrease in the concentration of all electron transport chain complexes (from CI to CIV) with age, with this trajectory especially evident in the decade following age 50 for CI and age 60 for CII, CIII, and CIV (Figure 2). In parallel, we also observed a decrease in the content of mitochondrial AIF which is consistent with the decrease in the amount of complex I because AIF, in addition to their apoptotic properties, has a specific role in the biogenesis and maintenance of complex I [27,28] (Figure 2). In contrast, the expression of complex V (ATP synthase) did not show any difference with age.

We then considered whether these changes in protein oxidative damage and mitochondrial complexes might be associated with changes/adaptions in cellular systems linked to stress resistance and cell survival. To this end, we measured the repressor element 1-silencing transcription factor REST, a regulator of neuroprotective stress response [29], and the serine/threonine kinase Akt, a member of the Akt signal transduction pathway that promotes cell survival, and the master regulator that senses cell nutrient and energy status, the mechanistic target of rapamycin mTOR [30]. Western blot analysis showed sustained expression of REST over the adult lifespan which was also observed for mRNA expression (Figure 3). Interestingly, a significant correlation was also observed, expressed through a quadratic equation, between the

REST content and the protein oxidation markers GSA and AASA (Figure 4). The protein expression of Akt increased significantly with age, especially at advanced ages, but this is not reflected in the mRNA levels, whereas mTOR protein expression and mRNA levels decrease significantly with age from the 60 years old (Figure 3).

In this line, the results obtained with immunohistochemistry for complex I, REST, Akt, and mTOR in human frontal cortex tissue confirm the temporal trajectories described above and demonstrate that the observed changes may be ascribed to neurons (Figure 5). Thus, the punctate cytoplasmic Complex I immunoreactivity detected in neurons, suggestive of their mitochondrial location, showed a decrease in staining across the decades. For REST, nuclear immunoreactivity was detected in neurons which were unaffected with age, indicative of sustained expression during normal brain aging. Finally, Akt and mTOR were detected at the cytoplasmic level, verifying a respectively increase or decrease immunoreactivity in neurons during brain aging, particularly from 60 onward.

Discussion

Available evidence suggests that changes in oxidative stress homeostasis is a conserved mechanism of age-related functional decline [13,14,31]. Thus, previous studies have reported increased levels of a) protein carbonyls, comparing young adults versus aged animal groups, in whole brain and specific regions in rats [32–34], and in the occipital lobe of human brain tissue [35]; b) 8-oxodG, a marker of DNA oxidation, in rat brain [36,37], and c) the advanced glycation endproducts Ne-carboxymethyl-lysine (CML), imidazolone and pentosidine, all of them using immunohistochemistry techniques, in aged and AD human brains [38–40]. The present study is the first to analyze selective and specific protein oxidative damage markers by mass spectrometry in frontal cortex from healthy humans covering an age range of 43 to 86 years old (n=41 individuals). Our results demonstrate that there is an increase in protein oxidative (GSA and AASA markers) and glycoxidative (CML and CEL markers) damage in human frontal cortex over the course of the adult lifespan. This increase seems to be selective because temporal trajectories for markers derived from the non-enzymatic modification of cysteine residues (CMC and SC) were maintained unchanged with age. A possible explanation for this dissociation in the behavior of different protein damage markers may be the cellular homeostatic mechanisms behind their formation. Thus, the increase in the oxidation markers could reflect an increase in the net flux of free radical generation, but also a decrease in the activity of protein turnover mechanisms; and for protein glycoxidation, the increased levels could express an increased oxidative stress status (again free radical generation and protein turnover), but also an increased level in the content of carbonyl compounds which act as a substrate for the formation of these markers which, in turn, reflect the concentration of carbohydrates that participate essentially in anaerobic glycolysis. In contrast, the unchanged content in markers derived from cysteine modification may be an indication of better preservation of cysteine residues and their functionality by specific repair mechanisms over the adult lifespan—mechanisms which are absent for other kind of markers.

Studies of gene expression reveal that decreased expression of mitochondrial genes is a conserved trait of the aging process in animal species ranging from *C.elegans* to humans [2,8–10,12,25]. Additionally, our results demonstrate a progressive loss in the content of mitochondrial electron transport chain (ETC) complexes from CI to CIV, and also that this decrease is effective from age 60 onward, concomitant with the breakpoint where the accrual of protein damage markers starts. Interestingly, there is a temporal gap in the progressive loss in the content of mitochondrial ETC complexes from CI to CIV, being evident in the decade following age 50 for CI, and age 60 for CII, CIII, and CIV. Inside mitochondria, electrons from reduced substrates move from CI and CII of the ETC through CIII and CIV to oxygen, forming water and causing protons to be pumped across the mitochondrial inner membrane. When glucose is metabolized through the TCA cycle (or fatty acids through β-oxidation), it generates electron donors. The main electron donor is NADH, which gives electrons to CI. The other electron donor generated by the TCA cycle is $FADH_2$, formed by succinate dehydrogenase, which donates electrons to CII. In this context, we propose the existence of an adaptive mechanism boosted to preserve the decline of mitochondrial bioenergetics capacity during brain aging based on a shift in brain metabolic control from glucose-driven bioenergetics to a compensatory and supplementary fatty acid β-oxidation pathway in order to maintain neuronal function and survival.

Consequently, the decrease in mitochondria-related gene expression and content of electron transport chain complexes could be on the basis of a normal decrement in mitochondrial function which could contribute to the age-dependent functional deficits in neurons and, in exacerbated cases, render neurons vulnerable to age-dependent neurodegenerative pathology. Another important element is to define the temporal trajectories of these physiological changes. A recent study showed that the transcriptional profile of the human frontal cortex from individuals ranging from 26 to 106 years of age defined a set of genes with reduced expression after age 40 [9]. In addition to this change in transcriptional profile, other studies analyzing the main lipid classes of different areas of human brain have also confirmed the occurrence of age-

related lipid alterations. Globally, all the described changes are perfectly aligned to the progressive and deleterious character of the aging process, and it seems that concentrations of most lipids in the human brain decrease after the age of 50 [4]. In the present study, we demonstrate that the breakpoint at which the temporal trajectories for oxidative and glycoxidative markers change in human frontal cortex expressing a progressive increase in protein damage is at 60 years old. Whether the described breakpoints (transcriptional profile at 40 years old, lipid composition at 50 years old, and protein damage at 60 years old) express a progressive and additional deleterious effect at molecular levels, or are independent pathways of the aging process, is a question that remains unanswered. But it is plausible to postulate that these changes might represent, from a given threshold, the molecular substrate determining a change in the normal temporal trajectory toward the onset of AD pathology.

The preservation of neural cells (particularly neurons) and cognitive functions throughout an entire healthy adult lifespan demands a cellular adaption involving robust stress-response mechanisms. In this context, a recent study showed that REST (also known as neuron-restrictive silencer factor, NRSF) - which regulates a network of genes that mediate cell stress resistance- is induced in human brain during aging, being associated with preservation of cognitive function and increased longevity [29]. By contrast, this gene network becomes affected at early stages of AD when REST is lost from the nucleus [29]. Our results confirm this observation and demonstrate that the presence of sustained and elevated REST levels at the nuclear level is present over the adult lifespan of healthy humans. In addition, the correlation between REST content and protein oxidation reinforces the idea that stressors like oxidative stress could be the trigger inducing REST expression as an adaptive response.

An additional mechanism may be mediated by the PI3-K/Akt/mTOR signaling pathway. All components of the PI3-K/Akt/mTOR signaling pathway are expressed in the brain at high levels, predominantly but not exclusively in neurons [30]. In post-mitotic neurons the integration of neuronal responses through this signaling pathway has significant functional effect on stress

responses, synaptic function, and cognitive processing, and it is involved in brain aging, and age-related neurodegenerative diseases [41–43]. mTOR is a conserved serine/threonine kinase which regulates metabolism in response to different factors such as nutrients, growth factors, and cellular energy conditions. An increasing number of studies show that disturbance in mTOR signaling in the brain affects multiple pathways including glucose metabolism, energy production, mitochondrial function, and autophagy. All these events could be key players in age-related cognitive decline as well as neurodegenerative diseases. Thus, signaling through the PI3-K/Akt/mTOR pathway and its activation is negatively affected in the brain of individuals with AD compared with healthy subjects [44–47]. In this line, an altered Akt subcellular location have been described in hippocampal and cortical neurons in the AD brain [44,48]. In addition, levels of mTOR signaling are also specifically increased in neurons predicted to develop neurofibrillary tangles [46]. Conversely, attenuation of the mTOR signal, through pharmacological and nutritional intervention, increases longevity and is associated with a healthy lifespan, including improvement in brain function [41,42]. Consequently, we infer that the lower mTOR expression observed in the frontal cortex with age in the present study for the first time is a physiological adaption aimed at preserving neuronal survival and conferring resistance to stress over the adult lifespan.

Here we show that oxidative and glycoxidative damage significantly increases during human brain aging, with a breakpoint at 60 years old. This trajectory is coincident with a decrease in the content of the mitochondrial respiratory chain complex I to IV. In addition, our findings suggest that the deterioration in oxidative stress homeostasis during aging induces an adaptive response of stress resistance mechanisms based on a sustained expression of REST, and increased and decreased content of Akt and mTOR, respectively, over the adult lifespan in order to preserve neuronal function.

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Conflict of Interest

None declared

Author Contributions

I.F. and R.P. designed the experiments. R.C., A.N., M.R., and R.P. analyzed the data. R.C., A.N., M.D.G., V.A., M.J., N.M.M., G.P.R., M.P.G.V, and M.P.O. performed the experiments. R.P. supervised the design and data interpretation. The manuscript was written by I.F. and R.P. and edited by R.P. All authors discussed the results and commented on the manuscript.

References

[1] J.R. Andrews-Hanna, A.Z. Snyder, J.L. Vincent, C. Lustig, D. Head, M.E. Raichle, R.L. Buckner, Disruption of large-scale brain systems in advanced aging., Neuron. 56 (2007) 924–35. doi:10.1016/j.neuron.2007.10.038.

[2] B.A. Yankner, T. Lu, P. Loerch, The aging brain., Annu. Rev. Pathol. 3 (2008) 41–66.

doi:10.1146/annurev.pathmechdis.2.010506.092044.

[3] N.A. Bishop, T. Lu, B.A. Yankner, Neural mechanisms of ageing and cognitive decline, Nature. 464 (2010) 529–535. doi:10.1038/nature08983.

[4] A. Naudí, R. Cabré, M. Jové, V. Ayala, H. Gonzalo, M. Portero-Otín, I. Ferrer, R. Pamplona, Lipidomics of Human Brain Aging and Alzheimer's Disease Pathology, 2015. doi:10.1016/bs.irn.2015.05.008.

[5] L.E. Hebert, J. Weuve, P.A. Scherr, D.A. Evans, Alzheimer disease in the United States (2010-2050) estimated using the 2010 census., Neurology. 80 (2013) 1778–83. doi:10.1212/WNL.0b013e31828726f5.

[6] C.K. Lee, R. Weindruch, T.A. Prolla, Gene-expression profile of the ageing brain in mice., Nat. Genet. 25 (2000) 294–7. doi:10.1038/77046.

[7] C.H. Jiang, J.Z. Tsien, P.G. Schultz, Y. Hu, The effects of aging on gene expression in the hypothalamus and cortex of mice., Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1930–4. doi:10.1073/pnas.98.4.1930.

[8] E.M. Blalock, K.-C. Chen, K. Sharrow, J.P. Herman, N.M. Porter, T.C. Foster, P.W. Landfield, Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment., J. Neurosci. 23 (2003) 3807–19. http://www.ncbi.nlm.nih.gov/pubmed/12736351 (accessed November 5, 2015).

[9] T. Lu, Y. Pan, S.-Y. Kao, C. Li, I. Kohane, J. Chan, B.A. Yankner, Gene regulation and DNA damage in the ageing human brain., Nature. 429 (2004) 883–91. doi:10.1038/nature02661.

[10] L. Erraji-Benchekroun, M.D. Underwood, V. Arango, H. Galfalvy, P. Pavlidis, P. Smyrniotopoulos, J.J. Mann, E. Sibille, Molecular aging in human prefrontal cortex is selective and continuous throughout adult life., Biol. Psychiatry. 57 (2005) 549–58. doi:10.1016/j.biopsych.2004.10.034.

[11] M.T. Lin, M.F. Beal, Mitochondrial dysfunction and oxidative stress in neurodegenerative

diseases., Nature. 443 (2006) 787–95. doi:10.1038/nature05292.

[12] J.M. Zahn, S. Poosala, A.B. Owen, D.K. Ingram, A. Lustig, A. Carter, A.T. Weeraratna, D.D. Taub, M. Gorospe, K. Mazan-Mamczarz, E.G. Lakatta, K.R. Boheler, X. Xu, M.P. Mattson, G. Falco, M.S.H. Ko, D. Schlessinger, J. Firman, S.K. Kummerfeld, W.H. Wood, A.B. Zonderman, S.K. Kim, K.G. Becker, AGEMAP: a gene expression database for aging in mice., PLoS Genet. 3 (2007) e201. doi:10.1371/journal.pgen.0030201.

[13] M. Jové, M. Portero-Otín, A. Naudí, I. Ferrer, R. Pamplona, Metabolomics of human brain aging and age-related neurodegenerative diseases., J. Neuropathol. Exp. Neurol. 73 (2014) 640–57. doi:10.1097/NEN.0000000000000091.

[14] R.S. Sohal, Role of oxidative stress and protein oxidation in the aging process., Free Radic. Biol. Med. 33 (2002) 37–44. http://www.ncbi.nlm.nih.gov/pubmed/12086680 (accessed February 1, 2016).

[15] I. Ferrer, Selection of controls in the study of human neurodegenerative diseases in old age., J. Neural Transm. 122 (2015) 941–7. doi:10.1007/s00702-014-1287-y.

[16] I. Ferrer, A. Martinez, S. Boluda, P. Parchi, M. Barrachina, Brain banks: benefits, limitations and cautions concerning the use of post-mortem brain tissue for molecular studies., Cell Tissue Bank. 9 (2008) 181–94. doi:10.1007/s10561-008-9077-0.

[17] R. Pamplona, E. Dalfó, V. Ayala, M.J. Bellmunt, J. Prat, I. Ferrer, M. Portero-Otín, Proteins in human brain cortex are modified by oxidation, glycoxidation, and lipoxidation. Effects of Alzheimer disease and identification of lipoxidation targets., J. Biol. Chem. 280 (2005) 21522–30. doi:10.1074/jbc.M502255200.

[18] P. Caro, J. Gomez, I. Sanchez, A. Naudi, V. Ayala, M. López-Torres, R. Pamplona, G. Barja, Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and mitochondrial DNA in rat kidney and brain mitochondria., Rejuvenation Res. 12 (2009) 421–34. doi:10.1089/rej.2009.0902.

[19] I. López-González, E. Aso, M. Carmona, M. Armand-Ugon, R. Blanco, A. Naudí, R. Cabré, M. Portero-Otin, R. Pamplona, I. Ferrer, Neuroinflammatory Gene Regulation, Mitochondrial Function, Oxidative Stress, and Brain Lipid Modifications With Disease Progression in Tau P301S Transgenic Mice as a Model of Frontotemporal Lobar Degeneration-Tau, J. Neuropathol. Exp. Neurol. 74 (2015) 975–999. doi:10.1097/NEN.0000000000000241.

[20] T.J. Hastie, R.J. Tibshirani, Generalized Additive Models, 1990. https://books.google.com/books?hl=ca&lr=&id=qa29r1Ze1coC&pgis=1 (accessed February 2, 2016).

[21] C.C. Sherwood, C.D. Stimpson, M.A. Raghanti, D.E. Wildman, M. Uddin, L.I. Grossman, M. Goodman, J.C. Redmond, C.J. Bonar, J.M. Erwin, P.R. Hof, Evolution of increased glia-neuron ratios in the human frontal cortex., Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 13606–11. doi:10.1073/pnas.0605843103.

[22] M. Somel, X. Liu, P. Khaitovich, Human brain evolution: transcripts, metabolites and their regulators., Nat. Rev. Neurosci. 14 (2013) 112–27. doi:10.1038/nrn3372.

[23] S.R. Thorpe, J.W. Baynes, Maillard reaction products in tissue proteins: new products and new perspectives., Amino Acids. 25 (2003) 275–81. doi:10.1007/s00726-003-0017-9.

[24] E.D. Merkley, T.O. Metz, R.D. Smith, J.W. Baynes, N. Frizzell, The succinated proteome., Mass Spectrom. Rev. 33 (2014) 98–109. doi:10.1002/mas.21382.

[25] P.M. Loerch, T. Lu, K.A. Dakin, J.M. Vann, A. Isaacs, C. Geula, J. Wang, Y. Pan, D.H. Gabuzda, C. Li, T.A. Prolla, B.A. Yankner, Evolution of the Aging Brain Transcriptome and Synaptic Regulation, PLoS One. 3 (2008) e3329. doi:10.1371/journal.pone.0003329.

[26] R. Pamplona, G. Barja, Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection., Biochim. Biophys. Acta. 1757 (2006) 496–508. doi:10.1016/j.bbabio.2006.01.009.

[27] N. Vahsen, C. Candé, J.-J. Brière, P. Bénit, N. Joza, N. Larochette, P.G. Mastroberardino,

M.O. Pequignot, N. Casares, V. Lazar, O. Feraud, N. Debili, S. Wissing, S. Engelhardt, F. Madeo, M. Piacentini, J.M. Penninger, H. Schägger, P. Rustin, G. Kroemer, AIF deficiency compromises oxidative phosphorylation., EMBO J. 23 (2004) 4679–89. doi:10.1038/sj.emboj.7600461.

[28] A.G. Porter, A.G.L. Urbano, Does apoptosis-inducing factor (AIF) have both life and death functions in cells?, Bioessays. 28 (2006) 834–43. doi:10.1002/bies.20444.

[29] T. Lu, L. Aron, J. Zullo, Y. Pan, H. Kim, Y. Chen, T.-H. Yang, H.-M. Kim, D. Drake, X.S. Liu, D. a Bennett, M.P. Colaiácovo, B. a Yankner, REST and stress resistance in ageing and Alzheimer's disease., Nature. 507 (2014) 448–54. doi:10.1038/nature13163.

[30] C. O' Neill, PI3-kinase/Akt/mTOR signaling: impaired on/off switches in aging, cognitive decline and Alzheimer's disease., Exp. Gerontol. 48 (2013) 647–53. doi:10.1016/j.exger.2013.02.025.

[31] F.L. Muller, M.S. Lustgarten, Y. Jang, A. Richardson, H. Van Remmen, Trends in oxidative aging theories., Free Radic. Biol. Med. 43 (2007) 477–503. doi:10.1016/j.freeradbiomed.2007.03.034.

[32] K.R. Brizzee, P.A. Cancilla, N. Sherwood, P.S. Timiras, The amount and distribution of pigments in neurons and glia of the cerebral cortex. Autofluorescent and ultrastructural studies., J. Gerontol. 24 (1969) 127–35. http://www.ncbi.nlm.nih.gov/pubmed/5789243 (accessed January 27, 2016).

[33] A. Dubey, M.J. Forster, H. Lal, R.S. Sohal, Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse., Arch. Biochem. Biophys. 333 (1996) 189–97. doi:10.1006/abbi.1996.0380.

[34] M.J. Forster, A. Dubey, K.M. Dawson, W.A. Stutts, H. Lal, R.S. Sohal, Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain., Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4765–9. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=39353&tool=pmcentrez&ren

dertype=abstract (accessed January 27, 2016).

[35] C.D. Smith, J.M. Carney, P.E. Starke-Reed, C.N. Oliver, E.R. Stadtman, R.A. Floyd, W.R. Markesbery, Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease., Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 10540–3. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=52964&tool=pmcentrez&rendertype=abstract (accessed December 11, 2015).

[36] A. Herrero, G. Barja, Effect of aging on mitochondrial and nuclear DNA oxidative damage in the heart and brain throughout the life-span of the rat., J. Am. Aging Assoc. 24 (2001) 45–50. doi:10.1007/s11357-001-0006-4.

[37] M.L. Hamilton, H. Van Remmen, J.A. Drake, H. Yang, Z.M. Guo, K. Kewitt, C.A. Walter, A. Richardson, Does oxidative damage to DNA increase with age?, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10469–74. doi:10.1073/pnas.171202698.

[38] A. Takeda, M. Wakai, H. Niwa, R. Dei, M. Yamamoto, M. Li, Y. Goto, T. Yasuda, Y. Nakagomi, M. Watanabe, T. Inagaki, Y. Yasuda, T. Miyata, G. Sobue, Neuronal and glial advanced glycation end product [Nepsilon-(carboxymethyl)lysine]] in Alzheimer's disease brains., Acta Neuropathol. 101 (2001) 27–35. http://www.ncbi.nlm.nih.gov/pubmed/11194938 (accessed January 27, 2016).

[39] T. Jono, T. Kimura, J. Takamatsu, R. Nagai, K. Miyazaki, T. Yuzuriha, T. Kitamura, S. Horiuchi, Accumulation of imidazolone, pentosidine and N(epsilon)-(carboxymethyl)lysine in hippocampal CA4 pyramidal neurons of aged human brain., Pathol. Int. 52 (2002) 563–71. http://www.ncbi.nlm.nih.gov/pubmed/12406185 (accessed January 27, 2016).

[40] H.-J. Lüth, V. Ogunlade, B. Kuhla, R. Kientsch-Engel, P. Stahl, J. Webster, T. Arendt, G. Münch, Age- and stage-dependent accumulation of advanced glycation end products in intracellular deposits in normal and Alzheimer's disease brains., Cereb. Cortex. 15 (2005) 211–20. doi:10.1093/cercor/bhh123.

[41] M.G. Garelick, B.K. Kennedy, TOR on the brain, Exp. Gerontol. 46 (2011) 155–163. doi:10.1016/j.exger.2010.08.030.

[42] M. Perluigi, F. Di Domenico, D.A. Butterfield, mTOR signaling in aging and neurodegeneration: At the crossroad between metabolism dysfunction and impairment of autophagy., Neurobiol. Dis. 84 (2015) 39–49. doi:10.1016/j.nbd.2015.03.014.

[43] J. Bockaert, P. Marin, mTOR in Brain Physiology and Pathologies., Physiol. Rev. 95 (2015) 1157–87. doi:10.1152/physrev.00038.2014.

[44] R.J. Griffin, A. Moloney, M. Kelliher, J.A. Johnston, R. Ravid, P. Dockery, R. O'Connor, C. O'Neill, Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer's disease pathology., J. Neurochem. 93 (2005) 105–17. doi:10.1111/j.1471-4159.2004.02949.x.

[45] A.M. Moloney, R.J. Griffin, S. Timmons, R. O'Connor, R. Ravid, C. O'Neill, Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling., Neurobiol. Aging. 31 (2010) 224–43. doi:10.1016/j.neurobiolaging.2008.04.002.

[46] J.-J. Pei, J. Hugon, mTOR-dependent signalling in Alzheimer's disease., J. Cell. Mol. Med. 12 (2008) 2525–32. doi:10.1111/j.1582-4934.2008.00509.x.

[47] K. Talbot, H.-Y. Wang, H. Kazi, L.-Y. Han, K.P. Bakshi, A. Stucky, R.L. Fuino, K.R. Kawaguchi, A.J. Samoyedny, R.S. Wilson, Z. Arvanitakis, J.A. Schneider, B.A. Wolf, D.A. Bennett, J.Q. Trojanowski, S.E. Arnold, Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline., J. Clin. Invest. 122 (2012) 1316–38. doi:10.1172/JCI59903.

[48] J.-J. Pei, S. Khatoon, W.-L. An, M. Nordlinder, T. Tanaka, H. Braak, I. Tsujio, M. Takeda, I. Alafuzoff, B. Winblad, R.F. Cowburn, I. Grundke-Iqbal, K. Iqbal, Role of protein kinase B in Alzheimer's neurofibrillary pathology., Acta Neuropathol. 105 (2003) 381–92. doi:10.1007/s00401-002-0657-y.

Figure 1. Relationship between age and different protein oxidative biomarkers. Graphics show the trend of each variable with age, analyzed by generalized additive model (GAM). Significance of the GAM analysis are: for GSA, R^2(adj)=0.556, p<0.00001, Deviance explained=57.7%; for AASA, R^2(adj)=0.549, p<0.00001, Deviance explained=57%; for CEL, R^2 (adj)=0.305, p<0.00028, Deviance explained=33.4%; for CML, R^2(adj)=0.156, p<0.012, Deviance explained=18.7%; for CMC, R^2 (adj)=0.053, p>0.05, Deviance explained=7.51%; and for 2-SC, R^2(adj)=0.0393, p>0.05, Deviance explained=5.88 %.

Figure 2. Representative immunoblots showing relative levels of the polypeptides measured corresponding to the mitochondrial respiratory complex I, II, III, IV, ATP synthase (Complex V), and AIF. Values are expressed as mean ± SEM from 5 different frontal cortex human samples normalized for porin protein levels as a mitochondrial marker.
[a] significantly different from 40-49 group, [b] significantly different from 50-59 group, [c] significantly different from 60-69 group, [d] significantly different from 70-79 group. *p<0.05, **p<0.01, ***p<0.001.

Figure 3. Representative immunoblots and mRNA relative expression of REST, Akt and mTOR. Values are expressed as mean ± SEM from 5 different brain human samples normalized for actin protein levels for REST and ratios of phosphorylated mTOR to total mTOR and phosphorylated Akt to total-Akt. [a]significantly different from 40-49 group, [b] significantly different from 50-59 group, [c] significantly different from 60-69 group, [d] significantly different from 70-79 group. *p<0.05, **p<0.01.

Figure 4. Relationship between the steady-state levels of specific protein damage markers (GSA and AASA, μmol/mol lysine) and the repressor element 1-silencing transcription factor (REST, % change) expression. These regressions were determined and tested for significance using the mean values for each decade of age. Values used for regression equation (expressed as mean±SEM) are: For GSA: 2888.62±467.82 (40-49yrs), 2858.47±512.36 (50-59yrs), 2831.7±411.66 (60-69yrs), 8492.43±910.06 (70-79yrs), 9237.66±1353.52 (80-89yrs); for AASA: 64.54±37.68 (40-49yrs), 43.32±15.85 (50-59yrs), 40.49±13.78 (60-69yrs), 275.41±21.51 (70-79yrs), 384.72±81.71 (80-89yrs); for REST: 1.00±0.19 (40-49yrs), 1.05±0.18 (50-59yrs), 1.08±0.11 (60-69yrs), 1.22±0.21 (70-79yrs), 1.59±0.56 (80-89yrs).

Figure 5. Representative images of Complex I, REST, Akt, and mTOR detection in human frontal cortex samples at each decade of age. Paraffin sections, slightly counterstained with haematoxylin. Bar = 25 μm.

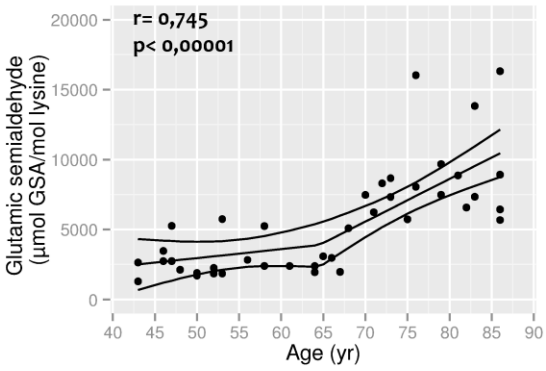
Table 1. Summary of cases examined.

Case	Gender	Age (yrs)	Post Mortem Delay	RIN Values
1	Man	43	4 h 35 min	7.3
2	Man	43	5 h 55 min	7.5
3	Woman	46	9 h 35 min	6.9
4	Man	46	15 h	7.5
5	Man	47	4 h 55 min	
6	Woman	48	4 h	
7	Woman	50	14 h 30 min	
8	Man	50	17 h 15 min	
9	Woman	52	5 h 45 min	6.6
10	Man	52	4 h 40 min	7.1
11	Man	53	3 h	
12	Man	53	7 h 25 min	
13	Man	56	3 h 45 min	7.1
14	Woman	58	4 h	
15	Man	58	4 h	7.3
16	Man	61	3 h 55 min	7.4
17	Woman	64	5 h	
18	Man	64	8 h 35 min	
19	Man	65	16 h	6.1

20	Woman	66	4 h 15min	7.1
21	Woman	67	5 h 20 min	5.8
22	Man	68	10 h 55 min	
23	Man	70	2 h	7.3
24	Woman	71	6 h	6.7
25	Woman	71	8 h 30 min	6.7
26	Man	72	3 h 35 min	7.5
27	Woman	73	4 h 30 min	
28	Woman	73	5 h 30 min	
29	Woman	75	3 h 25 min	
30	Man	76	4 h 15 min	
31	Man	76	6 h 30 min	
32	Woman	79	3 h 35 min	
33	Woman	79	5 h	
34	Woman	81	4 h	
35	Woman	82	3 h 05 min	6.9
36	Man	83	3 h 03 min	7
37	Man	83	4 h 30 min	7.3
38	Woman	86	4 h 15 min	
39	Woman	86	19 h 30 min	
40	Man	86	5 h 35 min	6
41	Man	86	18 h	

Highlights

- Protein oxidative and glycoxidative damage significantly increases during human brain aging, with a breakpoint at 60 years old.
- This trajectory is coincident with a decrease in the content of the mitochondrial respiratory chain complex I to IV.
- The deterioration in oxidative stress homeostasis during brain aging induces an adaptive response of stress resistance mechanisms over the adult lifespan in order to preserve neuronal function.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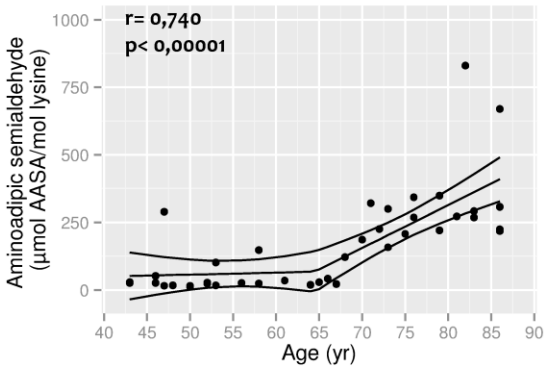
p< 0,00001
Glutamic semialdehyde (µmol GSA/mol lysine)
Age (yr)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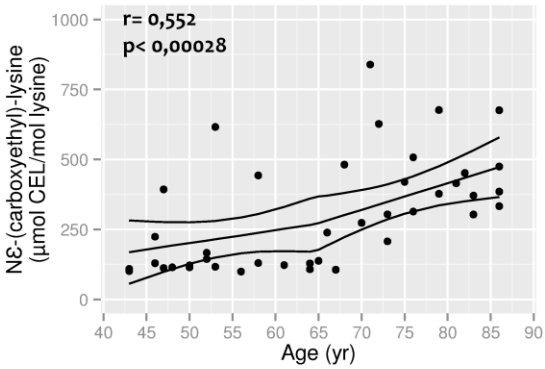
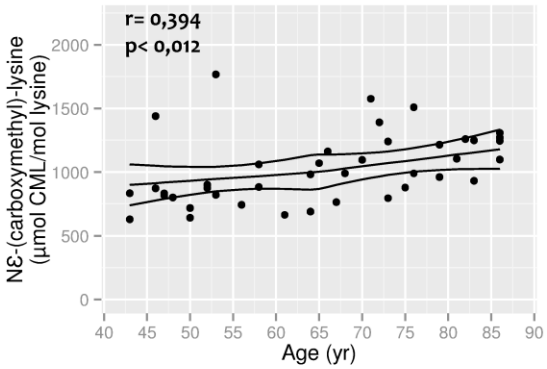
p< 0,00001
Aminoadipic semialdehyde (µmol AASA/mol lysine)
Age (yr)


r= 0,552
p< 0,00028
Nε-(carboxyethyl)-lysine (µmol CEL/mol lysine)
Age (yr)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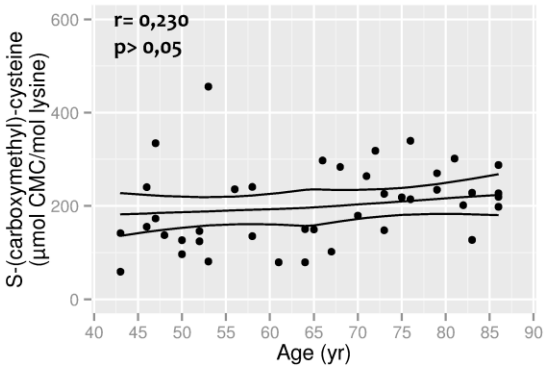
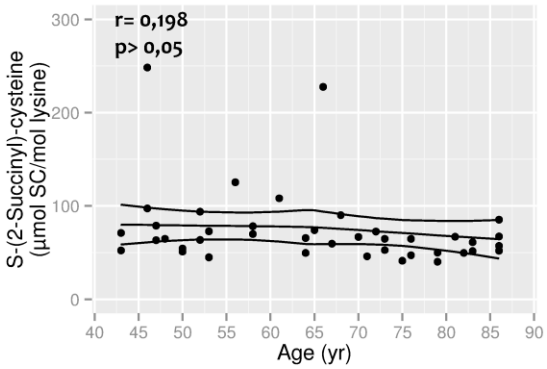
Nε-(carboxymethyl)-lysine (µmol CML/mol lysine)
Age (yr)


r= 0,230
p> 0,05
S-(carboxymethyl)-cysteine (µmol CMC/mol lysine)
Age (yr)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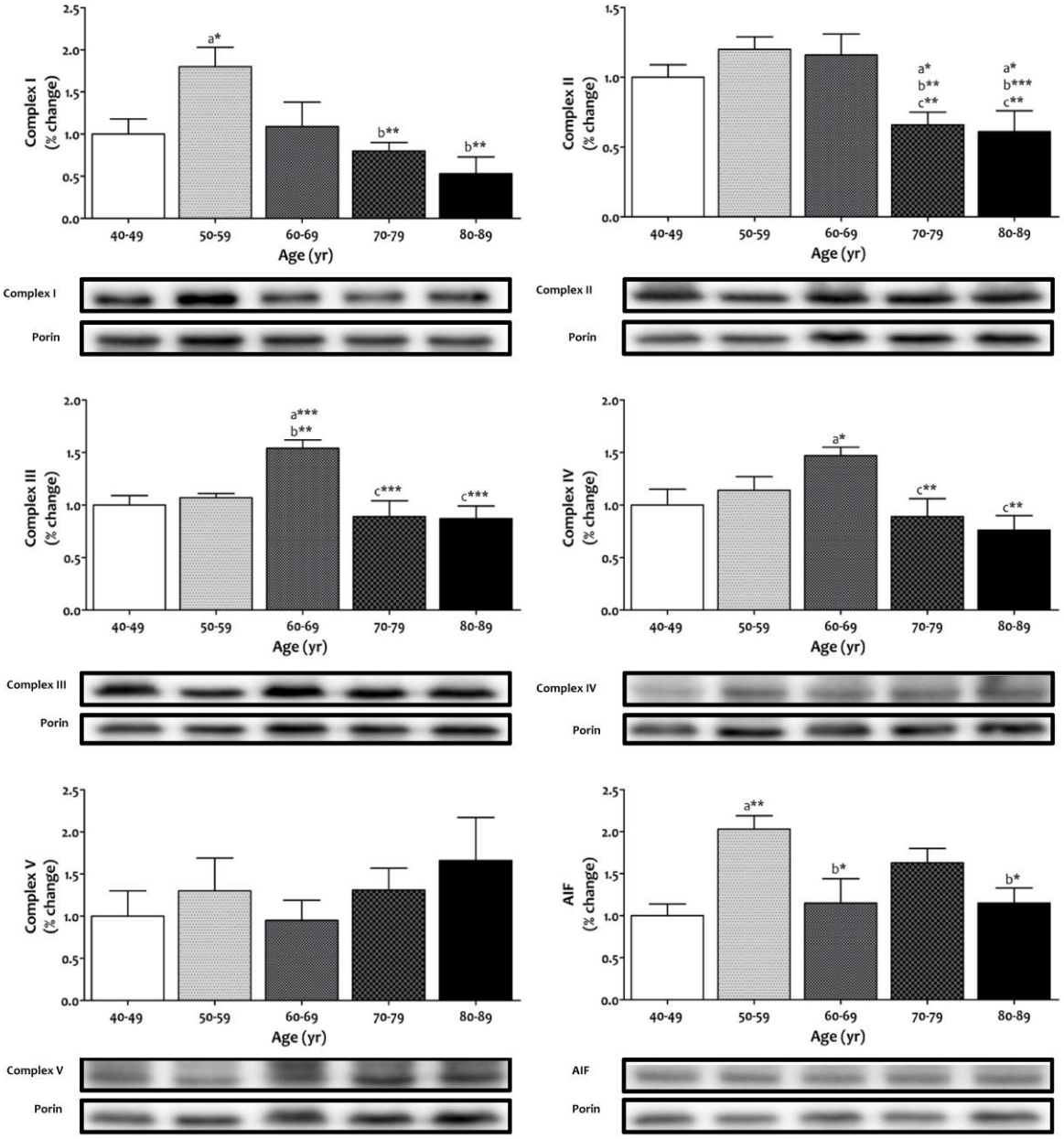
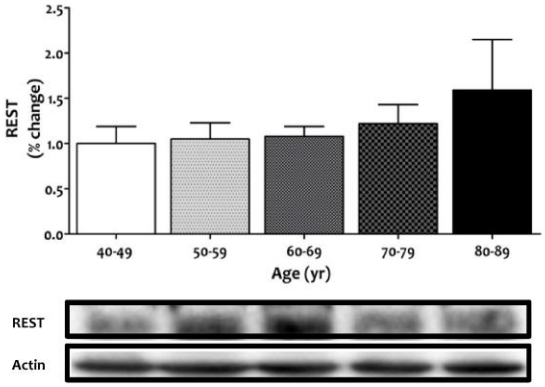
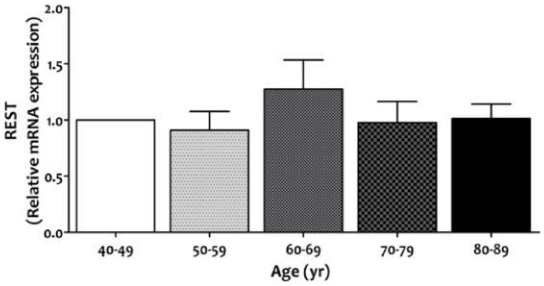
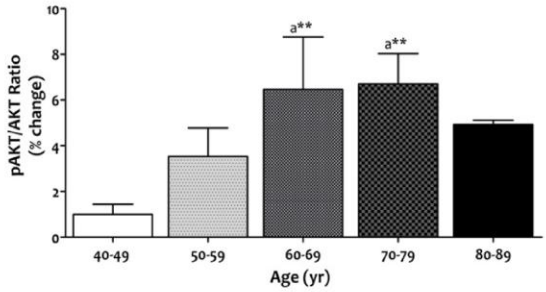
p> 0,05
S-(2-Succinyl)-cysteine (µmol SC/mol lysine)
Age (yr)

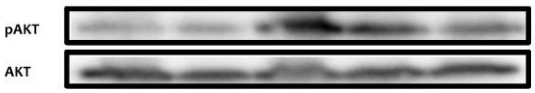

Complex I
(% change)
a*
b**
b**
40-49
50-59
60-69
70-79
80-89
Age (yr)
Complex I
Porin
Complex II
(% change)
a*
b**
c**
a*
b***
c**
Age (yr)
Complex II
Porin
Complex III
(% change)
a***
b**
c***
c***
Age (yr)
Complex III
Porin
Complex IV
(% change)
a*
c**
c**
Age (yr)
Complex IV
Porin
Complex V
(% change)
Age (yr)
Complex V
Porin
AIF
(% change)
a**
b*
b*
Age (yr)
AIF
Porin

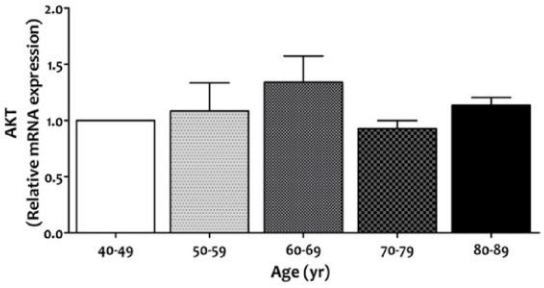

REST
(% change)
2.5
2.0
1.5
1.0
0.5
0.0
40-49
50-59
60-69
70-79
80-89
Age (yr)

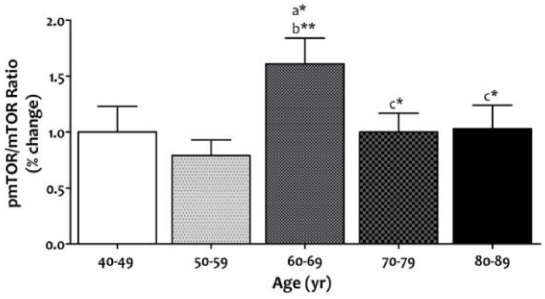
REST
Actin

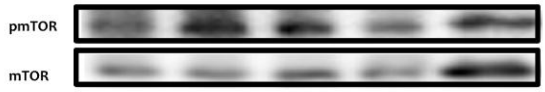

REST
(Relative mRNA expression)
2.0
1.5
1.0
0.5
0.0
40-49
50-59
60-69
70-79
80-89
Age (yr)

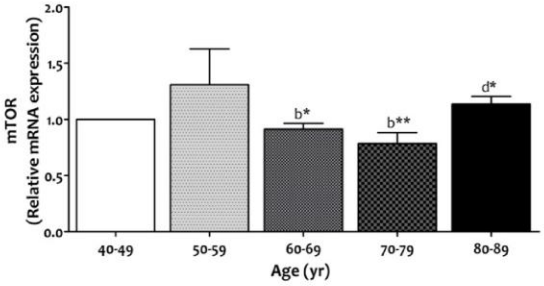

pAKT/AKT Ratio
(% change)
10
8
6
4
2
0
a**
a**
40-49
50-59
60-69
70-79
80-89
Age (yr)

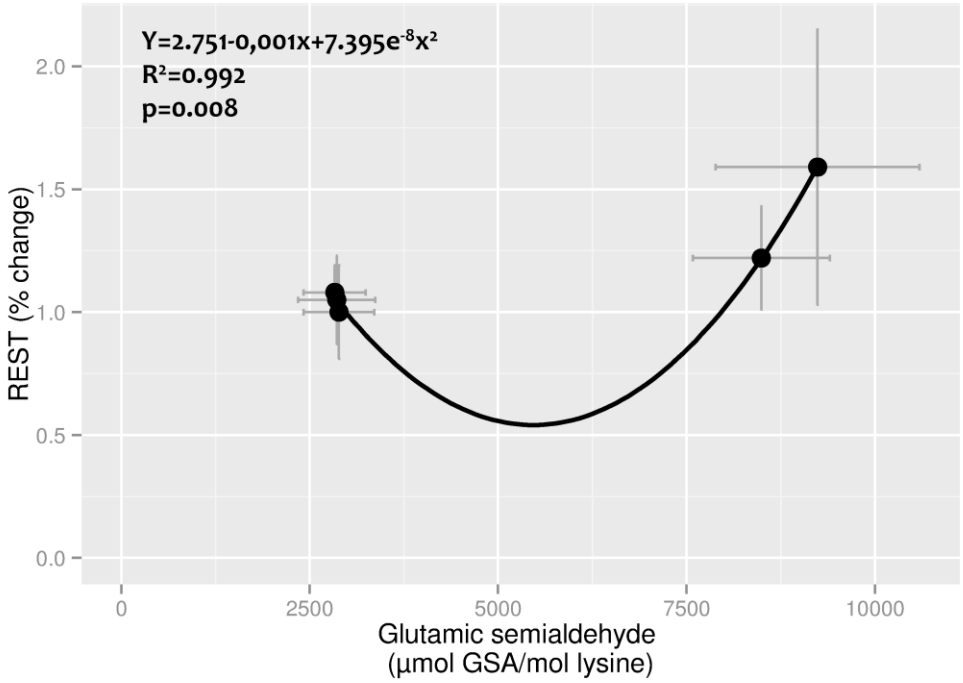

pAKT
AKT

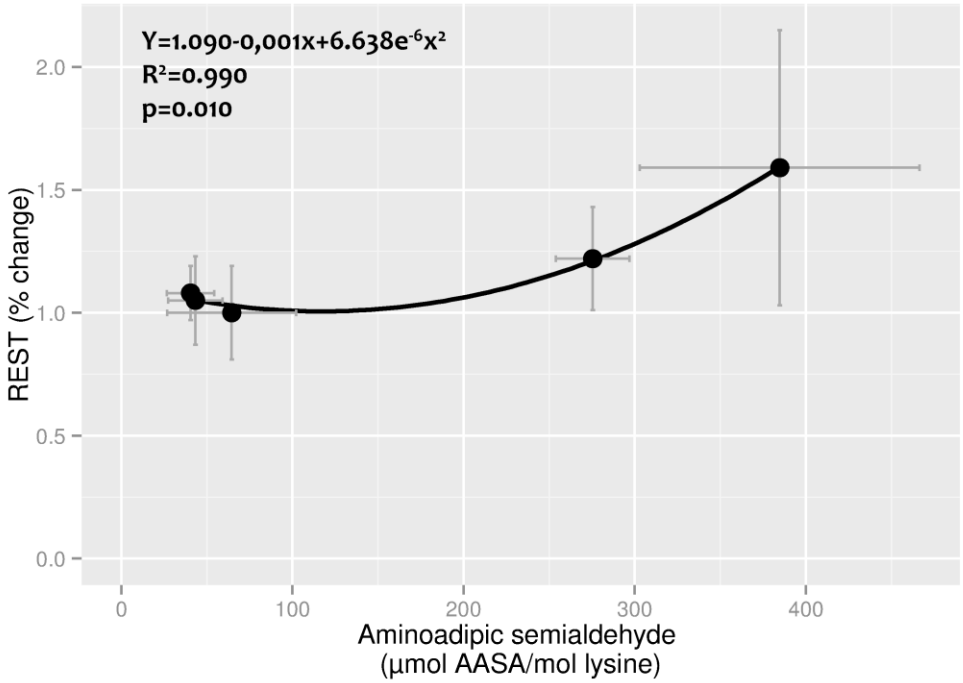

AKT
(Relative mRNA expression)
2.0
1.5
1.0
0.5
0.0
40-49
50-59
60-69
70-79
80-89
Age (yr)


pmTOR/mTOR Ratio
(% change)
2.0
1.5
1.0
0.5
0.0
a*
b**
c*
c*
40-49
50-59
60-69
70-79
80-89
Age (yr)


pmTOR
mTOR


mTOR
(Relative mRNA expression)
2.0
1.5
1.0
0.5
0.0
b*
b**
d*
40-49
50-59
60-69
70-79
80-89
Age (yr)


Y=2.751-0,001x+7.395e-8x²
R²=0.992
p=0.008
REST (% change)
2.0
1.5
1.0
0.5
0.0
0
2500
5000
7500
10000
Glutamic semialdehyde
(µmol GSA/mol lysine)


Y=1.090-0,001x+6.638e-6x²
R²=0.990
p=0.010
REST (% change)
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
Aminoadipic semialdehyde
(µmol AASA/mol lysine)