Lipoxidation End-Product Malondialdehyde-Lysine in Aging and Longevity

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The nonenzymatic adduction of malondialdehyde (MDA) to the protein amino groups leads to the formation of malondialdehyde-lysine (MDALys). The degree of unsaturation of biological membranes and the intracellular oxidative conditions are the main factors that modulate MDALys formation. The low concentration of this modification in the different cellular components, found in a wide diversity of tissues and animal species, is indicative of the presence of a complex network of cellular protection mechanisms that avoid its cytotoxic effects.

advanced lipoxidation end-products aging carbonyl-amine reaction cytotoxicity dietary restriction longevity metabolism reactive carbonyl species

1. Introduction

An enzymatic post-translational modification (PTM) is a chemical modification of one or more amino acids of a protein in a given biological system [1]. These modifications can be, either irreversible or reversible. Examples of PTMs are protein acetylation, glycosylation, methylation, phosphorylation, sumoylation, and ubiquitylation. Indeed, several hundred types of enzymatic PTMs have been described as affecting a significant portion of the cell proteome [2]. PTMs alter the structure and function of proteins in the cells [1]. All cells of living organisms utilize the PTMs to control their signaling networks and physiological processes, further expanding their protein functions. Among these functions are: Determination or regulation of catalytic activity, interaction with ligands, protein-protein interaction, protein folding, protein turnover, signaling function, and targeting specific subcellular compartments [1][3][4].

In contrast, a non-enzymatic PTM is a chemical modification, reversible or irreversible, mediated by reactive compounds on one or more amino acids of proteins, but as an inescapable event of endogenous chemical cell damage. Examples of non-enzymatic PTMs are glycation, glycoxidation, nitrosylation, oxidation, succination, and lipoxidation [5][6][7].

2. The Protein Adduct Malondialdehyde-Lysine

The reaction of malondialdehyde (MDA) with the amino group of the side chain of lysine (Lys) residues in proteins via a Schiff base reaction (reversible covalent adduct) leads to the formation of the malondialdehyde-lysine

(MDALys) adduct and the lys-MDA-lys cross-link [8][9][10][11][12][13][14][15]. MDA can also generate a Lys fluorescent adduct and Arg–Lys cross-link adduct [10][13][16][17][18]. This non-enzymatic reaction is called protein lipoxidation [5] and the generated products are called Advanced Lipoxidation End-products (ALEs) [5][19].

Although, MDA can be also generated enzymatically as a byproduct of the cyclooxygenase reaction in thromboxane and prostaglandin biosynthesis, this compound results mainly from the oxidative degradation of polyunsaturated fatty acids (PUFAs), being arachidonic (20:4n6) and docosahexaenoic (22:6n3) acids the main precursors [20]. In biological systems, MDA is a product of lipid peroxidation of cell membranes as a consequence of reaction of PUFAs and radical species [20][21][22][23]. Therefore, lipid peroxidation generates hydroperoxides, which undergo fragmentation to produce the reactive intermediate with three carbons in length called MDA [20]. More specifically, MDA is a reactive di-aldehydes (alkanedial) characterized by two carbonyl groups (the common group R-CHO consisting of a carbonyl center bonded to hydrogen), able to form two Schiff bases.

MDA is a ubiquitously generated product since lipid rich bilayers - for example both plasma and mitochondrial membranes - are present in all cells and provide an optimal environment for producing a large abundance of this compound. Compared with reactive oxygen species (ROS), MDA has a relatively long half-life (minutes-hours) and a non-charged structure, which makes it a potentially more destructive compound. The reason is that MDA can affect cell structures, located in its vicinity, and also distant macromolecular targets from the MDA source [24].

Under physiological conditions, MDA is not a highly reactive compound, increasing its reactivity at lower pH. At strong acidic conditions, MDA can react with amino acids such as glycine, leucine, valine, and the guanidino group of arginine, to yield different adducts. In vitro experiments, with conditions more similar to physiological ones, showed that MDA can react with a broad variety of amino acids such as histidine, tyrosine, and arginine exclusively at the alpha amino group, and even cysteine. However, the reaction of MDA with cysteine at pH 7.4 is virtually non-existent. Findings from different experimental conditions indicate that MDA is low reactive, and its reaction with the alpha-amino groups of amino acids is in fact not favored [8][9][20]. In the reaction of MDA with secondary amines, the epsilon amino group of lysine is the main target [10][20]. In comparison with free amino acids, proteins seem to be more readily modified by MDA under physiological conditions, probably due to the more favorable (but undetermined) environmental conditions provided by proteins [9][10][20]. In vitro experiments incubating MDA with proteins, such as albumin and RNase, demonstrated that the free epsilon-amino group of lysine is the main target of MDA, although other amino acids like histidine, tyrosine, arginine, and methionine might also be altered to some extent [9][10][11][20]. In vivo, the main compounds detected, characterized, and quantified in proteins are the adduct MDALys and the cross-link lys-MDA-lys (see next sections).

MDA can generate a diversity of adducts and intra- and inter-molecular cross-links resulting of the chemical and non-enzymatic modification of nucleophilic groups in macromolecules like proteins, but also nucleic acids and aminophospholipids (phosphatidylethanolamine (PE) and phosphatidylserine (PS)). Therefore, MDA can also react with the exocyclic amino groups of nucleosides to form alkylated products. Among DNA bases, the high nucleophilicity of guanine results in a higher vulnerability to generate adducts, MDA-deoxyguanosine (M1dG) being

the most common [25][26][27]. Finally, MDA can also react with amino groups of aminophospholipids to generate adducts as MDA-PE [28].

Considering the origin of MDALys, it is plausible to postulate that MDALys is an integrator biomarker of oxidative stress and lipid peroxidation [11][14][19][20][29].

3. Methods to Detect and Quantify MDALys in Proteins

Although the most used methods to detect MDALys adducts are based on mass spectrometry (MS)-based and antibody-based techniques (for more details, see [15]), other approaches which are currently in use are fluorescence-based methods [30][31], high-performance liquid chromatography (HPLC) [11], or nuclear magnetic resonance [13]. MS is the preferred method for the detection of MDA-dG detection, whereas MDA-aminophospholipids are analyzed using both fluorescence and MS based methods [28].

When an antibody-based technique is chosen, the recognition site is crucial. Thus, there are a number of antibodies (monoclonal and polyclonal) that recognized MDA-modified proteins which have been used for immunohistochemistry [32][33][34], immunoblot analysis [35][36][37][38][39], two-dimensional PAGE analysis [40], and ELISA [34][41][42]. All these approaches have the limitation that they do not per se provide information about the specific modified protein or the precise site of modification within the protein [15]. However, its excellent sensitivity allows a semi-quantitative approach to the degree of protein modification in a biological system, as well as visualizing the selectivity of modified proteins, which will require additional methods for their identification.

Other techniques, such as liquid chromatography-electrospray ionization or matrix-assisted laser desorption/ionization-MS (LC-ESI or MALDI-MS)-based proteomics analysis would be useful to overcome these limitations and obtain information about the specific site of MDA adducts formation [15][43]. This approach would be an excellent source of information in order to learn about the molecular mechanism, features related to specificity, and the meaning of the non-enzymatic modification.

4. The MDA-Lipoxidized Proteome

Despite the fact that any protein is potentially a target for modification by MDA – as studies carried out in vitro in proteins, such as hemoglobin, albumin, RNase, insulin B-chain, spermidine, and ubiquitin, among others, seem to suggest – our knowledge on the MDA-modified proteome is currently very limited, and is restricted to studies basically carried out in human brain and plasma.

Although MDA-modified proteins are mainly located in the mitochondria, they are also described in other locations like nucleus, cytosol and cell membrane, as well as extracellular compartments such as plasma. These observations demonstrate the facility of migration of MDA due to their chemical traits.

The analysis of the MDA-lipoxidized proteome indicates that these modifications are not specific of a biological process or molecular function and suggest a wide-ranging effect of this product in cell structure and metabolism. Therefore, proteins involved in energy metabolism (glycolysis, TCA cycle, oxidative phosphorylation, energy transduction, and fatty acid beta-oxidation), cytoskeleton, neurotransmission, proteostasis, plasma transport, and structural components of extracellular matrix are modified. These results reinforce the heterogeneity of this specific PTM and suggest that not all Lys residues can interact and react with MDA to generate MDALys. In line with this, it can be postulated that the specific structural traits and spatial location of Lys residues determine the generation of MDALys and, consequently, the molecular damage.

5. Metabolism of MDA-Modified Proteins

The MDALys concentration of a particular cell type, or even of a specific subcellular compartment, tissue, organ or animal species, is the result of a complex system of interactions with the participation of multiple mechanisms. As a starting point, there are two determining factors: the lipid composition of a cell membrane, and the homeostasis of oxidative stress. In relation to membrane lipids, we know that there is a direct relationship between the degree of unsaturation and their susceptibility to lipid peroxidation [24]. Therefore, the cell tries to maintain the integrity of the membrane without giving up its composition by using defense, repair, and replacement systems to reduce its vulnerability and the impact of oxidative stress [44][45]. This will determine the MDA levels generated. Regarding oxidative stress, the net flow of free radicals generated at the mitochondrial level plays a key role since these free radicals are responsible for damaging cellular components and, among them, lipids. These two biological characteristics are determinants of the MDA levels generated [20][24], and both traits are related to the aging process and the determination of longevity [45][46][47][48].

Once MDA is formed, a cellular response to maintain basal concentration of this and other aldehydes within physiological limits is initiated. This adaptive response involves different mechanisms, such as enzyme-mediated detoxification, urinary excretion, and antioxidant responses [23][24]. In relation to the latter, carbonyl species can work by sending regulatory signals to activate specific protein targets in order to decrease lipoxidation-derived damage and improve antioxidant defenses. This adaptive response is, at least, partially mediated by the carbonyl compound hydroxynonenal that (i) modifies and activates the uncoupling proteins (UCPs), resulting in a reduction in mitochondrial ROS production [49]; and (ii) induces 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 [50][51][52]. To date, this hydroxynonenal mediated antioxidant response is not described in MDA. In fact, the low or null reactivity of MDA with cysteine suggests that MDA does not have a regulatory signal activity. Although, the potential effects under special conditions of concentration or environment cannot be dismissed, it seems that MDA possesses a preponderantly cytotoxic role instead of a regulatory function. Furthermore, the non-reactivity of MDA toward glutathione significantly limits the capacity of enzymes designed to detoxify carbonyl compounds via conjugation with glutathione (GSH) to degrade it. Consequently, MDA demands alternative

enzymatic detoxification ways. Among them are the enzymes aldehyde dehydrogenase and aldoketoreductases [20][53][54], which participate in the maintenance of MDA levels within physiological levels.

The fact that MDALys is an adduct or a cross-link depends on whether the protein can be degraded [55] or accumulated forming aggregates. In line with this, evidence showed that MDALys can be degraded, as it has been detected in the urine of various animal species, such as mice, rats and humans [20][55][56][57][58]. Therefore, the balance between all these factors will determine the concentration of MDALys in a given biological system.

6. Cytotoxic Effects of MDALys Adducts

The molecular consequences of MDALys adducts formation in proteins mostly include detrimental structural and functional changes. Thus, MDALys formation induces alterations in physico-chemical properties such as conformation [5], charge [59], and solubility [60], formation of intra- and inter-molecular protein cross-links and aggregates [11][60][61], loss of enzymatic activity [15][62], and accelerated rate (for MDALys) or resistance to proteolysis (cross-links) [55]. When biological effects are considered, deleterious consequences such as immunogenicity (MDA generates immunoreactive materials in proteins) [41][61], binder to the receptor for advanced glycation end-products (RAGE) [59], and induction of monocyte activation and vascular complications [63] have been described. Additionally, MDA-adducts formation on nucleic acids induces DNA damage and mutagenesis [25][26][27], as well as alterations in physico-chemical and biological properties of the lipid bilayer when aminophospholipids are modified [28]. As an in vivo example of their cytotoxic effects at cellular level, a relevant recent study demonstrated that MDA causes neuronal mitochondrial dysfunction by directly promoting ROS generation and modifying mitochondrial proteins [64].

7. Protein Lipoxidation by MDA in Physiological and Pathological Models

Previous data showed that MDA-lipoxidation is detected in all tissues analyzed using immunoblotting and MS techniques, although the degree of modification varies significantly among them. In line with this, the presence of MDALys was described at both mitochondrial and tissue levels in a diversity of animal species. A relation of tissues, includes the brain, heart, liver, kidney, skeletal muscle, and plasma.

Notably, the higher MDALys concentration seems to be more present at mitochondrial levels in comparison to the tissue as a whole, probably as an expression of specific traits in mitochondria (high lipid content, high degree of membrane unsaturation, and high free radical generation) favoring MDALys formation. Likewise, it is remarkable that the higher MDALys concentration is reported in long-lived tissues like brain and heart which, in addition, have higher energy demands, and share identical traits to mitochondria: High lipid content, high degree of unsaturation, and high flux of mitochondrial free radical generation.

During the last 30 years, this post-translational modification has also been detected in several pathological models including metabolic diseases such as chronic iron overload [65], metabolic syndrome [66], and type 2 diabetes and

its complications [42][67][68][69]; in vascular diseases like atherosclerosis [70][71][72]; and in a diversity of neurodegenerative diseases such as Alzheimer's disease [32][40], Incidental Lewy Body Disease [33], Creutzfeldt-Jakob Disease [73], Pick's disease [36], Lewy Body diseases [60], familial Parkinson's disease [74], and X-adrenoleukodystrophy [75][76]. In all these cases the pathological state presented increased steady-state levels of MDALys ascribed to alteration in lipid profiles and/or oxidative stress.

Together, these findings indicate that increased MDALys content in tissue proteins is a direct consequence of increased intracellular MDA concentration. For this reason, MDALys can be proposed as a biomarker of lipoxidative stress.

8. Malondialdehyde-Lysine in Aging and Longevity

Studies based on different experimental paradigms link MDALys to aging and longevity. These paradigms include studies performed (i) On individuals during aging, (ii) using species with different longevity, or specific strains and mutants within a species with different species, and (iii) applying physiological treatments that modified the aging rate and longevity.

8.1. Changes in MDALys During Aging

There are several studies that directly relate the aging process with MDA lipoxidation-derived molecular damage accumulation. Globally, this lipoxidation damage, measured in tissue homogenate and mitochondria, increases with age, with tissues composed of long-lived, postmitotic cells (brain, heart, and skeletal muscle) being the most affected. Notably, in reference to the brain, the accumulation of MDALys seems to be region-specific but the reasons for this fact remain to be elucidated. Reinforcing these findings, lipofuscin, a complex age-pigment derived from lipoxidation reactions (including reactions derived from MDA) and considered a hallmark of aging [30], also shows an accumulation that correlates with age [77][78]. This age-related accrual of molecular oxidative damage is concomitant with the increase PUFA content during aging described in several tissues and animal species, as well as detrimental changes in oxidative stress, both factors favoring lipid peroxidation and the subsequent formation of the lipoxidation derived products MDALys. This increase in MDALys concentration at the tissue level can also be extended to changes with age in MDA-dG [79], and MDA-aminophospholipids (reviewed in [28]). Importantly, the degree of change with age varies in a tissue-dependent way. The changes described could occur on the basis of the age-related changes in membrane physico-chemical properties, such as fluidity leading to an increased membrane rigidity and loss of function, which has been systematically described in diverse studies [28][80][81][82][83][84]. As a consequence of this accumulation of MDALys with age, and considering the selective pool of proteins that seem to be modified, it can be proposed that specific cellular biological processes such as energy production and proteostasis, biological functions, which require the participation of cytoskeleton, and functional properties of the extracellular matrix may be preferentially affected during aging by this nonenzymatic modification. The answer as to whether the link between MDALys and agingg is causation or correlation requires of additional studies, but it must be considered that MDALys is only one of many indicators of more widespread chemical damage in biological systems.

8.2. MDALys and Animal Longevity

The connection between lipoxidation-derived damage and animal longevity was first reported by Pamplona and collaborators [85]. The study demonstrated that the MDALys concentration of heart mitochondria from a long-lived pigeon was significantly lower than the one detected in a short-lived rat. Later, these results were observed in a wide range of tissues and animal species, including both vertebrates (mammals and birds) and invertebrates, where a lower concentration of MDALys at both, mitochondria and tissue was detected in long-lived vertebrates (birds and mammals), compared to short-lived ones. In agreement with this, it was described that longevous species have a low degree of total tissue and mitochondrial fatty acid unsaturation resulting in a low sensitivity to in vivo and in vitro lipid peroxidation and a low mitochondrial free radical production [24][86][87]. Furthermore, lipofuscin also showed an accumulation rate that inversely correlates with longevity [78]. Altogether, these results suggest that the lipoxidative damage is an optimized feature associated with animal longevity.

8.3. MDALys in Experimental Studies of Longevity Extension by Nutritional and Pharmacological Interventions

The study of how “anti-aging” nutritional and pharmacological interventions affect the lipoxidation-derived molecular damage associated with aging rate and longevity is crucial to establishing a causative role for membranes, oxidative stress and lipoxidation-derived damage in the determination of longevity. In line with this, several studies applying dietary modification have been performed, and membrane fatty acid composition and lipoxidative-derived damage have been evaluated [88][89][90]. These studies were specifically designed to partially circumvent the homeostatic system of compensation of dietary-induced changes in membrane unsaturation which operates at cellular level. The findings obtained demonstrate that lowering the membrane unsaturation of cellular membranes protects tissues against lipid peroxidation and MDALys formation.

Available evidence from both nutritional and pharmacological interventions that extend longevity in experimental models reinforces the relationship between MDA-derived lipoxidation damage and longevity. So, dietary interventions such as caloric- (CR), protein- (PR) and methionine (MetR) restriction and pharmacological interventions such as rapamycin decreased the degree of membrane unsaturation (reviewed in [48][83][91][92]) and especially the level of MDALys in a variety of tissues (for instance, liver, heart, and brain) and animal species (mainly rodents). Furthermore, it has been demonstrated that CR is able to reduce the levels of lipofuscin in tissues of rodents and *C. elegans* [78][93][94][95][96].

References

1. Boyer, R. Posttranslational modification of proteins: Expanding nature’s inventory. Christopher T. Walsh, Roberts & Company Publishers, Greenwood Village, CO, 2005, 576 pp., ISBN 0-9747077-3-2, $98.00. Biochem. Mol. Biol. Educ. 2006, 34, 461–462.

2. Lothrop, A.P.; Torres, M.P.; Fuchs, S.M. Deciphering post-translational modification codes. FEBS Lett. 2013, 587, 1247–1257.
3. Prabakaran, S.; Lippens, G.; Steen, H.; Gunawardena, J. Post-translational modification: Nature's escape from genetic imprisonment and the basis for dynamic information encoding. Wiley Interdiscip. Rev. Syst. Biol. Med. 2012, 4, 565–583.
4. Santos, A.L.; Lindner, A.B. Protein Posttranslational Modifications: Roles in Aging and Age-Related Disease. Oxid. Med. Cell. Longev. 2017, 2017, 5716409.
5. Thorpe, S.R.; Baynes, J.W. Maillard reaction products in tissue proteins: New products and new perspectives. Amino Acids 2003, 25, 275–281.
6. Golubev, A.; Hanson, A.D.; Gladyshev, V.N. Non-enzymatic molecular damage as a prototypic driver of aging. J. Biol. Chem. 2017, 292, 6029–6038.
7. Jové, M.; Pradas, I.; Mota-Martorell, N.; Cabré, R.; Ayala, V.; Ferrer, I.; Pamplona, R. Succination of Protein Thiols in Human Brain Aging. Front. Aging Neurosci. 2020, 12, 52.
8. Chio, K.S.; Tappel, A.L. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. Biochemistry 1969, 8, 2827–2832.
9. Chio, K.S.; Tappel, A.L. Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids. Biochemistry 1969, 8, 2821–2826.
10. Kikugawa, K.; Beppu, M. Involvement of lipid oxidation products in the formation of fluorescent and cross-linked proteins. Chem. Phys. Lipids 1987, 44, 277–296.
11. Requena, J.R.; Fu, M.X.; Ahmed, M.U.; Jenkins, A.J.; Lyons, T.J.; Baynes, J.W.; Thorpe, S.R. Quantification of malondialdehyde and 4-hydroxynonenal adducts to lysine residues in native and oxidized human low-density lipoprotein. Biochem. J. 1997, 322 Pt 1, 317–325.
12. Miyata, T.; Inagi, R.; Asahi, K.; Yamada, Y.; Horie, K.; Sakai, H.; Uchida, K.; Kurokawa, K. Generation of protein carbonyls by glycoxidation and lipoxidation reactions with autoxidation products of ascorbic acid and polyunsaturated fatty acids. FEBS Lett. 1998, 437, 24–28.
13. Slatter, D.A.; Murray, M.; Bailey, A.J. Formation of a dihydropyridine derivative as a potential cross-link derived from malondialdehyde in physiological systems. FEBS Lett. 1998, 421, 180–184.
14. Refsgaard, H.H.; Tsai, L.; Stadtman, E.R. Modifications of proteins by polyunsaturated fatty acid peroxidation products. Proc. Natl. Acad. Sci. USA 2000, 97, 611–616.
15. Domingues, R.M.; Domingues, P.; Melo, T.; Pérez-Sala, D.; Reis, A.; Spickett, C.M. Lipoxidation adducts with peptides and proteins: Deleterious modifications or signaling mechanisms? J. Proteomics 2013, 92, 110–131.

16. Nalr, V.; Offerman, R.J.; Turner, G.A.; Pryor, A.N.; Baenzlger, N.C. Fluorescent 1.4-Dihydropyridines: The Malondlaldehyde connection. Tetrahedron 1988, 44, 2793–2803.
17. Itakura, K.; Uchida, K.; Osawa, T. A novel fluorescent malondialdehyde-lysine adduct. Chem. Phys. Lipids 1996, 84, 75–79.
18. Slatter, D.A.; Avery, N.C.; Bailey, A.J. Identification of a new cross-link and unique histidine adduct from bovine serum albumin incubated with malondialdehyde. J. Biol. Chem. 2004, 279, 61–69.
19. Pamplona, R. Advanced lipoxidation end-products. Chem. Biol. Interact. 2011, 192, 14–20.
20. Esterbauer, H.; Schaur, R.J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 1991, 11, 81–128.
21. Catalá, A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem. Phys. Lipids 2009, 157, 1–11.
22. Zimniak, P. Relationship of electrophilic stress to aging. Free Radic. Biol. Med. 2011, 51, 1087–1105.
23. Fritz, K.S.; Petersen, D.R. An overview of the chemistry and biology of reactive aldehydes. Free Radic. Biol. Med. 2013, 59, 85–91.
24. Pamplona, R. Membrane phospholipids, lipoxidative damage and molecular integrity: A causal role in aging and longevity. Biochim. Biophys. Acta 2008, 1777, 1249–1262.
25. Marnett, L.J. Oxy radicals, lipid peroxidation and DNA damage. Toxicology 2002, 181–182, 219–222.
26. Marnett, L.J.; Riggins, J.N.; West, J.D. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. J. Clin. Investig. 2003, 111, 583–593.
27. Wauchope, O.R.; Mitchener, M.M.; Beavers, W.N.; Galligan, J.J.; Camarillo, J.M.; Sanders, W.D.; Kingsley, P.J.; Shim, H.-N.; Blackwell, T.; Luong, T.; et al. Oxidative stress increases M1dG, a major peroxidation-derived DNA adduct, in mitochondrial DNA. Nucleic Acids Res. 2018, 46, 3458–3467.
28. Naudí, A.; Jové, M.; Ayala, V.; Cabré, R.; Portero-Otín, M.; Pamplona, R. Non-enzymatic modification of aminophospholipids by carbonyl-amine reactions. Int. J. Mol. Sci. 2013, 14, 3285–3313.
29. Hartley, D.P.; Kroll, D.J.; Petersen, D.R. Prooxidant-initiated lipid peroxidation in isolated rat hepatocytes: Detection of 4-hydroxynonenal- and malondialdehyde-protein adducts. Chem. Res. Toxicol. 1997, 10, 895–905.

30. Uchida, K. Lipofuscin-like fluorophores originated from malondialdehyde. Free Radic. Res. 2006, 40, 1335–1338.
31. Estévez, M.; Padilla, P.; Carvalho, L.; Martín, L.; Carrapiso, A.; Delgado, J. Malondialdehyde interferes with the formation and detection of primary carbonyls in oxidized proteins. Redox Biol. 2019, 26, 101277.
32. Dei, R.; Takeda, A.; Niwa, H.; Li, M.; Nakagomi, Y.; Watanabe, M.; Inagaki, T.; Washimi, Y.; Yasuda, Y.; Horie, K.; et al. Lipid peroxidation and advanced glycation end products in the brain in normal aging and in Alzheimer's disease. Acta Neuropathol. 2002, 104, 113–122.
33. Dalfó, E.; Portero-Otín, M.; Ayala, V.; Martínez, A.; Pamplona, R.; Ferrer, I. Evidence of oxidative stress in the neocortex in incidental Lewy body disease. J. Neuropathol. Exp. Neurol. 2005, 64, 816–830.
34. Ishii, T.; Kumazawa, S.; Sakurai, T.; Nakayama, T.; Uchida, K. Mass spectroscopic characterization of protein modification by malondialdehyde. Chem. Res. Toxicol. 2006, 19, 122–129.
35. Pamplona, R.; Portero-Otín, M.; Sanz, A.; Ayala, V.; Vasileva, E.; Barja, G. Protein and lipid oxidative damage and complex I content are lower in the brain of budgerigar and canaries than in mice. Relation to aging rate. Age (Dordr) 2005, 27, 267–280.
36. Muntané, G.; Dalfó, E.; Martínez, A.; Rey, M.J.; Avila, J.; Pérez, M.; Portero, M.; Pamplona, R.; Ayala, V.; Ferrer, I. Glial fibrillary acidic protein is a major target of glycoxidative and lipoxidative damage in Pick's disease. J. Neurochem. 2006, 99, 177–185.
37. Kichev, A.; Ilieva, E.V.; Piñol-Ripoll, G.; Podlesniy, P.; Ferrer, I.; Portero-Otín, M.; Pamplona, R.; Espinet, C. Cell death and learning impairment in mice caused by in vitro modified pro-NGF can be related to its increased oxidative modifications in Alzheimer disease. Am. J. Pathol. 2009, 175, 2574–2585.
38. Ilieva, E.V.; Kichev, A.; Naudí, A.; Ferrer, I.; Pamplona, R.; Portero-Otín, M. Mitochondrial dysfunction and oxidative and endoplasmic reticulum stress in argyrophilic grain disease. J. Neuropathol. Exp. Neurol. 2011, 70, 253–263.
39. Domínguez-González, M.; Puigpinós, M.; Jové, M.; Naudi, A.; Portero-Otín, M.; Pamplona, R.; Ferrer, I. Regional vulnerability to lipoxidative damage and inflammation in normal human brain aging. Exp. Gerontol. 2018, 111, 218–228.
40. Pamplona, R.; Dalfó, E.; Ayala, V.; Bellmunt, M.J.; Prat, J.; Ferrer, I.; Portero-Otín, M. Proteins in human brain cortex are modified by oxidation, glycoxidation, and lipoxidation. Effects of Alzheimer disease and identification of lipoxidation targets. J. Biol. Chem. 2005, 280, 21522–21530.
41. Uchida, K.; Sakai, K.; Itakura, K.; Osawa, T.; Toyokuni, S. Protein modification by lipid peroxidation products: Formation of malondialdehyde-derived N(epsilon)-(2-propenol)lysine in

proteins. Arch. Biochem. Biophys. 1997, 346, 45–52.

42. Choudhuri, S.; Roy, P.K.; Mitra, B.; Sen, S.; Sarkar, R.; Das, M.; Biswas, D.; Ghosh, P.; Biswas, A.; Chakraborty, S.; et al. Hyperlipidemia-Mediated Increased Advanced Lipoxidation End Products Formation, an Important Factor Associated with Decreased Erythrocyte Glucose-6-Phosphate Dehydrogenase Activity in Mild Nonproliferative Diabetic Retinopathy. Can. J. diabetes 2017, 41, 82–89.
43. Weißer, J.; Ctortecka, C.; Busch, C.J.; Austin, S.R.; Nowikovsky, K.; Uchida, K.; Binder, C.J.; Bennett, K.L. A Comprehensive Analytical Strategy To Identify Malondialdehyde-Modified Proteins and Peptides. Anal. Chem. 2017, 89, 3847–3852.
44. Hagen, R.M.; Rodriguez-Cuenca, S.; Vidal-Puig, A. An allostatic control of membrane lipid composition by SREBP1. FEBS Lett. 2010, 584, 2689–2698.
45. Pamplona, R.; Costantini, D. Molecular and structural antioxidant defenses against oxidative stress in animals. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2011, 301, R843–R863.
46. Pamplona, R.; Barja, G. Highly resistant macromolecular components and low rate of generation of endogenous damage: Two key traits of longevity. Ageing Res. Rev. 2007, 6, 189–210.
47. Barja, G. The mitochondrial free radical theory of aging. Prog. Mol. Biol. Transl. Sci. 2014, 127, 1–27.
48. Jové, M.; Mota-Martorell, N.; Pradas, I.; Galo-Licona, J.D.; Martín-Gari, M.; Obis, È.; Sol, J.; Pamplona, R. The Lipidome Fingerprint of Longevity. Molecules 2020, 25, 4343.
49. Echtay, K.S.; Esteves, T.C.; Pakay, J.L.; Jekabsons, M.B.; Lambert, A.J.; Portero-Otín, M.; Pamplona, R.; Vidal-Puig, A.J.; Wang, S.; Roebuck, S.J.; et al. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. EMBO J. 2003, 22, 4103–4110.
50. Conrad, M.; Schneider, M.; Seiler, A.; Bornkamm, G.W. Physiological role of phospholipid hydroperoxide glutathione peroxidase in mammals. Biol. Chem. 2007, 388, 1019–1025.
51. Maher, J.; Yamamoto, M. The rise of antioxidant signaling--the evolution and hormetic actions of Nrf2. Toxicol. Appl. Pharmacol. 2010, 244, 4–15.
52. Casañas-Sánchez, V.; Pérez, J.A.; Fabelo, N.; Quinto-Alemany, D.; Díaz, M.L. Docosahexaenoic (DHA) modulates phospholipid-hydroperoxide glutathione peroxidase (Gpx4) gene expression to ensure self-protection from oxidative damage in hippocampal cells. Front. Physiol. 2015, 6, 203.
53. Siu, G.M.; Draper, H.H. Metabolism of malonaldehyde in vivo and in vitro. Lipids 1982, 17, 349–355.
54. Marnett, L.J.; Buck, J.; Tuttle, M.A.; Basu, A.K.; Bull, A.W. Distribution and oxidation of malondialdehyde in mice. Prostaglandins 1985, 30, 241–254.

55. Mahmoodi, H.; Hadley, M.; Chang, Y.X.; Draper, H.H. Increased formation and degradation of malondialdehyde-modified proteins under conditions of peroxidative stress. Lipids 1995, 30, 963–966.
56. Mcgirr, L.G.; Hadley, M.; Draper, H.H. Identification of N alpha-acetyl-epsilon-(2-propenal) lysine as a urinary metabolite of malondialdehyde. J. Biol. Chem. 1985, 260, 15427–15431.
57. Draper, H.H.; McGirr, L.G.; Hadley, M. The metabolism of malondialdehyde. Lipids 1986, 21, 305–307.
58. Agarwal, S.; Wee, J.J.; Hadley, M.; Draper, H.H. Identification of a deoxyguanosine-malondialdehyde adduct in rat and human urine. Lipids 1994, 29, 429–432.
59. Mol, M.; Degani, G.; Coppa, C.; Baron, G.; Popolo, L.; Carini, M.; Aldini, G.; Vistoli, G.; Altomare, A. Advanced lipoxidation end products (ALEs) as RAGE binders: Mass spectrometric and computational studies to explain the reasons why. Redox Biol. 2019, 23, 101083.
60. Dalfó, E.; Ferrer, I. Early alpha-synuclein lipoxidation in neocortex in Lewy body diseases. Neurobiol. Aging 2008, 29, 408–417.
61. Vay, D.; Parodi, M.; Rolla, R.; Mottaran, E.; Vidali, M.; Bellomo, G.; Albano, E. Circulating antibodies recognizing malondialdehyde-modified proteins in healthy subjects. Free Radic. Biol. Med. 2001, 30, 277–286.
62. Yarian, C.S.; Rebrin, I.; Sohal, R.S. Aconitase and ATP synthase are targets of malondialdehyde modification and undergo an age-related decrease in activity in mouse heart mitochondria. Biochem. Biophys. Res. Commun. 2005, 330, 151–156.
63. Shanmugam, N.; Figarola, J.L.; Li, Y.; Swiderski, P.M.; Rahbar, S.; Natarajan, R. Proinflammatory effects of advanced lipoxidation end products in monocytes. Diabetes 2008, 57, 879–888.
64. Long, J.; Liu, C.; Sun, L.; Gao, H.; Liu, J. Neuronal mitochondrial toxicity of malondialdehyde: Inhibitory effects on respiratory function and enzyme activities in rat brain mitochondria. Neurochem. Res. 2009, 34, 786–794.
65. Sochaski, M.A.; Bartfay, W.J.; Thorpe, S.R.; Baynes, J.W.; Bartfay, E.; Lehotay, D.C.; Liu, P.P. Lipid peroxidation and protein modification in a mouse model of chronic iron overload. Metabolism. 2002, 51, 645–651.
66. Zagol-Ikapite, I.; Sosa, I.R.; Oram, D.; Judd, A.; Amarnath, K.; Amarnath, V.; Stec, D.; Oates, J.A.; Boutaud, O. Modification of platelet proteins by malondialdehyde: Prevention by dicarbonyl scavengers. J. Lipid Res. 2015, 56, 2196–2205.
67. Lopes-Virella, M.F.; Hunt, K.J.; Baker, N.L.; Virella, G. VADT Group of Investigators High levels of AGE-LDL, and of IgG antibodies reacting with MDA-lysine epitopes expressed by oxLDL and

MDA-LDL in circulating immune complexes predict macroalbuminuria in patients with type 2 diabetes. J. Diabetes Complicat. 2016, 30, 693–699.

68. Miyata, T.; Fu, M.X.; Kurokawa, K.; van Ypersele de Strihou, C.; Thorpe, S.R.; Baynes, J.W. Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: Is there oxidative stress in uremia? Kidney Int. 1998, 54, 1290–1295.
69. Miyata, T.; Sugiyama, S.; Suzuki, D.; Inagi, R.; Kurokawa, K. Increased carbonyl modification by lipids and carbohydrates in diabetic nephropathy. Kidney Int. Suppl. 1999, 71, S54–S56.
70. Yamada, S.; Kumazawa, S.; Ishii, T.; Nakayama, T.; Itakura, K.; Shibata, N.; Kobayashi, M.; Sakai, K.; Osawa, T.; Uchida, K. Immunochemical detection of a lipofuscin-like fluorophore derived from malondialdehyde and lysine. J. Lipid Res. 2001, 42, 1187–1196.
71. Kim, J.G.; Taylor, W.R.; Parthasarathy, S. Demonstration of the presence of lipid peroxide-modified proteins in human atherosclerotic lesions using a novel lipid peroxide-modified anti-peptide antibody. Atherosclerosis 1999, 143, 335–340.
72. Gonen, A.; Hansen, L.F.; Turner, W.W.; Montano, E.N.; Que, X.; Rafia, A.; Chou, M.-Y.; Wiesner, P.; Tsiantoulas, D.; Corr, M.; et al. Atheroprotective immunization with malondialdehyde-modified LDL is hapten specific and dependent on advanced MDA adducts: Implications for development of an atheroprotective vaccine. J. Lipid Res. 2014, 55, 2137–2155.
73. Freixes, M.; Rodríguez, A.; Dalfó, E.; Ferrer, I. Oxidation, glycoxidation, lipoxidation, nitration, and responses to oxidative stress in the cerebral cortex in Creutzfeldt-Jakob disease. Neurobiol. Aging 2006, 27, 1807–1815.
74. Gomez, A.; Ferrer, I. Involvement of the cerebral cortex in Parkinson disease linked with G2019S LRRK2 mutation without cognitive impairment. Acta Neuropathol. 2010, 120, 155–167.
75. Fourcade, S.; López-Erauskin, J.; Galino, J.; Duval, C.; Naudi, A.; Jove, M.; Kemp, S.; Villarroya, F.; Ferrer, I.; Pamplona, R.; et al. Early oxidative damage underlying neurodegeneration in X-adrenoleukodystrophy. Hum. Mol. Genet. 2008, 17, 1762–1773.
76. Morató, L.; Galino, J.; Ruiz, M.; Calingasan, N.Y.; Starkov, A.A.; Dumont, M.; Naudí, A.; Martínez, J.J.; Aubourg, P.; Portero-Otín, M.; et al. Pioglitazone halts axonal degeneration in a mouse model of X-linked adrenoleukodystrophy. Brain 2013, 136, 2432–2443.
77. Tsuchida, M.; Miura, T.; Aibara, K. Lipofuscin and lipofuscin-like substances. Chem. Phys. Lipids 1987, 44, 297–325.
78. Terman, A.; Brunk, U.T. Lipofuscin. Int. J. Biochem. Cell Biol. 2004, 36, 1400–1404.
79. Draper, H.H.; Agarwal, S.; Nelson, D.E.; Wee, J.J.; Ghoshal, A.K.; Farber, E. Effects of peroxidative stress and age on the concentration of a deoxyguanosine-malondialdehyde adduct in rat DNA. Lipids 1995, 30, 959–961.

80. Laganiere, S.; Yu, B.P. Anti-lipoperoxidation action of food restriction. Biochem. Biophys. Res. Commun. 1987, 145, 1185–1191.
81. Yu, B.P.; Suescun, E.A.; Yang, S.Y. Effect of age-related lipid peroxidation on membrane fluidity and phospholipase A2: Modulation by dietary restriction. Mech. Ageing Dev. 1992, 65, 17–33.
82. Laganiere, S.; Yu, B.P. Modulation of membrane phospholipid fatty acid composition by age and food restriction. Gerontology 1993, 39, 7–18.
83. Yu, B.P. Membrane alteration as a basis of aging and the protective effects of calorie restriction. Mech. Ageing Dev. 2005, 126, 1003–1010.
84. Hulbert, A.J.; Pamplona, R.; Buffenstein, R.; Buttemer, W.A. Life and death: Metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 2007, 87, 1175–1213.
85. Pamplona, R.; Portero-Otín, M.; Requena, J.R.; Thorpe, S.R.; Herrero, A.; Barja, G. A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria of the longevous pigeon than in the short-lived rat. Mech. Ageing Dev. 1999, 106, 283–296.
86. Pamplona, R.; Barja, G.; Portero-Otín, M. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: A homeoviscous-longevity adaptation? Ann. N. Y. Acad. Sci. 2002, 959, 475–490.
87. Pamplona, R.; Barja, G. An evolutionary comparative scan for longevity-related oxidative stress resistance mechanisms in homeotherms. Biogerontology 2011, 12, 409–435.
88. Herrero, A.; Portero-Otín, M.; Bellmunt, M.J.; Pamplona, R.; Barja, G. Effect of the degree of fatty acid unsaturation of rat heart mitochondria on their rates of H2O2 production and lipid and protein oxidative damage. Mech. Ageing Dev. 2001, 122, 427–443.
89. Portero-Otin, M.; Bellmunt, M.J.; Requena, J.R.; Pamplona, R. Protein modification by advanced Maillard adducts can be modulated by dietary polyunsaturated fatty acids. Biochem. Soc. Trans. 2003, 31, 1403–1405.
90. Pamplona, R.; Portero-Otin, M.; Sanz, A.; Requena, J.; Barja, G. Modification of the longevity-related degree of fatty acid unsaturation modulates oxidative damage to proteins and mitochondrial DNA in liver and brain. Exp. Gerontol. 2004, 39, 725–733.
91. Pamplona, R.; Barja, G. Mitochondrial oxidative stress, aging and caloric restriction: The protein and methionine connection. Biochim. Biophys. Acta 2006, 1757, 496–508.
92. Jové, M.; Ayala, V.; Ramírez-Núñez, O.; Naudí, A.; Cabré, R.; Spickett, C.M.; Portero-Otín, M.; Pamplona, R. Specific lipidome signatures in central nervous system from methionine-restricted mice. J. Proteome Res. 2013, 12, 2679–2689.

93. Enesco, H.E.; Kruk, P. Dietary restriction reduces fluorescent age pigment accumulation in mice. Exp. Gerontol. 1981, 16, 357–361.
94. De, A.K.; Chipalkatti, S.; Aiyar, A.S. Some biochemical parameters of ageing in relation to dietary protein. Mech. Ageing Dev. 1983, 21, 37–48.
95. Rao, G.; Xia, E.; Nadakavukaren, M.J.; Richardson, A. Effect of dietary restriction on the age-dependent changes in the expression of antioxidant enzymes in rat liver. J. Nutr. 1990, 120, 602–609.
96. Gerstbrein, B.; Stamatas, G.; Kollias, N.; Driscoll, M. In vivo spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in Caenorhabditis elegans. Aging Cell 2005, 4, 127–137.