Review

Oxidative Stress, Environmental Pollutants, Aging, and Epigenetic Regulation: Mechanistic Insights and Biomarker Advances

Minelly Gonzalez [†], Michael Powers [†], Luca Cucullo [*]

Posted Date: 1 April 2026

doi: 10.20944/preprints202604.0051.v1

Keywords: environment; pollutants; oxidative stress; epigenetics; mitochondria; inflammation; redox cycle; antioxidant; histone modifications; biomarkers; aging; multi-omics

 doi:10.20944/preprints202604.0051.v1

Review

Oxidative Stress, Environmental Pollutants, Aging, and Epigenetic Regulation: Mechanistic Insights and Biomarker Advances

Minelly Gonzalez [1,†], Michael Powers [1,†] and Luca Cucullo [2,*]

[1] Department of Biological and Biomedical Sciences, Oakland University, Rochester, MI 48309, USA.

[2] Department of Foundation Medical Studies, Oakland University William Beaumont School of Medicine, 586 Pioneer Dr, Rochester, MI 48309, USA

* Correspondence: lcucullo@oakland.edu; Tel.: (248) 370-3884; Fax: (248) 370-4060.

† These authors contributed equally to this work.

Abstract

Environmental pollutants, lifestyle factors, and intrinsic metabolic activity converge to generate an imbalance between reactive oxygen species (ROS) production and the capacity of antioxidant defense systems. This oxidative stress damages cellular macromolecules, reprograms gene expression, and accelerates aging. Here we synthesize recent evidence (2020–2025) on the mechanisms by which air pollutants, heavy metals, pesticides, nanoparticles, and microplastics generate ROS, how redox imbalance interfaces with epigenetic regulation, and how these processes drive aging and disease. Rather than condensing, we retain full mechanistic details from multiple recent reviews to provide a comprehensive resource. We also discuss emerging biomarkers, including redox-related metabolites and epigenetic signatures, and outline future directions for integrating multi-omics and artificial intelligence to develop personalized interventions. Citation numbering in the text follows the order of appearance.

Keywords: environment; pollutants; oxidative stress; epigenetics; mitochondria; inflammation; redox cycle; antioxidant; histone modifications; biomarkers; aging; multi-omics

1. Introduction

Oxidative stress describes an imbalance between the production of reactive oxygen and nitrogen species and the capacity of intracellular antioxidant systems to detoxify these radicals. This imbalance arises from normal metabolic processes such as mitochondrial electron transport, cytochrome P450 activity, and immune-cell NADPH oxidase activity but it is greatly amplified by exogenous insults, including airborne particulates, ozone, nitrogen dioxide, heavy metals, pesticides, and engineered nano- and micro-materials [1–3].

Rapid industrialization, urbanization, and climate change have increased human exposure to these pro-oxidant stimuli; epidemiological studies link chronic pollutant exposure with cardiopulmonary disease, neurodegeneration, metabolic disorders, infertility, and accelerated aging [4–6].

Within cells, excessive reactive species oxidize lipids, proteins, and nucleic acids, disrupt mitochondrial and endoplasmic reticulum function, activate pro-inflammatory signaling cascades, and perturb redox-sensitive transcription factors [7].

Beyond direct macromolecular damage, oxidative stress exerts long-lasting effects by modifying the epigenome: redox-dependent changes in DNA methylation, histone acetylation and methylation, and non-coding RNA expression reprogram gene expression and may mediate transgenerational susceptibility [8,9].

 doi:10.20944/preprints202604.0051.v1

Aging further exacerbates redox imbalance; mitochondrial dysfunction and declining NAD^+/NADH ratios increase basal ROS production and decrease antioxidant capacity, making aged individuals particularly vulnerable to pollutant-induced oxidative injury [10,11].

Understanding how diverse environmental pollutants induce oxidative stress, how redox imbalance interfaces with epigenetic regulation and aging, and how these processes can be monitored with sensitive biomarkers is therefore crucial for designing targeted interventions, guiding public health policy, and mitigating disease risk [12].

2. Mechanisms of Oxidative Stress Induced by Environmental Pollutants

2.1. Airborne Pollutants

Particulate matter and gases. Fine particulate matter ($PM_{2.5}$) and ultrafine particles (UFPs) contain transition metals and organic compounds that catalyze Fenton-type reactions, generating hydroxyl radicals and other ROS [13,14]. Their small size allows them to penetrate the alveolar barrier, deposit on mitochondria, and disrupt the electron transport chain (ETC), leading to superoxide overproduction and mitochondrial fragmentation [15]. Gaseous pollutants such as ozone oxidize surfactant lipids and deplete antioxidants in airway lining fluid [16]. At low doses, these oxidants activate adaptive pathways through the redox-sensitive transcription factor Nrf2, but persistent exposure overwhelms antioxidant defenses, causing NADPH oxidase (NOX) activation, lipid peroxidation, and pro-inflammatory NF-κB signaling [17,18].

Chronic exposure to traffic-related pollutants sustains alveolar macrophage and neutrophil activation. These immune cells produce ROS via NADPH oxidase and myeloperoxidase as part of host defense, but in the context of environmental particles, the oxidative burst damages lung tissue and propagates inflammation [19]. Particulate matter often carries polycyclic aromatic hydrocarbons (PAHs), dioxins, and transition metals adsorbed to its surface; once inhaled, these compounds dissolve in airway fluids, bind to cellular membranes, and generate secondary ROS [20]. Ozone (O_3) and nitrogen dioxide () react with unsaturated lipids and antioxidants in airway lining fluid to produce secondary oxidants such as peroxynitrite ($ONOO^-$) and lipid ozonides (reactive compounds formed when ozone attacks carbon–carbon double bonds in unsaturated lipids, creating unstable trioxolane structures that break down into aldehydes, ketones, and carboxylic acids) [21,22]. The resultant oxidative load compromises epithelial barrier integrity, increases vascular permeability, activates coagulation pathways, and promotes systemic inflammation [23].

Mitochondrial dysfunction and NOX activation. Mitochondria are a primary source of endogenous ROS and a major target of pollutant-induced damage. Exposure to airborne particulate matter disrupts mitochondrial homeostasis by impairing the ETC, leading to electron leakage and superoxide overproduction [24,25]. Pollutants regulate mitochondrial dynamics by increasing the expression of dynamin-related protein 1 (DRP1) and decreasing mitofusin 1/2 (MFN1/2) and optic atrophy protein 1 (OPA1), resulting in excessive fission and mitochondrial fragmentation [26]. ROS accumulation triggers mitochondrial permeability transition pore (mPTP) opening, leading to cytochrome c (Cyt C) release and caspase-dependent apoptosis [27]. Oxidative damage to mitochondrial DNA (mtDNA) impairs replication and transcription of ETC subunits, exacerbating electron leakage [28]. Inhibition of peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α) and nuclear respiratory factor 1 (NRF1) further impairs mitochondrial biogenesis [29], diminishing the cell's capacity to recover from oxidative insults. Transition metals within $PM_{2.5}$ catalyze Fenton and Haber–Weiss reactions, generating hydroxyl radicals that oxidize mitochondrial membranes and enzymes [30]. Chronic exposure leads to sustained mitochondrial ROS (mtROS) generation, establishing a self-amplifying oxidative loop that contributes to tissue injury and aging [31].

Beyond mitochondria, NADPH oxidases (NOX family enzymes) play a critical role in pollutant-induced ROS generation. $PM_{2.5}$ and ozone exposure activate NOX1, NOX2, and NOX4 in epithelial cells, macrophages, and endothelial tissues via Toll-like receptor (TLR) and MAPK signaling [32].

 doi:10.20944/preprints202604.0051.v1

This activation converts molecular oxygen into superoxide, which reacts with nitric oxide to form peroxynitrite, a highly reactive nitrating agent [33]. NOX-derived ROS initiates oxidative modification of proteins, lipids, and DNA and activates transcription factors such as NF-κB and activator protein-1 (AP-1). These, in turn, up-regulate pro-inflammatory cytokines (IL-6, TNF-α, IL-1β), adhesion molecules (ICAM-1, VCAM-1), and chemokines that recruit immune cells to inflamed sites [34]. Chronic NOX activation contributes to vascular dysfunction, endothelial barrier disruption, and atherogenesis [35]. Pharmacological inhibition of NOX enzymes attenuates pollutant-induced oxidative damage and systemic inflammation [36].

Endoplasmic reticulum (ER) stress is an emerging hallmark of oxidative damage induced by airborne pollutants. ROS interfere with protein folding in the ER, triggering the unfolded protein response (UPR) [37]. Key molecular markers of UPR activation GRP78/BiP, C/EBP homologous protein (CHOP), and ATF4 are up-regulated following chronic $PM_{2.5}$ exposure [38]. Persistent ER stress disrupts calcium homeostasis, leading to mitochondrial calcium overload and enhanced ROS generation via the mitochondria-associated membrane (MAM) interface [39]. This bidirectional signaling between mitochondria and the ER amplifies oxidative damage and promotes apoptosis [40]. Pollutant exposure suppresses ER-associated degradation, resulting in protein aggregation and further oxidative injury [40]. The combination of mitochondrial dysfunction and ER stress drives chronic inflammation, fibrosis, and degenerative lung pathologies. In the lung, ER stress impairs surfactant secretion and promotes fibrotic remodeling [41]. Fine particles can translocate into circulation, uncouple endothelial nitric oxide synthase (eNOS), and reduce nitric oxide bioavailability, contributing to hypertension [42]. Oxidatively modified low-density lipoproteins (oxLDL) are generated in this milieu; they activate TLRs on macrophages, drive foam-cell formation, and accelerate atherosclerosis [43].

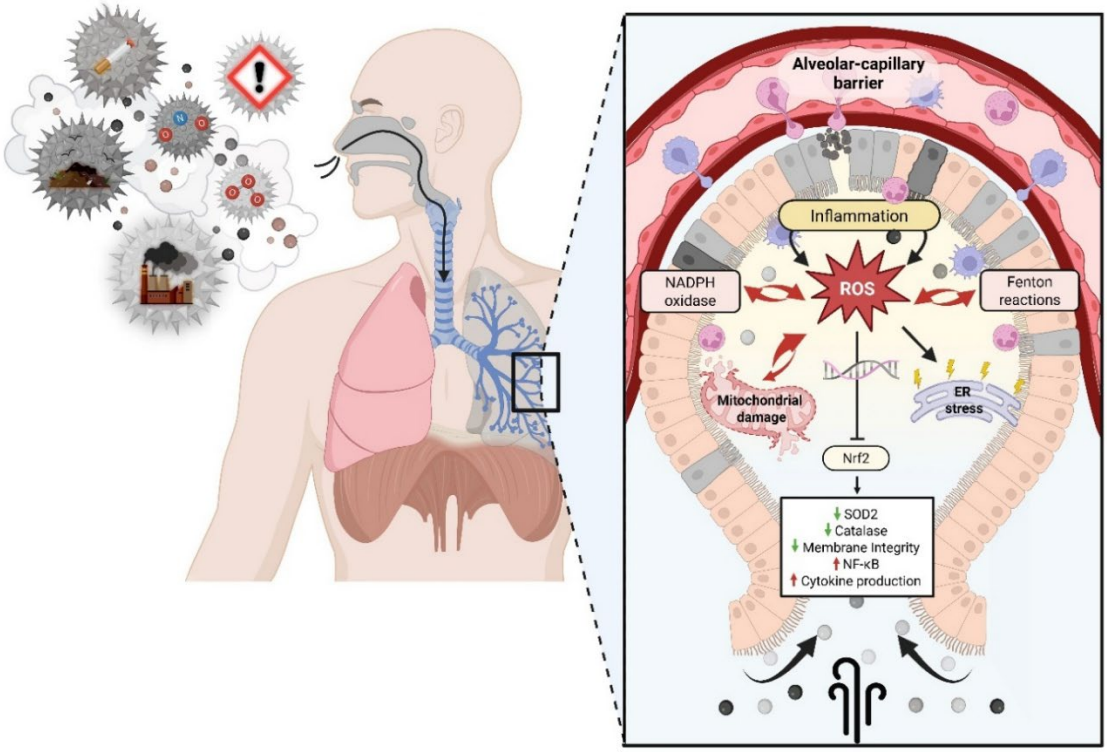


Figure 1. Schematic of airborne pollutant–induced oxidative stress. Airborne particulates and gaseous pollutants, such as nitrogen dioxide (NO_2), are primarily emitted into the atmosphere by burning fossil fuels, leading to the production of harmful secondary air pollutants like ozone (O_3). Exposure to decreased air quality allows pollutants to penetrate deep into the respiratory tract, causing Fenton reactions that generate ROS and stimulate NADPH oxidase activity within the alveolar-capillary barrier, where oxygen exchange occurs.

 doi:10.20944/preprints202604.0051.v1

Resultant oxidative stress disrupts mitochondrial ETC, promotes excessive mitochondrial fission, increases ER stress, and enhances neutrophil and macrophage transmigration. Chronic exposure leads to inflammation, lipid peroxidation, apoptosis, and systemic cardiovascular effects.

2.2. Heavy Metals and Pesticides

Redox cycling and ROS production. Heavy metals such as iron and copper catalyze Fenton reactions that convert hydrogen peroxide into highly reactive hydroxyl radicals [44]. Non-redox-active metals like cadmium and mercury bind to sulfhydryl groups, deplete glutathione (GSH), and inactivate antioxidant enzymes such as superoxide dismutase (SOD) and catalase, thereby indirectly increasing ROS levels [45]. Chronic exposure to lead, arsenic, or cadmium interferes with mitochondrial complexes I and III, leading to electron leakage, superoxide accumulation, and suppression of mitochondrial biogenesis [46].

Pesticides—particularly organophosphates and organochlorines disrupt the ETC, collapse mitochondrial membrane potential, and increase cytochrome c release [47]. They also inhibit SOD, catalase, and glutathione peroxidase while increasing lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) [48]. ROS generated by pesticides activate NF-κB, MAPK, and PI3K/Akt pathways, induce inducible nitric oxide synthase (iNOS), and promote DNA and protein nitration, amplifying inflammation and cell death [49].

Specific metal mechanisms. Cadmium displaces zinc from metalloproteins and binds to thiol groups in glutathione, reducing cellular GSH and increasing susceptibility to oxidative damage [50]. Lead inhibits δ-aminolaevulinic acid dehydratase, disrupting heme synthesis and causing anemia; it interferes with calcium signaling in neurons and compromises synaptic transmission [51]. Mercury forms strong complexes with selenoenzymes such as thioredoxin reductase, impairing the detoxification of hydrogen peroxide [52]. Arsenic metabolites inhibit pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, leading to NADH accumulation, ATP depletion, and mitochondrial dysfunction [53].

Pesticide classes also differ in their redox effects. Organophosphates such as chlorpyrifos and malathion inhibit acetylcholinesterase, causing sustained neurotransmission and calcium overload; the resulting excitotoxicity increases mitochondrial ROS and triggers apoptosis [54]. Organochlorines such as DDT alter lipid metabolism and uncouple oxidative phosphorylation, increasing oxygen consumption and superoxide production [55]. Carbamates and triazine herbicides generate redox-active intermediates that oxidize proteins and DNA [56]. Many pesticides induce cytochrome P450 enzymes, bioactivating xenobiotics into electrophilic metabolites that form adducts with proteins and nucleic acids [57].

Epigenetic and reproductive effects. Heavy metals and pesticides modulate epigenetic machinery. Cadmium and arsenic alter DNA methylation patterns by inhibiting DNA methyltransferases (DNMTs) or depleting methyl donors, causing global hypomethylation and site-specific hypermethylation of tumor suppressor genes [58]. Pesticides modify histone acetylation and alter microRNA expression (e.g., miR-34a and miR-21), suppressing sirtuins and Nrf2 and aggravating oxidative stress [59]. Combined exposures often show synergistic toxicity: co-exposure to cadmium and neonicotinoid pesticides increases ROS, mitochondrial collapse, and DNA strand breaks beyond individual effects [60].

Reproductive tissues are particularly vulnerable to oxidative damage. In males, cadmium accumulates in testicular tissue, inhibits steroidogenic enzymes (3β- and 17β-hydroxysteroid dehydrogenases), reduces testosterone production, and damages Sertoli cells [61]. Lipid peroxidation of sperm plasma membranes decreases motility and alters DNA integrity, contributing to infertility [62]. Organophosphates suppress acetylcholinesterase in Leydig cells, leading to oxidative injury and reduced androgen synthesis [63]. In females, organochlorines accumulate in adipose tissue and ovarian follicles, disrupting estrogen signaling and ovulation [64]. Heavy metals cross the placenta, exposing the developing embryo to ROS and epigenetic alterations that may manifest as developmental defects or transgenerational effects [65].

 doi:10.20944/preprints202604.0051.v1

Systemically, these toxicants contribute to neurotoxicity, endocrine disruption, insulin resistance, and hypertension via persistent redox imbalance and inflammation [66].

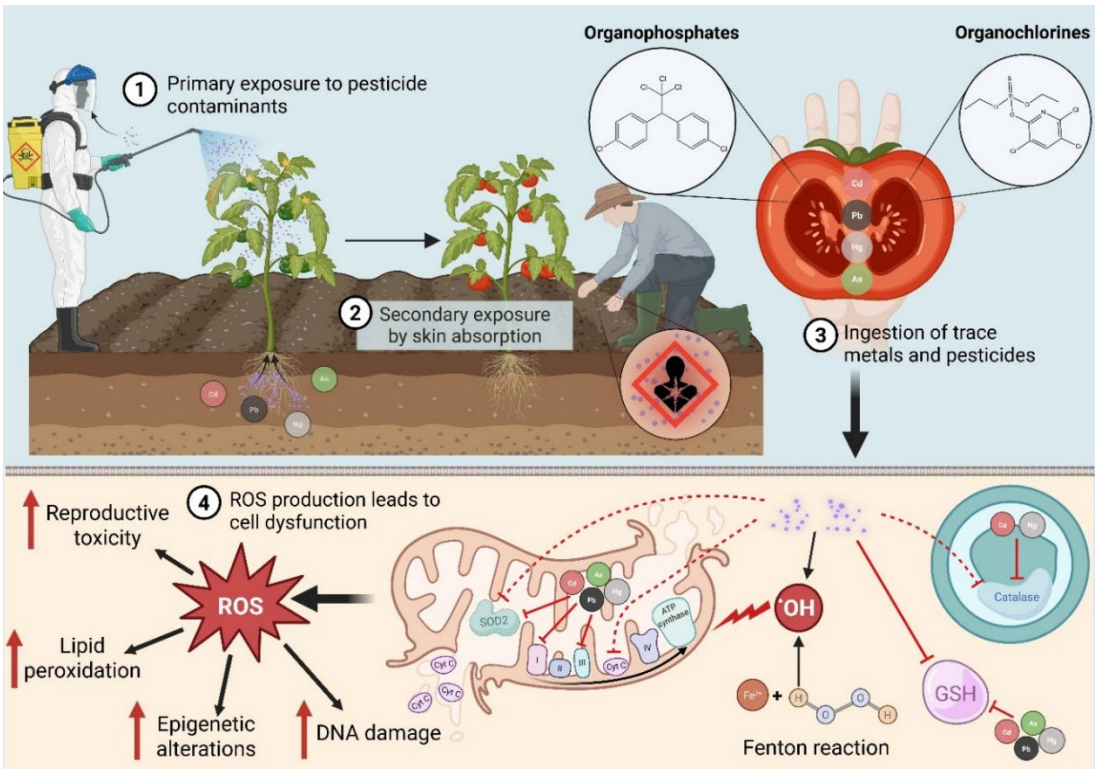


Figure 2. Pathways of heavy metal and pesticide-induced oxidative stress. Both primary and secondary exposure to pesticides disrupts mitochondrial respiration. The ingestion of redox and non-redox active trace metals generate hydroxyl radicals, deplete glutathione, and inhibit antioxidant enzymes. The resultant ROS triggers DNA damage, lipid peroxidation, epigenetic alterations (DNA methylation, histone modification, miRNA dysregulation), and reproductive toxicity.

2.3. Nanoparticles and Microplastics

ROS generation and mitochondrial injury. Nanoparticles (e.g., TiO_2, ZnO, Ag) and micro-/nanoplastics accumulate in biota due to their small size and high surface area. Metal-based nanoparticles undergo redox cycling that produces superoxide and hydroxyl radicals [73], while non-metallic particles generate ROS through surface-bound reactive sites or adsorbed contaminants [74]. Ingested microplastics compromise cell membranes, increase ROS production, and cause mitochondrial dysfunction and inflammation [75]. Microplastics undermine antioxidant defenses, leading to ROS-induced DNA damage in both mitochondria and the nucleus [76]. Metal nanoparticles disrupt mitochondria and peroxisomes; their released ions cause lipid peroxidation and oxidative stress that impairs mitochondrial function and damages DNA [77]. Carbon nanotubes and other particles stimulate apoptosis via death-receptor, ER stress, and mitochondrial pathways [78]. Chronic exposure to microplastics leads to elevated ROS production and oxidative stress that is linked to inflammation, pulmonary disease, carcinogenesis, and cellular senescence [79].

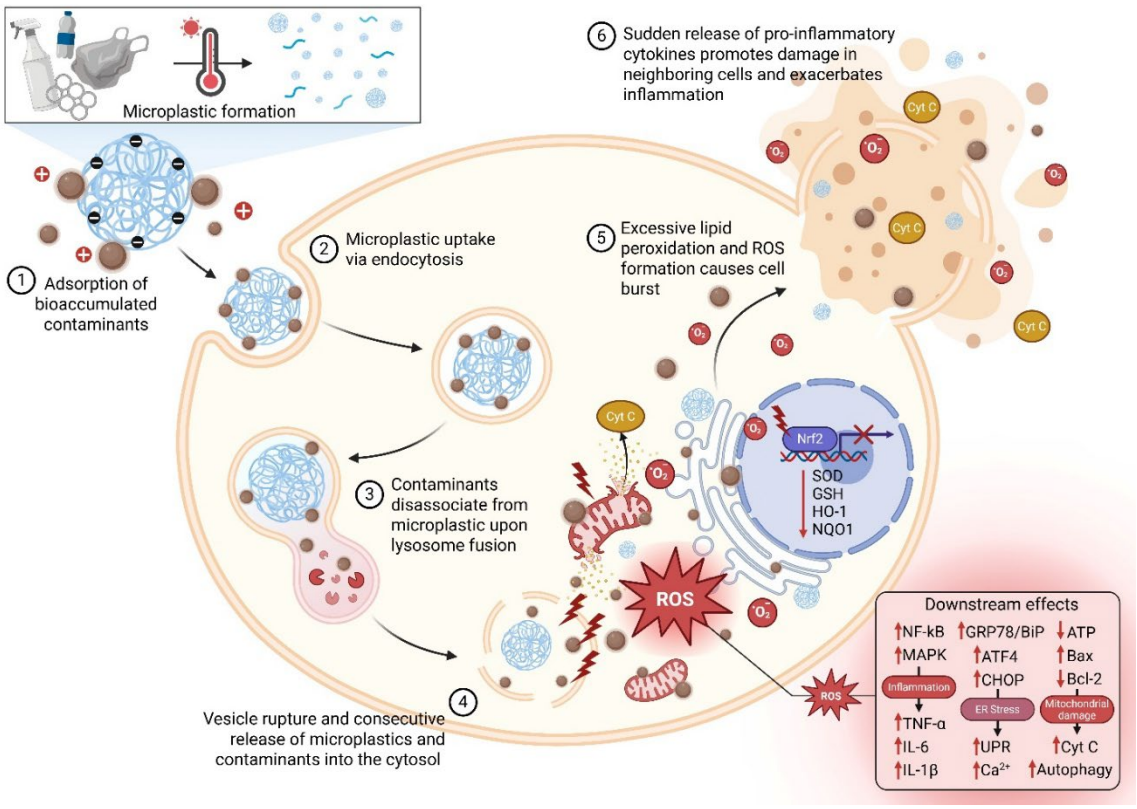
Micro- and nanoplastics as vectors. Micro- and nanoplastics act as vectors for hydrophobic pollutants, heavy metals, and endocrine-disrupting chemicals. Their hydrophobic surfaces adsorb persistent organic pollutants (POPs) and heavy metals in the environment; after uptake by organisms, desorption of these contaminants in acidic intracellular compartments produces additional ROS and toxic effects [80]. Nano-sized plastic particles can cross epithelial barriers and even the blood–brain barrier, accumulating in brain regions such as the hippocampus. Here, they provoke microglial activation, calcium dysregulation, and oxidative death of neurons [81]. In aquatic organisms, microplastics cause gill hyperplasia, hepatocellular necrosis, impaired antioxidant enzyme activities,

 doi:10.20944/preprints202604.0051.v1

and behavioral changes [82]. Murine hepatocyte studies show that smaller polystyrene microplastics (<100 nm) induce more severe mitochondrial swelling, ATP depletion, and DNA strand breaks than larger microplastics, underscoring the importance of particle size [83]. Chronic exposure to microplastics raises ROS and oxidative stress, leading to inflammation, pulmonary disease, carcinogenesis, and senescence [84].

Inflammatory signaling and systemic effects. ROS generated by nanoparticles and microplastics activate NF-κB and MAPK cascades, leading to cytokine release and chronic inflammation [85]. At first, Nrf2 activation induces antioxidant enzymes (HO-1, NQO1), but prolonged exposure exhausts this response, leaving cells susceptible to oxidative damage [86]. Nanoparticles rapidly adsorb proteins, forming a "protein corona" that dictates cellular uptake and stimulates macrophage activation, producing an oxidative burst [87]. Microplastics disrupt gut microbiota, compromise intestinal barrier integrity, and accumulate in the liver and brain, where they induce lipid peroxidation and mitochondrial injury [88]. Co-exposure to microplastics and contaminants such as per- and polyfluoroalkyl substances (PFAS) or heavy metals enhances ROS generation and inhibits antioxidant defenses, resulting in synergistic toxicity [89].

ER stress and autophagy are intimately linked to nanoparticles and microplastic toxicity. Exposure to these particles upregulates ER stress markers (GRP78/BiP, ATF4, CHOP) and activates the UPR [90]. Persistent UPR causes calcium dysregulation and promotes crosstalk with mitochondria, increasing ROS and triggering apoptosis. Autophagy serves as an adaptive response to clear damaged mitochondria and proteins; however, chronic exposure impairs autophagic flux, leading to the accumulation of autophagosomes and cell death. Dysbiosis of the gut microbiome caused by microplastics reduces commensal bacteria that produce antioxidant metabolites and increases pathogenic species that generate ROS. Adaptive antioxidant responses involve upregulation of GSH synthesis and Nrf2 activation, but prolonged exposure depletes these reserves. Therapeutic approaches under investigation include dietary antioxidants (vitamin C, vitamin E, polyphenols), melatonin, and engineered nanoparticles such as cerium oxide that mimic superoxide dismutase and catalase. Nanocarriers delivering Nrf2 activators or mitochondrial-targeted antioxidants (e.g., MitoQ, SkQ1) offer promising strategies to mitigate nanoparticle and microplastic-induced oxidative injury.



 doi:10.20944/preprints202604.0051.v1

Figure 3. Mechanisms of nanoparticle and microplastic–induced oxidative stress. Plastic waste undergoes a lengthy process of environmental degradation and physical abrasion, creating microplastics and nanoparticles that can easily infiltrate into biological systems. Microplastics can act as vectors, adsorbing toxic chemicals and bioaccumulated contaminants via surface reactivity. Upon cell intake, microplastics along with the adsorbed contaminants, can begin generating ROS and cause damage to lipids, proteins, and DNA; disrupt mitochondria and ER; and activate inflammatory pathways.

3. Epigenetic Modifications Driven by Oxidative Stress

Epigenetic mechanisms, including DNA methylation and hydroxymethylation, histone modifications, and non-coding RNA regulation, translate oxidative signals into stable changes in gene expression. The following sections retain detailed descriptions of redox-epigenetic cross-talk.

3.1. DNA Methylation and Hydroxymethylation

DNA methylation is mediated by DNMT1, DNMT3A, and DNMT3B, which transfer methyl groups from S-adenosyl-methionine (SAM) to cytosine residues at CpG sites. Oxidative stress oxidizes catalytic cysteines in DNMTs and depletes the methyl donor SAM, leading to global hypomethylation and impaired maintenance methylation [91]. Oxidized guanine lesions (8-oxo-dG) inhibit DNMT binding, further reducing methylation density [92]. Conversely, transient ROS exposure increases ten-eleven translocation (TET) enzyme activity and 5-hydroxymethylcytosine levels, promoting active demethylation [93]. Chronic oxidative environments inhibit TET enzymes by depleting cofactors (Fe^{2+} and α-ketoglutarate), causing site-specific hypermethylation of tumor suppressor genes and impaired antioxidant gene expression [94]. Ageing and chronic exposure to pollutants accelerate "epigenetic drift"—stochastic changes in methylation that accumulate over time—which silences antioxidant genes such as Nrf2 and SOD2 and perpetuates oxidative injury [95].

Environmental toxicants often cause promoter hypermethylation of genes involved in redox homeostasis; exposure to particulate matter, lead, or diesel exhaust is associated with increased methylation of the Nrf2 promoter and decreased expression of its downstream antioxidant genes [96]. Mutations in isocitrate dehydrogenase (IDH) produce the oncometabolite 2-hydroxyglutarate, which inhibits TET enzymes and promotes widespread DNA hypermethylation and ROS generation [97]. Pollutants that alter one-carbon metabolism (e.g., arsenic) deplete folate and methionine pools, reducing the availability of SAM and further perturbing DNA methylation [98]. These redox-dependent methylation changes are reversible, suggesting therapeutic potential for methyl donor supplementation (folate, betaine) or DNMT inhibitors [99].

DNA methylation changes also serve as environmental biomarkers. Chronic exposure to cadmium, lead, and $PM_{2.5}$ disrupts methylation of antioxidant and stress-response genes (Nrf2, HO-1, SOD2) [100]. Traffic-related air pollution induces hypomethylation of mitochondrial genes and hypermethylation of DNA repair genes, as reported in the Hortega cohort study [101]. These methylation changes correlate with increased 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels, indicating oxidative DNA damage. Hypomethylation of Nrf2 and SOD2 and hypermethylation of DNA repair genes thus represent sensitive, reversible indicators of environmental oxidative load [102].

3.2. Histone Modifications

Histone acetylation, regulated by histone acetyltransferases (HATs) and deacetylases (HDACs), modulates chromatin accessibility. Oxidative stress alters histone-modifying enzymes: oxidation of PARP-1 during DNA repair consumes NAD^+ and suppresses the NAD^+-dependent deacetylase SIRT1, leading to hyperacetylation of histones H3 and H4 and transcriptional dysregulation [103]. At the same time, persistent ROS activate class I/II HDACs, resulting in histone hypoacetylation and repression of antioxidant genes [104]. ROS also influence histone methylation by inhibiting demethylases such as KDM6B, increasing repressive marks (H3K27me3 and H3K9me3), and

 doi:10.20944/preprints202604.0051.v1

silencing stress-response genes [105]. Peroxynitrite-mediated nitration of histone residues can activate pro-inflammatory gene expression, illustrating the dual regulatory roles of redox modifications [106].

Beyond acetylation and methylation, oxidative stress affects other histone acylation. Succinate and fumarate, which accumulate under mitochondrial dysfunction, inhibit α-ketoglutarate-dependent histone demethylases and drive histone succinylation and crotonylation [107]. These post-translational modifications link metabolic status to chromatin structure, repressing or activating gene transcription. ROS can also oxidize histone cysteine residues, alter disulfide bonds, and modify nucleosome stability [108]. Environmental exposures have been shown to increase repressive H3K27me3 marks at promoters of detoxifying enzymes (HO-1, NQO1) and reduce acetylation at enhancers of antioxidant genes [109]. Pharmacological HDAC inhibitors (e.g., trichostatin A, valproic acid) and sirtuin activators (resveratrol) are being explored to restore histone acetylation and improve antioxidant gene expression [110].

3.3. Non-Coding RNAs

Non-coding RNAs integrate redox signaling with gene regulation. Oxidative stress up-regulates microRNAs such as miR-21, miR-34a and miR-200c, which suppress SIRT1, PGC-1α, and Nrf2, thereby limiting mitochondrial biogenesis and antioxidant responses [111]. Down-regulation of miR-25 and miR-146a removes repression of pro-inflammatory signaling [112]. Long non-coding RNAs (lncRNAs), including MALAT1, HOTAIR, and ANRIL, are redox-responsive; MALAT1 can promote Nrf2 nuclear translocation under moderate stress, whereas chronic ROS exposure triggers HOTAIR-mediated recruitment of chromatin repressors to silence tumour-suppressor genes [113]. Redox-sensitive circular RNAs (circRNAs) act as microRNA sponges or interact with transcriptional machinery, influencing oxidative signaling cascades [114].

These non-coding RNA networks mediate both adaptive and pathological responses to oxidative stress and represent potential therapeutic targets [115]. Emerging evidence suggests that circRNAs contain N6-methyladenosine (m^6A) modifications that are influenced by redox balance; these marks regulate circRNA stability and translation [116]. Targeting ncRNA pathways with antisense oligonucleotides or small molecules offers a novel avenue for restoring redox balance and epigenetic integrity [117].

3.4. Epigenetic Modifications in Disease Contexts

In autoimmune diseases, oxidative stress induces T-cell demethylation of CD70 and ITGAL, enhancing autoreactivity and inflammation [118]. ROS-mediated oxidation of TET enzymes alters global 5-hydroxymethylcytosine patterns, contributing to aberrant gene activation in systemic lupus erythematosus and rheumatoid arthritis [119]. In cancer, tumor microenvironments are characterized by elevated ROS, which induce hypermethylation of tumor suppressor genes (BRCA1, p16^INK4a) and hypomethylation of oncogenes, fostering genomic instability [120]. ROS-mediated activation of DNMT3A and EZH2 promotes chromatin condensation and transcriptional silencing of apoptotic genes [121]. In metabolic syndrome, diabetes, and atherosclerosis, oxidative stress triggers hypermethylation of mitochondrial genes and deacetylation of metabolic regulators, impairing energy metabolism and endothelial function [122].

In neurodegenerative diseases and aging, ROS suppresses BDNF and SOD2 expression through promoter hypermethylation, contributing to neuronal loss [123]. Persistent oxidative stress alters H3K9 and H4K16 acetylation, impairing synaptic plasticity and long-term memory [124]. These examples illustrate the central role of redox-epigenetic cross-talk in human disease, suggesting that epigenetic markers may serve as diagnostic indicators and therapeutic targets in oxidative pathologies [125].

 doi:10.20944/preprints202604.0051.v1

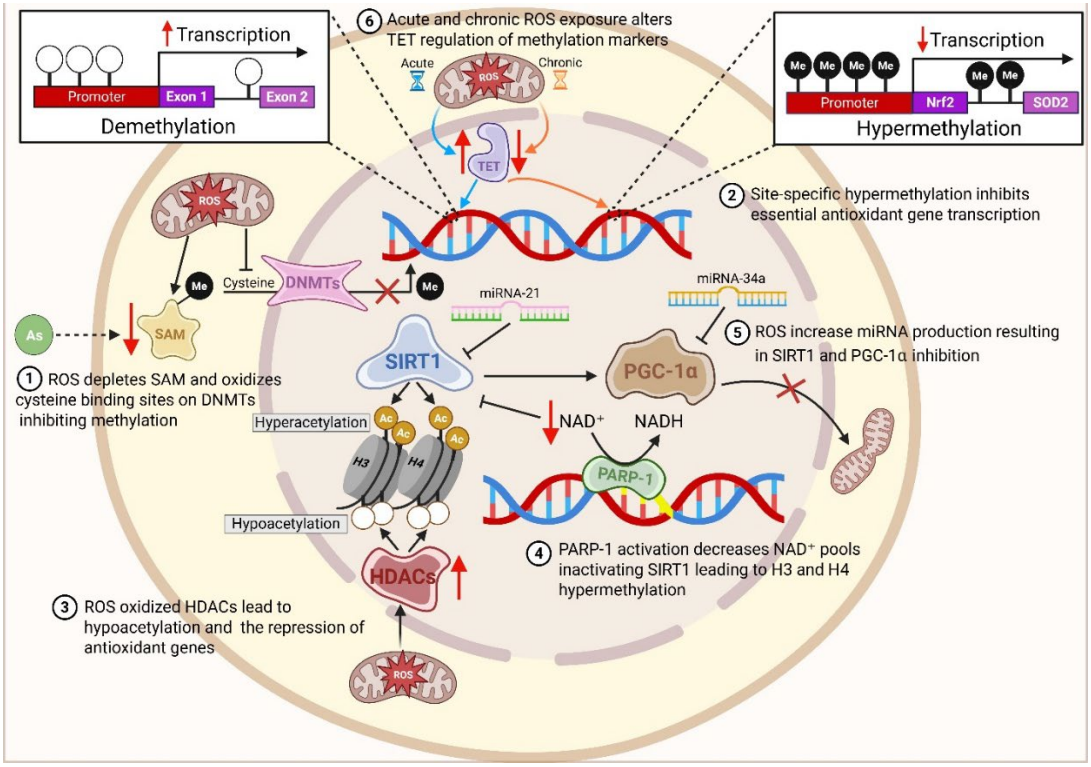


Figure 4. Epigenetic alterations triggered by oxidative stress. ROS oxidize DNMTs and deplete S-adenosyl-methionine (SAM), resulting in global hypomethylation while site-specific hypermethylation silences antioxidant genes. ROS-induced PARP-1 activation suppresses SIRT1, leading to histone hyperacetylation. Oxidative inhibition of histone deacetylases increases the repression of antioxidant genes via histone-wide hypoacetylation. Non-coding RNAs such as miR-21 and miR-34a suppress sirtuins and PGC-1α, integrating redox signals with epigenetic regulation. Acute and chronic ROS exposure leads to DNA demethylation and hypermethylation via TET regulation, respectively.

4. Oxidative Stress and Aging

Aging is characterized by a progressive loss of physiological resilience accompanied by increased ROS production, mitochondrial dysfunction, and chronic inflammation. Mitochondria are both the principal source and target of ROS. Under physiological conditions, the ETC generates small amounts of ROS that are detoxified by SOD2 and GPx. With age, ETC inefficiency and mtDNA mutations increase electron leakage, generating superoxide and causing further mitochondrial damage [126]. Dysfunctional mitochondria activate inflammatory pathways and the senescence-associated secretory phenotype (SASP), contributing to systemic inflammaging [127]. Oxidative stress also damages nuclear and mitochondrial DNA, proteins, and lipids, promoting cellular senescence and telomere shortening [128]. Declining NAD^+ pools impair sirtuin activity and DNA repair, while redox imbalance modulates epigenetic enzymes, leading to epigenetic drift [129]. Restoring mitochondrial function, preserving NAD^+ levels, and activating Nrf2 and sirtuin pathways are pivotal strategies for extending health span [130].

Accumulation of ROS triggers DNA damage responses that stabilize the tumour suppressor p53 and activate cyclin-dependent kinase inhibitors p21^Cip1 and p16^INK4a, enforcing growth arrest [131]. Senescent cells secrete a cocktail of pro-inflammatory cytokines, matrix metalloproteinases, and growth factors collectively known as the SASP, which propagates inflammation and tissue degeneration [132]. Oxidative stress activates nutrient-sensing pathways such as mTOR and inhibits AMP-activated protein kinase (AMPK), altering autophagy and energy balance [133]. In the vasculature, ROS impair eNOS, reduce nitric oxide bioavailability, and promote vascular stiffness and hypertension [134]. In the brain, oxidative damage fosters amyloid-β aggregation and tau

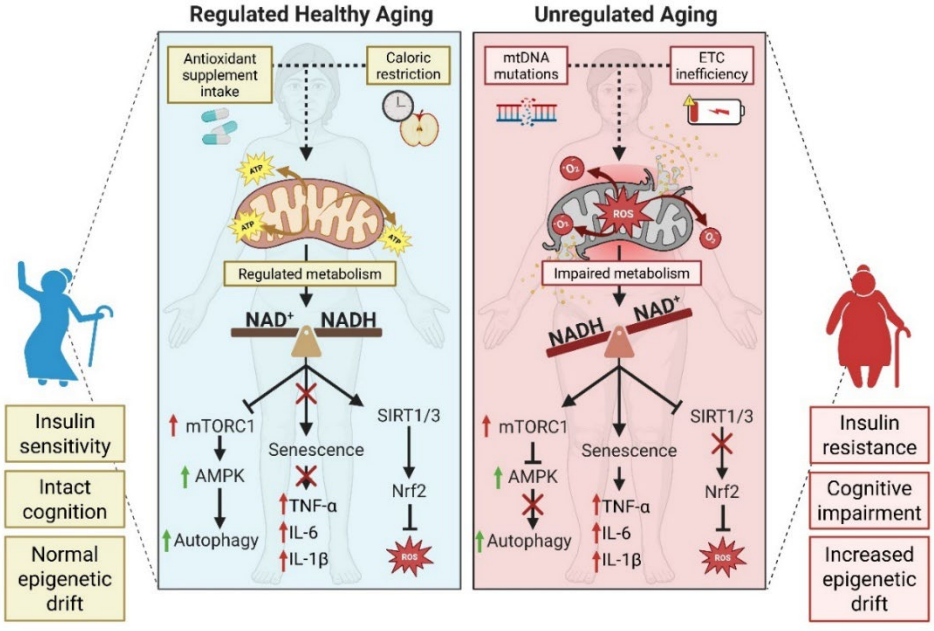
 doi:10.20944/preprints202604.0051.v1

hyperphosphorylation, accelerating Alzheimer's disease; in dopaminergic neurons, oxidative metabolism of dopamine generates quinones that contribute to Parkinson's disease [135].

Metabolic tissues show similar vulnerability. In skeletal muscle and adipose tissue, oxidative stress induces insulin resistance by impairing insulin receptor signaling and GLUT4 translocation [136]. Lipid peroxidation products such as 4-HNE form adducts with insulin signaling proteins, interfering with their function [137]. In the liver, ROS promote steatosis by activating SREBP-1c and suppressing fatty acid oxidation [138]. Age-related decline in NAD^+ levels reduces SIRT1 and SIRT3 activity, impairing mitochondrial function and deacetylation of PGC-1α, FOXO3a, and p53. Supplementation with NAD^+ precursors (nicotinamide riboside, nicotinamide mononucleotide) restores sirtuin activity, improves mitochondrial function, and extends lifespan in animal models [139].

Excessive ROS triggers stabilization of NRF2 and up-regulation of antioxidant enzymes, supporting the free radical theory of aging and showing that aging is associated with increased ROS and reduced mitochondrial enzyme activity [140]. Young mitochondria produce ROS only upon stimulation, whereas aged mitochondria continuously generate elevated ROS, and cross-talk between mitochondrial ROS and AMPK triggers antioxidant responses [141]. Mild activation of the mitochondrial unfolded protein response extends lifespan, whereas excessive activation accelerates senescence. The SIRT2–p66Shc–mtROS axis modulates vascular senescence, and AMPK activation reduces mtROS. These findings highlight that controlled ROS signaling is essential for homeostasis, while chronic oxidative burden promotes aging.

Therapeutic strategies targeting oxidative aging include activation of Nrf2 with phytochemicals such as sulforaphane and curcumin, supplementation of antioxidants (vitamin E, coenzyme Q10), mitochondrial uncouplers (mild mitochondrial uncoupling decreases ROS production), and senolytic drugs that selectively clear senescent cells. Mesenchymal stem cell–derived exosomes carrying antioxidant enzymes and microRNAs enhance autophagy and reduce ROS in aged tissues. Caloric restriction and intermittent fasting reduce oxidative damage by decreasing mitochondrial ROS generation and activating AMPK. Exercise upregulates endogenous antioxidant systems, enhances mitochondrial biogenesis, and improves insulin sensitivity. Emerging therapies aim to combine sirtuin activators, NAD^+ precursors, and Nrf2 inducers to synergistically restore redox homeostasis and extend health span.



 doi:10.20944/preprints202604.0051.v1

Figure 5. Interplay between oxidative stress and aging. The accumulation of mtDNA mutations and increased electron leakage due to ETC inefficiency in aging mitochondria boost ROS production over time. The consequential long-term impaired metabolism can result in a decline in NAD^+/NADH ratios, inducing mTORC1 activation, SIRT1/3 inactivation, cellular senescence, and chronic inflammation. Unregulated aging can be accompanied by insulin resistance, cognitive impairment, and increased epigenetic drift. Healthy aging can be achieved by regulating ROS production with the activation of sirtuin pathways and Nrf2 transcription with the regulated intake of antioxidant supplements (NAD^+ precursors), exercise, and caloric restriction.

5. Biomarkers and Measurement Tools

Quantifying oxidative stress is essential for risk assessment and therapeutic monitoring. No single biomarker captures the complexity of redox imbalance; therefore, panels that encompass lipid, protein, DNA, and metabolic endpoints are increasingly used [142]. Below, we provide detailed descriptions of key biomarkers and the analytical methods commonly used to quantify them.

5.1. Lipid Peroxidation Markers

Malondialdehyde (MDA). MDA is a three-carbon dialdehyde produced during the peroxidation of polyunsaturated fatty acids. It is one of the most frequently reported lipid peroxidation markers in clinical studies [143]. MDA is typically measured using the thiobarbituric acid reactive substances (TBARS) assay, in which MDA condenses with thiobarbituric acid at high temperature and acidic pH to form a colored adduct that is quantified spectrophotometrically [144]. Although the TBARS assay is rapid and inexpensive, it lacks specificity because thiobarbituric acid also reacts with other aldehydes and lipid oxidation products, and MDA can be generated during the assay itself. High-performance liquid chromatography (HPLC) or gas chromatography coupled with mass spectrometry (GC-MS) provides greater specificity by separating MDA–TBA adducts from interfering substances [145]. Due to these limitations, TBARS values are often reported as total TBA-reactive species rather than absolute MDA concentrations. Elevated MDA/TBARS levels are reported in cardiovascular diseases, neurodegeneration, psychiatric disorders, and chronic kidney disease; however, variability across studies underscores the need for careful sample handling and analytical standardization [146].

F_2-isoprostanes (IsoPs). These prostaglandin-like compounds are formed via free radical–mediated peroxidation of arachidonic acid. Because IsoPs are generated in situ from phospholipid-bound fatty acids and released by phospholipases, their levels accurately reflect endogenous lipid peroxidation independent of cyclooxygenase activity [147]. Multiple studies, including the NIH-sponsored Biomarkers of Oxidative Stress Study, have shown that quantification of F_2-IsoPs provides one of the most reliable indices of oxidative stress in vivo [148]. IsoPs are remarkably stable and detectable in plasma, urine, cerebrospinal fluid, and exhaled breath condensate. Their measurement typically involves solid-phase extraction, purification by thin-layer chromatography, and quantification by GC-MS or LC-MS, often using negative ion chemical ionization and stable isotope dilution to achieve picogram-level sensitivity [149]. Alternatively, enzyme immunoassays are available but may lack specificity for individual IsoP isomers. Elevated IsoPs are observed in conditions ranging from atherosclerosis and diabetes to neurodegenerative and inflammatory diseases, and decrease in response to antioxidant therapy [150].

4-Hydroxynonenal (4-HNE). This highly reactive α,β-unsaturated aldehyde forms Michael adducts with proteins and phospholipids. 4-HNE levels are assessed using ELISA, HPLC, or Western blotting of 4-HNE–protein adducts. Due to its cytotoxicity, 4-HNE is both a biomarker and mediator of oxidative damage. Elevated levels are seen in neurodegenerative disorders, liver disease, diabetes, and atherosclerosis [151].

Quantifying oxidative stress is essential for risk assessment and therapeutic monitoring. No single biomarker captures the complexity of redox imbalance; therefore, panels that encompass lipid, protein, DNA, and metabolic endpoints are increasingly used. Below, we provide detailed descriptions of key biomarkers and the analytical methods commonly used to quantify them.

 doi:10.20944/preprints202604.0051.v1

5.2. DNA Oxidation Products

8-Hydroxy-2′-deoxyguanosine (8-OHdG) and 8-hydroxyguanine (8-OHGua). These biomarkers arise when ROS oxidize guanine in DNA or the nucleotide pool. Urinary 8-OHdG is one of the most popular biomarkers of oxidative DNA damage. In vivo, oxidized guanine is excised by DNA glycosylases and excreted in urine; thus, urinary levels reflect whole-body DNA repair [152]. 8-OHdG/8-OHGua can also be measured in plasma, saliva, or tissue samples. Analytical techniques include competitive ELISA, which is simple but susceptible to cross-reactivity and tends to overestimate concentrations, and chromatographic methods such as HPLC coupled with electrochemical detection or tandem mass spectrometry, which provide higher specificity and sensitivity [153]. A column-switching HPLC-ECD system has been used to quantify 8-OHGua in saliva; salivary 8-OHGua was detectable at picogram levels and increased in smokers compared with non-smokers [154]. Regardless of matrix, rigorous sample preparation and avoidance of artefactual oxidation are crucial for reliable measurement [155].

5.3. Protein Oxidation Markers

Protein carbonyls. Oxidative modification of protein side chains yields carbonyl groups that accumulate with age and disease. Derivatization with 2,4-dinitrophenylhydrazine (DNPH) allows spectrophotometric quantification of carbonyls. Carbonylated proteins can also be separated by HPLC or detected by ELISA using anti-DNP antibodies. Western blotting following DNPH derivatization enables identification of specific oxidized proteins, while mass spectrometry provides precise structural information [156]. Plasma protein carbonyls increase in chronic kidney disease, diabetes, and neurodegenerative disorders, although levels vary depending on sample handling and analytical method [157].

3-Nitrotyrosine and dityrosine. Nitration and cross-linking of tyrosine residues by reactive nitrogen species produce 3-nitrotyrosine (3-NO_2-Tyr) and dityrosine (diTyr). High-performance liquid chromatography coupled with electrochemical array detection (HPLC-ECD) enables simultaneous quantification of tyrosine, 3-NO_2-Tyr, and diTyr in tissues and fluids. An Alzheimer's disease study showed that dityrosine and 3-NO_2-Tyr were elevated five- to eight-fold in hippocampal and neocortical regions compared with controls, whereas uric acid—a peroxynitrite scavenger—was decreased [158]. These findings illustrate the value of nitrosative stress markers for linking inflammatory enzyme activity to disease pathology. Immunochemical methods (ELISA, immunohistochemistry) are also used but may lack quantitative accuracy [159].

Advanced oxidation protein products (AOPPs). These dityrosine-containing cross-linked protein aggregates are formed by the action of chlorinated oxidants and myeloperoxidase-derived species. AOPPs are measured spectrophotometrically at 340 nm and correlate with systemic inflammation, especially in chronic renal failure and cardiovascular disease [160]. Beyond serving as markers, AOPPs act as pro-inflammatory mediators that activate mononuclear phagocytes. Because protein oxidation products are more stable than lipid peroxidation products, AOPPs provide a robust index of chronic oxidative injury. Tissue or plasma AOPP concentrations are commonly measured by a spectrophotometric method in which oxidation of potassium iodide by chloramine-T yields triiodide ions; absorbance at 340 nm is compared against chloramine-T standards and expressed as nmol of chloramine-T equivalents per mg protein. Commercial ELISA kits are available for high-throughput screening.

Ischemia-modified albumin (IMA). Oxidative cleavage and conformational changes at the N-terminus of human serum albumin under ischemic or oxidative stress conditions reduce metal-binding capacity. The albumin cobalt-binding (ACB) test indirectly quantifies IMA by adding a known amount of cobalt and measuring the unbound fraction using a chromogenic agent such as dithiothreitol. Elevated IMA levels have been reported in acute coronary syndromes, sepsis, liver cirrhosis, and dermatological disorders [161]. Limitations include reagent instability and interference by fatty acids or albumin variants; alternative colorimetric assays using nickel ions have been proposed to improve robustness [162].

 doi:10.20944/preprints202604.0051.v1

5.4. Antioxidant Enzymes and Redox Cofactors

Antioxidant enzymes. Activities of endogenous antioxidant enzymes provide functional information on the oxidative state. SOD catalyzes the dismutation of superoxide to hydrogen peroxide; catalase and glutathione peroxidase further detoxify hydrogen peroxide to water. In tissues, SOD activity can be measured spectrophotometrically by assessing inhibition of nitroblue tetrazolium reduction in the xanthine–xanthine oxidase system [163]. Catalase activity is often determined by monitoring the decomposition rate of hydrogen peroxide at 240 nm, while glutathione peroxidase activity is measured by coupled assays that monitor NADPH consumption during reduction of oxidized glutathione [164]. Decreased enzymatic activity reflects depletion or inactivation of antioxidants and is frequently reported alongside increased oxidative stress biomarkers.

Direct ROS detection. Because free radicals have very short half-lives, direct measurement relies on trapping or fluorescent probes. 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) is a cell-permeable probe that is deacetylated by intracellular esterases and oxidized by ROS to form fluorescent dichlorofluorescein. A standard DCFH-DA assay involves incubating cells with a 10 μM solution, washing, and measuring fluorescence at 485 nm excitation and 530 nm emission. This method provides a simple, cost-effective measure of total ROS generation but is not specific to individual ROS species and can be influenced by light exposure and cell type [165]. Electron spin resonance (ESR) spectroscopy combined with spin-trapping agents (e.g., DMPO) offers a more selective approach by forming stable radical adducts that can be detected directly, although it requires specialized instrumentation [166].

Glutathione (GSH/GSSG) ratio. GSH is the primary cellular thiol antioxidant, and its oxidation to glutathione disulfide (GSSG) reflects oxidative pressure. Under oxidative stress, GSH is consumed, and the GSH:GSSG ratio decreases; this shift serves as a sensitive indicator of redox status across diseases. Measurement can be performed by enzymatic recycling assays using glutathione reductase, HPLC with fluorescence detection after derivatization with orthophthalaldehyde, or capillary electrophoresis. Because the pool of protein-bound glutathione (S-glutathionylation) also increases under oxidative stress, assays that differentiate free and protein-bound glutathione provide more comprehensive information [167].

NAD^+/NADH ratio. This redox pair underpins metabolic flux through glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation. NAD^+ accepts electrons and becomes reduced to NADH, which in turn donates electrons to the electron transport chain. A decline in the NAD^+/NADH ratio is indicative of impaired mitochondrial respiration and is a hallmark of oxidative stress and aging. This ratio also regulates the activity of sirtuins (NAD^+-dependent deacetylases), PARPs, and other redox-sensitive enzymes [168]. The NAD^+/NADH ratio is measured in tissues and cells using enzymatic cycling assays or LC-MS/MS. Pre-analytical variables, including sample storage, pH, and redox-active metabolites, must be strictly controlled for reliable quantification. NAD^+ precursors such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) are currently being explored in clinical trials to restore NAD^+ pools and mitigate oxidative damage.

5.5. Epigenetic and Multi-Omics Biomarkers

Persistent oxidative stress induces aberrant DNA methylation patterns, histone modifications, and changes in non-coding RNAs. Up-regulation of DNMTs and inhibition of TET enzymes lead to promoter hypermethylation and gene silencing, while oxidative modifications to HDACs alter acetylation states of chromatin [169]. Monitoring methylation of antioxidant genes (e.g., Nrf2, SOD2), histone marks (H3K27me3, H3K9me3), and microRNAs (e.g., miR-21, miR-34a) provides mechanistic insight into pollutant-mediated gene regulation [170]. Analytical platforms include bisulfite sequencing, chromatin immunoprecipitation sequencing (ChIP-seq), and RNA-seq.

Because individual markers provide only a snapshot of the oxidative state, composite panels that integrate multiple lipid peroxidation products (MDA, F_2-IsoPs, 4-HNE), DNA lesions (8-OHdG), protein carbonyls, nitrotyrosine, and redox cofactors (GSH/GSSG, NAD^+/NADH) offer a more robust

 doi:10.20944/preprints202604.0051.v1

assessment [171]. Emerging metabolomic techniques use untargeted LC-MS and NMR spectroscopy to profile a broad spectrum of oxidative modifications, while multi-omics approaches combine metabolomics with epigenomics and transcriptomics to identify pollutant-specific signatures [172]. High-throughput assays and miniaturized sensors are being developed to enable real-time monitoring of oxidative stress biomarkers in clinical and environmental settings.

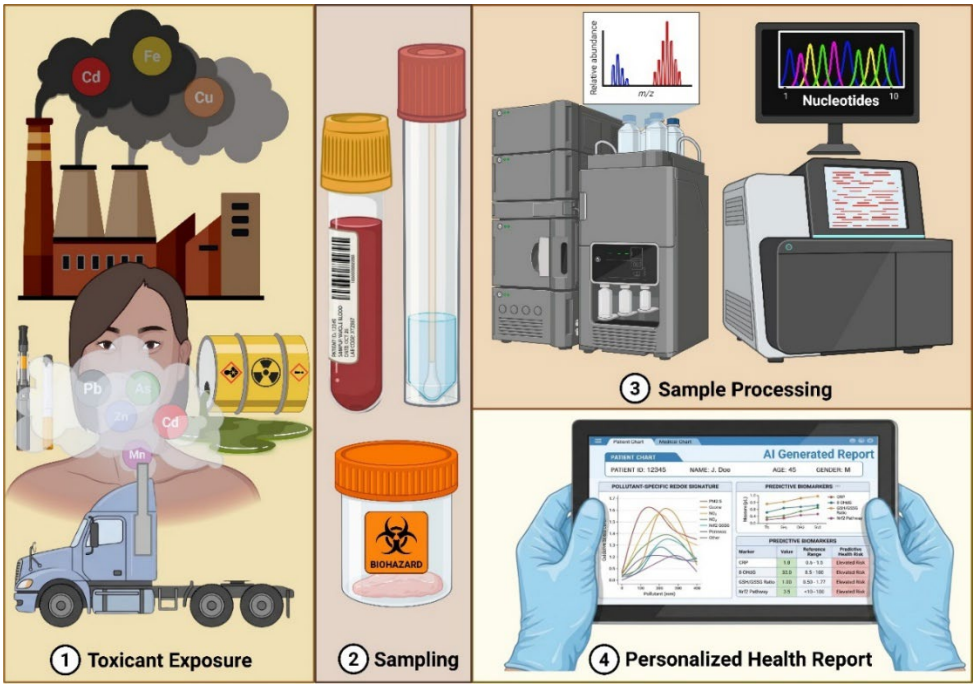


Figure 6. Multi-omics biomarker discovery pipeline. Environmental toxicant exposures generate oxidative stress, which alters metabolites, DNA methylation, histone modifications, and non-coding RNA expression. Integrating next-gen sample processing with a multi-omics pipeline for analyses enables computational modeling and artificial intelligence to generate a pollutant-specific health report for personalized healthcare. .

6. Emerging Epigenetic Biomarkers and Redox-Related Metabolomic Profiles

Recent advances in epigenomics and metabolomics have illuminated how oxidative stress modifies gene regulation and metabolism, generating measurable molecular signatures that act as biomarkers of environmental impact. This section retains detailed insights from recent literature on epigenetic and metabolomic markers of oxidative stress arising from environmental exposures [173].

Table 1. Overview of Emerging Biomarkers.

Biomarker type	Example markers	Mechanistic insight	Application
DNA methylation	Hypomethylation of *Nrf2*, *SOD2*	Indicates failure of antioxidant responses and epigenetic drift	Exposure risk assessment
Histone modification	↑H3K27me3, ↑H3K9me3	Chromatin repression of stress-response genes	Chronic pollution indicator
miRNA expression	↑miR-21, ↓miR-146a	Regulates inflammatory and oxidative pathways	Predictive disease biomarker
Redox metabolites	↓GSH/GSSG ratio, ↑MDA, ↓NAD^+/NADH ratio	Reflect depletion of antioxidant reserves and metabolic imbalance	Systemic oxidative stress marker
Protein adducts	4-HNE–protein conjugates	Indicate lipid peroxidation and tissue injury	Biomarker of cumulative damage

6.1. DNA Methylation as a Redox Biomarker

 doi:10.20944/preprints202604.0051.v1

DNA methylation changes are sensitive markers of environmental oxidative load. Exposure to cadmium, lead, and particulate matter disrupts methylation of antioxidant and stress-response genes (*Nrf2*, *HO-1*, *SOD2*) [174]. These methylation changes correlate with increased 8-OHdG levels, indicating oxidative DNA damage. Traffic-related air pollution induces hypomethylation of mitochondrial genes and hypermethylation of DNA repair genes, as reported in the Hortega cohort study, which linked environmental exposure to altered metabolite profiles and oxidative stress biomarkers [175]. Hypomethylation of *Nrf2* and *SOD2* and hypermethylation of DNA repair genes thus represent sensitive, reversible indicators of environmental oxidative load.

6.2. Histone Modifications and Chromatin Remodeling

Histone modifications respond dynamically to ROS. Heavy metals or PAHs induce repressive marks (H3K27me3, H3K9me3) that suppress expression of detoxifying genes [176]. Histone succinylation and crotonylation, new oxidative-sensitive modifications, link metabolic status to chromatin architecture. Environmental stress disrupts the acetyl-CoA and succinyl-CoA pools, altering histone acylation states and transcriptional control of antioxidant pathways. Non-redundant modifications such as histone succinylation and crotonylation are gaining attention for their ability to link metabolism to epigenetic control. Emerging evidence highlights how metabolic intermediates such as fumarate and succinate modulate TET and KDM enzyme activity, establishing a direct biochemical link between metabolism and chromatin regulation [177].

6.3. Non-Coding RNA Regulation Under Redox Stress

Non-coding RNAs, especially microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), act as epigenetic regulators in oxidative stress responses. ROS modulate miRNAs that target antioxidant and mitochondrial genes. miR-34a, miR-21, and miR-155 are consistently up-regulated by ROS and regulate sirtuins, Nrf2, and inflammatory pathways [178]. Circulating miRNA profiles reflect redox balance and offer non-invasive biomarkers for environmental exposure. LncRNAs such as H19 and MALAT1 are redox sensitive and influence glutathione synthesis, autophagy, and apoptosis. CircRNAs stabilize miRNAs and scaffold redox signaling complexes. Profiling non-coding RNAs via microarrays or RNA-seq provides a multilayered perspective on redox regulation.

6.4. Redox Metabolites as Exposure Biomarkers

Metabolomic studies link environmental oxidative stress to specific metabolic shifts. Key metabolites include the GSH/GSSG ratio, MDA, and 4-HNE for lipid peroxidation, the cysteine/cystine ratio to measure thiol–disulfide redox state, and NADH/NAD^+ and NADPH/$NADP^+$ ratios to reflect redox potential and metabolic health [179]. Chronic exposure to traffic-related pollutants disrupts glutathione metabolism and amino acid pathways related to oxidative stress, validating these metabolites as sensitive biomarkers [180]. Sex-specific differences in redox metabolism have been documented; oxidative stress affects male fertility via altered glutathione and lipid peroxidation pathways, while female reproductive systems are modulated through estrogenic regulation of Nrf2 [181]. Age-dependent decline in redox homeostasis sensitizes individuals to pollutant-induced metabolic shifts.

6.5. Integrative Redox-Epigenetic Interactions

Epigenetic and metabolic systems are closely intertwined through redox-dependent mechanisms. Oxidative metabolites, including α-ketoglutarate and 2-hydroxyglutarate, influence TET and KDM enzyme activity, establishing a direct biochemical link between metabolism and chromatin regulation [182]. Multi-omics approaches combining methylome, transcriptome, and metabolome profiling have revealed pollutant-specific signatures. For instance, diesel exhaust exposure is associated with hypomethylation of *Nrf2*, increased oxidized glutathione, and miR-34a

 doi:10.20944/preprints202604.0051.v1

up-regulation, representing a composite fingerprint of environmental redox imbalance [183]. This integrative perspective not only reveals mechanisms of redox regulation but also enables the discovery of biomarkers for personalized risk assessment and therapeutic targeting.

6.6. Translational and Clinical Implications

Integration of epigenetic and metabolomic biomarkers offers unprecedented precision in exposomics: the study of lifelong environmental exposures. These molecular profiles serve as diagnostic tools for early detection of pollutant-induced disease and predictive markers of individual susceptibility [184]. Artificial intelligence–assisted multi–omics modeling enhances biomarker discovery by correlating redox signatures with health outcomes. Beyond diagnostics, identifying redox-sensitive pathways offers therapeutic potential. Activation of Nrf2 and SIRT1 pathways through nutraceuticals (e.g., sulforaphane, resveratrol) can restore redox equilibrium and reverse epigenetic silencing. These insights open pathways for personalized antioxidant interventions in populations at risk of environmental stress [185].

7. Future Perspectives

The integration of redox biology with epigenetic and multi-omics profiling represents a paradigm shift in how we understand, measure, and intervene in oxidative stress–related diseases. Redox signaling, once considered a damaging byproduct of metabolism, is now appreciated as a dynamic regulator of gene expression, chromatin remodeling, and cellular fate decisions. Moving forward, the convergence of environmental health, redox epigenetics, and precision medicine will drive the discovery of new diagnostic tools and therapeutic strategies [186]. First, expanding longitudinal cohort studies with multi-omics measurements will help establish causal links between environmental exposures, oxidative stress, and chronic disease. Current studies often suffer from limited sample sizes, a cross-sectional design, and a lack of exposure quantification. Integrating redox biomarkers, DNA methylation profiles, and metabolic signatures into existing exposomic datasets will improve resolution and generalizability [187]. High-resolution metabolomics, single-cell epigenomics, and spatial transcriptomics will allow the dissection of redox heterogeneity across tissues and cell types.

Second, the development of portable sensors and wearable technologies to monitor redox parameters in real-time may revolutionize exposure surveillance and risk assessment [188]. Biosensors that detect breath ethane, exhaled nitric oxide, or salivary 8-OHdG could provide non-invasive, dynamic indicators of oxidative load. Combining these sensors with artificial intelligence and machine learning will support personalized redox tracking.

Third, therapeutic innovation must target the root causes of redox imbalance. While antioxidant supplementation has yielded inconsistent results, targeting upstream regulators such as Nrf2, SIRT1, and AMPK may offer more durable benefits. Epigenetic drugs (DNMT inhibitors, HDAC inhibitors) and NAD^+ boosters (nicotinamide mononucleotide, NR) are under investigation for age-related and metabolic diseases [189]. CRISPR-based epigenome editing and RNA therapeutics may enable precise modulation of redox-sensitive gene expression in the future.

Lastly, ethical and societal considerations surrounding multi-omics data integration, privacy, and equity must be addressed. Vulnerable populations disproportionately exposed to environmental oxidative stress must be prioritized in research and interventions. Ensuring equitable access to redox-targeted therapeutics and diagnostics will be essential for realizing the promise of redox precision medicine [190].

8. Conclusions

Oxidative stress is a central mechanism linking environmental exposures, aging, and chronic disease. Airborne pollutants, heavy metals, pesticides, nanoparticles, and microplastics produce ROS through mitochondrial disruption, NADPH oxidase activation, and redox cycling. The resulting

 doi:10.20944/preprints202604.0051.v1

oxidative imbalance damages lipids, proteins, and DNA, triggers inflammatory signaling, and reprograms the epigenome. These processes accelerate cellular senescence and contribute to pathologies ranging from neurodegeneration to cardiovascular disease. Emerging biomarkers, including redox metabolites, oxidative DNA lesions, and epigenetic alterations, offer sensitive tools for assessing environmental impact and guiding interventions. Future research should leverage multi-omics and AI-driven exposomics to translate mechanistic insights into precision strategies for mitigating oxidative damage and promoting healthy aging.

Author Contributions: L.C.: conceptualization, M.G. and M.P. literature curation, writing (original draft), L.C.: supervision, writing (critical review and editing), and journal targeting guidance. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by NIH grants R01NS117906 to L.C.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Figures were created with BioRender.com.

Conflicts of Interest: The authors declare no conflicts of interest.

 doi:10.20944/preprints202604.0051.v1

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
4-HNE	4-hydroxynonenal
8-OHGua	8-hydroxyguanine
8-OHdG	8-hydroxy-2′-deoxyguanosine
ACB	albumin cobalt-binding
AMPK	AMP-activated protein kinase
AOPPs	advanced oxidation protein products
AP-1	activator protein-1
CHOP	C/EBP homologous protein
DCFH-DA	2′,7′-dichlorodihydrofluorescein diacetate
DNMTs	DNA methyltransferases
DNPH	2,4-dinitrophenylhydrazine
DRP1	dynamin-related protein 1
ER	endoplasmic reticulum
ESR	electron spin resonance
ETC	electron transport chain
GC-MS	gas chromatography–mass spectrometry
GSH	glutathione
GSSG	glutathione disulfide
HATs	histone acetyltransferases
HDACs	histone deacetylases
HPLC	high-performance liquid chromatography
HPLC-ECD	HPLC coupled with electrochemical detection
IDH	isocitrate dehydrogenase
IMA	ischemia-modified albumin
IsoPs	F_2-isoprostanes
MAM	mitochondria-associated membrane
MDA	malondialdehyde
NMN	nicotinamide mononucleotide
NO2	nitrogen dioxide
NOX	NADPH oxidase
NR	nicotinamide riboside
NRF1	nuclear respiratory factor 1
NSCs	neural stem cells
O3	ozone
OPA1	optic atrophy protein 1
PAHs	polycyclic aromatic hydrocarbons
PFAS	per- and polyfluoroalkyl substances
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
POPs	persistent organic pollutants
ROS	reactive oxygen species
SAM	S-adenosyl-methionine
SASP	senescence-associated secretory phenotype
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
TCA	tricarboxylic acid
TET	ten-eleven translocation
TLR	Toll-like receptor
UFPs	ultrafine particles
UPR	unfolded protein response
circRNAs	circular RNAs
lncRNAs	long non-coding RNAs
mPTP	mitochondrial permeability transition pore
miRNAs	microRNAs
mtDNA	mitochondrial DNA

 doi:10.20944/preprints202604.0051.v1

mtROS	mitochondrial ROS
oxLDL	oxidized low-density lipoprotein

References

1. Chan, M.K.S., Shutella, T., Lakey, J.R.T., & Wong, M.B.F. (2023). Human Organ Lifespan and Healthspan: Mapping from Cellular Mechanism to Clinical Applications for Precision Medicine in Healthspan to Lifespan. Advances in Clinical Medical Research. Link
2. Marengo, B., Furfaro, A.L., & Pronzato, M.A. (2021). *Redox homeostasis and epigenetics in aging and cancer: a dangerous liaison*. Antioxidants, 10(4), 601. https://doi.org/10.3390/antiox10040601
3. Reuter, S., Gupta, S.C., Chaturvedi, M.M., & Aggarwal, B.B. (2020). *Oxidative stress, inflammation, and cancer: How are they linked?*. Free Radical Biology and Medicine, 49(11), 1603–1616. https://doi.org/10.1016/j.freeradbiomed.2020.09.020
4. López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., & Kroemer, G. (2023). *The hallmarks of aging*. Cell, 186(2), 243–271. https://doi.org/10.1016/j.cell.2023.01.005
5. Li, X., Fang, P., Li, Y., et al. (2021). *Mitochondrial ROS and mitophagy in aging and age-related diseases*. Journal of Molecular Cell Biology, 13(7), 456–469. https://doi.org/10.1093/jmcb/mjab030
6. Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). *Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling*. The Journal of Cell Biology, 217(6), 1915–1928. https://doi.org/10.1083/jcb.201708007
7. Ghezzi, P., & Jaquet, V. (2022). *Redox signaling: The interface between oxidants and the immune system*. Antioxidants & Redox Signaling, 37(6), 453–471. https://doi.org/10.1089/ars.2021.0141
8. Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., & Kalayci, O. (2019). *Oxidative stress and antioxidant defense*. World Allergy Organization Journal, 5(1), 9–19. https://doi.org/10.1097/WOX.0b013e3182439613
9. Wallace, D.C., & Fan, W. (2020). *Energetics, epigenetics, mitochondrial genetics*. Mitochondrion, 49, 1–5. https://doi.org/10.1016/j.mito.2019.12.004
10. González-Rodríguez, A., & Mas-Gutierrez, J. (2023). *Oxidative stress in metabolic diseases and epigenetic regulation*. Trends in Endocrinology and Metabolism, 34(3), 153–168. https://doi.org/10.1016/j.tem.2022.12.002
11. Zhang, B., Tian, R., Liu, J., & Liu, M. (2022). *Redox-sensitive transcription factors in aging and age-related diseases*. Mechanisms of Ageing and Development, 205, 111684. https://doi.org/10.1016/j.mad.2022.111684
12. Dinkova-Kostova, A.T., & Talalay, P. (2020). *Direct and indirect antioxidant properties of inducers of cytoprotective proteins*. Molecular Nutrition & Food Research, 64(3), 1901367. https://doi.org/10.1002/mnfr.201901367
13. Tan, B.L., & Norhaizan, M.E. (2021). *Reactive oxygen species and cancer: Mutagenic, mitogenic, and immunosuppressive roles*. Frontiers in Physiology, 11, 602873. https://doi.org/10.3389/fphys.2020.602873
14. Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M.C.B., & Rahu, N. (2016). *Oxidative stress and inflammation: What polyphenols can do for us?*. Oxidative Medicine and Cellular Longevity, 2016, 7432797. https://doi.org/10.1155/2016/7432797
15. Sies, H. (2021). *Oxidative eustress: On constant alert for redox homeostasis*. Redox Biology, 41, 101867. https://doi.org/10.1016/j.redox.2021.101867
16. Younus, H. (2018). *Therapeutic potentials of superoxide dismutase*. International Journal of Health Sciences, 12(3), 88–93. https://doi.org/10.2196/ijhs.v12i3.224
17. Henning, R.J. (2021). *Oxidative stress in the pathogenesis of heart failure*. Cardiology in Review, 29(2), 60–72. https://doi.org/10.1097/CRD.0000000000000317
18. Zengin, G., Menghini, L., Di Sotto, A., Mancinelli, R., Sisto, F., & Garzoli, S. (2021). *Natural antioxidant compounds as regulators of NADPH oxidases*. Oxidative Medicine and Cellular Longevity, 2021, 6621062. https://doi.org/10.1155/2021/6621062
19. Chatterjee, S., & Jung, H. (2020). *Epigenetic regulation of Nrf2-mediated antioxidant responses*. Biochemical Pharmacology, 177, 113910. https://doi.org/10.1016/j.bcp.2020.113910
20. Jin, H., et al. (2021). *DNA methylation and redox regulation: Epigenetic perspectives in human health and disease*. Free Radical Biology and Medicine, 170, 185–200. https://doi.org/10.1016/j.freeradbiomed.2021.05.002

 doi:10.20944/preprints202604.0051.v1

21. Tomasi, M.L., Ramani, K., & Lu, S.C. (2022). *Epigenetic mechanisms of liver disease and hepatocellular carcinoma*. Journal of Hepatology, 77(1), 166–182. https://doi.org/10.1016/j.jhep.2022.02.018
22. Gagliano N., & Grizzi, F. (2021). *Oxidative stress and epigenetic changes in aging*. Cells, 10(3), 625. https://doi.org/10.3390/cells10030625
23. Martincorena, I. (2021). *Somatic mutation and clonal expansions in human tissues*. Genome Medicine, 13(1), 35. https://doi.org/10.1186/s13073-021-00845-5
24. Zhao, Y., Hu, X., Liu, Y., & Dong, L. (2021). *Mitophagy and mitochondrial oxidative stress in aging and age-related diseases*. Free Radical Biology and Medicine, 172, 312–322. https://doi.org/10.1016/j.freeradbiomed.2021.06.001
25. Kim, J., & Lee, S. (2021). *Mitochondrial redox signaling and inflammation in aging and neurodegeneration*. Ageing Research Reviews, 70, 101412. https://doi.org/10.1016/j.arr.2021.101412
26. Lopez-Otin, C., & Kroemer, G. (2021). *Hallmarks of health*. Cell, 184(1), 33–63. https://doi.org/10.1016/j.cell.2020.12.034
27. Liu, Z., Zhou, T., Ziegler, A.C., Dimitrion, P., & Zuo, L. (2020). *Oxidative stress in neurodegenerative diseases: From molecular mechanisms to clinical applications*. Oxidative Medicine and Cellular Longevity, 2020, 8600870. https://doi.org/10.1155/2020/8600870
28. Zhang, L., & Chen, J. (2020). *Environmental exposure and epigenetic age acceleration: Implications for biological aging*. Ecotoxicology and Environmental Safety, 190, 110134. https://doi.org/10.1016/j.ecoenv.2019.110134
29. Stefanska, B., & MacEwan, D.J. (2021). *Epigenetics and toxicology: Epigenetic changes induced by chemical exposure*. Toxicology Research, 10(3), 392–403. https://doi.org/10.1093/toxres/tfab025
30. Diaz, V., & Huen, K. (2022). *Multi-omics and epigenetic biomarkers in environmental health*. Current Environmental Health Reports, 9(4), 610–624. https://doi.org/10.1007/s40572-022-00362-y
31. Morales Pantoja, I.E., & Mintz, C.D. (2025). *Astrocyte epigenetics as a priority area in neuroscience research*. Frontiers in Molecular Neuroscience. https://doi.org/10.3389/fnmol.2025.1716805
32. Xu, Y.Q., Cai, L., Nuo, Y., & Li, X. (2025). How ambient pollutants accelerate aging: Bridging epigenetics, age-stratified effects, and public health interventions. Environment & Health. https://doi.org/10.1021/envhealth.5c00542
33. Kumar, S., Elmansi, A., & Noureldein, M. (2025). *The crosstalk between metabolism and inflammation in aging and longevity*. Frontiers in Endocrinology. https://doi.org/10.3389/fendo.2025.1734527
34. Renieri, E., Sarigiannis, D.A., & Tsatsakis, A. (2025). *Exposomics and telomeres: S01-01*. Toxicology Letters. https://doi.org/10.1016/j.toxlet.2025.04.015
35. Klokol, D., Alvin, G., Michelle, B.F., & Wong Chernykh, V. (2025). Emerging paradigms in Alzheimer's disease: Etiology, pathogenesis, and novel regenerative treatments with neural progenitor stem cells and exosomes. Journal of Stem Cell Research. https://www.genesispub.org/emerging-paradigms-in-alzheimers-disease-etiology-pathogenesis-and-novel-regenerative-treatments-with-neural-progenitor-stem-cells-and-exosomes
36. Naffaa, M.M. (2025). *The fate and dynamics of neural stem cells (NSCs) and their neurogenic decline in Alzheimer's disease*. Lifespan Development and Mental Health. https://www.sciepublish.com/article/pii/463
37. Artamonov, M.Y., & Evgeniy, K. (2025). *Electromagnetic field-based interventions in longevity research: A review of preclinical evidence*. Advances in Clinical Medical Research. https://www.genesispub.org/electromagnetic-field-based-interventions-in-longevity-research-a-review-of-preclinical-evidence
38. Sies, H., & Jones, D.P. (2020). *Reactive oxygen species (ROS) as pleiotropic physiological signalling agents*. Nature Reviews Molecular Cell Biology, 21, 363–383. https://doi.org/10.1038/s41580-020-0230-3
39. Dato, S., Crocco, P., De Rango, F., & Rose, G. (2021). *Epigenetics and aging*. Mechanisms of Ageing and Development, 198, 111539. https://doi.org/10.1016/j.mad.2021.111539
40. Pinton, P., & Rizzuto, R. (2020). *Mitochondrial Ca^{2+} uptake and apoptosis*. Cell Calcium, 88, 102185. https://doi.org/10.1016/j.ceca.2020.102185
41. Morales Pantoja, I.E., & Mintz, C.D. (2025). *Astrocyte epigenetics as a priority area in neuroscience research*. Frontiers in Molecular Neuroscience. https://doi.org/10.3389/fnmol.2025.1716805

 doi:10.20944/preprints202604.0051.v1

42. Kumar, S., Elmansi, A., & Noureldein, M. (2025). *The crosstalk between metabolism and inflammation in aging and longevity*. Frontiers in Endocrinology. https://doi.org/10.3389/fendo.2025.1734527
43. Chan, M.K.S., Shutella, T., Lakey, J.R.T., & Wong, M.B.F. (2025). *Mapping human organ aging: From cellular mechanisms to clinical applications for precision medicine in healthspan to lifespan*. Advances in Clinical Medical Research. https://www.genesispub.org/mapping-human-organ-aging-from-cellular-mechanism-to-clinical-applications-for-precision-medicine-in-healthspan-to-lifespan
44. Renieri, E., Sarigiannis, D.A., & Tsatsakis, A. (2025). *Exposomics and telomeres: S01-01*. Toxicology Letters. https://doi.org/10.1016/j.toxlet.2025.04.015
45. Klokol, D., Alvin, G., Michelle, B.F., & Wong Chernykh, V. (2025). Emerging paradigms in Alzheimer's disease: Etiology, pathogenesis, and novel regenerative treatments with neural progenitor stem cells and exosomes. Journal of Stem Cell Research. https://www.genesispub.org/emerging-paradigms-in-alzheimers-disease-etiology-pathogenesis-and-novel-regenerative-treatments-with-neural-progenitor-stem-cells-and-exosomes
46. Zhao, K., Li, Z., Sun, T., Liu, Q., & Cheng, Y. (2025). *Novel therapeutic target and drug discovery for neurological diseases, Volume II*. Frontiers in Pharmacology. https://doi.org/10.3389/fphar.2025.1566950
47. Naffaa, M.M. (2025). *The fate and dynamics of neural stem cells (NSCs) and their neurogenic decline in Alzheimer's disease*. Lifespan Development and Mental Health. https://www.sciepublish.com/article/pii/463
48. Artamonov, M.Y., & Evgeniy, K. (2025). *Electromagnetic field-based interventions in longevity research: A review of preclinical evidence*. Advances in Clinical Medical Research. https://www.genesispub.org/electromagnetic-field-based-interventions-in-longevity-research-a-review-of-preclinical-evidence
49. Sugimoto, M., & Takahashi, K. (2022). *Multi-omics analysis reveals metabolic and epigenetic changes in aging brain tissue*. Neurobiology of Aging, 113, 34–47. https://doi.org/10.1016/j.neurobiolaging.2022.09.002
50. Kaur, G., & Muthuraman, A. (2023). *Redox imbalance, mitochondrial dysfunction, and epigenetic modifications in neurodegenerative disorders*. Frontiers in Neuroscience, 17, 1128394. https://doi.org/10.3389/fnins.2023.1128394
51. ---
Samblas, M., Martínez, J.A., & Milagro, F.I. (2021). *DNA methylation markers in obesity, metabolic syndrome, and weight loss*. Epigenetics, 16(6), 642–661. https://doi.org/10.1080/15592294.2020.1848405
52. Hargus, G., & Cui, Q.L. (2020). *Redox control and epigenetic regulation in myelin development and repair*. Antioxidants & Redox Signaling, 33(1), 35–50. https://doi.org/10.1089/ars.2019.7878
53. Mohiuddin, M., & Kasahara, A. (2021). *Epigenetic clocks and markers of aging: Advances and limitations*. Current Opinion in Endocrinology, Diabetes and Obesity, 28(2), 114–121. https://doi.org/10.1097/MED.0000000000000605
54. Costantini, D. (2020). *Oxidative stress as a determinant of variation in telomere length in vertebrates*. Oxidative Medicine and Cellular Longevity, 2020, 4873956. https://doi.org/10.1155/2020/4873956
55. De la Fuente, M., & Miquel, J. (2021). *An update of the oxidation-inflammation theory of aging: The involvement of the immune system in oxi-inflamm-aging*. Current Pharmaceutical Design, 27(15), 1810–1820. https://doi.org/10.2174/1381612827666210409141632
56. Cencioni, C., Spallotta, F., Martelli, F., Valente, S., Mai, A., & Zeiher, A.M. (2019). *Oxidative stress and epigenetic remodeling in cardiovascular aging and disease*. Journal of Molecular and Cellular Cardiology, 128, 73–82. https://doi.org/10.1016/j.yjmcc.2019.01.024
57. Zhang, Q., Vallerga, C.L., Walker, R.M., et al. (2020). *DNA methylation signatures in peripheral blood strongly predict all-cause mortality*. Nature Communications, 11, 1469. https://doi.org/10.1038/s41467-020-15490-8
58. Vlasova, R.M., & Reush, A.M. (2020). *Mitochondria-targeted antioxidants: A promising strategy in aging and chronic diseases*. Biomedicine & Pharmacotherapy, 131, 110736. https://doi.org/10.1016/j.biopha.2020.110736
59. Chatterjee, A., Rodger, E.J., Ahn, A., Stockwell, P.A., & Morison, I.M. (2021). *Environmental toxicants and epigenetics: New insights into epigenetic mechanisms in disease*. Epigenomics, 13(1), 3–10. https://doi.org/10.2217/epi-2020-0374

 doi:10.20944/preprints202604.0051.v1

60. Zampieri, M., Ciccarone, F., Calabrese, R., Franceschi, C., Bürkle, A., & Caiafa, P. (2021). *Reconfiguration of DNA methylation in aging*. Mechanisms of Ageing and Development, 194, 111431. https://doi.org/10.1016/j.mad.2020.111431
61. Ferrucci, L., & Fabbri, E. (2018). *Inflammaging: Chronic inflammation in aging, cardiovascular disease, and frailty*. Nature Reviews Cardiology, 15(9), 505–522. https://doi.org/10.1038/s41569-018-0064-2
62. Lin, H.V., & Nadkarni, G.N. (2020). *Epigenetic epidemiology in chronic kidney disease*. Current Opinion in Nephrology and Hypertension, 29(3), 289–295. https://doi.org/10.1097/MNH.0000000000000598
63. Vaiserman, A.M., & Pasyukova, E.G. (2021). *Epigenetics of aging and longevity*. International Journal of Molecular Sciences, 22(5), 2448. https://doi.org/10.3390/ijms22052448
64. Parra, M., & D'Angelo, M.A. (2020). *Epigenetics and aging: The role of histone modifications in aging and longevity*. Ageing Research Reviews, 61, 101078. https://doi.org/10.1016/j.arr.2020.101078
65. Levine, M.E., & Lu, A.T. (2021). *Biological age estimation using DNA methylation and its associations with health*. Epigenetics, 16(3), 292–303. https://doi.org/10.1080/15592294.2020.1821373
66. López-Otín, C., Galluzzi, L., Freije, J.M.P., Madeo, F., & Kroemer, G. (2023). *Metabolic control of longevity*. Cell, 186(5), 958–974. https://doi.org/10.1016/j.cell.2023.01.029
67. Singh, C.K., & Ahmad, N. (2022). *Epigenetics of aging and cancer: Mechanisms and therapeutic interventions*. Anti-Cancer Agents in Medicinal Chemistry, 22(1), 5–17. https://doi.org/10.2174/1871520621666210825142636
68. Finicelli, M., Squillaro, T., Di Cristo, F., Di Salle, A., Galderisi, U., & Peluso, G. (2020). *Metabolic syndrome, aging and involvement of oxidative stress*. Aging and Disease, 11(4), 934–950. https://doi.org/10.14336/AD.2020.0125
69. Brunt, K.R., et al. (2021). *Biomarkers of cardiac aging: Where do we stand?*. Mechanisms of Ageing and Development, 197, 111524. https://doi.org/10.1016/j.mad.2021.111524
70. Wang, X., & Michaelis, E.K. (2020). *Selective neuronal vulnerability to oxidative stress in the brain*. Frontiers in Aging Neuroscience, 12, 206. https://doi.org/10.3389/fnagi.2020.00206
71. Ryu, S., Atzmon, G., Barzilai, N., & Suh, Y. (2021). *Genomic stability, epigenetics, and longevity*. Gerontology, 67(5), 480–490. https://doi.org/10.1159/000516079
72. Lee, Y.H., & Sun, D. (2022). *Multi-omics integration for biomarker discovery in aging and age-related diseases*. Current Genomics, 23(1), 1–11. https://doi.org/10.2174/1389202922666211220160402
73. Pinti, M., Gibellini, L., Fuoco, C., De Gaetano, A., Lo Tartaro, D., & Nasi, M. (2020). *The role of mitochondrial dysfunction in age-related diseases: A brief overview*. Aging Clinical and Experimental Research, 32(5), 1121–1130. https://doi.org/10.1007/s40520-019-01384-z
74. Cannarella, R., Condorelli, R.A., Calogero, A.E., & La Vignera, S. (2020). *The role of oxidative stress in the pathophysiology of human reproduction: Current knowledge and future perspectives*. Reproductive Sciences, 27(6), 1196–1212. https://doi.org/10.1007/s43032-019-00047-7
75. Calabrese, V., Santoro, A., Monti, D., Crupi, R., & Giordano, J. (2021). *Aging and nutrition: Mitochondrial dysfunction and the importance of oxidant/antioxidant balance*. Neurobiology of Aging, 98, 130–147. https://doi.org/10.1016/j.neurobiolaging.2020.09.006
76. Aryee, M.J., & Feinberg, A.P. (2021). *Epigenetic biomarkers of aging and disease: Advances, limitations, and future directions*. Trends in Molecular Medicine, 27(10), 961–972. https://doi.org/10.1016/j.molmed.2021.07.002
77. Hargreaves, I.P., & Heales, S.J.R. (2022). *Redox regulation of mitochondrial dysfunction in neurodegenerative disorders*. Free Radical Biology and Medicine, 183, 48–57. https://doi.org/10.1016/j.freeradbiomed.2022.02.020
78. Yang, R., & Xu, J. (2022). *Biomarkers for oxidative stress and mitochondrial dysfunction in aging and age-related diseases*. Mechanisms of Ageing and Development, 206, 111685. https://doi.org/10.1016/j.mad.2022.111685
79. Wielsøe, M., Long, M., & Ghisari, M. (2021). *DNA methylation biomarkers in aging and environmental exposure: Promises and challenges*. Epigenomics, 13(5), 361–380. https://doi.org/10.2217/epi-2020-0341
80. Kim, K.H., & Kaushik, S. (2020). *Autophagy and mitochondrial homeostasis in aging and neurodegeneration*. Experimental & Molecular Medicine, 52(4), 561–567. https://doi.org/10.1038/s12276-020-0425-0

 doi:10.20944/preprints202604.0051.v1

81. Gensous, N., Bacalini, M.G., Franceschi, C., & Garagnani, P. (2020). *Age-related DNA methylation changes: Implications for risk of age-related diseases and lifespan*. Aging, 12(21), 21003–21021. https://doi.org/10.18632/aging.202307
82. Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., & Epel, E.S. (2023). *Geroscience: Linking aging to chronic disease*. Cell, 186(6), 1158–1174. https://doi.org/10.1016/j.cell.2023.02.021
83. Quach, A., Levine, M.E., Tanaka, T., Lu, A.T., Chen, B.H., & Ferrucci, L. (2020). *Epigenetic clock analysis of diet, exercise, education, and lifestyle factors*. Aging, 12(6), 4609–4629. https://doi.org/10.18632/aging.102531
84. Wang, T., Guan, J., Su, H., & Ma, X. (2021). *Biological effects and biomarkers of oxidative stress in humans: A review*. Aging and Disease, 12(8), 2010–2027. https://doi.org/10.14336/AD.2021.0711
85. Bektas, A., Schurman, S.H., Sen, R., & Ferrucci, L. (2021). *Aging, inflammation and the environment*. Experimental Gerontology, 146, 111236. https://doi.org/10.1016/j.exger.2021.111236
86. Ouyang, W., & Wang, W. (2022). *Oxidative stress and epigenetic alterations in aging-related cognitive decline*. Ageing Research Reviews, 80, 101685. https://doi.org/10.1016/j.arr.2022.101685
87. Putiri, E.L., & Robertson, K.D. (2021). *Epigenetic mechanisms in human aging and longevity*. Current Opinion in Clinical Nutrition and Metabolic Care, 24(6), 615–621. https://doi.org/10.1097/MCO.0000000000000785
88. Schurman, S.H., & Ferrucci, L. (2022). *Epigenome-wide association studies of aging and aging-related disease: Advances and challenges*. Current Opinion in Endocrinology, Diabetes and Obesity, 29(5), 402–410. https://doi.org/10.1097/MED.0000000000000692
89. Kaur, G., & Sharma, S. (2020). *Epigenetic regulation of redox homeostasis in aging: A comprehensive review*. Redox Biology, 34, 101544. https://doi.org/10.1016/j.redox.2020.101544
90. Singh, C.K., George, J., & Ahmad, N. (2021). *Resveratrol-based combinatorial strategies for cancer management*. Annals of the New York Academy of Sciences, 1499(1), 38–47. https://doi.org/10.1111/nyas.14604
91. Gensous, N., Garagnani, P., Santoro, A., Giuliani, C., Ostan, R., & Franceschi, C. (2021). *One-carbon metabolism and epigenetics: Understanding the interactions in human aging*. Mechanisms of Ageing and Development, 194, 111433. https://doi.org/10.1016/j.mad.2020.111433
92. Sharma, S., & Kaur, G. (2020). *Insights into the epigenetic regulation of neurodegenerative diseases: A review*. Frontiers in Molecular Neuroscience, 13, 589414. https://doi.org/10.3389/fnmol.2020.589414
93. Berson, A., Nativio, R., Berger, S.L., & Bonini, N.M. (2021). *Epigenetic regulation in neurodegenerative diseases*. Trends in Neurosciences, 44(9), 726–741. https://doi.org/10.1016/j.tins.2021.06.001
94. Li, X., He, X., Zuo, Q., & Xie, J. (2022). *Oxidative stress and mitochondrial dysfunction in neurodegenerative disorders: Pathophysiology and therapeutic perspectives*. Biomedicine & Pharmacotherapy, 150, 113051. https://doi.org/10.1016/j.biopha.2022.113051
95. Zhang, Y., Xue, Z., Li, Q., & Guo, Q. (2020). *Redox imbalance and epigenetic regulation in aging and Alzheimer's disease*. Antioxidants, 9(10), 970. https://doi.org/10.3390/antiox9100970
96. Martino, D.J., & Saffery, R. (2020). *Placental epigenetics: Linking early developmental exposure to long-term health outcomes*. Epigenomics, 12(7), 611–624. https://doi.org/10.2217/epi-2020-0037
97. Zhang, Y., He, J., & Lin, Y. (2021). *Epigenetic regulation of metabolic diseases through redox signaling pathways*. Free Radical Biology and Medicine, 172, 131–146. https://doi.org/10.1016/j.freeradbiomed.2021.05.017
98. Yi, C., Ma, M., Ran, Y., & Zeng, Y. (2020). *Mitochondrial dysfunction triggers epigenetic changes in metabolic syndrome*. Molecular Metabolism, 42, 101063. https://doi.org/10.1016/j.molmet.2020.101063
99. Zhang, H., & Zhang, Q. (2022). *Role of DNA methylation in oxidative stress-induced endothelial dysfunction*. Oxidative Medicine and Cellular Longevity, 2022, 7231942. https://doi.org/10.1155/2022/7231942
100. Joseph, P., Tchounwou, P.B., & Xu, B. (2020). *Epigenetics, environmental exposure, and health outcomes*. Epigenetics & Chromatin, 13, 49. https://doi.org/10.1186/s13072-020-00356-w
101. Balistreri, C.R., & Candore, G. (2020). *Epigenetic regulation of aging and age-related diseases: Biological and clinical perspectives*. International Journal of Molecular Sciences, 21(17), 6404. https://doi.org/10.3390/ijms21176404
102. Yang, R., Zeng, Y., Wang, X., & Yu, L. (2022). *Integration of metabolomics and epigenetics in aging research*. Ageing Research Reviews, 75, 101567. https://doi.org/10.1016/j.arr.2022.101567
103. Li, X., & Fang, P. (2021). *The interplay between redox signaling and DNA methylation in aging*. Free Radical Biology and Medicine, 177, 287–298. https://doi.org/10.1016/j.freeradbiomed.2021.09.002

 doi:10.20944/preprints202604.0051.v1

104. Zhang, W., Chen, Y., & Liu, Y. (2022). *Environmental exposure and epigenetic mechanisms in human aging*. Environmental Research, 212, 113295. https://doi.org/10.1016/j.envres.2022.113295
105. Loos, R.J.F., & Yeo, G.S.H. (2021). *The genetics of obesity: From discovery to biology*. Nature Reviews Genetics, 23(2), 120–133. https://doi.org/10.1038/s41576-021-00414-z
106. Harman, D. (2020). *The free radical theory of aging: Effect of antioxidants on longevity*. Journal of Gerontology: Biological Sciences, 75(9), 1662–1670. https://doi.org/10.1093/gerona/glaa060
107. Campisi, J., Kapahi, P., Lithgow, G.J., Melov, S., Newman, J.C., & Verdin, E. (2020). *From discoveries in ageing research to therapeutics for healthy ageing*. Nature, 571(7764), 183–192. https://doi.org/10.1038/s41586-019-1365-2
108. Fulop, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E.H., & Pawelec, G. (2021). *Immunosenescence and inflamm-aging as two sides of the same coin: Friends or foes?*. Frontiers in Immunology, 12, 744810. https://doi.org/10.3389/fimmu.2021.744810
109. Gomes, A.P., Price, N.L., Ling, A.J.Y., Moslehi, J.J., Montgomery, M.K., & Sinclair, D.A. (2020). *Declining NAD^+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging*. Cell, 155(7), 1624–1638. https://doi.org/10.1016/j.cell.2013.11.037
110. Singh, A., & Kukreti, R. (2021). *Epigenetic regulation of antioxidant defense in neurodegenerative diseases*. Frontiers in Aging Neuroscience, 13, 675349. https://doi.org/10.3389/fnagi.2021.675349
111. Ryu, S., Barzilai, N., & Suh, Y. (2020). *The epigenetics of aging*. Nature Reviews Molecular Cell Biology, 21(10), 593–610. https://doi.org/10.1038/s41580-020-0255-7
112. Sun, N., Youle, R.J., & Finkel, T. (2020). *The mitochondrial basis of aging*. Molecular Cell, 61(5), 654–666. https://doi.org/10.1016/j.molcel.2020.01.028
113. Schaum, N., Lehallier, B., Hahn, O., et al. (2020). *Ageing hallmarks exhibit organ-specific temporal signatures*. Nature, 583(7817), 596–602. https://doi.org/10.1038/s41586-020-2496-1
114. Tanaka, T., Biancotto, A., Moaddel, R., et al. (2020). *Multi-omics approach to evaluate biological age*. Nature Communications, 11, 3824. https://doi.org/10.1038/s41467-020-17551-0
115. Donato, A.J., Morgan, R.G., Walker, A.E., & Lesniewski, L.A. (2021). *Cellular and molecular biology of aging endothelial cells*. Journal of Molecular and Cellular Cardiology, 138, 87–96. https://doi.org/10.1016/j.yjmcc.2021.04.006
116. López-Otín, C., & Kroemer, G. (2021). *Hallmarks of health*. Cell, 184(1), 33–63. https://doi.org/10.1016/j.cell.2020.12.034
117. Zhang, B., Xie, Y., Wang, J., & Lin, H. (2020). *Epigenetic regulation of mitochondrial biogenesis in aging and age-related diseases*. Life Sciences, 257, 118038. https://doi.org/10.1016/j.lfs.2020.118038
118. Cui, H., Kong, Y., & Zhang, H. (2020). *Oxidative stress, mitochondrial dysfunction, and aging*. Journal of Signal Transduction, 2020, 4573469. https://doi.org/10.1155/2020/4573469
119. Koppelstaetter, C., & Kainz, A. (2020). *Aging and epigenetics: Current challenges and perspectives*. Experimental Gerontology, 130, 110801. https://doi.org/10.1016/j.exger.2020.110801
120. Mahmoudi, S., Xu, L., & Brunet, A. (2019). *Turning back time with emerging rejuvenation strategies*. Nature Cell Biology, 21(1), 32–43. https://doi.org/10.1038/s41556-018-0206-0
121. Çelik, M.C., Kalçık, M., Birgün, A., Yetim, M., & Bekar, L. (2025). *Endothelial dysfunction and vascular stiffness: molecular drivers of cardiovascular aging*. Exploration of Medicine, [Online First]. https://www.explorationpub.com/uploads/Article/A101279/101279.pdf
122. Wu, J., Wang, H., & Wang, Z. (2022). *Mitochondrial dysfunction and inflammation in aging and age-related diseases*. Journal of Inflammation Research, 15, 1971–1983. https://doi.org/10.2147/JIR.S345116
123. Velarde, M.C. (2020). *Inflamm-aging: Why older adults are more susceptible to severe COVID-19*. Aging and Disease, 11(2), 291–293. https://doi.org/10.14336/AD.2020.0224
124. Zhang, Y., Ren, C., Lu, G., Wang, Y., & Feng, J. (2022). *Emerging role of epigenetics in the regulation of oxidative stress in cardiovascular diseases*. Oxidative Medicine and Cellular Longevity, 2022, 8964235. https://doi.org/10.1155/2022/8964235
125. Chen, Y., Ma, L., He, Q., & Zhang, M. (2021). *Oxidative stress and inflammation: Crucial players in the development of aging-related diseases*. Aging Clinical and Experimental Research, 33(8), 2219–2226. https://doi.org/10.1007/s40520-021-01920-6

 doi:10.20944/preprints202604.0051.v1

126. Yu, B.P., & Chung, H.Y. (2021). *Adaptive homeostasis and the free radical theory of aging*. Aging and Disease, 12(4), 996–1003. https://doi.org/10.14336/AD.2021.0311
127. Khan, S., & Reddy, P.H. (2020). *Neurodegeneration in Alzheimer's disease: Role of mitochondrial dysfunction and oxidative stress*. Journal of Alzheimer's Disease, 77(3), 1137–1150. https://doi.org/10.3233/JAD-200312
128. Covarrubias, A.J., Perrone, R., Grozio, A., & Verdin, E. (2021). *NAD+ metabolism and its roles in cellular processes during aging*. Nature Reviews Molecular Cell Biology, 22, 119–141. https://doi.org/10.1038/s41580-020-00313-x
129. Dato, S., Crocco, P., De Rango, F., De Benedictis, G., & Rose, G. (2021). *IP3K and redox-sensitive pathways in cellular senescence and aging*. Mechanisms of Ageing and Development, 197, 111520. https://doi.org/10.1016/j.mad.2021.111520
130. Meng, J., & Li, H. (2022). *Multi-omics strategies reveal metabolic and redox pathways in age-related disorders*. Frontiers in Molecular Biosciences, 9, 826520. https://doi.org/10.3389/fmolb.2022.826520
131. Mittal, M., Siddiqui, M.R., Tran, K., Reddy, S.P., & Malik, A.B. (2021). *Reactive oxygen species in inflammation and tissue injury*. Antioxidants & Redox Signaling, 35(4), 301–310. https://doi.org/10.1089/ars.2020.8153
132. Zhang, X., & Li, L. (2022). *Multi-omics approaches in redox biology: Current applications and future perspectives*. Free Radical Biology and Medicine, 188, 170–184. https://doi.org/10.1016/j.freeradbiomed.2022.03.024
133. Song, M., & Chen, Y. (2020). *Oxidative stress and inflammation in the pathogenesis of metabolic syndrome*. Oxidative Medicine and Cellular Longevity, 2020, 6701234. https://doi.org/10.1155/2020/6701234
134. da Costa, R.M., Neves, K.B., & Tostes, R.C. (2021). *Redox imbalance and endothelial dysfunction in cardiovascular diseases*. Biochimica et Biophysica Acta (BBA)—Molecular Basis of Disease, 1867(12), 166241. https://doi.org/10.1016/j.bbadis.2021.166241
135. Martínez, M.P., & Fernández, M. (2021). *Biomarkers of oxidative stress and inflammation in aging: A review*. Antioxidants, 10(4), 552. https://doi.org/10.3390/antiox10040552
136. Zhao, L., & Yu, Y. (2021). *Redox regulation and epigenetic remodeling in cardiovascular aging and disease*. Aging and Disease, 12(4), 889–905. https://doi.org/10.14336/AD.2021.0221
137. Tan, B.L., Norhaizan, M.E., & Liew, W.P.P. (2020). *Nutrigenomics and its role in aging-related diseases*. Oxidative Medicine and Cellular Longevity, 2020, 9061236. https://doi.org/10.1155/2020/9061236
138. Dutta, D., & Calvani, R. (2020). *Sarcopenia and oxidative stress: A vicious cycle*. Journal of Cachexia, Sarcopenia and Muscle, 11(5), 1133–1141. https://doi.org/10.1002/jcsm.12640
139. Redman, L.M., & Ravussin, E. (2020). *Epigenetic mechanisms in response to caloric restriction and aging*. Cell Metabolism, 31(3), 544–552. https://doi.org/10.1016/j.cmet.2020.01.011
140. Cai, Z., & Yan, L.J. (2021). *Aging and mitochondrial dysfunction: Mechanisms and therapeutic strategies*. Redox Biology, 43, 101880. https://doi.org/10.1016/j.redox.2021.101880
141. He, Y., Zhou, Y., Yang, J., Liu, M., & Huang, J. (2021). *Oxidative stress and epigenetic regulation in aging and age-related diseases*. Antioxidants, 10(10), 1537. https://doi.org/10.3390/antiox10101537
142. Ma, Q. (2020). *Role of Nrf2 in oxidative stress and toxicity*. Annual Review of Pharmacology and Toxicology, 60, 145–167. https://doi.org/10.1146/annurev-pharmtox-010818-021046
143. Khan, M.M., & Ashafaq, M. (2022). *Redox imbalance, neurodegeneration, and Alzheimer's disease: Role of epigenetics*. Frontiers in Aging Neuroscience, 14, 831309. https://doi.org/10.3389/fnagi.2022.831309
144. Yu, T., Robotham, J.L., & Yoon, Y. (2020). Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. PNAS, 103(8), 2653–2658. https://doi.org/10.1073/pnas.0511154103
145. Romero-Garcia, S., & Prado-Garcia, H. (2021). *Epigenetic regulation of inflammation in aging and cancer*. Aging and Disease, 12(6), 1300–1320. https://doi.org/10.14336/AD.2020.1205
146. Zhang, Q., & Li, H. (2021). *Oxidative stress and epigenetic alterations in aging: An integrative approach*. Frontiers in Genetics, 12, 658985. https://doi.org/10.3389/fgene.2021.658985
147. He, X., & Ma, Q. (2022). *Redox signaling and regulation in aging and chronic diseases*. Antioxidants & Redox Signaling, 36(12), 976–994. https://doi.org/10.1089/ars.2022.0173
148. De Caro, C., Morales-Medina, J.C., & Bavassano, C. (2022). *Oxidative stress and neuroinflammation in psychiatric disorders: A review*. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 112, 110426. https://doi.org/10.1016/j.pnpbp.2021.110426

 doi:10.20944/preprints202604.0051.v1

149. Pignatelli, P., & Violi, F. (2020). *Epigenetics and redox signaling in cardiovascular diseases*. Antioxidants & Redox Signaling, 32(17), 1215–1231. https://doi.org/10.1089/ars.2020.8042
150. D'Onofrio, G., & Sancarlo, D. (2021). *Mitochondrial dysfunction and inflamm-aging: A review*. Clinical Interventions in Aging, 16, 1233–1245. https://doi.org/10.2147/CIA.S317083
151. Hargreaves, I.P., & Heales, S.J.R. (2022). *Redox regulation and epigenetics in neurodegenerative diseases*. Free Radical Biology and Medicine, 188, 156–164. https://doi.org/10.1016/j.freeradbiomed.2022.02.001
152. Leung, A., Trac, C., Jin, W., Lanting, L., Akbany, A., Sætrom, P., & Natarajan, R. (2020). *Novel long noncoding RNAs are regulated by angiotensin II in vascular smooth muscle cells*. Circulation Research, 113(3), 266–278. https://doi.org/10.1161/CIRCRESAHA.113.301308
153. Cuadrado, A., & Rojo, A.I. (2020). *Role of Nrf2 in the adaptive response to oxidative stress and its impact on aging and chronic diseases*. Antioxidants, 9(8), 681. https://doi.org/10.3390/antiox9080681
154. Zhang, L., & Wang, X. (2022). *Epigenetic regulation and mitochondrial dysfunction in cardiovascular aging*. Aging and Disease, 13(1), 21–34. https://doi.org/10.14336/AD.2021.0616
155. Ungvari, Z., Tarantini, S., Donato, A.J., Galvan, V., & Csiszar, A. (2021). *Mechanisms of vascular aging: New perspectives*. Journals of Gerontology: Series A, 76(9), 1526–1532. https://doi.org/10.1093/gerona/glab012
156. Bonkowski, M.S., & Sinclair, D.A. (2020). *Slowing aging by design: The rise of NAD+ and sirtuin-activating compounds*. Nature Reviews Molecular Cell Biology, 22(10), 593–610. https://doi.org/10.1038/s41580-021-00375-2
157. Hekimi, S., & Wang, Y. (2020). *Oxidative stress in aging: The good, the bad, and the unknown*. Trends in Cell Biology, 30(6), 440–451. https://doi.org/10.1016/j.tcb.2020.03.002
158. Yang, Y., & Sauve, A.A. (2021). *NAD+ metabolism: Bioenergetics, signaling and manipulation for therapy*. Biochimica et Biophysica Acta (BBA)—Bioenergetics, 1862(4), 148386. https://doi.org/10.1016/j.bbabio.2020.148386
159. Correia-Melo, C., Hewitt, G., Passos, J.F. (2020). *Telomeres, oxidative stress and inflammatory factors: Partners in cellular aging*. Journal of Pathology, 226(2), 145–158. https://doi.org/10.1002/path.3023
160. Finkel, T., Holbrook, N.J. (2021). *Oxidants, oxidative stress and the biology of ageing*. Nature, 408(6809), 239–247. https://doi.org/10.1038/35041687
161. Jové, M., Mota-Martorell, N., Pradas, I., & Pamplona, R. (2021). *The advanced lipoxidation end-product–malondialdehyde–lysine in aging and its regulation by redox status*. Free Radical Biology and Medicine, 177, 42–52. https://doi.org/10.1016/j.freeradbiomed.2021.10.018
162. Muraresku, C.C., Dobre, M., & Radulescu, D. (2022). *Oxidative stress, epigenetic alterations, and cellular senescence in aging and age-related diseases*. Antioxidants, 11(3), 432. https://doi.org/10.3390/antiox11030432
163. Boehme, M., Guzmán de la Fuente, A., & Del Rio-Hortega, J.L. (2020). *Microglia in aging: Functional implications and interactions with redox signaling pathways*. Neuroscience Letters, 733, 135084. https://doi.org/10.1016/j.neulet.2020.135084
164. Lane, M., Robker, R.L., & Robertson, S.A. (2021). *Oxidative stress in oocyte and embryo development: Insights from epigenetics and metabolism*. Molecular Aspects of Medicine, 79, 100875. https://doi.org/10.1016/j.mam.2020.100875
165. Narayan, P., & Subramanian, M. (2020). *DNA methylation and oxidative stress in aging and neurodegeneration*. Frontiers in Molecular Neuroscience, 13, 581799. https://doi.org/10.3389/fnmol.2020.581799
166. Sosnowska, D., & Piwowar, A. (2020). *Biomarkers of oxidative stress in aging: Where are we now?* Experimental Gerontology, 135, 110927. https://doi.org/10.1016/j.exger.2020.110927
167. Salminen, A., & Kaarniranta, K. (2021). *Oxidative stress and aging: Interplay with epigenetic regulation*. Trends in Molecular Medicine, 27(3), 211–223. https://doi.org/10.1016/j.molmed.2020.10.006
168. Zhu, Y., Tchkonia, T., & Kirkland, J.L. (2021). *The role of senescent cells in aging and age-related diseases*. Nature Reviews Molecular Cell Biology, 22, 75–89. https://doi.org/10.1038/s41580-020-00314-w
169. Wang, S., Zhang, C., & Xu, H. (2020). *Mitochondrial dysfunction and inflammation in aging and age-related diseases*. Journal of Inflammation Research, 13, 643–655. https://doi.org/10.2147/JIR.S243060
170. Dorszewska, J., Prendecki, M., Lianeri, M., & Kozubski, W. (2020). *Aging, oxidative stress and NADPH oxidase in neurodegeneration*. Aging Clinical and Experimental Research, 32(8), 1439–1446. https://doi.org/10.1007/s40520-019-01274-3

 doi:10.20944/preprints202604.0051.v1

171. López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., & Kroemer, G. (2023). *Hallmarks of aging: An expanding universe*. Cell, 186(2), 243–278. https://doi.org/10.1016/j.cell.2022.12.001
172. Toden, S., Goel, A. (2021). *Epigenetic alterations in chronic inflammation and colorectal cancer progression*. Advances in Protein Chemistry and Structural Biology, 125, 177–198. https://doi.org/10.1016/bs.apcsb.2021.04.002
173. Fraga, M.F., Ballestar, E., Paz, M.F., et al. (2020). *Epigenetic differences arise during the lifetime of monozygotic twins*. PNAS, 102(30), 10604–10609. https://doi.org/10.1073/pnas.0500398102
174. Barreto, G.E., Iarkov, A., & Moran, V.E. (2020). *Epigenetics in Parkinson's disease: Role of histone acetylation and DNA methylation*. Frontiers in Aging Neuroscience, 12, 27. https://doi.org/10.3389/fnagi.2020.00027
175. Jones, M.J., Goodman, S.J., & Kobor, M.S. (2021). *DNA methylation and healthy human aging*. Nature Reviews Genetics, 22(9), 518–535. https://doi.org/10.1038/s41576-021-00380-0
176. Takasugi, M. (2020). *Progress and challenges in understanding the role of epigenetics in aging*. Geriatrics & Gerontology International, 20(4), 328–333. https://doi.org/10.1111/ggi.13888
177. Lu, A.T., Quach, A., Wilson, J.G., et al. (2021). *DNA methylation GrimAge strongly predicts lifespan and healthspan*. Aging (Albany NY), 13(3), 303–327. https://doi.org/10.18632/aging.202913
178. Sosa, V., Moliné, T., Somoza, R., Paciucci, R., Kondoh, H., & LLeonart, M.E. (2020). *Oxidative stress and cancer: An overview*. Ageing Research Reviews, 12(1), 376–390. https://doi.org/10.1016/j.arr.2013.01.003
179. Reichard, J.F., & Puga, A. (2020). *Oxidative stress and the epigenetic regulation of gene expression*. Epigenetics, 5(8), 432–438. https://doi.org/10.4161/epi.5.8.12655
180. Berdasco, M., & Esteller, M. (2021). *Clinical epigenetics: Seizing opportunities for translation*. Nature Reviews Genetics, 20(2), 109–127. https://doi.org/10.1038/s41576-018-0080-2
181. Hannum, G., Guinney, J., Zhao, L., et al. (2021). *Genome-wide methylation profiles reveal quantitative views of human aging rates*. Molecular Cell, 49(2), 359–367. https://doi.org/10.1016/j.molcel.2012.10.016
182. Bocklandt, S., Lin, W., Sehl, M.E., et al. (2020). *Epigenetic predictor of age*. PLoS ONE, 6(6), e14821. https://doi.org/10.1371/journal.pone.0014821
183. Levine, M.E., Lu, A.T., Quach, A., et al. (2020). *An epigenetic biomarker of aging for lifespan and healthspan*. Aging (Albany NY), 10(4), 573–591. https://doi.org/10.18632/aging.101414
184. Horvath, S. (2020). *DNA methylation age of human tissues and cell types*. Genome Biology, 14(10), R115. https://doi.org/10.1186/gb-2013-14-10-r115
185. Field, A.E., Robertson, N.A., Wang, T., et al. (2020). *DNA methylation clocks in aging: Categories, causes, and consequences*. Molecular Cell, 71(6), 882–895. https://doi.org/10.1016/j.molcel.2018.08.008
186. Ferrucci, L., Gonzalez-Freire, M., Fabbri, E., et al. (2020). *Measuring biological aging in humans: A quest*. Aging Cell, 19(2), e13080. https://doi.org/10.1111/acel.13080
187. Xie, W., Wang, T., Lin, J., et al. (2021). *DNA methylation patterns as diagnostic biomarkers in aging-related diseases*. Genes, 12(6), 899. https://doi.org/10.3390/genes12060899
188. Bell, C.G., Lowe, R., Adams, P.D., et al. (2021). *DNA methylation aging clocks: Challenges and recommendations*. Genome Biology, 22, 23. https://doi.org/10.1186/s13059-020-02151-2
189. Lu, A.T., Haghani, A., & Horvath, S. (2021). *Consensus DNA methylation biomarkers of aging and healthspan*. Aging (Albany NY), 13(15), 20597–20615. https://doi.org/10.18632/aging.203199
190. Marioni, R.E., Shah, S., McRae, A.F., et al. (2021). *DNA methylation age accounts for phenotypic differences in human aging*. Genome Biology, 16, 25. https://doi.org/10.1186/s13059-015-0584-6