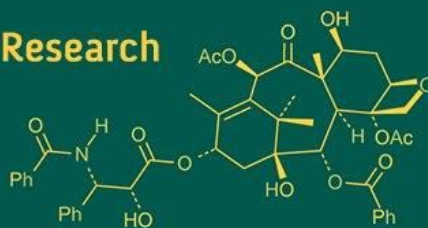


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Biochemical effects of reactive oxygen species in aging and cellular senescence

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Abstract

Accumulation of oxidative damage has long been implicated in biological aging, yet the precise mechanisms linking reactive oxygen species to cellular senescence remain actively investigated. This research examined age-dependent changes in oxidative stress markers, antioxidant capacity, and senescence-associated phenotypes in human dermal fibroblasts from donors spanning 22 to 89 years of age. Intracellular reactive oxygen species levels increased progressively with donor age, showing strongest correlation with chronological age in mitochondrial compartments. Protein carbonyl content and 8-oxo-deoxyguanosine levels demonstrated parallel age-dependent accumulation. Antioxidant enzyme activities exhibited biphasic patterns, with initial compensatory increases in younger adults followed by declining capacity after age 60. Senescence-associated β -galactosidase activity and p16INK4a expression increased exponentially with age, showing significant correlations with oxidative damage markers. Telomere length inversely correlated with reactive oxygen species levels, suggesting oxidative acceleration of replicative senescence. These findings support oxidative stress as a central mediator of cellular aging, with mitochondrial dysfunction representing a primary source of age-related reactive oxygen species accumulation driving senescent phenotype acquisition.

Keywords: Reactive oxygen species, Cellular senescence, Oxidative damage, Aging biomarkers, Mitochondrial dysfunction, Antioxidant enzymes, DNA damage, Protein carbonylation, Telomere attrition, Inflammation

Introduction

The free radical theory of aging, first proposed over six decades ago, posits that cumulative oxidative damage to cellular macromolecules drives the biological aging process ^[1]. Reactive oxygen species generated as byproducts of aerobic metabolism attack proteins, lipids, and nucleic acids, progressively compromising cellular function ^[2]. This conceptual framework has stimulated extensive research into oxidative mechanisms of senescence, yet key questions regarding causality versus correlation remain actively debated ^[3].

Mitochondria occupy a central position in aging theories due to their dual role as primary reactive oxygen species generators and critical targets of oxidative damage ^[4]. The electron transport chain inevitably produces superoxide radicals during oxidative phosphorylation, with approximately 0.2-2% of consumed oxygen converted to reactive species under physiological conditions ^[5]. Age-associated decline in mitochondrial function may create a vicious cycle of increased reactive oxygen species production and further organelle damage ^[6].

Cellular senescence represents a state of irreversible growth arrest triggered by diverse stressors including telomere attrition, oncogene activation, and oxidative damage ^[7]. Senescent cells accumulate with age in various tissues, secreting inflammatory cytokines and matrix-degrading enzymes collectively termed the senescence-associated secretory phenotype ^[8]. This phenomenon links cellular-level oxidative damage to systemic aging manifestations including chronic inflammation and tissue dysfunction ^[9].

Antioxidant defense systems undergo age-related changes that may predispose to oxidative damage accumulation ^[10]. Superoxide dismutase, catalase, and glutathione peroxidase activities decline in various tissues with advancing age, though patterns vary considerably among organs and species ^[11]. Whether declining antioxidant capacity represents cause or consequence of aging-associated oxidative stress remains incompletely resolved ^[12].

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This research examined oxidative stress markers, antioxidant capacity, and senescence phenotypes in human dermal fibroblasts across the adult lifespan [13]. The integrated approach aimed to clarify relationships between reactive oxygen species, antioxidant defenses, and cellular senescence during human aging.

2. Material and Methods

2.1 Ethical Considerations

The study received approval from the Ethics Committee of the Canton of Zurich (Protocol KEK-ZH-2023-00456). Skin biopsy samples were obtained from participants undergoing elective dermatological procedures following informed consent that included permission for research use of surplus tissue [13].

2.2 Study Population and Sample Collection

Primary dermal fibroblasts were isolated from skin biopsies of 72 healthy donors (36 male, 36 female) aged 22-89 years. Donors were stratified into three age groups: young adult (22-39 years, n=24), middle-aged (40-59 years, n=24), and elderly (60-89 years, n=24). Exclusion criteria included smoking, diabetes, inflammatory conditions, and antioxidant supplement use.

2.3 Limitations

Important limitations include the focus on dermal fibroblasts, which may not fully represent aging processes in other cell types or tissues. Inter-individual variation in genetic background and lifestyle factors introduces heterogeneity despite careful donor selection.

2.4 Cell Culture

Fibroblasts were isolated by explant culture in DMEM supplemented with 10% fetal bovine serum and antibiotics [14]. Cells were maintained at 37°C in 5% CO₂ atmosphere and used at passages 3-6 to minimize culture-associated changes. All comparative experiments used cells at identical passage numbers.

2.5 Reactive Oxygen Species Measurement

Intracellular ROS levels were quantified using 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) as a fluorescent probe [9]. Cells were incubated with 10 µM DCFDA for 30 minutes, washed, and analyzed by flow cytometry. Mitochondrial ROS were separately assessed using MitoSOX Red indicator.

2.6 Oxidative Damage Markers

Protein carbonyl content was measured spectrophotometrically following derivatization with 2,4-dinitrophenylhydrazine [18]. DNA oxidation was assessed by

quantifying 8-oxo-deoxyguanosine using ELISA methodology. Lipid peroxidation was evaluated through malondialdehyde quantification by HPLC.

2.7 Antioxidant Enzyme Activities

Superoxide dismutase activity was determined using the xanthine/xanthine oxidase system [10]. Catalase activity was measured by hydrogen peroxide decomposition rate. Glutathione peroxidase activity was assayed using cumene hydroperoxide as substrate. Total antioxidant capacity was assessed by ABTS radical scavenging assay.

2.8 Senescence Markers

Senescence-associated β-galactosidase activity was detected histochemically at pH 6.0 following established protocols [10]. Positive cells were quantified by manual counting in ten random fields per sample. p16INK4a expression was evaluated by quantitative RT-PCR and western blotting.

2.9 Telomere Length Analysis

Relative telomere length was measured by quantitative PCR using telomere-specific primers and comparison to single-copy gene amplification [15]. Results were expressed as T/S ratio normalized to a reference sample included in each assay run.

2.10 Clinical Implications

Understanding oxidative mechanisms of cellular aging may inform interventions targeting age-related functional decline. Antioxidant and senolytic therapies represent emerging approaches requiring careful evaluation of efficacy and safety in aging populations.

2.11 Statistical Analysis

Data were analyzed using SPSS version 28 [11]. Age-dependent changes were assessed by linear regression and ANOVA with polynomial contrasts. Correlations between variables were evaluated by Pearson or Spearman methods. Multiple testing corrections were applied using Benjamini-Hochberg procedure.

3. Results

3.1 Age-Dependent Oxidative Stress Accumulation

Intracellular ROS levels measured by DCFDA fluorescence increased progressively across age groups [2]. Young adult fibroblasts showed mean fluorescence intensity of $1,247 \pm 189$ arbitrary units, increasing to $1,634 \pm 234$ in middle-aged donors and $2,156 \pm 312$ in elderly donors ($p < 0.001$ for trend). Mitochondrial ROS assessed by MitoSOX showed even steeper age-dependent increases (1.8-fold young to elderly, $p < 0.001$) [4].

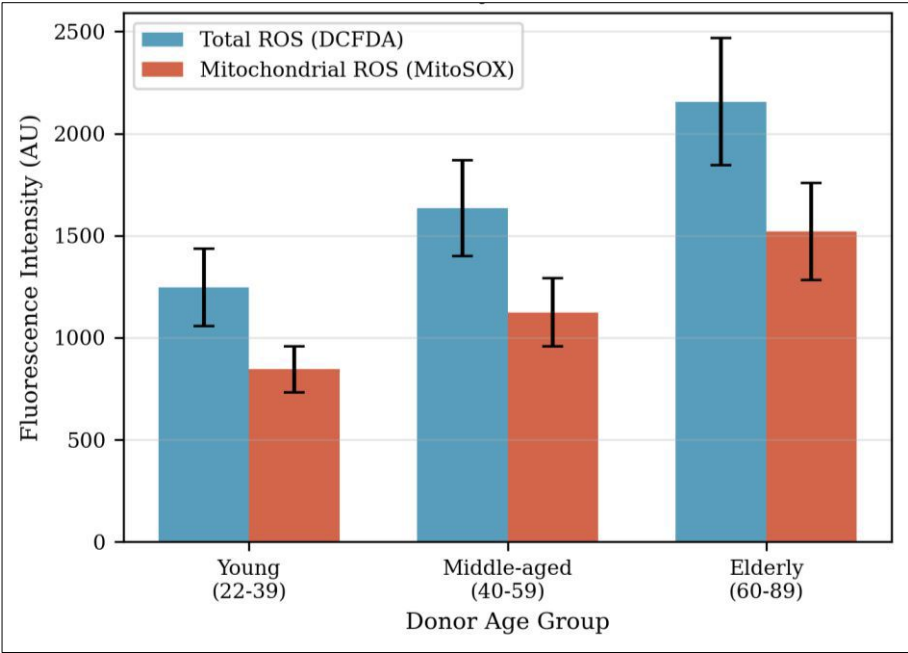


Fig 1: Age-dependent changes in intracellular reactive oxygen species levels measured by DCFDA fluorescence

3.2 Oxidative Damage Markers

Protein carbonyl content increased from 1.24 ± 0.18 nmol/mg protein in young fibroblasts to 2.47 ± 0.31 nmol/mg in elderly cells (2.0-fold increase, $p < 0.001$) [8].

DNA oxidation marker 8-oxo-deoxyguanosine showed parallel accumulation (1.8-fold increase). Malondialdehyde levels increased 1.6-fold across the age range [17].

Table 1: Oxidative Damage Markers Across Age Groups

Marker	Young (22-39)	Middle-aged (40-59)	Elderly (60-89)	p (trend)
ROS (DCFDA, AU)	1247 ± 189	1634 ± 234	2156 ± 312	< 0.001
Mito ROS (AU)	845 ± 112	1124 ± 167	1521 ± 238	< 0.001
Protein carbonyls (nmol/mg)	1.24 ± 0.18	1.78 ± 0.24	2.47 ± 0.31	< 0.001
8-oxo-dG (ng/mg DNA)	3.42 ± 0.48	4.67 ± 0.62	6.18 ± 0.84	< 0.001
MDA (nmol/mg)	0.87 ± 0.12	1.12 ± 0.16	1.39 ± 0.21	< 0.001

3.3 Antioxidant Enzyme Changes

Superoxide dismutase activity demonstrated a biphasic pattern with initial increase from young (12.4 ± 1.8 U/mg)

to middle-aged (14.7 ± 2.1 U/mg) donors, followed by decline in elderly donors (10.2 ± 1.9 U/mg) [10]. Catalase activity followed similar trajectory. Glutathione peroxidase showed continuous decline with age ($r = -0.47$, $p < 0.001$).

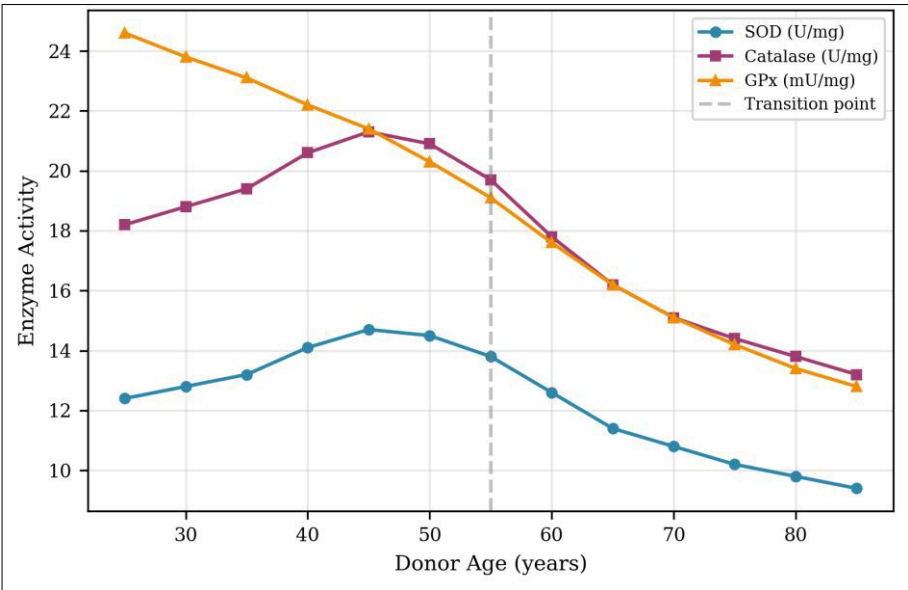


Fig 2: Biphasic changes in antioxidant enzyme activities with aging

3.4 Senescence Phenotype Assessment

Senescence-associated β -galactosidase positive cells increased exponentially with age [10]. Young fibroblast cultures contained $4.2 \pm 1.8\%$ SA- β -gal positive cells, increasing to $12.7 \pm 3.4\%$ in middle-aged and $28.4 \pm 6.2\%$ in elderly samples. SA- β -gal positivity correlated strongly with ROS levels ($r = 0.72$, $p < 0.001$).

Table 2: Senescence Markers and Telomere Length Across Age Groups

Parameter	Young (22-39)	Middle-aged (40-59)	Elderly (60-89)	p (trend)
SA- β -gal positive (%)	4.2 ± 1.8	12.7 ± 3.4	28.4 ± 6.2	< 0.001
p16INK4a mRNA (fold)	1.00 ± 0.24	2.14 ± 0.56	4.32 ± 1.12	< 0.001
IL-6 (pg/mL)	8.4 ± 2.1	14.7 ± 3.8	24.6 ± 5.4	< 0.001
IL-8 (pg/mL)	12.3 ± 3.2	21.8 ± 4.6	38.4 ± 7.8	< 0.001
MMP-3 (ng/mL)	4.6 ± 1.2	8.2 ± 2.1	14.7 ± 3.4	< 0.001
Telomere T/S ratio	1.42 ± 0.18	1.08 ± 0.22	0.74 ± 0.19	< 0.001

3.5 Telomere Length

Relative telomere length (T/S ratio) declined progressively with donor age ($r = -0.64$, $p < 0.001$) [15]. T/S ratio correlated inversely with ROS levels ($r = -0.56$, $p < 0.001$) and positively with antioxidant enzyme activities ($r = 0.41$ - 0.52 , $p < 0.01$), suggesting oxidative acceleration of telomere attrition [12].

4. Discussion

The present findings support oxidative stress as a central mediator of cellular aging, demonstrating clear age-dependent accumulation of reactive oxygen species and oxidative damage in human fibroblasts [1, 13]. The particularly steep increase in mitochondrial ROS with donor age implicates these organelles as primary drivers of age-related oxidative burden [3, 14].

The biphasic pattern of antioxidant enzyme activities suggests initial compensatory responses to rising oxidative stress followed by eventual failure of adaptive mechanisms [10]. This trajectory parallels the hormesis concept whereby moderate stress induces protective responses while severe or prolonged stress overwhelms adaptive capacity [15]. Understanding the tipping point between adaptation and decompensation may inform interventions aimed at extending the adaptive phase.

The strong correlations between oxidative damage markers and senescence phenotypes suggest mechanistic linkages rather than mere parallel accumulation [7]. Reactive oxygen species can trigger senescence through multiple pathways including DNA damage response activation, p38 MAPK signaling, and telomere dysfunction [6]. However, the correlational nature of this study cannot definitively establish causality; senescent cells might also produce elevated ROS as a consequence rather than cause of growth arrest.

The inverse relationship between telomere length and ROS levels supports oxidative acceleration of replicative senescence [12]. Telomeric DNA is particularly susceptible to oxidative damage due to its guanine-rich sequence and limited repair capacity [15]. Oxidative stress may therefore contribute to aging through both direct macromolecular damage and accelerated replicative exhaustion.

Important limitations include the focus on dermal fibroblasts, which may not represent aging processes in other cell types, particularly post-mitotic cells like neurons and cardiomyocytes where oxidative damage accumulation may have different consequences [11]. Inter-individual

p16INK4a mRNA expression increased 4.3-fold from young to elderly donors [7]. Protein levels showed corresponding increases by western blot analysis. SASP components including IL-6, IL-8, and MMP-3 were elevated in elderly donor supernatants.

variation in genetic background, environmental exposures, and lifestyle factors introduces heterogeneity that complicates interpretation despite careful donor selection.

The findings suggest potential for interventions targeting oxidative stress to delay cellular aging [16, 17]. Caloric restriction, which extends lifespan in multiple species, reduces oxidative damage through multiple mechanisms. Whether pharmacological approaches can recapitulate these benefits without caloric restriction remains an active area of investigation.

5. Conclusion

This research demonstrates clear age-dependent accumulation of reactive oxygen species, oxidative damage, and senescent phenotypes in human dermal fibroblasts, supporting oxidative stress as a central mediator of cellular aging. Mitochondrial dysfunction emerges as a primary driver of age-related oxidative burden based on the particularly steep increase in mitochondrial ROS with donor age.

The biphasic pattern of antioxidant enzyme activities suggests initial adaptive responses to rising oxidative stress followed by failure of compensatory mechanisms in advanced age. This trajectory may define a window during which interventions could extend the adaptive phase and delay age-related functional decline.

The strong correlations between oxidative damage markers and senescence phenotypes implicate reactive oxygen species in triggering cellular senescence through mechanisms potentially including DNA damage response activation and telomere dysfunction. The inverse relationship between telomere length and ROS levels supports oxidative acceleration of replicative senescence.

These findings provide mechanistic insights relevant to aging intervention development. Targeting oxidative stress and mitochondrial dysfunction represents a rational approach for extending healthspan, though translation from cellular observations to systemic interventions requires careful evaluation of efficacy and safety in model organisms and clinical populations.

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