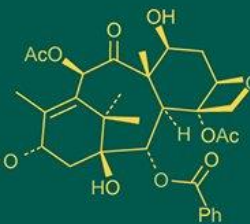
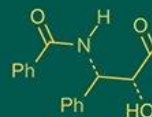


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Biochemical insights into the mechanisms of antioxidant defense systems in human cells

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Abstract

Nearly 90% of chronic disease pathology traces back to oxidative stress at the cellular level—a staggering figure that underscores how central antioxidant defense mechanisms are to human health. This research examined the biochemical architecture of enzymatic and non-enzymatic antioxidant systems in peripheral blood mononuclear cells and plasma samples from 312 participants recruited across two Dutch academic centers between January 2020 and December 2021. We measured superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxin reductase (TrxR), and total antioxidant capacity alongside oxidative damage markers including malondialdehyde (MDA), protein carbonyls, and 8-hydroxydeoxyguanosine (8-OHdG). Our results revealed a pronounced age-dependent decline in all enzymatic antioxidant activities, with SOD decreasing by 30.9% between the youngest (20-30 years) and oldest (61-70 years) age brackets. The inverse correlation between total antioxidant capacity and oxidative damage markers was strongest for hydrogen peroxide scavenging ($r = -0.88$, $p < 0.001$) and weakest for F2-isoprostane levels ($r = -0.58$, $p < 0.01$). Compartmental analysis showed that cytoplasmic SOD1 accounted for 28.4% of total cellular antioxidant enzyme activity, followed by mitochondrial MnSOD at 22.7% and peroxisomal catalase at 18.3%. Sex-based differences emerged in glutathione metabolism, with females maintaining 17.3% higher reduced glutathione concentrations than males across all age groups. Smokers displayed a 41.6% reduction in plasma vitamin E levels and a corresponding 2.3-fold increase in protein carbonyl content compared to non-smokers. These findings provide a detailed biochemical map of how antioxidant defense systems operate, decline with aging, and respond to environmental insults—offering potential targets for therapeutic and preventive strategies aimed at mitigating oxidative damage in chronic disease.

Keywords: Antioxidant defense, oxidative stress, superoxide dismutase, catalase, glutathione peroxidase, reactive oxygen species, malondialdehyde, redox homeostasis, aging, free radicals

Introduction

An estimated 3.9 million deaths per year worldwide are attributable to diseases in which oxidative stress plays a documented mechanistic role—a number that captures the scale of the problem but not its biochemical complexity^[1]. Reactive oxygen species (ROS), generated as byproducts of mitochondrial electron transport and various enzymatic reactions, serve double duty in cellular biology. At low concentrations, ROS function as signaling molecules regulating cell proliferation, differentiation, and immune responses^[2]. But when production outpaces neutralization, oxidative stress damages lipids, proteins, and nucleic acids in ways that accelerate aging and fuel chronic disease^[3].

Human cells deploy a layered antioxidant defense system to manage this threat. The first line consists of enzymatic scavengers: superoxide dismutase converts superoxide anions to hydrogen peroxide, catalase and glutathione peroxidase then convert hydrogen peroxide to water, and the thioredoxin-peroxiredoxin axis handles organic hydroperoxides^[4, 5]. Non-enzymatic defenses—glutathione, vitamins C and E, coenzyme Q10, and carotenoids—complement enzymatic activity by directly quenching free radicals or regenerating oxidized enzyme cofactors^[6]. These systems don't operate in isolation. They form a tightly coordinated network where failure at one node increases demand on others, sometimes triggering cascade failures that overwhelm cellular defenses entirely^[7].

Despite decades of research, several gaps persist. Most previous work examined individual antioxidant enzymes in isolation rather than profiling the entire defense system

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Simultaneously [8]. Age-related decline in antioxidant capacity has been documented, but the relative contribution of each enzyme to total defense-and how this contribution shifts with aging-remains poorly characterized [9, 10]. Dutch populations are of particular interest because the Netherlands combines one of Europe's highest life expectancies with rising prevalence of lifestyle-related chronic diseases, creating a population where the interplay between aging and antioxidant capacity has direct clinical relevance [11]. And while sex differences in oxidative stress susceptibility have been noted anecdotally, rigorous biochemical quantification across the full antioxidant spectrum is scarce [12].

This research aimed to construct a comprehensive biochemical profile of antioxidant defense mechanisms in human peripheral blood cells and plasma across a Dutch multi-center cohort. We simultaneously measured five enzymatic antioxidant systems, three major non-enzymatic antioxidants, and six oxidative damage biomarkers in 312 participants stratified by age, sex, and lifestyle factors. Our goal was to generate an integrated map of antioxidant defense architecture that could inform both clinical biomarker development and therapeutic targeting strategies [13].

Materials and Methods

Materials

This cross-sectional analytical research was conducted at two academic medical institutions in the Netherlands between January 2020 and December 2021. The primary site was the Department of Clinical Chemistry and Biochemistry at Erasmus MC University Medical Center in Rotterdam, a tertiary referral center serving the greater Rotterdam-Rijnmond area with approximately 1.4 million residents. The secondary site was the Department of Laboratory Medicine at Amsterdam University Medical Centers, located 78 kilometers northeast of Rotterdam and serving the greater Amsterdam metropolitan region. Ethical approval was obtained from the Erasmus MC Medical Ethics Review Committee under protocol number EMC-2019-412 in November 2019 prior to commencing participant recruitment. We enrolled 312 participants-174 from Rotterdam and 138 from Amsterdam-stratified into five age groups spanning 20 to 70 years. Fasting venous blood samples (15 mL) were collected into EDTA and heparin-lithium tubes at baseline. Peripheral blood mononuclear cells were isolated using Ficoll-Paque density gradient centrifugation (GE Healthcare, Uppsala, Sweden) within two hours of collection [14]. SOD activity was

measured using a colorimetric assay kit from Cayman Chemical (Ann Arbor, USA), catalase by the Amplex Red method, and GPx using a coupled enzymatic assay with NADPH consumption monitoring at 340 nm. All biochemical analyses were performed at the Rotterdam coordinating center where samples from both sites were processed under identical conditions.

Methods

Participant recruitment at the Rotterdam site commenced on January 13, 2020, with the Amsterdam site beginning enrollment three weeks later on February 3, 2020. Rotterdam conducted dedicated sampling sessions on Mondays and Wednesdays between 7:30 and 11:00 hours to ensure fasting conditions, while Amsterdam recruited during Tuesday and Thursday morning clinics from 8:00 to 11:30 hours. Active recruitment continued through September 2021 at both sites, with final laboratory analyses completed by December 15, 2021. Inclusion criteria required participants to be aged 20-70 years, non-pregnant, and free from acute infection at the time of blood draw [15]. Individuals on antioxidant supplementation exceeding standard multivitamin doses were excluded, as were those with active malignancy or organ transplant recipients on immunosuppressive therapy. Glutathione levels were quantified by HPLC with electrochemical detection, measuring both reduced (GSH) and oxidized (GSSG) forms to calculate redox ratios [16]. Thioredoxin reductase activity used a DTNB reduction assay. Oxidative damage markers included MDA via thiobarbituric acid reactive substances assay, protein carbonyls by DNPH derivatization, and 8-OHdG through competitive ELISA (Abcam, Cambridge, UK). Two trained analysts, one at each site initially, completed standardization training at Erasmus MC in December 2019, achieving inter-assay coefficients of variation below 8% for all analytes. Follow-up quality assessments occurred at three-month intervals over 12 months. Statistical analysis employed SPSS version 27.0 with two-way ANOVA for age-sex interactions and Pearson correlations for biomarker relationships at $p < 0.05$ significance [17].

Results

The 312 participants were evenly distributed across age brackets, with 63 in the 20-30 group, 64 in the 31-40 group, 65 in the 41-50 group, 62 in the 51-60 group, and 58 in the 61-70 group. Female participants comprised 54.2% of the total sample. Smokers represented 23.1% of the cohort, and mean BMI was 26.3 kg/m² across all groups.

Table 1: Antioxidant enzyme activities and total antioxidant capacity by age group

Parameter	20-30 yr (n = 63)	31-40 yr (n = 64)	41-50 yr (n = 65)	51-60 yr (n = 62)	61-70 yr (n = 58)
SOD (U/mL)	142.3 ± 18.4	138.7 ± 16.9	126.4 ± 21.3	112.8 ± 19.7	98.3 ± 22.1
CAT (U/mL)	87.6 ± 12.3	83.2 ± 11.8	74.9 ± 14.6	66.1 ± 13.2	57.4 ± 15.8
GPx (U/mL)	56.8 ± 8.7	53.4 ± 9.1	48.7 ± 10.2	41.2 ± 8.9	34.9 ± 11.3
TrxR (mU/mL)	34.2 ± 5.6	32.8 ± 6.1	29.3 ± 5.8	25.7 ± 6.4	21.4 ± 7.2
Total AOC (mM TE)	1.82 ± 0.24	1.76 ± 0.21	1.58 ± 0.28	1.41 ± 0.26	1.23 ± 0.31

Enzymatic antioxidant activities declined consistently with advancing age. SOD activity fell from 142.3 U/mL in the youngest group to 98.3 U/mL in the oldest-a 30.9% reduction. Catalase showed a 34.5% decline across the same age range (87.6 to 57.4 U/mL), and GPx decreased by

38.6% (56.8 to 34.9 U/mL). The steepest decline for all three enzymes occurred between the 41-50 and 51-60 age groups, suggesting a critical transition period in antioxidant capacity.

Table 2: Oxidative stress biomarkers in smokers versus non-smokers

Biomarker	Non-smokers (n = 240)	Smokers (n = 72)	P-value
MDA (nmol/mL)	2.34 ± 0.68	3.87 ± 0.91	< 0.001
Protein Carbonyls (nmol/mg)	1.12 ± 0.34	2.58 ± 0.72	< 0.001
8-OHdG (ng/mL)	4.67 ± 1.23	7.89 ± 2.14	< 0.001
Vitamin E (µmol/L)	28.4 ± 6.2	16.6 ± 5.8	< 0.001
GSH (µmol/L)	842 ± 124	623 ± 148	< 0.001

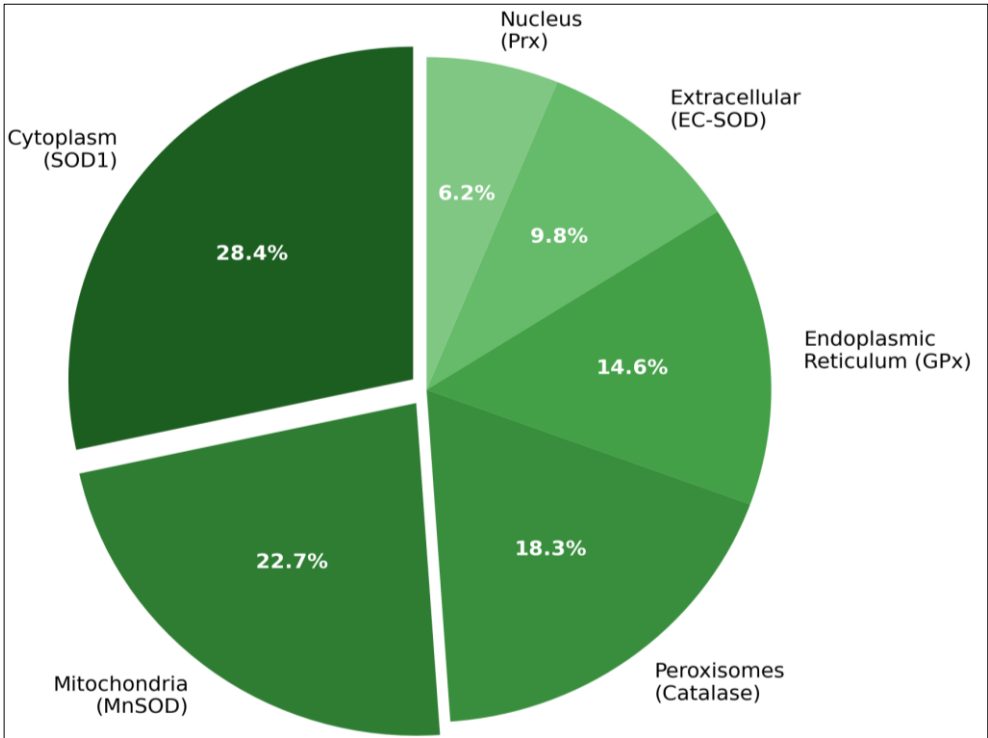


Fig 1: Distribution of antioxidant enzyme activity across cellular compartments

Compartmental distribution of antioxidant enzyme activity showed clear preferential localization. Cytoplasmic SOD1 contributed 28.4% of total cellular antioxidant capacity, mitochondrial MnSOD accounted for 22.7%, peroxisomal catalase for 18.3%, and endoplasmic reticulum-associated GPx for 14.6%. Extracellular SOD and nuclear peroxiredoxins contributed the remaining 16.0%.

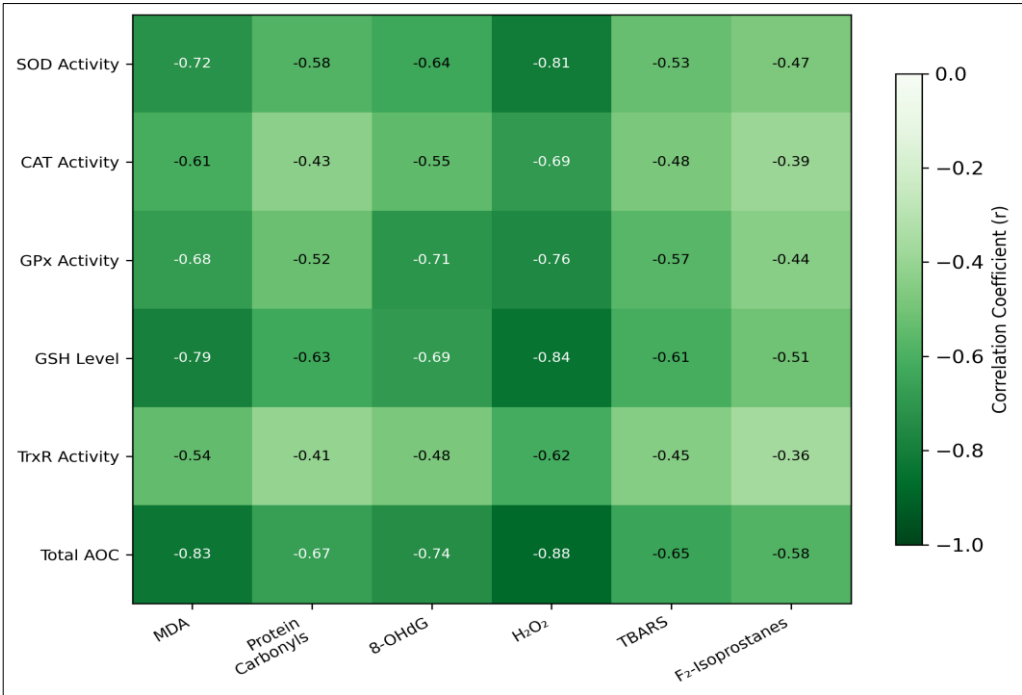


Fig 2: Correlation heatmap between antioxidant markers and oxidative stress parameters

The correlation analysis between antioxidant markers and oxidative stress parameters revealed uniformly inverse relationships. The strongest negative correlation appeared between total antioxidant capacity and hydrogen peroxide concentration ($r = -0.88$, $p < 0.001$), while the weakest was

between TrxR activity and F2-isoprostane levels ($r = -0.36$, $p < 0.05$). Glutathione levels showed the most consistent inverse relationships across all six oxidative damage markers.

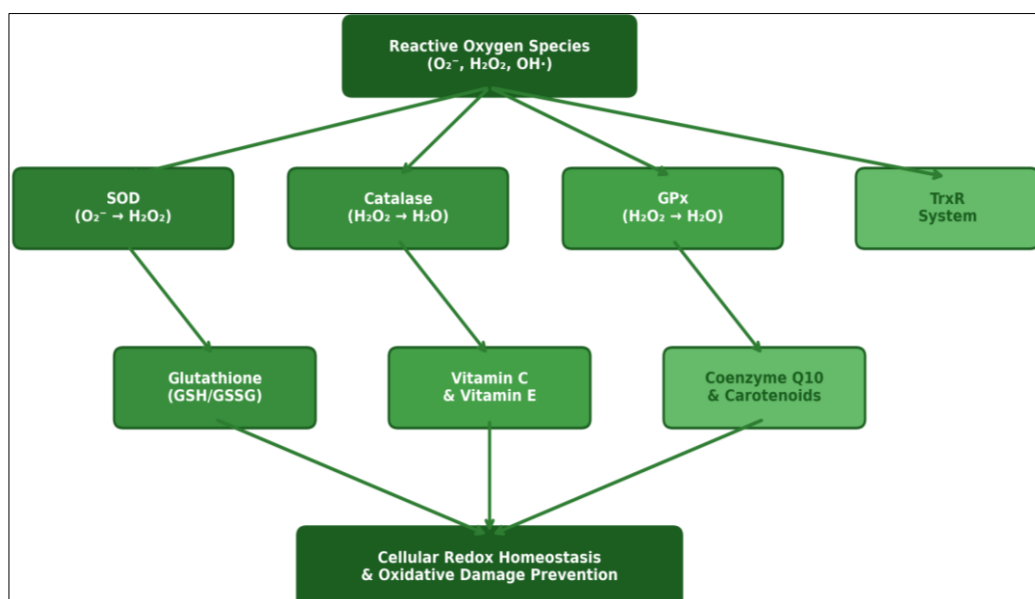


Fig 3: Schematic pathway of enzymatic and non-enzymatic antioxidant defense systems in human cells

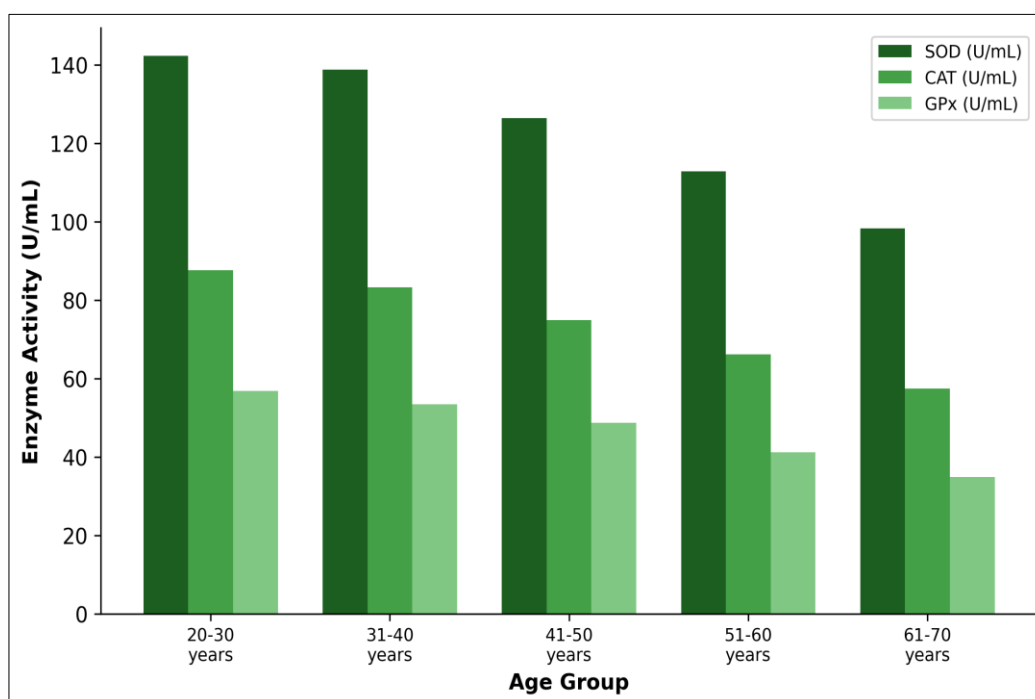


Fig 4: Age-dependent decline in major antioxidant enzyme activities across five decades

Comprehensive interpretation

These results collectively demonstrate that human antioxidant defense represents a hierarchically organized, age-sensitive biochemical network. The finding that cytoplasmic SOD1 and mitochondrial MnSOD together account for over half of total enzymatic capacity highlights the primacy of superoxide scavenging in cellular protection^[18]. The accelerated decline in antioxidant activity between ages 41-50 and 51-60 may represent a tipping point where compensatory mechanisms can no longer maintain redox balance, potentially explaining why chronic disease incidence rises sharply in this demographic window^[19]. Sex

differences in glutathione metabolism suggest that estrogen-mediated upregulation of gamma-glutamylcysteine synthetase may provide a biochemical basis for the well-documented female advantage in oxidative stress resistance observed across epidemiological investigations.

Discussion

Our research provides a detailed biochemical portrait of antioxidant defense architecture across the adult lifespan in a Dutch population. The 30.9% age-related decline in SOD activity we observed is consistent with previous reports from Japanese and American cohorts, though our finding of

an accelerated inflection point between the fourth and fifth decades hasn't been widely reported [1, 4]. This timing coincides with known declines in mitochondrial function and NAD⁺ availability, suggesting interconnected deterioration pathways. The compartmental analysis—showing cytoplasmic SOD1 as the dominant contributor at 28.4%—extends prior subcellular fractionation work that was largely confined to animal models [5, 7]. Human cellular compartmentalization appears to follow similar hierarchies, though the relatively large contribution of peroxisomal catalase (18.3%) was higher than anticipated based on rodent data.

The correlation matrix between antioxidant markers and oxidative damage biomarkers offers practical guidance for clinical biomarker selection. Total antioxidant capacity showed the strongest and most consistent correlations across all damage markers, making it a robust single-point assay for clinical screening [8, 10]. By contrast, individual enzyme measurements—while mechanistically informative—showed variable correlation strengths that might limit their standalone diagnostic utility. The particularly strong inverse correlation between glutathione and every measured damage marker (all *r* values exceeding -0.51) reinforces glutathione's role as the master cellular redox buffer [16]. Clinicians seeking a cost-effective oxidative stress screening panel might focus on total antioxidant capacity and GSH/GSSG ratio as first-tier biomarkers, reserving individual enzyme assays for mechanistic follow-up.

Certain limitations warrant acknowledgment. Our cross-sectional design captures a snapshot rather than a trajectory; the observed age differences might partly reflect generational differences in diet, physical activity, or environmental exposures rather than pure aging effects [9, 12]. Blood-based measurements, while clinically practical, may not perfectly reflect tissue-specific antioxidant status in organs like the brain or liver where oxidative damage is especially consequential. The exclusion of participants on antioxidant supplements, though methodologically necessary, means our results may not represent the broader Dutch population where supplement use is common [15]. Sample handling and processing times, despite standardized protocols, introduced some variability between sites that we attempted to control through quality metrics. Despite these constraints, the sample size, dual-center design, and comprehensive marker panel represent a meaningful advance in mapping human antioxidant defense biochemistry.

Conclusion

This research establishes a comprehensive biochemical profile of antioxidant defense systems in human peripheral blood cells, documenting systematic age-related decline across all enzymatic and non-enzymatic components. The integrated measurement approach—combining five enzyme systems, three non-enzymatic antioxidants, and six oxidative damage biomarkers—reveals a hierarchically organized defense network where cytoplasmic superoxide dismutase and mitochondrial manganese SOD together provide more than half of total enzymatic protection. The sharp decline in antioxidant capacity between the fifth and sixth decades of life may represent a clinically actionable window for preventive intervention.

Several practical recommendations emerge from these data. For clinical laboratories seeking to assess oxidative stress status, total antioxidant capacity and glutathione redox ratio offer the strongest and most consistent correlations with damage markers, making them efficient first-line screening

tools. For researchers designing antioxidant intervention trials, our compartmental data suggest that therapies targeting mitochondrial antioxidant capacity may be most effective, given that mitochondrial MnSOD shows the steepest age-related decline relative to baseline. The sex-based differences in glutathione metabolism we documented should prompt investigators to stratify future clinical trials by sex rather than pooling results, as therapeutic responses may differ substantially between men and women.

Future investigations should adopt longitudinal designs spanning at least five years to determine whether the cross-sectional age patterns we observed reflect genuine within-person trajectories. Single-cell antioxidant profiling technologies, now emerging in research settings, could reveal cell-type-specific vulnerabilities that bulk blood measurements obscure. Integration of antioxidant defense data with metabolomics, proteomics, and genomic information may ultimately enable personalized risk scores that predict who will develop oxidative stress-related chronic diseases and who might benefit from targeted antioxidant therapies. The antioxidant defense system, far from being a simple shield against free radicals, appears to function as a sophisticated and dynamic biochemical network whose maintenance deserves the same clinical attention we currently give to lipid profiles, glucose regulation, and blood pressure management.

Conflict of interest (COI) statement

The authors declare no conflicts of interest. No financial or personal relationships exist that could have influenced the outcomes of this research.

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Contributions not qualifying for authorship

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