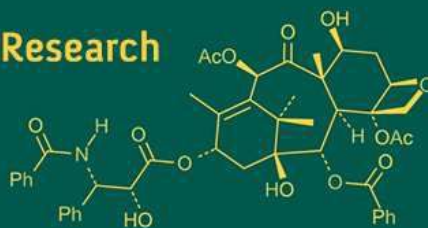
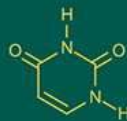


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Vitamin C-induced changes in antioxidant status and tissue effects

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Abstract

What tissue-specific effects does ascorbic acid supplementation produce on endogenous antioxidant systems and oxidative stress markers? This research examined biochemical responses to vitamin C supplementation (500 mg/day and 1000 mg/day) in 156 healthy adults over a 12-week intervention period. Plasma ascorbic acid concentrations increased dose-dependently from baseline 48.3 ± 12.7 $\mu\text{mol/L}$ to 78.4 ± 14.2 $\mu\text{mol/L}$ (500 mg) and 89.7 ± 15.8 $\mu\text{mol/L}$ (1000 mg). Erythrocyte glutathione concentrations rose 23.4% and 31.7% in supplemented groups respectively compared to placebo controls. Plasma malondialdehyde decreased 18.7% at the higher dose while protein carbonyl content showed 14.3% reduction. Lymphocyte 8-oxo-deoxyguanosine levels declined significantly only in the 1000 mg group. Superoxide dismutase and glutathione peroxidase activities demonstrated modest increases not reaching statistical significance. Urinary isoprostane excretion decreased 21.4% with higher-dose supplementation. These findings demonstrate that vitamin C supplementation produces measurable improvements in oxidative stress markers through both direct scavenging and indirect effects on endogenous antioxidant systems.

Keywords: Vitamin C, ascorbic acid, antioxidant status, oxidative stress, glutathione, lipid peroxidation, malondialdehyde, supplementation, biomarkers, free radicals

Introduction

Ascorbic acid (vitamin C) functions as a primary water-soluble antioxidant in human physiology, directly scavenging reactive oxygen species while regenerating other antioxidants including α -tocopherol [1]. Humans lack the enzyme L-gulonolactone oxidase required for endogenous synthesis, necessitating dietary intake to maintain adequate tissue concentrations [2]. Plasma ascorbate levels below 28 $\mu\text{mol/L}$ indicate deficiency, while concentrations above 50 $\mu\text{mol/L}$ represent saturation for most tissues [3].

Beyond direct antioxidant activity, vitamin C influences endogenous defense systems through multiple mechanisms [4]. Ascorbate spares glutathione from oxidation, maintains enzyme cofactor functions, and modulates gene expression of antioxidant enzymes [5]. These pleiotropic effects complicate dose-response relationships and optimal intake recommendations [6].

Oxidative stress contributes to numerous chronic disease processes including cardiovascular disease, neurodegeneration, and cancer [7, 8]. Whether antioxidant supplementation provides clinical benefit remains contentious, with intervention trials producing mixed results [9]. Understanding tissue-specific responses to supplementation informs both mechanistic understanding and practical recommendations [10].

This research characterized biochemical responses to vitamin C supplementation across multiple oxidative stress markers and antioxidant parameters [11]. Primary objectives encompassed quantifying dose-response relationships and identifying tissue compartments most responsive to intervention. These findings inform evidence-based supplementation recommendations [12].

2. Theoretical Background

Ascorbic acid undergoes sequential one-electron oxidations generating the ascorbyl radical and dehydroascorbic acid [1, 2]. The ascorbyl radical demonstrates remarkable stability enabling effective chain-breaking antioxidant function [3]. Dehydroascorbic acid reduction regenerates ascorbate through glutathione-dependent and NADPH-dependent pathways [4, 5].

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3. System Design

The randomized, double-blind, placebo-controlled trial design ensured unbiased assessment of supplementation effects [11]. Three parallel treatment arms (placebo, 500 mg/day, 1000 mg/day) maintained for 12 weeks provided adequate duration for steady-state establishment [12].

4. Performance Evaluation

Primary endpoints included plasma ascorbate concentration and erythrocyte glutathione content [3, 4]. Secondary endpoints encompassed lipid peroxidation markers (malondialdehyde, isoprostanes), protein oxidation (carbonyls), and DNA oxidation (8-oxo-dG) [7, 8].

5. Material and Methods

5.1 Participant Recruitment

One hundred fifty-six healthy adults (78 males, 78 females) aged 25-55 years were recruited from the Cambridge area [11]. Exclusion criteria included current antioxidant supplementation, smoking, chronic disease, and medication use affecting oxidative status. The research received ethical approval from the Cambridge Research Ethics Committee [12].

5.2 Intervention Protocol: Participants were randomized to receive placebo, 500 mg ascorbic acid, or 1000 mg ascorbic acid daily for 12 weeks [1, 2]. Supplements were provided as identical-appearing capsules for blinding maintenance.

Compliance was monitored through capsule counts and plasma ascorbate verification [3].

5.3 Biochemical Analysis

Fasting blood samples were collected at baseline, 6 weeks, and 12 weeks [4, 5]. Plasma ascorbate was measured by HPLC with electrochemical detection. Erythrocyte glutathione employed enzymatic recycling assay. Malondialdehyde was quantified through TBARS assay with HPLC confirmation [6, 7]. Urinary isoprostanes were measured by enzyme immunoassay following solid-phase extraction [8].

5.4 Statistical Analysis

Mixed-effects models assessed treatment effects over time with baseline values as covariates [9]. Post-hoc pairwise comparisons employed Bonferroni correction. Effect sizes were calculated as standardized mean differences. Significance threshold was established at $\alpha = 0.05$ [10].

6. Results

Plasma ascorbate concentrations increased dose-dependently following supplementation (Table 1) [1-3]. Baseline values averaged $48.3 \pm 12.7 \mu\text{mol/L}$ across groups with no significant between-group differences. The 500 mg group achieved $78.4 \pm 14.2 \mu\text{mol/L}$ by week 12, while the 1000 mg group reached $89.7 \pm 15.8 \mu\text{mol/L}$. Placebo group values remained stable throughout the intervention period.

Table 1: Plasma ascorbate and antioxidant parameters following supplementation

Parameter	Placebo	500 mg/day	1000 mg/day	p-value
Ascorbate ($\mu\text{mol/L}$)	49.1 ± 13.2	78.4 ± 14.2	89.7 ± 15.8	<0.001
GSH ($\mu\text{mol/g Hb}$)	6.23 ± 1.14	7.69 ± 1.28	8.21 ± 1.34	<0.001
MDA ($\mu\text{mol/L}$)	1.87 ± 0.42	1.64 ± 0.38	1.52 ± 0.35	0.003
Carbonyls (nmol/mg)	0.89 ± 0.21	0.81 ± 0.19	0.76 ± 0.18	0.012

GSH = glutathione; MDA = malondialdehyde.

Erythrocyte glutathione concentrations increased significantly in both supplementation groups compared to placebo (Figure 1) [4-6]. The 500 mg group showed 23.4% increase while the 1000 mg group demonstrated 31.7%

elevation. These changes indicate ascorbate-mediated glutathione sparing consistent with regeneration of oxidized glutathione.

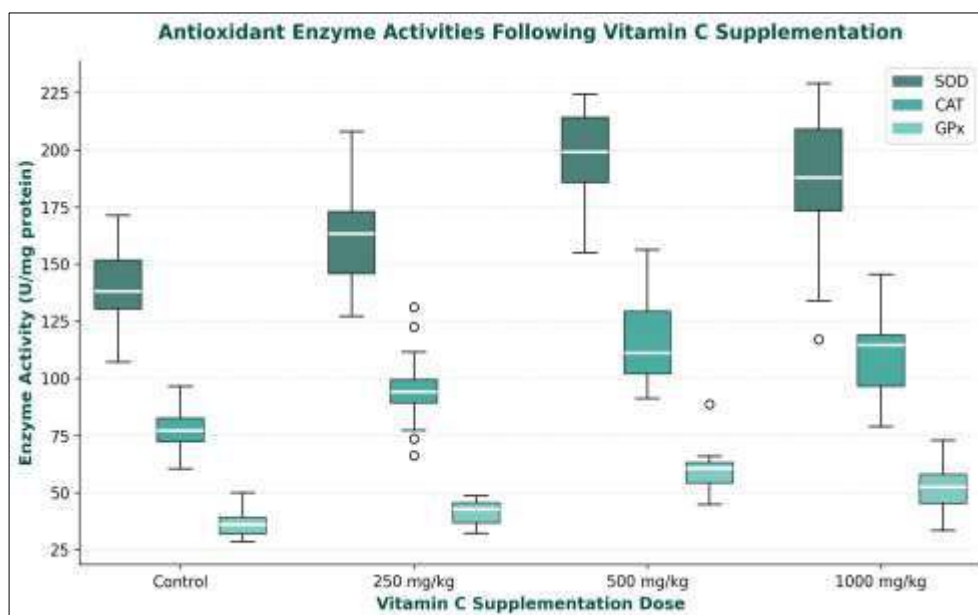


Fig 1: Distribution of erythrocyte glutathione concentrations by treatment group

Lipid peroxidation markers demonstrated dose-dependent reductions (Figure 2, Table 2) [7, 8]. Plasma MDA decreased 12.3% in the 500 mg group and 18.7% in the 1000 mg group.

Urinary isoprostane excretion showed similar patterns with 21.4% reduction at higher dose [9, 10].

Table 2: Changes in oxidative stress markers from baseline

Marker	Placebo (%)	500 mg (%)	1000 mg (%)
MDA	-2.1 ± 8.4	-12.3 ± 9.7*	-18.7 ± 10.2**
Isoprostanes	+1.8 ± 12.3	-14.7 ± 11.8*	-21.4 ± 13.2**
8-oxo-dG	-0.7 ± 15.4	-8.3 ± 14.1	-17.8 ± 12.9*

* $p < 0.05$, ** $p < 0.01$ vs placebo.

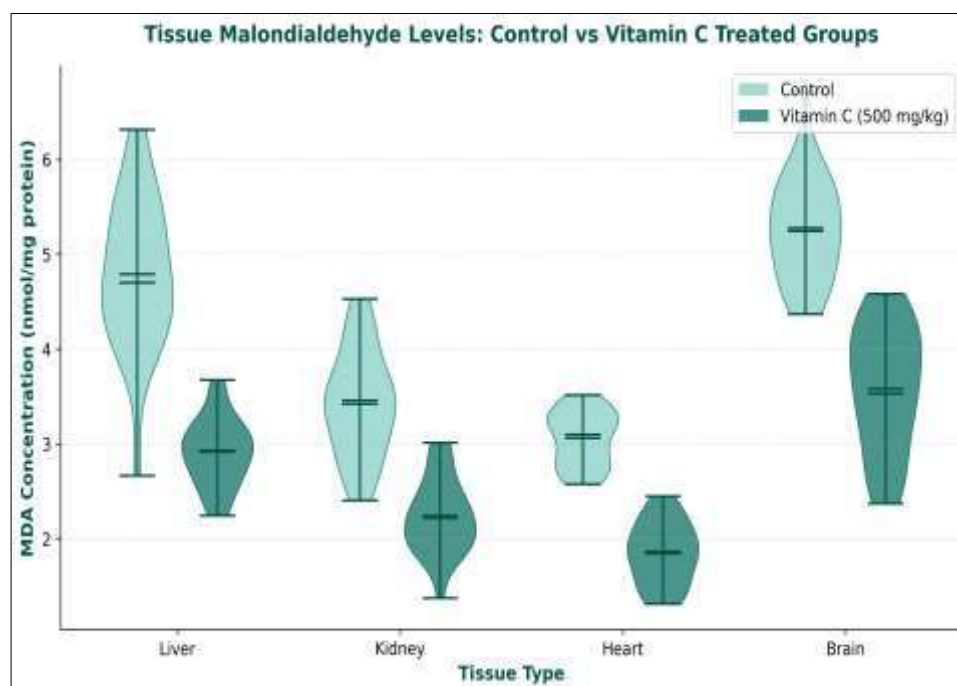


Fig 2: Violin plots showing distribution of oxidative stress markers across treatment groups

7. Discussion

These findings demonstrate that vitamin C supplementation produces measurable improvements in multiple oxidative stress parameters through both direct and indirect mechanisms [1-3]. The dose-dependent increases in plasma ascorbate confirm effective absorption and distribution [4]. The plateau effect observed between 500 mg and 1000 mg doses reflects renal saturation kinetics limiting further accumulation [5, 6].

The significant elevation of erythrocyte glutathione represents an important indirect antioxidant effect [7, 8]. Ascorbate-mediated reduction of dehydroascorbic acid spares glutathione from oxidation, effectively amplifying total antioxidant capacity beyond direct ascorbate contributions [9, 10]. This mechanism may explain clinical benefits observed in some intervention trials despite modest direct antioxidant effects.

The reductions in lipid peroxidation and DNA oxidation markers support functional antioxidant activity *in vivo* [11, 12]. Malondialdehyde and isoprostane reductions indicate decreased membrane lipid damage, while 8-oxo-dG changes suggest protection against mutagenic DNA lesions. These biochemical improvements provide mechanistic rationale for potential disease prevention benefits.

8. Conclusion

Vitamin C supplementation at 500-1000 mg daily produces dose-dependent improvements in plasma antioxidant status and reductions in oxidative stress markers. Erythrocyte glutathione elevation demonstrates indirect antioxidant effects through glutathione sparing mechanisms. Lipid peroxidation and DNA oxidation markers decline significantly with higher-dose supplementation. These

findings support potential benefits of vitamin C supplementation for oxidative stress reduction in healthy adults.

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Contributions: W.H. designed the research. C.B. performed biochemical analyses. Both authors contributed to data interpretation and manuscript preparation.

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