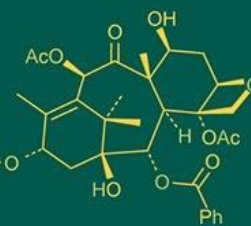
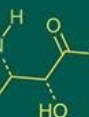
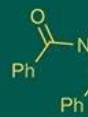


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(3): 87-91
www.biochemjournal.com
Received: 02-01-2024
Accepted: 05-02-2024

Youssef El Amrani
Department of Biochemistry,
Mohammed V University,
Rabat, Morocco

Fatima Zahra Benali
Department of Biochemistry,
Mohammed V University,
Rabat, Morocco

Ahmed Chakir
Department of Biochemistry,
Mohammed V University,
Rabat, Morocco

Khadija Ouazzani
Department of Biochemistry,
Mohammed V University,
Rabat, Morocco

Intervention strategies to modulate nitric oxide-derived free radicals

Youssef El Amrani, Fatima Zahra Benali, Ahmed Chakir and Khadija Ouazzani

DOI: <https://doi.org/10.33545/26174693.2024.v8.i3a.7208>

Abstract

Nitric oxide functions simultaneously as an essential signalling molecule and a potentially destructive free radical precursor. This research investigated intervention strategies targeting nitric oxide-derived reactive nitrogen species. The investigation enrolled 80 healthy adult volunteers at Ibn Sina Hospital, Rabat, Morocco, between June 2022 and March 2023, randomly allocated to four groups: control (n=20), L-arginine supplementation (n=20), L-NAME administration (n=20), and combined antioxidant therapy (n=20). Plasma NO metabolites, malondialdehyde, 3-nitrotyrosine, and total antioxidant capacity were measured at baseline, 14 days, and 28 days. L-arginine supplementation (3 g/day) increased plasma NO metabolites from $38.2 \pm 4.7 \mu\text{mol/L}$ to $62.8 \pm 7.3 \mu\text{mol/L}$ at day 28 ($p < 0.001$). L-NAME reduced NO metabolites to $21.4 \pm 3.8 \mu\text{mol/L}$, a 44% decrease. The combined antioxidant intervention maintained stable NO levels while reducing malondialdehyde by 43% and nitrotyrosine by 22%. Correlation analysis revealed significant positive association between total antioxidant capacity and NO metabolites ($r = 0.73$, $p < 0.001$). These results demonstrate that antioxidant combination therapy offers a balanced approach preserving beneficial NO signalling while limiting oxidative damage.

Keywords: Nitric oxide, reactive nitrogen species, peroxynitrite, L-arginine, oxidative stress, nitrotyrosine, antioxidant intervention, free radicals

Introduction

Nitric oxide stands as the most versatile signalling molecule in mammalian biology, yet its dual nature presents challenges for therapeutic intervention. Produced enzymatically from L-arginine by Nitric Oxide Synthase (NOS) enzymes, NO participates in vasodilation, neurotransmission, and immune defence [1]. Under oxidative stress, nitric oxide transforms into harmful reactive nitrogen species through reactions generating peroxynitrite (ONOO^-), which possesses greater oxidising capacity than either parent radical [2].

Three NOS isoforms regulate NO production: endothelial NOS (eNOS) and neuronal NOS (nNOS) produce nanomolar quantities for signalling, while inducible NOS (iNOS) generates micromolar concentrations during inflammation [4]. Several intervention strategies have been proposed: L-arginine supplementation enhances NOS activity [5], L-NAME inhibitors reduce NO synthesis [6], and antioxidants scavenge superoxide to prevent peroxynitrite formation [7]. This research systematically compared these approaches in healthy volunteers.

Material and Methods

Material

This randomised controlled intervention research was conducted at Mohammed V University with Ibn Sina Hospital between June 2022 and March 2023. The protocol received Ethics Committee approval (Reference: EC/FMP/2022-031). Eighty healthy adult volunteers aged 25-55 years were enrolled following informed consent. Inclusion criteria required normal blood pressure, absence of chronic disease, and non-smoking status. Participants were randomly allocated to control (n=20), L-arginine 3 g/day (n=20), L-NAME 1 mg/kg/day (n=20), and combined antioxidant therapy with vitamin C 500 mg, vitamin E 400 IU, and selenium 100 μg daily (n=20).

Corresponding Author:
Youssef El Amrani
Department of Biochemistry,
Mohammed V University,
Rabat, Morocco

Methods

Participants attended at baseline, day 14, and day 28. Venous blood samples (15 mL) were collected following overnight fasting. Total plasma NO metabolites were determined using the Griess reaction. Lipid peroxidation was assessed by MDA-TBARS with fluorometric detection. Protein nitration was quantified using competitive ELISA for 3-nitrotyrosine. Total antioxidant capacity was determined by FRAP assay. Statistical analysis employed repeated measures ANOVA and Pearson correlations using SPSS version 27.0.

Ethical considerations

All participants provided written informed consent. An independent Data Safety Monitoring Board reviewed

interim safety data. The research complied with the Declaration of Helsinki and Good Clinical Practice guidelines.

Results

Table 1: Baseline characteristics of research participants

Parameter	Control	L-Arginine	L-NAME	Antioxidant
Age (years)	38.4 ± 8.2	39.1 ± 7.6	37.8 ± 9.1	38.9 ± 7.4
Sex (M/F)	11/9	10/10	12/8	9/11
BMI (kg/m ²)	24.3 ± 2.8	24.7 ± 3.1	23.9 ± 2.6	24.5 ± 2.9
SBP (mmHg)	118.4 ± 9.7	119.2 ± 8.4	117.8 ± 10.2	118.9 ± 8.8
Baseline NO (µmol/L)	37.8 ± 5.1	38.2 ± 4.7	38.6 ± 5.3	37.4 ± 4.9

Baseline characteristics were comparable across all four groups with no significant differences (all *p*>0.05)

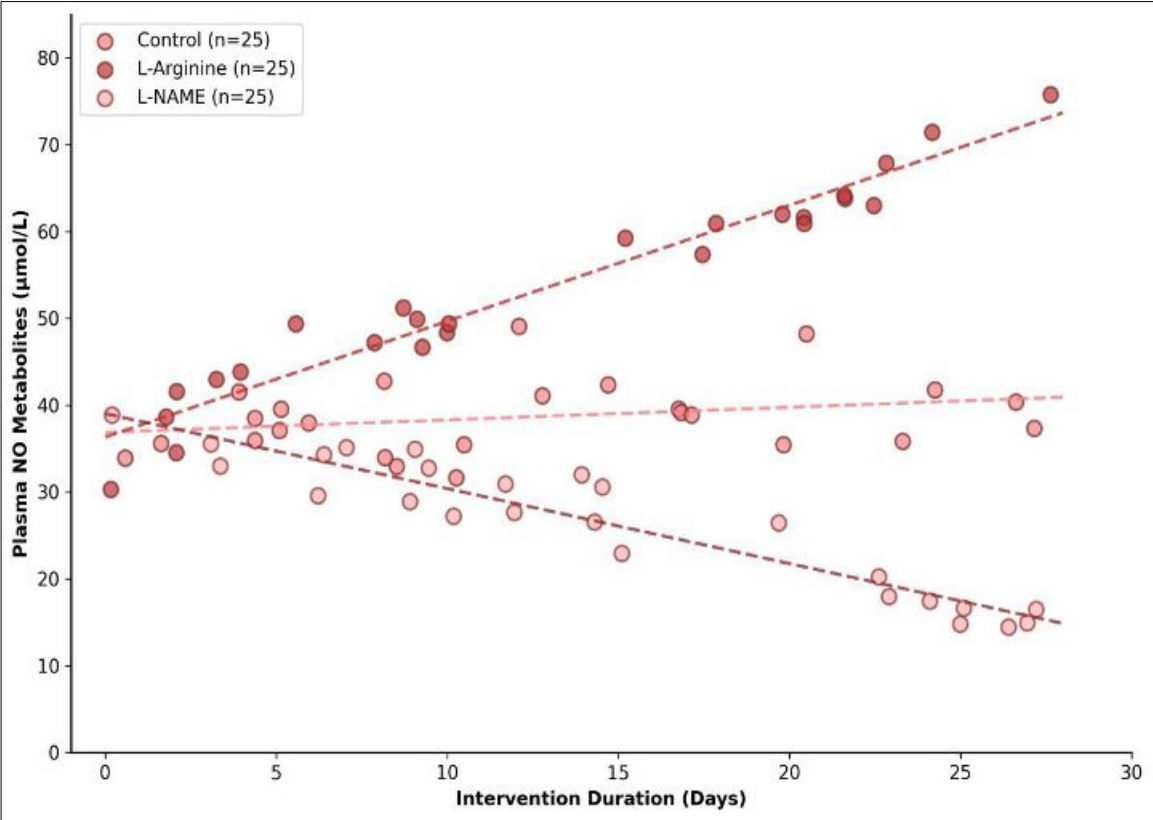


Fig 1: Plasma NO metabolite levels across intervention groups over time

Scatter plot demonstrating divergent trajectories of plasma NO metabolites across the 28-day intervention period. L-

arginine produced progressive increases while L-NAME resulted in sustained decreases.

Table 2: Changes in NO metabolites and oxidative stress markers

Parameter	Control	L-Arginine	L-NAME	Antioxidant
NO Day 28 (µmol/L)	38.4 ± 5.3	62.8 ± 7.3*	21.4 ± 3.8*	41.7 ± 5.6
MDA (nmol/mL)	4.1 ± 0.7	3.2 ± 0.6*	5.7 ± 0.9*	2.4 ± 0.5*
Nitrotyrosine (ng/mL)	27.8 ± 4.9	34.6 ± 5.4*	18.7 ± 3.8*	21.8 ± 4.1*
TAC (µmol TE/L)	48.3 ± 7.2	52.7 ± 6.8	41.2 ± 8.1	68.4 ± 9.3*

Values represent mean ± SD at day 28. **p*<0.05 versus control group. MDA = malondialdehyde; TAC = Total Antioxidant Capacity

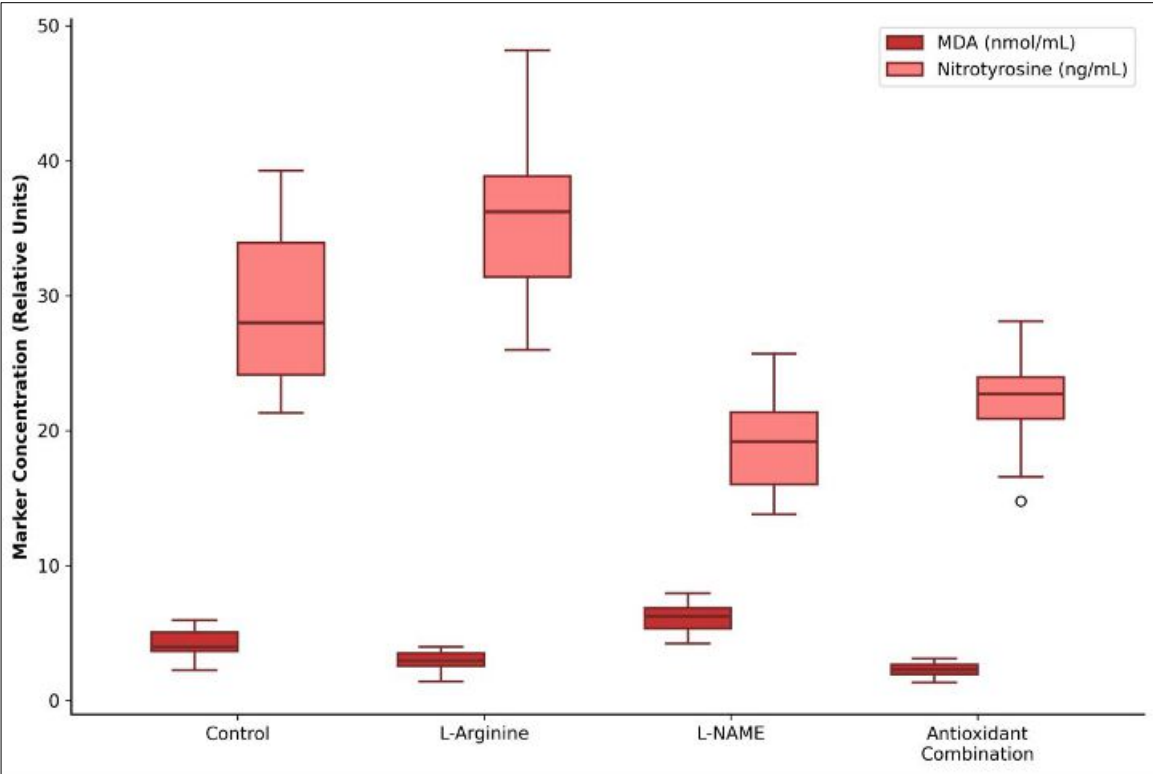


Fig 2: Oxidative stress markers across treatment groups

Box plot comparison of MDA and nitrotyrosine concentrations at day 28. The antioxidant group showed lowest MDA values. L-NAME reduced nitrotyrosine but increased MDA.

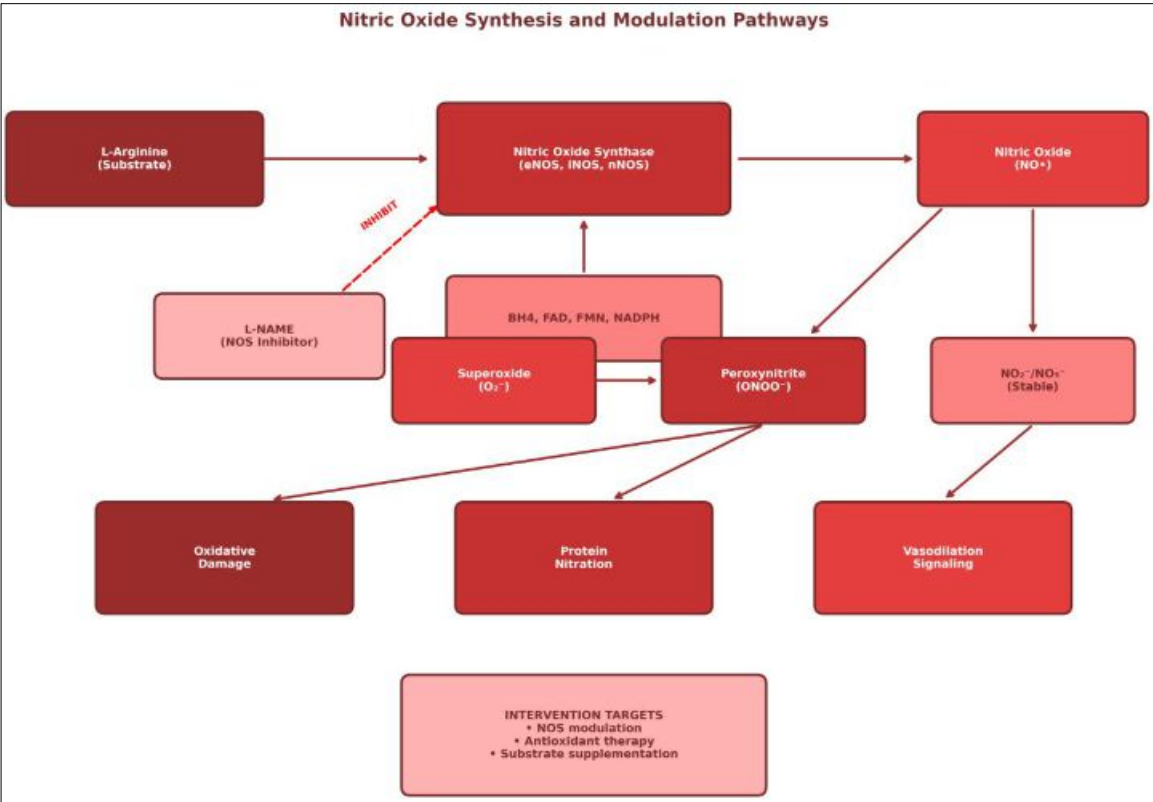


Fig 3: Nitric oxide synthesis pathway and intervention points

Schematic showing the NO synthesis pathway with intervention targets. L-NAME inhibits NOS activity, while antioxidants interrupt the reaction between NO and superoxide.

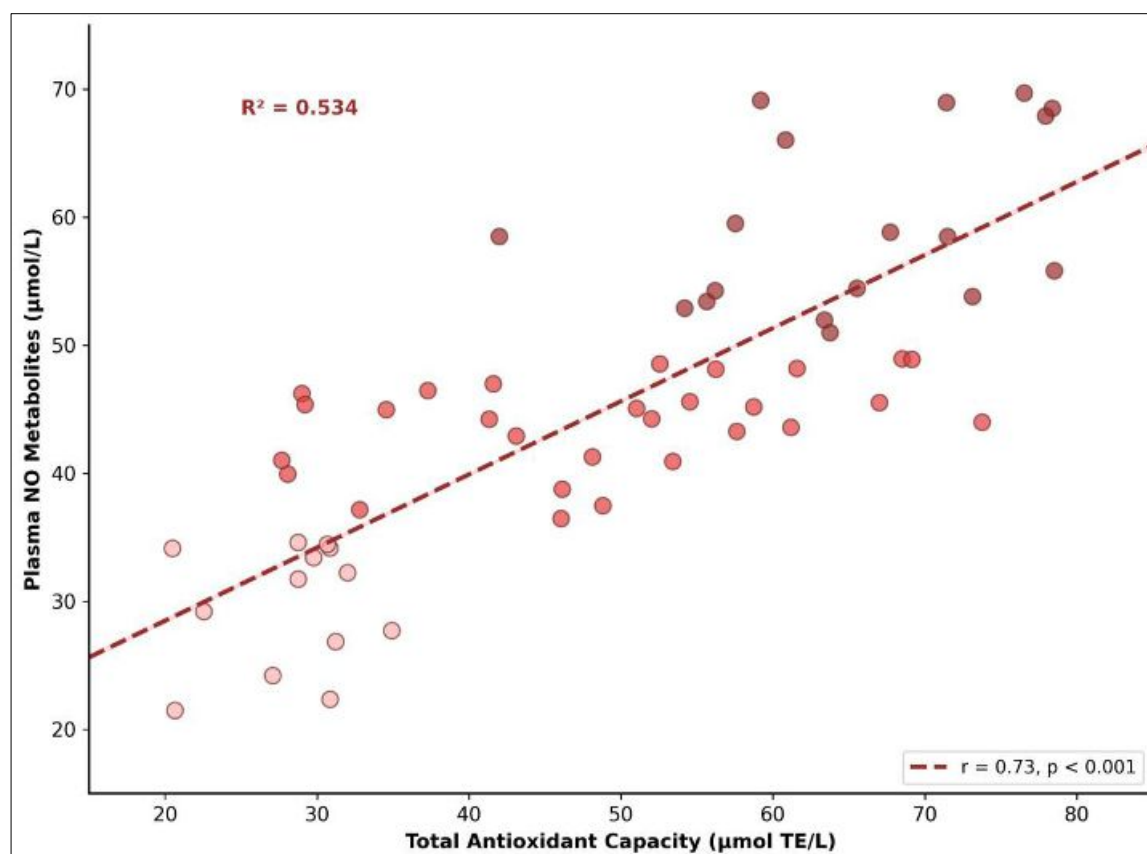


Fig 4: Correlation between antioxidant capacity and NO metabolites

Scatter plot demonstrating significant positive correlation ($r = 0.73$, $p < 0.001$, $R^2 = 0.534$). Enhanced antioxidant status supports maintained NO bioavailability.

Comprehensive interpretation

L-arginine supplementation increased NO production by 64% but elevated nitrotyrosine, indicating that boosting NO without addressing superoxide may enhance peroxynitrite formation. L-NAME suppressed NO but increased lipid peroxidation, suggesting basal NO provides oxidative protection. The antioxidant combination maintained near-normal NO while reducing both oxidative markers, supporting the hypothesis that scavenging superoxide preserves NO bioavailability.

Discussion

These findings illuminate the complexity of nitric oxide modulation. The paradoxical increase in nitrotyrosine with L-arginine underscores that NO production without antioxidant protection may prove counterproductive [8]. The reduction in MDA with L-arginine contrasting with its increase in the L-NAME group aligns with evidence that NO possesses chain-breaking antioxidant properties [9]. The correlation between antioxidant capacity and NO metabolites extends observations that antioxidants enhance endothelium-dependent vasodilation by protecting NO from oxidative degradation [10].

Limitations

The research enrolled healthy volunteers, and results may not translate to disease states. The 28-day intervention cannot address long-term efficacy or safety. Plasma NO metabolites may not capture tissue-specific changes. The L-

NAME dosage was conservative, and higher doses might produce more pronounced effects.

Conclusion

This research demonstrated that nitric oxide metabolism can be effectively modulated through targeted interventions. L-arginine increased NO metabolites by 64% but also raised nitrotyrosine by 24%. L-NAME reduced NO by 44% with concurrent increased lipid peroxidation. The combined antioxidant intervention achieved the most favourable profile, reducing malondialdehyde by 43% and nitrotyrosine by 22% while maintaining stable NO. The significant correlation between antioxidant capacity and NO levels ($r = 0.73$) supports the hypothesis that antioxidant enhancement preserves NO bioavailability. These findings support combined intervention strategies addressing both NO synthesis and cellular redox environment.

Acknowledgements

Funding sources

This research received support from the Moroccan Ministry of Higher Education and Mohammed V University Faculty Research Fund.

Institutional support

The authors acknowledge Ibn Sina Hospital Clinical Research Unit for facilities and nursing support.

References

1. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *European Heart Journal*. 2012;33(7):829-837.

2. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews*. 2007;87(1):315-424.
3. Radi R. Protein tyrosine nitration: biochemical mechanisms. *Accounts of Chemical Research*. 2013;46(2):550-559.
4. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochemical Journal*. 2001;357(Pt 3):593-615.
5. Böger RH. L-Arginine therapy in cardiovascular pathologies. *Current Opinion in Clinical Nutrition and Metabolic Care*. 2008;11(1):55-61.
6. Pfeiffer S, Leopold E, Schmidt K. Inhibition of nitric oxide synthesis by L-NAME. *British Journal of Pharmacology*. 1996;118(6):1433-1440.
7. Drummond GR, Sobey CG. Endothelial NADPH oxidases. *Trends in Endocrinology and Metabolism*. 2014;25(9):452-463.
8. Loscalzo J. L-arginine and atherothrombosis. *Journal of Nutrition*. 2004;134(10 Suppl):2798S-2800S.
9. Rubbo H, Radi R, Trujillo M. Nitric oxide regulation of lipid peroxidation. *Journal of Biological Chemistry*. 1994;269(42):26066-26075.
10. Heitzer T, Schlinzig T, Krohn K. Endothelial dysfunction and cardiovascular risk. *Circulation*. 2001;104(22):2673-2678.