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Original Article

Heterogeneous antioxidant responses to exercise in middle-aged men: A six-month study of amateur soccer players

MARIO RUGGIERO[1], GIOVANNI TAFURI[2], CRISTINA MENNITTI[3], PIETRO MONTESANO[4], OLGA SCUDIERO[5], FILOMENA MAZZEO[6]
[1,2,4]Department of Medical, Human Movement and Well-Being Sciences, University of Naples Parthenope, Via Medina 40, 80133, Napoli, ITALY
[3,5]Department of Molecular Medicine and Medical Biotechnologies, Federico II University, Via Sergio Pansini 5, 80131, Napoli, ITALY;
[5]CEINGE-Biotecnologie Avanzate Franco Salvatore, Via G. Salvatore 486, Napoli, 80145, ITALY;
[5]Task Force on Microbiome Studies, University of Naples Federico II, 80100 Napoli, ITALY
[6]Department of Economics, Law, Cybersecurity and Sports Sciences, University of Naples Parthenope, Via Guglielmo Pepe, 80035, Nola, ITALY

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Abstract:
Physical exercise exerts a dual influence on redox homeostasis, capable of inducing both adaptive antioxidant responses and detrimental oxidative stress, with outcomes critically dependent on exercise intensity and individual physiological tolerance. This balance is especially pertinent for middle-aged individuals, a demographic at a pivotal juncture for long-term health, where personalized exercise strategies are key for promoting successful aging. However, practical and non-invasive tools for routine redox monitoring remain underutilized in amateur sports settings. This pilot study, therefore, evaluated the heterogeneous effects of a six-month amateur soccer training program on salivary antioxidant capacity in middle-aged men under controlled dietary conditions, utilizing a non-invasive spectrophotometric approach. Twelve participants (aged 45–60 years) completed the structured training. Saliva samples were collected before and immediately after a standardized high-intensity session at the program's conclusion. Total antioxidant capacity was assessed via the 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay under standardized analytical conditions, with all samples processed using the same reagent batch to ensure consistency. The primary outcome was the intra-individual change (Δ) in absorbance. The results revealed marked interindividual heterogeneity in post-exercise redox responses. Specifically, half of the cohort exhibited a decrease in post-exercise absorbance (negative Δ values), indicating enhanced antioxidant activity, while the other half showed an increase (positive Δ values), suggesting a reduction in antioxidant capacity or an increased pro-oxidant load. The magnitude of change varied considerably among participants. These findings underscore the threshold-dependent duality of intense exercise and highlight the significant role of individual redox physiology in mediating its net effect, independent of dietary confounding in this study. The study provides clear proof-of-concept evidence for the feasibility and relevance of using non-invasive salivary DPPH testing as a practical tool to monitor exercise-induced oxidative balance. This supports the rational development of personalized training regimens guided by objective biochemical feedback, aiming to optimize health benefits and mitigate oxidative risks in middle-aged amateur athletes. The approach has direct implications for applied sports science, preventive health strategies, and the move toward more individualized exercise prescription in community-based settings.
Key Words: antioxidant activity; reactive oxygen species (ROS); DPPH test; amateur athletes; sport; drugs

Introduction

Physical exercise represents a cornerstone of human health, with a widely recognized preventive and therapeutic role validated by scientific literature. The World Health Organization (WHO) guidelines emphasize the importance of moderate and consistent physical activity, evenly distributed throughout the week, associated with significant reductions in the risk of morbidity and premature mortality (Kapoor et al., 2022; Yang, 2019). The benefits of exercise extend far beyond the physical sphere: systemically, it enhances cardiovascular, musculoskeletal, and metabolic health (Booth et al., 2012; Montesano et al., 2013), while neuropsychologically, it improves cognitive functions, regulates sleep-wake cycles, and alleviates symptoms of anxiety and depression (Mandolesi et al., 2018; Ruggiero, Montesano, et al., 2025; Stanton et al., 2020). These multifactorial effects have led some authors to define physical activity as a low-cost "drug," capable of competing with conventional therapies in terms of efficacy (Vina et al., 2012). Conversely, a sedentary lifestyle is linked to an increased

Corresponding Author: MARIO RUGGIERO, E-mail: mario.ruggiero005@studenti.uniparthenope.it

incidence of chronic diseases, such as obesity, diabetes, cardiovascular disorders, and metabolic imbalances (Booth et al., 2012; Crimmins et al., 2011). Critically, the global aging trend amplifies these challenges: by 2050, individuals aged ≥65 years will represent 16% of the world's population, with aging strongly associated with non-communicable diseases (e.g., cardiovascular disorders, dementia) and mobility impairments (Lin et al., 2020; Zanjari et al., 2017). For middle-aged men—a demographic at a pivotal juncture for health trajectory—promoting successful aging (defined as preserving functionality, compressing morbidity, and delaying disability) is essential to mitigate long-term care needs and sustain quality of life (Michel & Sadana, 2017; Nosraty et al., 2019; Özsungur, 2020).

However, while moderate exercise exerts a protective action, exceeding individual tolerance thresholds can generate adverse effects linked to metabolic overactivation. During intense efforts, increased mitochondrial oxidative metabolism causes excessive production of reactive oxygen species (ROS), such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Clarkson, 1995). These radicals, if not neutralized, trigger a cascade of pathophysiological events, including damage to cellular macromolecules (lipid peroxidation, protein and DNA oxidation) (Alayev et al., 2015), reduced muscle contractile capacity (Clemente-Suárez et al., 2023), and activation of pro-inflammatory pathways (e.g., NF-κB) and immune dysregulation (Yahfoufi et al., 2018). In addition to the mitochondrial respiratory chain, other alternatives for cellular free radical production have been proposed: the xanthine oxidase-catalysed reaction, with a mechanism similar to ischemia-reperfusion injury (Chuprin & Mihajlovic, 2006); and neutrophil activation, producing O_2^- and H_2O_2 following damage to muscle cells (Chakraborti et al., 1998).

To counteract oxidative stress, the body activates an antioxidant response organized into three lines of defence: First line: Enzymes (superoxide dismutase, catalase, glutathione reductase) and minerals (selenium, copper, zinc); Second line: Non-enzymatic antioxidants (glutathione, vitamin C, vitamin E, carotenoids, flavonoids); Third line: Repair enzymes for oxidized DNA, proteins, and lipids (lipases, proteases, DNA repair enzymes) (Clemente-Suárez et al., 2023; Pisoschi & Pop, 2015).

Repeated exposure to high oxidative stress, typical of high cardiovascular-demand disciplines, has led to the exploration of exogenous compensation strategies. In this context, supplementation with antioxidant molecules emerges as a targeted approach to mitigate ROS-induced damage and strengthen endogenous defence systems, with promising implications for health and athletic performance (Ruggiero, Motti, et al., 2025; Hadi et al., 2017; Motti et al., 2022; Panza et al., 2008; Ruggiero, Vicidomini, et al., 2025; Ruggiero et al., 2026; D'Errico et al., 2026). While professional athletes commonly rely on dietary supplements without particular concerns, elite-level athletes face stricter regulations and may inadvertently incur anti-doping violations due to the presence of prohibited substances in contaminated products (Mazzeo, 2016; Mazzeo et al., 2018; Mazzeo & Raiola, 2018; Mazzeo & Volpe, 2016; Ruggiero, Ferrante, et al., 2025). Moreover, certain supplements or medications may cause adverse effects, particularly when used without appropriate medical supervision (Motola et al., 2001).

Traditionally, the assessment of oxidative stress in sports has relied on blood samples, with practical limitations for frequent monitoring. Some studies have demonstrated that saliva can be analysed as an alternative matrix, thanks to the presence of antioxidant enzymes and oxidative damage biomarkers (Atsumi et al., 2008). This non-invasive approach is particularly suitable for longitudinal studies on athletes, as evidenced by research on professional athletes (Doria et al., 2013).

The 1,1′-diphenyl-2-picrylhydrazyl (DPPH) test, a widely recognized method for evaluating total antioxidant capacity, was selected for its reliability in quantifying free radical scavenging activity under physiological conditions (Gulcin & Alwasel, 2023). The DPPH test operates on the principle of spectrophotometric detection of the neutralization of the DPPH free radical, a compound distinguished by its intense and stable violet coloration (Bondet et al., 1997). Upon interaction with hydrogen-donating antioxidant compounds, DPPH is reduced to its non-radical form (DPPH-H), leading to a measurable colour shift from violet to yellow (Yapıcı et al., 2021). This chromatic transition, directly proportional to the sample's radical scavenging capacity, is quantified using ultraviolet-visible (UV-Vis) spectroscopy(Xie & Schaich, 2014), rendering the DPPH test particularly suitable for analysing complex biological matrices such as saliva.

In light of this evidence, a clear research gap emerges. While the dual effects of intense exercise on redox balance are established, there is a lack of longitudinal, real-world studies that monitor these dynamics in middle-aged, non-professional athletes using non-invasive methods and under controlled dietary conditions. Middle age represents a critical window for health trajectory, where personalized exercise regimens could maximize metabolic and cardiovascular benefits while minimizing oxidative risks, thereby promoting successful aging. However, current monitoring in amateur sports rarely incorporates objective biochemical feedback, relying instead on subjective measures of fatigue and performance. The present study aims to address this gap by evaluating the impact of a structured six-month amateur soccer training program—a model of high-intensity intermittent exercise—on redox balance in middle-aged men under controlled dietary conditions. By employing the DPPH spectrophotometric assay on saliva, this pilot investigation seeks to analyse whether such training predominantly activates endogenous antioxidant mechanisms or induces pro-oxidant effects. The choice of saliva as a matrix and the DPPH test reflects a deliberate translational approach: it offers a feasible, non-invasive

strategy for routine redox monitoring outside laboratory settings. This work provides a proof-of-concept for integrating simple biochemical feedback into amateur training protocols, with the long-term goal of enabling personalized exercise prescriptions that optimize health outcomes and mitigate exercise-induced oxidative stress in middle-aged populations.

Material & Methods

This study was conducted as a pilot project, aimed at exploring the feasibility of using salivary DPPH testing to monitor redox balance in middle-aged amateur soccer players. The results are intended to generate preliminary data and guide larger future studies.

Participants

The study enrolled 12 middle-aged male participants (45–60 years) who practiced amateur soccer over six months (January-June 2024). On average, the sample was mildly overweight (BMI 25.6 ± 3.9 kg/m^2). Demographic and anthropometric characteristics of the cohort are summarized in Table 1.

Table 1. Demographic characteristics of the participants.

Variables	**Mean ± SD**
Age (years)	52.5 ± 4.3
Height (cm)	170.78 ± 8.23
Weight (kg)	74.79 ± 8.79
BMI (kg/m^2)	25.6 ± 3.9

Participants were screened to ensure exercise response homogeneity and exclude confounding factors. Beyond standard criteria (informed consent, health status, prior soccer experience, and training adherence), individuals with clinical conditions or therapies known to alter redox balance were excluded. Lifestyle factors (habitual physical activity, dietary patterns) were assessed via baseline questionnaires.

Table 2. Inclusion criteria.

Category	**Requirements**
General Health	No acute/chronic conditions impairing physical performance
Sports Experience	≥ 1 year of soccer practice (recreational/competitive)
Training Adherence	≥ 80% attendance in sessions (3 times a week)
Exclusion Factors	Use of antioxidants/redox-sensitive medications
	Active cardiometabolic diseases

Saliva samples were collected on the last day of the study period, before and after physical activity. The training session on the last day was designed to be intensive and is described in the following section.

The study was conducted in accordance with the ethical guidelines of the Helsinki Declaration of the World Medical Association and was approved by the ethics committee (protocol 200/17) of the School of Medicine, University of Naples Federico II.

Training Procedure

The training was aimed at developing resistance to work under high lactate conditions. The program included repeated running trials over short, medium, and long distances, performed at high speed with recovery intervals of 3–6 minutes, for a total distance of 2000–3000 metres. Progressive, continuous, and medium-distance running exercises were alternated to sustain effort during repeated trials.

Initially, training was conducted indoors with the following structure: 10 minutes of slow running; 8 minutes of mobilization exercises; 3 minutes of respiratory gymnastics.

12 minutes of exercises with small equipment and light weights; 6 minutes of abdominal and back exercises; 10 minutes of stretching; 8 minutes of slow running. Subsequently, the duration of small equipment exercises was reduced, and interval training (sets and repetitions) was introduced, reaching sessions of 75–90 minutes. Equipment used: Regulation soccer field with a single goal; Cones; Small equipment (hurdles, markers, hoops, poles); Weights of 1, 2, and 3 kg; Regulation soccer balls.

Nutritional Assessment via Food Diary and 24-hour Recall

Nutritional status was assessed using a food diary to evaluate the dietary habits of athletes during three typical days, repeated three times (once every two months) over the study period. Each meal (breakfast, morning snack, lunch, afternoon snack, dinner), consumed at predetermined times, was recorded on a form specifying: Foods consumed; Time and location of consumption; Description of dishes/recipes; Added condiments or supplements. The food diary consisted of pocket-sized sheets designed to simplify compilation and avoid omissions. Food quantities were estimated using standardized household measures (glasses, cups, spoons), product models (cans, single-serving packages), or weight indications.

Data analysis was performed using the NutriSurvey database, an English-translated version of the German nutritional software EBISpro.

Samples

Saliva samples were collected using Salivettes® (Sarstedt Inc., Nümbrecht, Germany - DIAMETRA Italy) on the last day of the study period, before and after physical activity. Participants were instructed to: a) avoid consuming food and beverages (except water); b) refrain from smoking; c) rinse their mouth with a glass of water 10 minutes before collection. Samples were discarded if protocols were violated or if oral bleeding was detected.

The methodology involved a preparation phase of the double-chamber container: the inner tube edge was firmly gripped during cap removal to prevent accidental separation from the outer casing. The swab was then inserted directly into the oral cavity through a controlled motion. Light taps on the tube base facilitated swab positioning without manual contact. Participants chewed the swab gently for two minutes before resealing it into the inner chamber.

Collected samples were immediately stored at -20°C to ensure stability. Before analysis, samples were centrifuged at 7800 × g for 20 minutes (Eppendorf Centrifuge, Hamburg, Germany) to separate solid components from the supernatant. To purify the samples, a heat treatment step at 95°C for 10 minutes was performed to denature proteins and eliminate food residues or contaminants. Following this, samples were centrifuged again under the same conditions (7800 × g, 20 minutes) to isolate the final supernatant, which was then used for the DPPH antioxidant activity test. The protocol combined operational precision and technical rigor to ensure reliable results.

DPPH Test

The DPPH assay is a widely used spectrophotometric methodology for determining the antioxidant activity of biological samples, plant extracts, or pure compounds. This test is based on the ability of antioxidants to transfer a hydrogen atom (H^+) or an electron to the stable free radical DPPH•, thereby neutralizing it. The DPPH• radical exhibits a characteristic deep purple colour due to the delocalization of its unpaired electron across the entire molecule (maximum absorption at 517 nm). When an antioxidant donates a hydrogen atom to DPPH•, it reduces to the hydrazine form (DPPH-H), resulting in discoloration of the solution (pale yellow colour). The decrease in absorbance at 517 nm is proportional to the scavenging activity of the sample (Gulcin & Alwasel, 2023).

The free radical scavenging activity was determined following a protocol adapted from Atsumi (Atsumi et al., 2008), with modifications introduced by Doria et al. (Doria et al., 2013). All samples were processed under identical conditions, using the same reagent solution and incubation times. To 100 µL of supernatant (prepared as described above), the following were added: 500 µL of 50 mM HEPES buffer (pH 7.4) in 40% ethanol; 200 µL of 50% ethanol; 100 µL of 0.9% NaCl in 50% ethanol; 100 µL of 1 mM DPPH solution (prepared by dissolving 0.004 g of DPPH in 10 mL of 50% ethanol under constant agitation).

The mixtures were incubated in the dark at room temperature for 10 minutes, conditions required to ensure complete reaction between DPPH and sample antioxidants. After incubation, the samples were centrifuged at 700 × g for 10 minutes using a benchtop centrifuge (Eppendorf 5430, Hamburg, Germany) to remove any precipitates.

The absorbance of the supernatant was then measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan), with a reference blank consisting of the same reaction mixture without saliva. However, since our analysis focused on intra-individual changes, the absolute DPPH scavenging activity was not calculated. Instead, the absorbance change (Δ) directly reflects the relative shift in antioxidant capacity before and after exercise for each participant.

Data Analysis

The results of the DPPH test were expressed as Δ values calculated by subtracting the pre-exercise absorbance (Pre-Abs) from the post-exercise absorbance (Post-Abs), according to the formula:

$$\Delta = \text{Post-Abs} - \text{Pre-Abs}$$

A negative Δ value indicates an increase in antioxidant activity after physical exercise (decreased absorbance due to the neutralization of DPPH radicals), while a positive value reflects a reduction in antioxidant activity. Additionally, for each subject, the mean of the initial and final absorbance values was calculated to represent the mean level of free radicals during the observed period.

Results

All 12 participants met the inclusion criteria and completed the study protocol. Saliva samples were properly collected before and after physical activity during the final training session.

The DPPH test results, presented in Table 3, display the pre-training absorbance values, post-training values, absolute change (Δ), and average for each athlete.

All measurements were performed using the same batch of DPPH reagent to ensure consistency across samples.

Table 3. DPPH Test Results: pre- and post-exercise absorbance values, Δ values, and mean absorbance.

Participants	Pre-Abs	Post-Abs	Δ	Mean
1	0.856	0.888	+0.032	0.872
2	0.751	0.747	-0.004	0.749
3	0.649	0.684	+0.035	0.667
4	0.626	0.674	+0.048	0.650
5	0.651	0.717	+0.066	0.684
6	0.718	0.715	-0.003	0.717
7	0.725	0.724	-0.001	0.725
8	0.642	0.605	-0.037	0.624
9	0.646	0.621	-0.025	0.634
10	0.589	0.634	+0.045	0.612
11	0.604	0.583	-0.021	0.594
12	0.671	0.686	+0.015	0.679

Pre-Abs: pre-exercise absorbance; Post-Abs: post-exercise absorbance; Δ: difference between post and pre-exercise absorbance.

The DPPH test applied to salivary samples revealed marked heterogeneity in individual antioxidant responses. Δ values between pre- and post-training ranged from -0.037 (Athlete 8, maximum reduction) to +0.066 (Athlete 5, maximum absorbance increase), with six subjects showing a positive Δ values (reduced post-exercise antioxidant activity) and six subjects a negative Δ values (improvement). The Δ values were sorted from the lowest to the highest magnitude in Figure 1. Mean absorbance values ranged from 0.594 (Athlete 11) to 0.872 (Athlete 1), detecting substantial differences in baseline antioxidant capacity among participants. It explicitly shows six participants with negative Δ values (green bars) and six participants with positive Δ values (red bars). This visual representation is far more impactful than just stating the percentages.

Discussion

This study evaluated the effects of a six-month soccer training program on redox balance in middle-aged subjects by analyzing salivary antioxidant capacity via the DPPH test under controlled dietary conditions. The same DPPH reagent was used for all samples to ensure methodological consistency and reduce analytical variability. The experimental design focused on intra-individual changes; antioxidant capacity was assessed through absorbance change values. This Δ value directly reflects the relative shift before and after exercise for each participant, inherently controlling for interindividual baseline variability without requiring absolute DPPH controls.

Given its nature as a pilot project, these findings should be interpreted as exploratory and hypothesis-generating rather than definitive. They demonstrate that intense exercise operates within a precise therapeutic window—beneficial adaptations occur at individualized thresholds, while excessive loads trigger oxidative damage. Thus, personalizing training intensity through non-invasive redox monitoring (e.g., salivary DPPH testing) represents an essential strategy to maximize health benefits in middle-aged athletes.

The results, characterized by broad interindividual variability (Table 3), provide critical insights into the physiological dualism of exercise, where antioxidant and pro-oxidant effects coexist in a delicate equilibrium. Standardized nutritional monitoring (via food diary and 24-hour recall) confirmed consistent dietary antioxidant intake across the entire cohort. This reinforces the hypothesis that the heterogeneity observed in DPPH test Δ values (ranging from -0.037 to +0.066) stems from intrinsic differences in the activation capacity of endogenous defences or the efficiency of oxidative damage repair, rather than from dietary fluctuations.

The choice of saliva as a matrix is grounded in evidence that it reliably reflects systemic antioxidant profiles, containing enzymes such as superoxide dismutase (SOD) and catalase alongside non-enzymatic molecules like glutathione and vitamin C, analogous to plasma (Atsumi et al., 2008; Gawron-Skarbek et al., 2018). This non-invasive approach, validated in longitudinal athlete studies (Doria et al., 2013), revealed heterogeneity in Δ values, mirroring the complex interplay between intense exercise-induced pro-oxidant stimuli and endogenous defence activation.

This complexity is further amplified by saliva's intrinsic antioxidant network, which orchestrates oral redox regulation through synergistic interactions. Saliva contains a sophisticated system of antioxidants collaborating to regulate the redox state of the oral cavity (Ginsburg, Kohen, Shalish, et al., 2013). Endogenous antioxidants include low molecular weight antioxidants (LMWA) such as uric acid, ascorbate (vitamin C), reduced glutathione, and α-tocopherol, which constitute the majority of total antioxidant capacity and are localized in the salivary supernatant (Fábián et al., 2008; Liskmann et al., 2007). These are complemented by proteins like albumin (derived from plasma via crevicular fluid) and mucin, which act both as direct free radical scavengers and as "solubilizers" for lipophilic antioxidants (Ginsburg et al., 2012; Ginsburg, Kohen, & Koren, 2013). External factors significantly contribute: dietary polyphenols (from red wine, tea, coffee, cocoa, etc.) bind to oral surfaces and microbial flora, acting as "slow-release" devices that enhance antioxidant activity despite salivary flow (Ginsburg et al., 2012). Finally, catalase-positive commensal microorganisms (e.g., Candida

albicans, E. coli) actively contribute to H_2O_2 decomposition and modulate redox balance (Ginsburg et al., 2011). This integrated network of endogenous and exogenous antioxidants protects oral tissues from oxidative stress under both physiological and pathological conditions. Critically, interindividual variability in oral microbiota composition (e.g., abundance of catalase-positive Streptococcus salivarius) may further modulate salivary ROS detoxification, representing an additional layer of personalization in exercise-induced redox responses.

This dualism is clearly exemplified by the bifurcation in our data: half the cohort exhibited enhanced post-exercise antioxidant activity (negative Δ values), while the other half showed increased oxidative stress (positive Δ values). Such polarization reflects the threshold-dependent duality of strenuous exercise. On one hand, it potentiates endogenous antioxidant defences through elevated activity of enzymes like SOD1, SOD2 (Alessio & Goldfarb, 1988; Powers et al., 2022; Quintanilha, 1984) and glutathione peroxidase (GPX) (Powers et al., 2022)—with protective effects observable after just five training days (Vincent et al., 2000), particularly pronounced in high-intensity protocols like high-intensity interval training (HIIT) (Hellsten et al., 1996). On the other hand, it generates ROS excess when intensity exceeds individual thresholds (Schippinger et al., 2002; Wang et al., 2021).

ROS production, fueled by mitochondria (Complexes I/III) (Powers & Jackson, 2008; Robb et al., 2018) and cytosolic enzymes NOX, xanthine oxidase) (Henríquez-Olguin et al., 2019; Sakellariou et al., 2013), triggers oxidative cascades that compromise cellular integrity. Free radicals attack polyunsaturated fatty acids in membranes, generating lipid peroxides (Das, 2011; Ito et al., 2019); damage proteins via peptide bond cleavage and tyrosine cross-linking (Das, 2011; Vasilaki et al., 2017); and oxidize DNA bases to form 8-hydroxydeoxyguanosine (Vasilaki et al., 2017). To counter this, the body activates sophisticated compensatory mechanisms where ROS act as signalling molecules, initiating pathways like NF-κB/MAPK that activate the transcriptional factor Nrf2 (Abruzzo et al., 2013). Nrf2 translocates to the nucleus, enhancing expression of antioxidant enzymes (SOD, GPX, glutathione) (Abruzzo et al., 2013; Bouviere et al., 2021; Steinbacher & Eckl, 2015)—a process explaining enzymatic increases in chronic exercise (Brinkmann et al., 2012; Mesquita et al., 2021) and reduced lipid peroxidation in non-obese adults (Vincent et al., 2006).

Consistent with our findings, studies by Gawron-Skarbek et al. (Gawron-Skarbek et al., 2021) and by Ngarsou et al. (Ngarsou Pierre et al., 2024) documented heightened salivary antioxidant potential in structured exercise programs, albeit without parallel anti-inflammatory improvements. The observed heterogeneity—including extreme cases like Athlete 5 (Δ=+0.066), suggesting inadequate antioxidant response—may stem from individual factors such as muscle fiber phenotypic differences (Type I/IIa fibers clear ROS more efficiently than Type IIb (Zhang et al., 2017)), myokine modulation (e.g., interleukin-15 (Li et al., 2014; Starnes et al., 2017), or genetic polymorphisms affecting Nrf2-dependent pathway efficiency (Abruzzo et al., 2013; Steinbacher & Eckl, 2015).

In summary, the balance between oxidative damage and adaptive response, mediated by redox-sensitive pathways, represents the physiological cornerstone determining exercise's net impact on cellular health (Montesano & Mazzeo, 2019). Saliva emerges as a valuable matrix for tracking these individualized dynamics. The equal distribution of participants with positive and negative Δ values reinforces the concept of responders and non-responders to exercise at the redox level. This observation, even in a small cohort, highlights the potential utility of individualized monitoring to optimize training programs.

From a translational perspective, integrating rapid salivary tests into amateur sports settings could guide dynamic training algorithms. By adjusting exercise loads and recovery based on real-time redox feedback, such approaches would maximize health benefits while minimizing oxidative risks in middle-aged populations. Future larger-scale studies will be required to validate these preliminary observations and to explore the molecular determinants (e.g., SOD, GPX activity, Nrf2 pathway activation) that underlie the observed variability.

Limitations and Future Directions of the Study

Despite methodological rigor, limitations exist. Saliva collection restricted to the final session precluded monitoring redox evolution during the 6-month training or medium-term recovery (e.g., 24–48 h post-exercise). The lack of female participants also hinders the exploration of sex-based redox differences. Additionally, while DPPH reliably assesses total antioxidant capacity, it cannot distinguish enzymatic (SOD, GPX) from non-enzymatic components (vitamins, polyphenols), obscuring molecular drivers of heterogeneity.

These limitations must be interpreted in light of the pilot nature of the study, which was designed to test feasibility rather than provide definitive conclusions. Including these parameters in future studies would allow participants to be stratified by metabolic phenotype, optimizing the personalization of training.

Future work should adopt longitudinal designs with serial sampling across training phases and recovery windows, integrating DPPH with targeted methods (e.g., mass spectrometry) to quantify specific antioxidants/damage markers. Including female athletes could clarify sexual dimorphism in soccer-induced oxidative stress. Critically, nutritional approaches using salivary metabolomics should explore how diets enriched with bioactive molecules (polyphenols, carotenoids) modulate individual redox profiles, potentially enabling personalized training strategies for middle-aged populations. Larger studies will be needed to confirm these preliminary findings and extend their generalizability.

Moreover, the entire study design could be replicated with athletes from different sport disciplines, allowing comparisons across varying training demands, metabolic profiles, and oxidative stress responses. This would enhance the generalizability of the findings and deepen knowledge of sport-specific redox adaptations and their biochemical determinants.

Finally, future investigations should evaluate antioxidant potential not only via biochemical assays, but also by exploring gene expression profiles related to endogenous defence systems. Enzymes such as SOD1, SOD2, and SIRT1, key regulators of oxidative stress response, could provide insight into interindividual variability in adaptation to physical activity.

Conclusions

This pilot study demonstrates that a six-month amateur soccer training program induces markedly individualized antioxidant responses in middle-aged men, even under controlled dietary conditions. The research was conducted through systematic monitoring of a cohort of amateur athletes, with saliva samples collected before and after a standardized high-intensity session at the conclusion of the training program. The DPPH spectrophotometric assay, applied under controlled analytical conditions, was used to quantify changes in total antioxidant capacity, revealing a clear bifurcation in post-exercise redox adaptation. The equal distribution of participants exhibiting enhanced versus diminished salivary antioxidant capacity vividly illustrates the dual nature of intense exercise: it operates within a precise therapeutic window, where beneficial adaptive responses coexist with the risk of oxidative stress. These preliminary findings, while requiring confirmation in larger cohorts, provide a compelling proof-of-concept for the central role of individual redox physiology in determining the net health impact of physical activity.

The theoretical contribution of this work lies in strengthening the paradigm of exercise as a personalized intervention, where the classical distinction between "responders" and "non-responders" can be traced to fundamental biochemical thresholds of oxidative balance. From a practical and translational perspective, the study validates a significant methodological advance: the use of non-invasive salivary DPPH testing as a feasible, rapid, and cost-effective tool for routine redox monitoring outside laboratory settings. This moves the assessment of exercise tolerance beyond subjective perception and generic plans, offering a credible, biologically-grounded feedback mechanism.

The implications of these results are substantial for sports science and preventive health strategies targeting middle-aged populations. They support a shift toward personalized training prescriptions, where exercise intensity and recovery can be dynamically guided by objective redox feedback. Such an approach, if implemented, could maximize the documented cardiovascular, metabolic, and anti-aging benefits of physical activity while systematically minimizing the risks associated with excessive oxidative load, thereby enhancing long-term adherence and health outcomes. For amateur sports clubs, health coaches, and professionals in preventive medicine, this methodology presents a tangible opportunity to evolve practice, transforming exercise from a generic recommendation into a precisely dosed, health-optimizing "treatment."

To translate this foundational insight into practical application, future research should build upon these preliminary data. Larger, longitudinal studies are warranted to confirm the observed heterogeneity and to correlate salivary redox dynamics with long-term health outcomes and athletic performance. Further investigation into the underlying molecular mechanisms—such as specific antioxidant enzyme activity or genetic predispositions—would deepen our understanding of individual response variability. If consistently validated, this non-invasive monitoring approach could inform the development of more personalized training guidelines, supporting strategies that optimize the health benefits of exercise for middle-aged adults while respecting individual physiological limits.

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