

Sport-specific oxidative stress and post-exercise metabolic responses in elite athletes: a pilot comparative study of boxing, biathlon, and modern pentathlon

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Abstract

Problem Statement. Oxidative stress biomarkers may provide insight into athletes' adaptation to high training loads. However, findings across sport disciplines remain inconsistent. Accordingly, the submitted manuscript required substantial methodological, linguistic, statistical, and bibliographic revision to satisfy the Journal of Physical Education and Sport standards. **Approach.** This pilot comparative study included 36 highly qualified male athletes during the special preparatory phase of the annual training cycle: boxers (n = 12), biathletes (n = 12), and pentathletes (n = 12). Blood catalase (CAT) activity and malondialdehyde (MDA) concentration were measured at rest and following sport-specific control loads, while blood lactate was assessed at 1 and 5 min of recovery. The revised analysis used one-way ANOVA to compare the sports at each time point, Holm-adjusted pairwise tests, and Hedges' g effect sizes. **Purpose.** To compare CAT, MDA, and short-term lactate recovery across three Olympic disciplines with distinct energetic profiles and to present the findings in a cautious, journal-compliant form enriched with current literature and a publication-ready summary figure. **Results.** CAT differed significantly among sports before exercise, $F(2, 33) = 105.11$, $p < .001$, $\eta^2 = .864$, and after exercise, $F(2, 33) = 13.10$, $p < .001$, $\eta^2 = .442$. Post-load CAT was higher in modern pentathletes than in boxers ($p_{\text{Holm}} < .001$) and biathletes ($p_{\text{Holm}} < .001$), whereas boxers and biathletes did not differ ($p = .667$). MDA differed markedly at rest, $F(2, 33) = 475.61$, $p < .001$, $\eta^2 = .966$, and after exercise, $F(2, 33) = 506.84$, $p < .001$, $\eta^2 = .968$; boxers had the highest concentrations and biathletes the lowest values at both time points. Lactate at 1 min and 5 min recovery did not differ significantly between sports ($p = .302$ and $p = .260$, respectively). Descriptively, biathletes showed the strongest CAT increase (+98.4%) and fastest lactate clearance (-6.05%), whereas boxers showed a CAT decrease (-8.2%) and the highest MDA concentrations. **Conclusions.** CAT and MDA appear useful for profiling sport-related oxidative stress responses, but the findings should be interpreted as exploratory because of the small sample and non-identical sport-specific exercise protocols. Larger studies with standardized external-load monitoring and repeated-measures modelling are needed.

Key words: catalase; malondialdehyde; antioxidant defense; elite athletes; lipid peroxidation; lactate recovery

Introduction

Exercise-induced oxidative stress is a normal component of adaptation, yet excessive reactive oxygen species production can impair recovery, membrane stability, and metabolic efficiency. In sport science, catalase (CAT) and malondialdehyde (MDA) are frequently used to characterize antioxidant defense and lipid peroxidation, respectively. Contemporary mechanistic and review literature indicates that exercise redox responses depend on the balance between adaptive signaling and excessive oxidative load, as well as on exercise mode, duration, and training status (Powers & Jackson, 2008; Radak et al., 2013; He et al., 2016; Gomez-Cabrera et al., 2021; Thirupathi et al., 2021; Clemente-Suárez et al., 2023).

Meta-analytic work further suggests that structured exercise often reduces lipid peroxidation and improves selected antioxidant indices, although CAT-related responses are less uniform across protocols and populations (de Sousa et al., 2017; Simioni et al., 2018; Wang et al., 2023; Xie et al., 2025; Zhou et al., 2022). Accordingly, any sport-specific interpretation should be cautious and context dependent rather than universal.

Cross-sport comparisons remain practically relevant because oxidative and inflammatory profiles differ across athletic disciplines. Comparative research in elite and trained cohorts has shown that oxidative markers vary according to sport demands and seasonal context, including distinctions between team, endurance, and combat

settings (Hadzovic-Dzuvo et al., 2014; Sohail et al., 2020; Luti et al., 2022; Meng & Su, 2025; Karlsson et al., 2024). In the Ukrainian sports-science context, recent work has also emphasized blood-based monitoring of adaptation in race walking and triathlon-related settings (Stankevich et al., 2024; Stankevich et al., 2025).

The reviewer of the original submission correctly noted several weaknesses: an overextended conclusion, insufficiently precise statistical reporting, lack of pairwise p-values after correction for multiple comparisons, stylistic problems, and an overly strong claim about novelty. The revised manuscript addresses those points by restructuring the text to the JPES template, clarifying the role of diet control and ethics, tightening the discussion, integrating current DOI-bearing literature, limiting unsupported claims, and reporting corrected between-group statistics with effect sizes.

The purpose of the study was to compare CAT activity, MDA concentration, and early lactate recovery in elite boxers, biathletes, and modern pentathletes tested during the special preparatory phase of the annual training cycle.

Materials and Methods

Participants.

Thirty-six highly qualified male athletes aged 19-30 years participated in the study: boxing (n = 12), biathlon (n = 12), and modern pentathlon (n = 12). Athletes had 7-11 years of training experience and held the sport ranks Candidate Master of Sport, Master of Sport, International Master of Sport, or Honored Master of Sport. All participants were in the special preparatory phase of the preparatory period, completed approximately 25-35 h of training per microcycle, and were clinically healthy at the time of testing.

Nutritional control and ethics.

To address the reviewer's concern regarding diet, the athletes were fed centrally and the balance of the diet was monitored during the study period. No additional medication or antioxidant supplementation was used. Morning fasting blood samples were collected to determine baseline CAT and MDA. The study complied with the Helsinki Declaration and was approved by the Biomedical Ethics Commission of the National University of Ukraine on Physical Education and Sport, Kyiv, Ukraine (No. 2, 16.11.2024). Written informed consent was obtained from all participants.

Procedures and measures.

The testing design reflected the ecological demands of each sport. Boxers completed a specific control session based on an effective competitive activity model that included repeated high-intensity sparring bouts over approximately 1.5 h. Biathletes and modern pentathletes performed a graded treadmill protocol with mobile spirometric monitoring to model competition-related work. Because the external loads were sport-specific rather than mechanically identical, the study should be interpreted as a comparison of response profiles under discipline-relevant control sessions rather than a strict laboratory equivalence trial.

Capillary blood was sampled before exercise and after the control load. Lactate was determined at 1 min and 5 min of recovery with an LP-400 photometer (Dr. Lange, Germany). MDA was assessed by the thiobarbituric acid colorimetric method. CAT activity was determined by standard biochemical spectrophotometric procedures and is reported as mmol H₂O₂*min⁻¹*mg Hb⁻¹. Descriptive participant and protocol information is summarized in Table I.

Statistical analysis.

Data are reported as mean +/- standard error of the mean (SEM). Normality was assessed with the Shapiro-Wilk test. To correct the weakness of the original manuscript, between-sport comparisons at each matched time point were analysed with one-way ANOVA followed by Holm-adjusted pairwise comparisons. Effect sizes were expressed as Hedges g for pairwise differences and eta² for omnibus effects. The significance level was set at p < .05. Because the available dataset for revision was organized as summary values for each group and because pre-post covariance was not available in the manuscript tables, within-group changes are interpreted descriptively and the inferential emphasis is placed on corrected between-group comparisons.

Table I. Participant characteristics and protocol description

Variable	Boxing	Biathlon	Modern pentathlon
Sample size	12	12	12
Sex	Male	Male	Male
Age	19-30 years	19-30 years	19-30 years
Training experience	7-11 years	7-11 years	7-11 years
Training phase	Special preparatory phase	Special preparatory phase	Special preparatory phase
Control session	Sport-specific sparring-based load (~1.5 h)	Graded treadmill load with spirometric monitoring	Graded treadmill load with spirometric monitoring
Blood variables	CAT, MDA, lactate	CAT, MDA, lactate	CAT, MDA, lactate
Diet/supplementation	Centralized diet; no antioxidant supplements	Centralized diet; no antioxidant supplements	Centralized diet; no antioxidant supplements

Note. CAT = catalase; MDA = malondialdehyde.

Results

Catalase activity. As shown in Table II, resting CAT activity differed substantially between sports, $F(2, 33) = 105.11$, $p < .001$, $\eta^2 = .864$. Boxers had higher resting CAT than biathletes ($p_{\text{Holm}} < .001$, $g = 5.81$) and slightly higher CAT than modern pentathletes ($p = .035$, $g = 0.73$), whereas biathletes had lower resting CAT than modern pentathletes ($p_{\text{Holm}} < .001$, $g = -5.17$). After exercise, the between-sport difference remained significant, $F(2, 33) = 13.10$, $p < .001$, $\eta^2 = .442$. Modern pentathletes demonstrated higher post-load CAT than boxers ($p_{\text{Holm}} < .001$, $g = -2.20$) and biathletes ($p_{\text{Holm}} < .001$, $g = -1.69$), while boxers and biathletes did not differ ($p = .667$). Descriptively, CAT decreased in boxers (-8.2%), increased moderately in modern pentathletes (+23.3%), and almost doubled in biathletes (+98.4%).

Malondialdehyde. MDA showed very large between-sport effects both before and after exercise (Table II). At rest, the omnibus comparison was significant, $F(2, 33) = 475.61$, $p < .001$, $\eta^2 = .966$. Boxers had markedly higher MDA than biathletes ($p_{\text{Holm}} < .001$, $g = 9.75$) and modern pentathletes ($p_{\text{Holm}} < .001$, $g = 9.35$), and modern pentathletes also had higher MDA than biathletes ($p_{\text{Holm}} < .001$, $g = -2.70$). After exercise, the pattern persisted, $F(2, 33) = 506.84$, $p < .001$, $\eta^2 = .968$, with significant differences among all pairwise comparisons (all $p_{\text{Holm}} < .001$). Descriptively, post-load MDA increased by 42.7% in boxers, 31.7% in biathletes, and 90.9% in modern pentathletes.

Blood lactate recovery. Blood lactate did not differ significantly between sports at 1 min recovery, $F(2, 33) = 1.24$, $p = .302$, $\eta^2 = .070$, or at 5 min recovery, $F(2, 33) = 1.40$, $p = .260$, $\eta^2 = .078$ (Table III). Nevertheless, the recovery pattern was directionally different: biathletes showed a 6.05% decrease from 1 min to 5 min, modern pentathletes a minimal decrease of 0.57%, and boxers a 4.84% increase. These descriptive changes should be interpreted cautiously because the corrected between-sport comparisons were not statistically significant.

Figure 1 provides a publication-ready summary of the main findings by visualizing resting CAT and MDA values, relative pre-to-post changes, and the absence of significant between-sport differences in lactate recovery.

Table II. Oxidative stress markers before and after exercise across sports (mean +/- SEM)

Variable/time point	Boxing	Biathlon	Modern pentathlon	One-way ANOVA	Holm-adjusted pairwise comparisons
CAT pre-load	4.25 +/- 0.15	1.92 +/- 0.05	3.87 +/- 0.14	$F(2,33)=105.11$ $p<.001$ $\eta^2=.864$	B vs Bi: $p<.001$ B vs MP: $p=.035$ Bi vs MP: $p<.001$
CAT post-load	3.90 +/- 0.12	3.81 +/- 0.20	4.77 +/- 0.10	$F(2,33)=13.10$ $p<.001$ $\eta^2=.442$	B vs Bi: $p=.667$ B vs MP: $p<.001$ Bi vs MP: $p<.001$
MDA pre-load (nmol*L-1)	136.46 +/- 3.37	31.21 +/- 2.60	51.04 +/- 1.27	$F(2,33)=475.61$ $p<.001$ $\eta^2=.966$	B vs Bi: $p<.001$ B vs MP: $p<.001$ Bi vs MP: $p<.001$
MDA post-load (nmol*L-1)	194.84 +/- 3.98	41.09 +/- 2.90	97.45 +/- 3.40	$F(2,33)=506.84$ $p<.001$ $\eta^2=.968$	B vs Bi: $p<.001$ B vs MP: $p<.001$ Bi vs MP: $p<.001$

Note. B = boxing; Bi = biathlon; MP = modern pentathlon; CAT reported in mmol H₂O₂*min⁻¹*mg Hb⁻¹. Effect sizes from the revision memo were very large for most MDA contrasts and moderate-to-very-large for CAT contrasts.

Table III. Blood lactate during early recovery after exercise (mean +/- SEM, mmol*L-1)

Time point	Boxing	Biathlon	Modern pentathlon	One-way ANOVA	Holm-adjusted pairwise comparisons
1 min recovery	10.13 +/- 0.27	10.58 +/- 0.17	10.51 +/- 0.20	$F(2,33)=1.24$ $p=.302$ $\eta^2=.070$	All $p>.45$
5 min recovery	10.62 +/- 0.30	9.94 +/- 0.16	10.45 +/- 0.39	$F(2,33)=1.40$ $p=.260$ $\eta^2=.078$	All $p>.35$
Percent change (1 to 5 min)	+4.84%	-6.05%	-0.57%	Descriptive	Not inferentially significant

Note. Pairwise p -values are Holm-adjusted. The lactate pattern suggests faster recovery in biathletes, but the between-sport contrasts were not statistically significant.

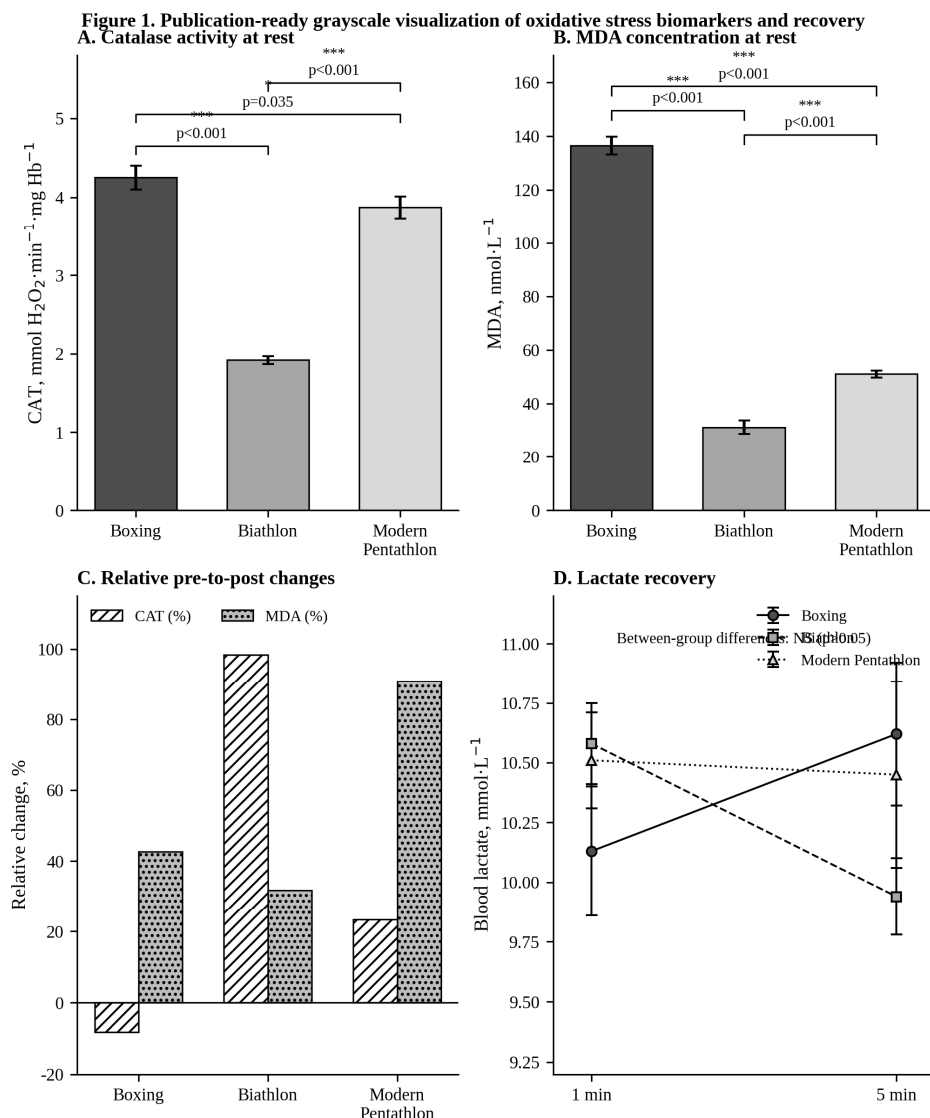


Figure 1. Grayscale visualization of oxidative stress biomarkers and recovery profiles in athletes from three sport disciplines. Panel A shows resting catalase (CAT) activity (mean $\pm$ SEM) with between-group significance derived from one-way ANOVA followed by Holm-corrected pairwise comparisons: boxing vs biathlon, $p<0.001$; boxing vs modern pentathlon, $p=0.035$; biathlon vs modern pentathlon, $p<0.001$. Panel B shows resting malondialdehyde (MDA) concentration (mean $\pm$ SEM); all between-group comparisons were significant after correction (all $p<0.001$). Panel C summarizes relative pre-to-post changes (%) in CAT and MDA. Panel D presents blood lactate during recovery (1 and 5 min; mean $\pm$ SEM), with no significant between-group differences at either time point (all $p>0.05$). * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS, not significant.

Discussion

The updated analysis confirms that the clearest between-sport contrasts concerned oxidative stress markers rather than lactate recovery. Boxers demonstrated the highest MDA concentrations both before and after exercise, whereas biathletes showed the lowest oxidative burden and the most favourable descriptive CAT increase. This pattern is compatible with cross-sport evidence showing that oxidative and inflammatory profiles differ according to discipline-specific demands and recovery context (Hadzovic-Dzuvo et al., 2014; Sohail et al., 2020; Luti et al., 2022; Meng & Su, 2025).

Recent athlete-focused studies also support the view that redox markers are shaped by both training background and seasonal timing. Higher CAT activity and lower lipid-peroxidation markers have been described in adolescent female athletes, endurance-trained men, and elite cross-country skiers, whereas seasonal transition or rest periods can still modify oxidative and inflammatory responses in trained cohorts (Bilici et al., 2025; Stojiljković et al., 2024; Jerome et al., 2024; Cichoń-Woźniak et al., 2024; Karlsson et al., 2024). Within that

broader literature, the present manuscript contributes pilot data from three Olympic sports examined during the same preparatory stage, not proof of unique novelty.

Our findings should not be interpreted as evidence that one discipline is universally better adapted than another. Exercise mode, duration, and intensity influence oxidative responses, and both acute and chronic adaptations depend on the balance between reactive oxygen species signaling and antioxidant buffering. Mechanistic and synthesis papers consistently emphasize that CAT, MDA, and related markers respond heterogeneously across protocols and populations (Powers & Jackson, 2008; Radak et al., 2013; He et al., 2016; Thirupathi et al., 2021; Lu et al., 2021; Xie et al., 2025; Zhou et al., 2022). The sport-specific control sessions used here therefore improve ecological validity but also limit direct physiological equivalence.

From a practical perspective, the results favour individualized monitoring rather than indiscriminate antioxidant intervention. Applied work in soccer and other team-sport settings suggests that targeted conditioning or well-managed recovery can improve performance while lowering or stabilizing oxidative stress markers, but high-dose or unsystematic antioxidant supplementation may blunt desirable training signals (Niknam et al., 2025; Meng & Su, 2024; Mason et al., 2020; Higgins et al., 2020; Williamson & Davison, 2020; Reljic, 2025). In our regional research context, blood-based antioxidant monitoring has also been proposed as a useful adjunct to functional evaluation in Ukrainian athletes (Stankevich et al., 2024; Stankevich et al., 2025).

Several limitations remain important. First, the sample size was small ($n = 12$ per group), which limits generalizability. Second, the exercise protocols were discipline-specific and therefore ecologically valid but not mechanically identical. Third, the revised statistics correct the original reporting by adding omnibus tests, multiplicity adjustment, and effect sizes, yet the manuscript tables still provide only summary-level pre-post data and not raw repeated measures. For that reason, the strongest conclusions should be restricted to between-group differences at matched time points. Fourth, the diet was controlled centrally, but exact intake was not quantified in the manuscript. Future research should combine larger samples, harmonized external-load monitoring, repeated-measures mixed modelling, and broader biomarker panels; recent reviews and meta-analyses indicate that protocol characteristics strongly affect redox outcomes and should therefore be modelled explicitly (Daud et al., 2022; Wang et al., 2023; Clemente-Suárez et al., 2023; Simioni et al., 2018).

Conclusions

1. Principal Findings and Redox Profile Characterization. The study identified distinct, sport-specific patterns of oxidative stress and enzymatic antioxidant response across elite athletes in boxing, biathlon, and modern pentathlon during the special preparatory phase. Omnibus comparisons demonstrated very large between-sport differences for malondialdehyde (MDA; $\eta^2 = 0.966$ at rest, $\eta^2 = 0.968$ post-exercise) and moderate-to-large differences for catalase activity (CAT; $\eta^2 = 0.864$ at rest, $\eta^2 = 0.442$ post-exercise). Boxers exhibited the most pronounced pro-oxidant burden, characterized by the highest MDA concentrations both at baseline and post-load, accompanied by a slight decrease in post-exercise CAT activity (-8.2%). Conversely, biathletes demonstrated a highly protective antioxidant profile with the lowest lipid peroxidation levels, a marked exercise-induced doubling of CAT activity (+98.4%), and superior early lactate clearance (-6.05%). Modern pentathletes displayed an intermediate baseline profile but achieved the highest absolute post-exercise CAT levels alongside a pronounced relative increase in MDA (+90.9%), reflecting the complex multi-component metabolic demands of their discipline.

2. Theoretical and Methodological Significance. From a theoretical perspective, these findings provide compelling evidence that exercise-induced redox responses are not uniform across elite disciplines but are intimately governed by the underlying bioenergetic structure, mechanical characteristics, and specific training adaptations of each sport. The data substantiate the concept that specialized endurance training (biathlon) fosters robust enzymatic antioxidant buffering capacity, whereas high-intensity intermittent impact loads (boxing) impose substantial cumulative free-radical stress that exceeds baseline enzymatic buffering. Methodologically, the combination of lipid peroxidation assessment (MDA), enzymatic scavenger profiling (CAT), and post-exercise lactate recovery dynamics establishes an informative, multi-dimensional framework for tracking athlete readiness and exercise strain.

3. Practical Applications and Sports Science Solutions. For coaching staff, sports physicians, and practitioners, the study delivers proven, applicable solutions for individualized training management: (a) routine monitoring of blood CAT and MDA serves as an objective diagnostic tool to detect excessive oxidative strain, mitigate non-functional overreaching, and optimize recovery intervals between intensive microcycles; (b) in combat sports and high-intensity disciplines exhibiting elevated baseline peroxidation, recovery protocols should prioritize tailored nutritional interventions and structured restorative modalities; (c) unguided, high-dose antioxidant supplementation should be avoided, as physiological ROS signaling remains essential for driving adaptive mitochondrial and enzymatic upregulation, especially in endurance-oriented disciplines.

4. Recommendations and Directions for Future Research. Given the pilot nature of this study, small cohort sizes ($n = 12$ per group), and the use of ecologically valid yet discipline-specific exercise protocols, these results should be interpreted as foundational response profiles. Future investigations should expand cohort sizes,

incorporate longitudinal repeated-measures designs across different phases of the annual cycle (macrocycle tracking), and evaluate comprehensive biomarker panels (e.g., superoxide dismutase, glutathione peroxidase, total antioxidant capacity, and inflammatory cytokines) coupled with synchronized external-load GPS and power monitoring to further advance evidence-based athletic preparation.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used OpenAI GPT-based language assistance via the Genspark platform in order to improve academic English. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflict of interest

The authors declare no conflict of interest.

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Author Contributions

Conceptualization, L.S. and Y.K.; methodology, L.S., Y.K., H.O., and R.T.; investigation, V.Y., Z.S., D.K., and N.V.; data curation, T.O. and R.T.; formal analysis, Y.K., R.B., and T.O.; validation, N.V. and R.B.; resources, Y.K. and L.S.; visualization, T.O. and R.B.; writing-original draft preparation, Y.K., H.O., and L.S.; writing-review and editing, N.V., R.T., V.Y., and R.B.; supervision, Y.K.; project administration, Y.K. and L.S. All authors have read and agreed to the published version of the manuscript.

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