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Effect of kickboxing training on total sulfhydryl (–SH) and ischemia-modified albumin (IMA) levels: a comparative study between athletes and sedentary individuals

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ABSTRACT

This study aimed to investigate the impact of kickboxing training on oxidative stress biomarkers, specifically total sulfhydryl (–SH) and ischemia-modified albumin (IMA) levels. The study was designed as a quantitative, cross-sectional, comparative investigation. The sample consisted of 45 healthy male university students aged 18–24 years from the Van province, Türkiye. Participants were divided into two groups: kickboxing athletes and sedentary controls. The experimental group (n = 20) consisted of male kickboxing athletes with a minimum of seven years of training experience, ranked among the top three in the Turkish Kickboxing Championship, and currently training at least four days per week. The control group (n = 25) consisted of sedentary male individuals with no history of regular exercise in the past six months, no alcohol or tobacco use, no chronic diseases, and adherence to a balanced diet. Blood samples were analyzed using validated biochemical methods. The results showed significantly higher –SH and lower IMA levels in the kickboxing group ($p < 0.05$). Effect size analysis indicated large differences for both markers (Cohen's $d = 1.25$ for –SH and $d = 1.00$ for IMA), suggesting that kickboxing training has a substantial impact on oxidative stress parameters. These findings highlight the physiological benefits of regular high-intensity physical activity in promoting cellular resilience and adaptive responses.

KEYWORDS

Kickboxing; Oxidative Stress; Total Sulfhydryl; Ischemia-Modified Albumin; Antioxidant Defense

1. INTRODUCTION

Regular physical activity plays a fundamental role in enhancing physiological resilience, improving biochemical functions, and promoting overall health (Powers & Jackson, 2008). One of its key systemic effects is the modulation of oxidative stress—a condition arising from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, which may lead to cellular damage and dysfunction (Halliwell & Gutteridge, 2015).

Antioxidants are compounds that neutralize free radicals generated during metabolic processes (Yılmaz, 2010). Among the biomarkers of antioxidant status, Total Sulfhydryl (–SH) groups represent thiol (-SH) moieties in plasma proteins and reflect the organism's redox capacity (Erel & Neselioğlu, 2014). Elevated –SH levels are considered a protective response against oxidative damage (Dalkılıç, 2006; Eryılmaz et al., 2020).

Ischemia-Modified Albumin (IMA), another key biomarker, is formed when oxidative stress alters the N-terminal region of the albumin molecule, impairing its ability to bind transition metals such as cobalt and nickel (Bar-Or et al., 2000; Bhagavan et al., 2003). Albumin's metal-binding function is highly dependent on the structural integrity of its N-terminal domain (Can & Yosunkaya, 2017; Sbarouni et al., 2011).

Kickboxing, a high-intensity combat sport, activates both aerobic and anaerobic energy systems simultaneously. While such exertion may acutely elevate oxidative stress and free radical production (Slimani et al., 2017), regular training is believed to enhance antioxidant defenses and mitigate these effects (Networks, 2024).

Given the sensitivity of IMA to hypoxic conditions and its role as an early marker of oxidative damage (Akbaş, 2022), systematic evaluation of both –SH and IMA in combat athletes may offer deeper insights into training-induced biochemical adaptations. However, studies examining these markers concurrently in kickboxing athletes remain limited.

By evaluating these biomarkers, the research aims to contribute to the understanding of oxidative stress regulation in combat sports and support biomarker-based approaches to athlete health and performance. The study hypothesizes that individuals engaged in regular kickboxing training will exhibit significantly higher –SH and lower IMA levels compared to sedentary individuals.

2. METHODS

2.1. Study Design and Participants

This study was designed as a quantitative, cross-sectional, and comparative investigation. The sample consisted of 45 healthy male university students aged between 18 and 24 years, residing in Van province. All participants lived in university dormitories and followed a similar, controlled nutrition program to minimize dietary variability as a confounding factor. To ensure homogeneity and eliminate potential hormonal influences—particularly the antioxidant effects of estrogen—only male participants were included. This approach aimed to reduce sex-related biochemical variability in oxidative stress markers. Participants were divided into two groups based on their physical activity status:

Experimental Group (n = 20): Male kickboxing athletes with a minimum of seven years of training experience, ranked in the top three in the Turkish Kick Boxing Championship, and currently training at least four days per week.

Control Group (n = 25): Sedentary male individuals with no history of regular exercise in the past six months, no use of alcohol or tobacco, no chronic diseases, and adherence to a balanced diet. Control group members were selected from the same geographic region and shared similar socioeconomic and cultural backgrounds with the experimental group.

The sample size was determined based on biochemical parameters reported in similar studies, using a 95% confidence interval. The goal was to include a sufficient number of participants to detect statistically significant differences between groups. Preliminary evaluations indicated that a minimum of 20 participants per group would be adequate to ensure narrow confidence intervals for the key biochemical markers –SH and IMA and to achieve statistical power in group comparisons. These sample sizes are considered sufficient to ensure the statistical reliability and validity of the study findings. Nevertheless, future studies should perform a priori power analyses to provide a more rigorous justification of sample adequacy.

2.2. Training Protocol of the Experimental Group

All athletes in the experimental group were individuals who had been training in clubs affiliated with the Turkish Kickboxing Federation for a minimum of seven years. Each athlete had placed within the top three in national championships and participated in regular training sessions at least four days per week, with a daily duration of 90–120 minutes. The training regimen incorporated technical-tactical drills, anaerobic interval running, endurance, strength, and flexibility components.

The type and intensity of training are summarized below to ensure reproducibility and facilitate replication in future research settings:

Table 1. Training protocol for kickboxing athlete

Total Training Duration	8 weeks (3 sessions per week)
Average Daily Training Duration	90 minutes
Training Content	Warm-up: 10–15 minutes of general mobilization, dynamic stretching, light cardio Technical-tactical applications (30–40 min) Strength or conditioning exercises (20–30 min) Anaerobic sprint or interval loads (15–20 min) Cool-down: 10–15 minutes of light cardio and static stretching
Intensity	70–90% of Maximum Heart Rate (HRmax)
Monitored Parameters	Heart rate, Rating of Perceived Exertion (RPE), exercise diary records

2.3. Measurements and Procedure

2.3.1. Anthropometric Measurements

Height was measured in centimeters using a precision Seca stadiometer (accuracy: 0.01 meters). Participants stood barefoot in an upright position, with head alignment adjusted according to the Frankfurt plane.

2.3.2. Blood Sample Collection and Biochemical Analysis

To minimize the acute effects of exercise on hematological parameters and oxidative stress markers, all participants refrained from physical activity for three days prior to blood collection. This rest period was implemented to enhance the reliability and standardization of biochemical analyses.

Blood samples were collected into standard biochemistry tubes and gently mixed 4–5 times. After resting at room temperature for 20 minutes, the samples were centrifuged at 3500 rpm for 10 minutes to separate serum from cellular components. The resulting serum samples were stored at −70°C until analysis.

All procedures were conducted at the Biochemistry Laboratory of Van Yüzüncü Yıl University Dursun Odabaş Medical Center. Biochemical analyses were performed under the supervision of expert academicians from the Faculty of Science. The study focused on evaluating serum levels of –SH and IMA. Samples were barcoded and recorded in the laboratory system, and the initial data served as the baseline for subsequent comparisons.

2.3.3. Biochemical Measurement Methods

–SH levels were measured using a spectrophotometric method based on the reaction of sulfhydryl groups with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), commonly known as Ellman's reagent (Sedlak & Lindsay, 1968; Ellman, 1959). The absorbance of the yellow-colored product was read at 412 nm using a Shimadzu UV-1800 spectrophotometer. –SH concentrations were calculated via standard curve and expressed in µmol/L. All samples were analyzed in duplicate, and intra-assay variability was monitored using quality control sera.

IMA levels were assessed using the Albumin-Cobalt Binding (ACB) test, which detects structural changes in albumin due to ischemia-induced oxidative stress (Bar-Or et al., 2000). In this method, serum samples were incubated with cobalt chloride, followed by dithiothreitol (DTT), and absorbance was measured at 470 nm. IMA values were expressed in absorbance units (ABSU), and all measurements were performed in duplicate to ensure consistency. Calibration standards and internal controls were included in each batch to validate assay performance. Serum samples were stored at −70°C until analysis, and all procedures adhered to ethical and technical standards for human biochemical research.

To account for individual variation in albumin levels, D-IMA values were calculated using the following formula:

D-IMA = (IMA value / Group median albumin value) × 100

Group-specific median albumin values were 4.60 g/dL for the kickboxing group and 4.30 g/dL for the sedentary group. This normalization approach enhances intergroup comparability and reflects methodological rigor (Roy et al., 2004; Duman et al., 2015).

Total Sulfhydryl (–SH) Measurement

–SH levels were determined using the spectrophotometric method described by Ellman (1959), which is based on the reaction of sulfhydryl groups with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), commonly known as Ellman's reagent. In this assay, DTNB reacts with free thiol groups in the serum to produce a yellow-colored product, 5-thio-2-nitrobenzoic acid (TNB), which can be quantified spectrophotometrically. The absorbance was measured at 412 nm using a Shimadzu UV-1800 spectrophotometer. –SH concentrations were calculated using a standard curve and expressed in micromoles per liter (µmol/L). All samples were analyzed in duplicate, and intra-assay variability was monitored using quality control sera.

Ischemia Modified Albumin (IMA) Measurement

IMA levels were assessed using the Albumin Cobalt Binding (ACB) test, as originally described by Bar-Or et al. (2000). This method is based on the reduced ability of albumin to bind cobalt ions in the presence of oxidative stress. In the assay, serum samples were incubated with a known concentration of cobalt chloride ($CoCl_2$), followed by the addition of dithiothreitol (DTT), which binds to unbound cobalt ions. The resulting color change was measured spectrophotometrically at 470 nm. IMA levels were expressed in absorbance units (ABSU), and all measurements were performed in duplicate to ensure consistency. Calibration standards and internal controls were included in each batch to validate assay performance. Serum samples were stored at −70°C until analysis, and all procedures adhered to ethical and technical standards for human biochemical research.

2.3.4. Biochemical Analysis

Total Sulfhydryl (–SH) Measurement

In this study, –SH levels were measured using a spectrophotometric technique based on the protocol originally described by Sedlak and Lindsay (1968), and adapted from the Ellman method. This method relies on the reaction between sulfhydryl groups and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), resulting in the formation of 2-nitro-5-mercaptobenzoic acid anion, which exhibits a characteristic absorbance at 412 nm.

Analytical Procedure

1. Blood samples collected in EDTA tubes were centrifuged at 3500 rpm for 5 minutes.
2. 0.5 mL of plasma was transferred into clean centrifuge tubes.
3. 1.5 mL of 0.2 M Tris buffer (pH 8.2) was added.
4. 0.1 mL of 0.01 M DTNB reagent was added.
5. Samples were vortexed briefly to ensure homogeneity.
6. 7.9 mL of methanol was added to each tube.
7. Tubes were incubated for 15 minutes at room temperature.
8. Samples were centrifuged at 4500 rpm for 10 minutes.

9. The absorbance of the supernatant was measured at 412 nm using a calibrated spectrophotometer.
10. Control (blank) tubes were prepared by replacing DTNB with distilled water.

–SH concentrations were calculated using standard curves and expressed in micromoles per liter (µmol/L). All measurements were performed in duplicate to ensure analytical precision.

2.4. Statistical Analyses

Descriptive statistics for continuous variables were presented as mean ± standard deviation (SD). The normality of data distribution was assessed using the Kolmogorov–Smirnov test, while the homogeneity of variances was evaluated using Levene's test. Since the data met the assumptions of normality and homogeneity, independent samples t-tests were used for pairwise group comparisons. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA).

2.5. Ethical Approval

The research protocol was reviewed and approved by the Van Yüzüncü Yıl University Non-Interventional Clinical Research Ethics Committee (Decision No: 2024/10/36, Date: 20/09/2024). All procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Prior to participation, the study's purpose, scope, and procedures were explained to all individuals, and informed consent was obtained.

3. RESULTS

Descriptive statistics and group comparisons for age, height, and body weight are presented in Table 2.

Table 2. Demographic characteristics of participants

Variable	Group	N	Mean ± SD	Min	Max	*p* value
Age (years)	Kickboxing Athletes	20	20.8 ± 1.9	18.0	25.00	0.902
	Sedentary Controls	25	20.9 ± 2.3	18.0	25.00	
Height (cm)	Kickboxing Athletes	20	174.0 ± 9.0	160.0	190.0	0.874
	Sedentary Controls	25	175.0 ± 11.0	158.0	196.0	
Weight (kg)	Kickboxing Athletes	20	71.3 ± 12.9	53.0	94.00	0.991
	Sedentary Controls	25	71.3 ± 14.4	54.0	95.00	

Note. *Data are presented as mean ± standard deviation (SD); p-values were calculated using independent samples t-test to compare the kickboxing and sedentary groups.*

The results of the independent samples t-test revealed no statistically significant differences between the kickboxing and sedentary groups in terms of age (p = 0.902), height (p = 0.874), or body weight (p = 0.991). These findings confirm the demographic comparability of the sample and support the validity of subsequent biochemical comparisons. Descriptive statistics and group comparison results for serum IMA and –SH levels are presented in Table 3.

Table 1. Descriptive statistics of IMA and –SH levels in groups and group comparison results

Biomarker	Group	N	Mean ± SD	95% Confidence Interval	*p* value
İMA (µmol/L)	Kickboxing Athletes	20	0.2755 ± 0.0289	0.2620 – 0.2890	0.001
	Sedentary Controls	25	0.5561 ± 0.0222	0.5469 – 0.5652	
(–SH) activity (U/mL)	Kickboxing Athletes	20	0.2888 ± 0.0739	0.2542 – 0.3234	0.001
	Sedentary Controls	25	0.1198 ± 0.0056	0.1175 – 0.1221	

The independent samples t-test revealed statistically significant differences between the kickboxing and sedentary groups for both biomarkers. Specifically, IMA levels were significantly lower in the kickboxing group (0.2755 ± 0.02888 µmol/L) compared to the sedentary group (0.5561 ± 0.02217 µmol/L), with a p-value of 0.001. Conversely, –SH activity was significantly higher in the kickboxing group (0.2888 ± 0.07386 U/mL) than in the sedentary group (0.1198 ± 0.00558 U/mL), also with a p-value of 0.001.

These findings suggest that individuals engaged in regular kickboxing training exhibit enhanced antioxidant capacity and reduced ischemia-related oxidative stress compared to sedentary individuals. To further assess the magnitüde of the observed differences in oxidative stress biomarkers, effect sizes were calculated using Cohen's d. This approach complements the p-values by quantifying the practical significance of the differences between groups. The results are presented in Table 4.

Table 2. Effect Size (Cohen's d) for between-group differences in oxidative stress biomarkers

Biomarker	Kickboxing Athletes	Sedentary Controls	*p* value	Cohen's d	Effect Size Interpretation
IMA (µmol/L)	0.2755 ± 0.02888	0.5561 ± 0.02217	0.001	1.00	Large
(–SH) (U/mL	0.2888 ± 0.07386	0.1198 ± 0.00558	0.001	1.25	Large

Note. *Values are presented as mean ± standard deviation (SD); p-values were calculated using independent samples t-test*

Cohen's d values were calculated using pooled standard deviations. Both biomarkers showed large effect sizes, indicating substantial physiological differences between groups.

Serum albumin levels were found to be significantly higher in kickboxing athletes compared to the sedentary control group (p = 0.003). This difference supports the impact of regular physical activity on biochemical parameters related to oxidative stress. The mean and median values of albumin concentrations are presented in Table 5. For the calculation of D-IMA, group median values were used as reference points to minimize individual variation and enhance comparability.

Table 3. Serum albumin levels and group comparison results

Biomarker	Group	N	Mean ± SD (g/dL)	Median (g/dL)	95% Confidence Interval	*p* value
Albumin	Kickboxing Athletes	20	4.62 ± 0.31	4.60	4.47 – 4.77	0.003
	Sedentary Controls	25	4.28 ± 0.22	4.30	4.18 – 4.38	-

Note. *Values are presented as mean ± standard deviation (SD). Albumin concentrations are expressed in grams per deciliter (g/dL). p-values were calculated using independent samples t-test. Median values were used for D-IMA normalization.*

D-IMA was calculated using the following formula:

D-IMA = (IMA value / Group median albumin value) × 100

This formula was applied to reduce individual variation in albumin levels and enhance intergroup comparability.

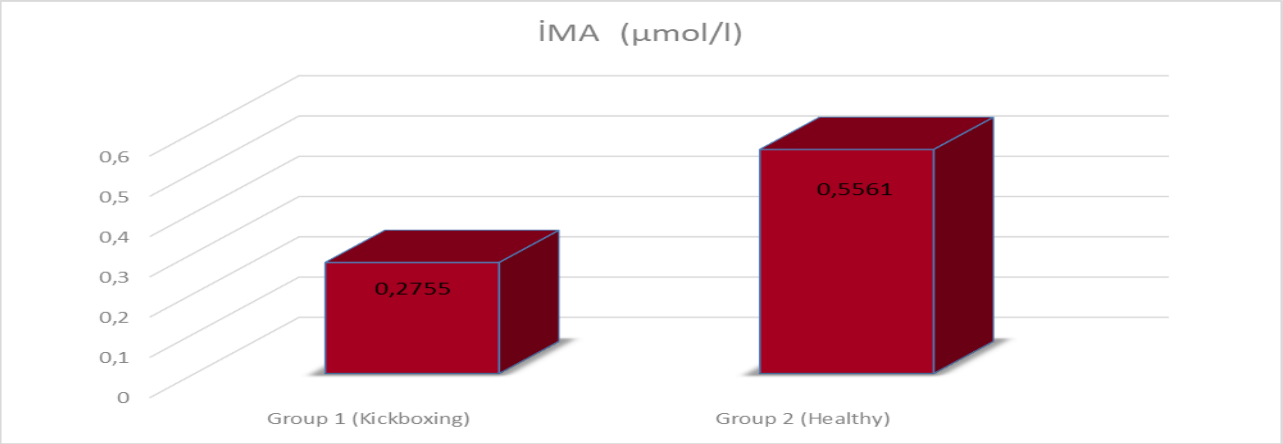

Figure 1. Comparison of Serum Ischemia-Modified Albumin (IMA) levels between experimental and control groups

As shown in Figure 1, IMA levels were significantly lower in kickboxing athletes compared to sedentary individuals. This visual representation reinforces the statistical finding (p = 0.001) and illustrates the protective effect of regular high-intensity training against ischemia-induced oxidative stress.

The results demonstrated that serum IMA levels were significantly lower in individuals who regularly participate in kickboxing (0.2755 µmol/L) compared to sedentary individuals (0.5561 µmol/L). This finding suggests that regular engagement in high-intensity physical activity may exert a protective effect by reducing ischemia-related oxidative stress at the cellular level. The lower IMA concentrations observed in the kickboxing group may reflect improved vascular function and enhanced antioxidant capacity.

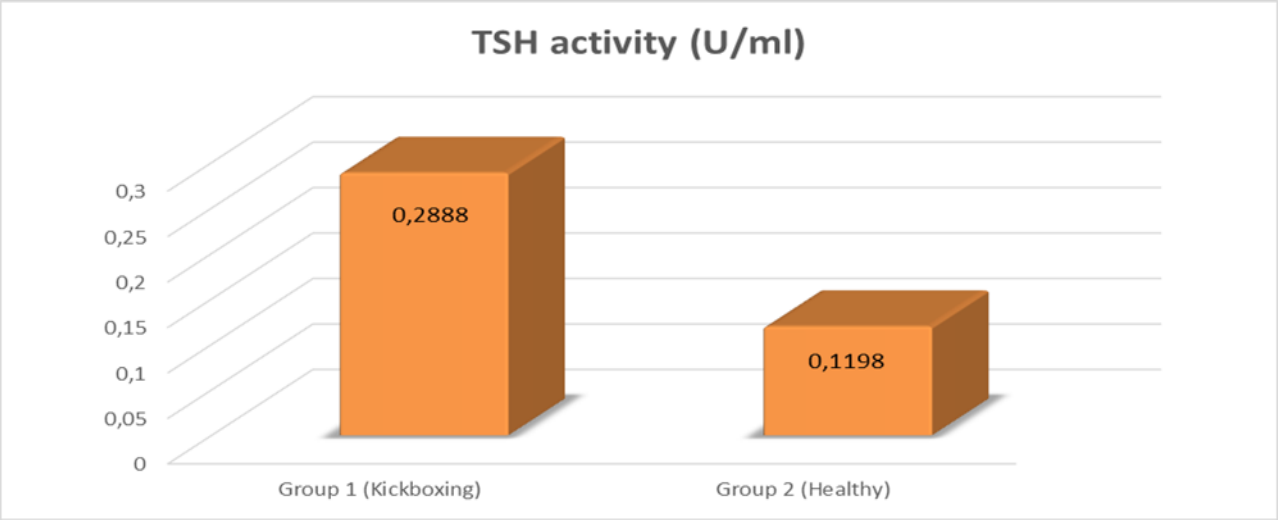

Figure 2. Comparison of Serum Total Sulfhydryl (–SH) levels between experimental and control groups

Figure 2 clearly demonstrates the elevated –SH activity in kickboxing athletes. This increase in sulfhydryl groups supports the hypothesis that long-term combat sport training enhances endogenous antioxidant defenses.

The data revealed that –SH levels were significantly higher in the kickboxing group (0.2888 U/mL) than in the sedentary group (0.1198 U/mL), with a p-value of 0.001. This elevation in (–SH) activity among physically active individuals indicates a strengthened endogenous antioxidant defense system. The increased availability of sulfhydryl groups may contribute to enhanced neutralization of reactive oxygen species, thereby offering protection against oxidative damage induced by free radicals.

4. DISCUSSION

This study examined the effects of regular kickboxing training on oxidative stress biomarkers—Total Sulfhydryl (–SH) and Ischemia-Modified Albumin (IMA) in young adult males. The results demonstrated that kickboxing athletes exhibited significantly higher –SH levels and lower

IMA concentrations compared to sedentary controls, indicating a favorable shift in redox balance associated with long-term high-intensity training.

Elevated –SH levels suggest an upregulation of thiol-based antioxidant mechanisms, which play a crucial role in neutralizing reactive oxygen species (ROS) and maintaining cellular homeostasis. These findings align with previous research showing enhanced antioxidant capacity in athletes engaged in combat and endurance sports (Özdemir et al., 2020; Kılıç et al., 2019). However, differences between endurance and combat sports should be discussed in greater detail, as exercise modality may influence hormonal and oxidative dynamics.

Previous research has demonstrated that endurance sports, such as marathon running and cycling, are often associated with sustained elevations in oxidative stress biomarkers due to prolonged oxygen consumption and mitochondrial ROS production (Lippi et al., 2005; Seo et al., 2006). In contrast, combat sports typically involve intermittent high-intensity bouts interspersed with recovery phases, which may allow more efficient activation of adaptive antioxidant mechanisms (Slimani et al., 2017). For example, endurance athletes frequently show increased IMA concentrations after exhaustive exercise, reflecting transient ischemic stress (Lippi et al., 2005), whereas combat athletes may exhibit lower IMA levels despite similar training intensity, likely due to improved vascular adaptation and enhanced thiol-based antioxidant responses (Memmedov et al., 2022). These differences suggest that exercise modality plays a critical role in shaping redox homeostasis, with endurance training imposing continuous oxidative challenges and combat sports promoting a balance between oxidative stress and adaptive recovery.

The reduced IMA levels observed in the kickboxing group further support the hypothesis that chronic exercise may attenuate ischemia-related oxidative modifications of albumin, a plasma protein highly sensitive to oxidative stress (Bar-Or et al., 2000).

Kickboxing, characterized by intermittent high-intensity bouts and rapid recovery phases, likely stimulates cardiovascular and metabolic adaptations that improve tissue oxygenation and reduce transient ischemic episodes. These physiological changes may explain the lower IMA concentrations, as albumin undergoes less structural alteration under improved circulatory conditions.

From a methodological perspective, the use of –SH and IMA as biomarkers provides a practical and non-invasive approach to assess oxidative stress status in athletes, due to their sensitivity to redox changes and feasibility in field-based assessments. Nevertheless, the cross-

sectional design significantly restricts causal inference. Therefore, findings should be interpreted with caution regarding cause-effect relationships.

Although the sample size aligns with previous studies, a formal power analysis would provide a more robust justification for its adequacy.

The inclusion of only male participants is understandable; however, this limits the generalizability of the findings to female populations. Future studies should incorporate gender diversity to enhance external validity.

Additionally, uncontrolled variables such as nutrition, sleep quality, and training load may have influenced the oxidative stress parameters.

The normalization of IMA values using group median albumin concentrations (D-IMA) provides a more refined assessment of ischemic stress independent of albumin variability. This methodological adjustment enhances the interpretability of IMA data, especially in physically active populations where albumin levels may fluctuate due to training load. From a clinical perspective, D-IMA may offer enhanced sensitivity in detecting subtle ischemic changes in athletes undergoing high-intensity training. Its application could extend beyond sports science into cardiology and emergency medicine, where rapid and reliable ischemia detection is critical.

Future studies should adopt longitudinal designs to monitor temporal changes in oxidative stress parameters, include female athletes to explore sex-specific physiological responses, and expand the biomarker panel to incorporate enzymatic antioxidants (e.g., SOD, GPx, CAT) and inflammatory markers. Molecular-level analyses, such as gene expression profiling of antioxidant-related pathways (e.g., HSP70, Nrf2), may further elucidate the mechanisms underlying exercise-induced oxidative modulation (Powers et al., 2011; Radák et al., 2008).

5. CONCLUSIONS

This study examined the impact of regular kickboxing training on oxidative stress biomarkers –SH and IMA in young adult males. The results demonstrated that kickboxing athletes had significantly higher –SH levels and lower IMA concentrations compared to sedentary controls, suggesting that long-term participation in high-intensity combat sports may enhance endogenous antioxidant capacity and reduce ischemia-related oxidative stress.

The elevated –SH levels reflect a strengthened thiol-based antioxidant defense, while the reduced IMA values indicate improved albumin integrity and diminished oxidative modification.

İmportant, the use of normalized IMA (D-IMA) values provided a more refined assessment of ischemic stress by accounting for individual variation in albumin levels. These biochemical adaptations may contribute to enhanced cellular resilience and recovery in physically active individuals.

Given the sensitivity of IMA to hypoxic and ischemic conditions, its application as a non-invasive biomarker in exercise physiology appears promising. Future research should adopt longitudinal designs to monitor dynamic changes over time, include female participants to assess sex-based physiological responses, and incorporate molecular-level analyses—such as gene expression profiling of antioxidant enzymes (e.g., SOD, GPx, HSP70)—to deepen understanding of exercise-induced oxidative modulation. Additionally, expanding the biomarker panel and integrating nutritional and recovery-related variables may offer a more comprehensive view of redox balance in combat sport athletes.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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