Effect of HIIT versus endurance training on oxidative stress markers

Efecto del entrenamiento HIIT frente al entrenamiento de resistencia sobre los marcadores de estrés oxidativo

Authors

Lotfi Nizar [1]
Madani Mohamed [2]

[1, 2] Normal Superior School (ENS), Hassan II University, Morocco

Corresponding author:
Lotfi Nizar
nizarlotfi99@gmail.com

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Abstract

Introduction. To avoid conceptual ambiguity, endurance training in this study is referred to as MICT, which reflects sustained efforts at moderate intensity (zone 2 of Seiler's triphasic model). By contrast, HIIT is classified as vigorous intermittent exercise (zone 3). This differentiation is essential for comparing the physiological adaptations of both modalities (Seiler, 2010). Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) production and antioxidant capacity, can cause cellular damage and contribute to chronic diseases. Biomarkers such as malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) are used to assess this imbalance. Physical exercise, particularly high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), modulates redox homeostasis, but their comparative effects remain underexplored.

Objective. To compare the effects of a HIIT protocol versus an moderate-intensity continuous training (MICT) protocol on oxidative stress markers (MDA, CAT, SOD) in healthy athletes, enhancing understanding of exercise-induced physiological adaptations.

Methodology**.** Twenty healthy male athletes (aged 18–35 years) were randomized into two groups (MICT, n=10; HIIT, n=10) following a 4-week training protocol (3 sessions/week, 60–80% VO_2max). MDA, CAT, and SOD levels were measured via colorimetry before and after exercise at the start and end of the program. Repeated-measures ANOVA assessed the effects of exercise, training program, and their interactions, with a significance threshold of $p<0.05$.

Findings**.** Both training modalities increased CAT activity (MICT: +5.4% at rest, +10.0% post-exercise; HIIT: +6.4% at rest, +11.0% post-exercise) with no inter-group differences. SOD levels increased in the MICT group at rest (+5.3%) and post-exercise (+6.0%), but only post-exercise in the HIIT group (+8.4%). MDA levels decreased at rest in both groups (MICT: -15.2%; HIIT: -17.3%) and post-exercise in the HIIT group (-13.0%), but not in the MICT group post-exercise.

Keywords

Oxidative stress, high-intensity interval training, moderate-intensity continuous training (MICT), malondialdehyde, catalase, superoxide dismutase, physiological adaptations, athletic performance.

Resumen

Introducción. Para evitar ambigüedades conceptuales, en este estudio el entrenamiento de resistencia se denomina MICT, lo que refleja esfuerzos sostenidos de intensidad moderada (zona 2 del modelo trifásico de Seiler). En cambio, el HIIT se clasifica como ejercicio intermitente vigoroso (zona 3). Esta diferenciación es esencial para comparar las adaptaciones fisiológicas de ambas modalidades (Seiler, 2010). El estrés oxidativo, que surge de un desequilibrio entre la producción de especies reactivas de oxígeno (ROS) y la capacidad antioxidante, puede causar daño celular y contribuir a enfermedades crónicas. Se utilizan biomarcadores como el malondialdehído (MDA), la catalasa (CAT) y la superóxido dismutasa (SOD) para evaluar este desequilibrio. El ejercicio físico, particularmente el entrenamiento interválico de alta intensidad (HIIT) y el entrenamiento continuo de intensidad moderada (MICT), modula la homeostasis redox, pero sus efectos comparativos siguen poco explorados.
Objetivo. Comparar los efectos de un protocolo de HIIT frente a un protocolo de entrenamiento continuo de intensidad moderada (MICT) sobre los marcadores de estrés oxidativo (MDA, CAT, SOD) en atletas sanos, para mejorar la comprensión de las adaptaciones fisiológicas inducidas por el ejercicio.
Metodología. Veinte atletas varones sanos (18–35 años) fueron asignados aleatoriamente a dos grupos (MICT, n=10; HIIT, n=10) y siguieron un protocolo de entrenamiento de 4 semanas (3 sesiones/semana, 60–80 % del VO_2máx). Los niveles de MDA, CAT y SOD se midieron mediante colorimetría antes y después del ejercicio al inicio y al final del programa. Se utilizó un ANOVA de medidas repetidas para evaluar los efectos del ejercicio, del programa de entrenamiento y sus interacciones, con un nivel de significación de $p<0,05$. Resultados. Ambas modalidades de entrenamiento aumentaron la actividad de CAT (MICT: +5,4 % en reposo, +10,0 % post-ejercicio; HIIT: +6,4 % en reposo, +11,0 % post-ejercicio) sin diferencias intergrupales. Los niveles de SOD aumentaron en el grupo MICT en reposo (+5,3 %) y post-ejercicio (+6,0 %), pero solo post-ejercicio en el grupo HIIT (+8,4 %). Los niveles de MDA disminuyeron en reposo en ambos grupos (MICT: -15,2 %; HIIT: -17,3 %) y post-ejercicio en el grupo HIIT (-13,0 %), pero no en el grupo MICT post-ejercicio.

Palabras clave

Entrenamiento interválico de alta intensidad (HIIT); entrenamiento continuo de intensidad moderada (MICT); malondialdehído (MDA); catalasa (CAT); superóxido dismutasa (SOD); adaptaciones fisiológicas.

Introduction

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant systems to neutralize them, is a complex physiological phenomenon (Sies, 2015). Excessive ROS accumulation can lead to macromolecular damage, such as lipid peroxidation, protein oxidation, and DNA lesions, contributing to various chronic pathologies (Halliwell & Gutteridge, 2015). To assess the extent of this imbalance, specific biomarkers are commonly quantified. Malondialdehyde (MDA) is a final product of membrane lipid peroxidation, serving as a reliable indicator of oxidative damage (Del Rio et al., 2005). Concurrently, endogenous antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), form the first line of cellular defense against ROS, their activity reflecting the body's ability to manage oxidative stress (Ighodaro & Akinloye, 2018).

Physical exercise is a potent modulator of redox homeostasis. While regular, moderate-intensity physical activity is widely recognized for its health benefits, including enhanced antioxidant defenses and reduced chronic oxidative stress (Powers & Jackson, 2008), acute exercise sessions, particularly those of high intensity or long duration, can transiently increase ROS production and induce acute oxidative stress (Finaud et al., 2006). However, this acute response is often considered an adaptive stimulus, essential for long-term improvements in the body’s antioxidant systems (Radak et al., 2008).

Over recent decades, high-intensity interval training (HIIT) has gained popularity due to its efficiency in rapidly improving cardiorespiratory fitness and metabolic health (Gibala et al., 2006). HIIT is characterized by alternating short periods of maximal or near-maximal effort with active or passive recovery phases. In contrast, traditional moderate-intensity continuous training (MICT) involves continuous efforts at moderate intensity. Despite growing interest in these two training modalities, a comprehensive understanding of their comparative impacts on oxidative stress markers remains an active area of research, with sometimes divergent results (Laursen & Jenkins, 2009).

This study distinguishes between moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT). MICT involves steady-state efforts at moderate intensity (zone 2), whereas HIIT consists of vigorous efforts interspersed with recovery phases (zone 3), as described by Seiler (2010).

The existing literature on the effect of HIIT on oxidative stress is nuanced. Some studies have reported that a single HIIT session can induce acute oxidative stress due to its high intensity (Skovgaard & Bangsbo, 2015). Nevertheless, chronic adaptations from regular HIIT have been associated with improved antioxidant capacity and reduced oxidative stress. For instance, Bogdanis et al. (2013) observed that short-term HIIT (nine sessions) reduced oxidative stress and increased antioxidant activity (Bogdanis et al., 2013). Similarly, Sarkar (2021) reported significant improvements in antioxidant status following a HIIT protocol (Sarkar, 2021). Other studies suggest that short-term HIIT may not always induce significant oxidative stress or muscle damage, highlighting the variability in individual and protocol-specific responses (Zouhal et al., 2008).

Moderate-intensity continuous training (MICT) is also known to modulate oxidative stress. Although prolonged MICT exercise can increase ROS production (Nikolaidis & Kyparos, 2012), regular training is often associated with strengthened antioxidant defenses (Radak et al., 2008; Bloomer & Goldfarb, 2004). Mallett (2024) emphasized the complexity of physiological responses of oxidative stress markers to MICT exercise, indicating that adaptations may depend on the intensity, duration, and frequency of training (Mallett, 2024).

Given the fundamental differences in the physiological stimuli induced by HIIT and moderate-intensity continuous training (MICT), it is critical to compare their respective effects on oxidative balance. The variability in training protocols and studied populations in the current literature makes it challenging to establish a unified conclusion on the comparative effects of these two modalities on oxidative stress markers.

Therefore, this study aims to compare the effects of a HIIT protocol versus an moderate-intensity continuous training (MICT) protocol on oxidative stress markers (MDA, CAT, SOD, and GPx) in healthy participants. The findings of this research will contribute to a better understanding of the physiological adaptations induced by different exercise modalities and their implications for health, performance, and prevention of oxidative stress-related diseases.

Method

This section explains how the research was done. The design of the same is described and it is explained how it was put into practice, justifying the choice of the methods used. This section should contain the type of quantitative research, the scope or depth of the research (exploratory, correlational and/or explanatory), population and sample, and the techniques used should be added. This section is fundamental, because it is the one that will allow the scientific community to reproduce the result. Most of this section should be written in the past tense, in a descriptive style.

Participants

To enhance comparability with other studies, participants were categorized using the framework proposed by McKay et al. (2022). According to this classification, they fall into level 2 (developmental athletes): individuals training ~3 times per week with the intention to compete at the local level, but not classified as elite performers.

Twenty healthy male athletes, aged 18 to 35 years, were recruited and randomized into two groups: MICT (n=10) and HIIT (n=10). Inclusion criteria included being aged 18 to 30 years, absence of chronic diseases, not taking medications likely to affect metabolism or oxidative stress, and being non-smokers. Participants followed a 4-week training protocol, with three sessions per week at an intensity of 60-80% of VO_2max, measured beforehand through a progressive effort test, for both groups. The study was approved by the local ethics committee, and all participants provided written informed consent before participating, in accordance with the ethical principles outlined in the Declaration of Helsinki. Data confidentiality was ensured throughout the study.

Training Protocols

MICT Group

The MICT group began the protocol with an initial test to determine the maximum number of laps (on a 250-meter track) they could complete in 45 minutes. This number of laps served as an individualized baseline for subsequent training sessions. During the first three sessions (week 1), participants were required to replicate the same number of laps in the same time frame (45 minutes). For the following weeks (weeks 2 to 6), the goal was to maintain the same number of laps while progressively reducing the allotted time by 3 minutes each week. This approach aimed to increase the relative intensity of the training by decreasing the time to cover the same distance, thereby progressively increasing the average speed. For example, in week 2, participants had to complete the same number of laps in 42 minutes, then in 39 minutes in week 3, and so forth.

HIIT Group (HIIT)

The HIIT group (HIIT) followed a high-intensity interval training protocol. For the first week, participants performed 8 laps on a 250-meter track at full speed, with a 4-minute passive recovery period between each lap. The effort intensity was maximal for each lap, aiming to achieve the highest possible speed. From week 2 onward, the recovery time between laps was reduced by 1 minute each week while maintaining the same number of laps and the instruction to run at full speed. For example, in week 2, recovery was 3 minutes, then 2 minutes in week 3, and so on until week 6. The progressive reduction in recovery time, combined with maximal effort, increased the overall physiological load and training intensity

Blood Sampling and Measurement of Oxidative Stress Markers

Blood samples were collected at two key points in the protocol to assess oxidative stress markers:

- Acute Phase (Start of the Protocol): A first sample (T0) was taken before the initial training session. A second sample (T1) was collected immediately after this session.
- Chronic Phase (End of 6 Weeks): A sample (T2) was taken before the final training session of week 6. A final sample (T3) was collected immediately after this session.

Blood samples were collected in appropriate tubes, centrifuged at 3000 rpm for 10 minutes at 4°C to separate plasma/serum, then aliquoted and stored at -80°C until analysis. The following parameters were measured:

Malondialdehyde (MDA): Indicator of lipid peroxidation. The assay was performed by colorimetry using the OxiSelect™ MDA Adduct ELISA Kit from Cell Biolabs (Reference: STA-330), based on the reaction with thiobarbituric acid (TBA) to form a measurable MDA-TBA adduct by spectrophotometry at 532 nm.

Catalase (CAT): Antioxidant enzyme involved in the decomposition of hydrogen peroxide. CAT activity was measured by colorimetry using the Catalase Assay Kit from Cayman Chemical (Reference: 707002), which quantifies the rate of hydrogen peroxide decomposition at 240 nm.

Superoxide Dismutase (SOD): Antioxidant enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. SOD activity was determined by colorimetry using the SOD Assay Kit from Sigma-Aldrich (Reference: 19160), based on the inhibition of the nitroblue tetrazolium (NBT) reduction reaction, measured by spectrophotometry at 450 nm.

Statistical Analysis

Data were expressed as means ± standard deviations, with 95% confidence intervals. A repeated-measures ANOVA was used to assess the main effects of the training program (time: before vs. after), acute exercise (state: rest vs. post-exercise), and group type (MICT vs. HIIT), as well as their interactions. Post-hoc comparisons adjusted by Bonferroni were performed to identify specific differences, with a significance threshold set at $p < 0.05$. The partial effect size (η^2) was calculated to estimate the effect size. Analyses were conducted using SPSS software (version 26).

Results

Catalase Variation

Descriptive statistics for catalase levels (U/mL) are presented in Table 1. Data are expressed as means ± standard deviations, with 95% confidence intervals (CI). Changes by athlete group are illustrated in Figure 1. Analyses of the effects of exercise, training program, and their interactions are presented in Tables 2 and 3.

Table 1. Descriptive Statistics for Catalase Before and After Exercise During the Training Program

Groups	Beginning of the Training Program				End of the Training Program			
	At Rest		Post-Exercise		At Rest		Post-Exercise	
	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI
MICT	44.82 ± 3.89	[42.04, 47.60]	49.82 ± 3.60	[47.25, 52.39]	47.22 ± 3.75	[44.54, 49.90]	54.82 ± 3.89	[52.04, 57.60]
Resistance	40.62 ± 3.65	[38.01, 43.22]	45.62 ± 3.94	[42.80, 48.43]	43.22 ± 3.80	[40.49, 45.94]	50.62 ± 3.65	[48.01, 53.22]
Total	42.72 ± 4.26	[40.72, 44.71]	47.72 ± 4.26	[45.72, 49.71]	45.22 ± 4.21	[43.25, 47.19]	52.72 ± 4.26	[50.72, 54.71]

Effect of Exercise on Catalase Levels and Differences Between the Two Groups

The evaluation of the overall and differential effects of physical exercise on catalase levels is presented in Tables 2 and 3.

Acute exercise significantly increased catalase levels in both groups, both at the start and end of the training program. At the start, the mean increase was 5.00 U/mL for the MICT group (from 44.82 ± 3.89, 95% CI [42.04, 47.60] to 49.82 ± 3.60, 95% CI [47.25, 52.39]; $F(1,9)=562.50$, $p<0.001$, $\eta^2=0.984$) and HIIT group (from 40.62 ± 3.65, 95% CI [38.01, 43.22] to 45.62 ± 3.94, 95% CI [42.80, 48.43]; $F(1,9)=562.50$, $p<0.001$, $\eta^2=0.984$). At the end, the increase was 7.60 U/mL for the MICT group (from 47.22 ± 3.75, 95% CI [44.54, 49.90] to 54.82 ± 3.89, 95% CI [52.04, 57.60]; $F(1,9)=2166.00$, $p<0.001$, $\eta^2=0.996$) and 7.40 U/mL for the HIIT group (from 43.22 ± 3.80, 95% CI [40.49, 45.94] to 50.62 ± 3.65, 95% CI [48.01, 53.22]; F(1,9)=2053.50, p<0.001, η^2=0.996). No significant difference was observed between the groups in the magnitude of the exercise-induced increase at either time point (F(1,18)=0.75, p=0.3979, η^2=0.040).

Main Effect of the Training Program on Catalase Levels

The training program significantly increased catalase levels in both groups, both at rest and post-exercise. For the MICT group, rest levels increased from 44.82 ± 3.89 (95% CI [42.04, 47.60]) to 47.22 ± 3.75 (95% CI [44.54, 49.90]; +5.4%, F(1,9)=216.00, p<0.001, η^2=0.960) and post-exercise from 49.82 ± 3.60 (95% CI [47.25, 52.39]) to 54.82 ± 3.89 (95% CI [52.04, 57.60]; +10.0%, F(1,9)=562.50, p<0.001, η^2=0.984). For the HIIT group, rest levels increased from 40.62 ± 3.65 (95% CI [38.01, 43.22]) to 43.22 ± 3.80 (95% CI [40.49, 45.94]; +6.4%, F(1,9)=253.50, p<0.001, η^2=0.966) and post-exercise from 45.62 ± 3.94 (95% CI [42.80, 48.43]) to 50.62 ± 3.65 (95% CI [48.01, 53.22]; +11.0%, F(1,9)=562.50, p<0.001, η^2=0.984). The overall effect of the program was significant (F(1,18)=4275.00, p<0.001, η^2=0.996).

Interaction Effects of Training Program × Group on Catalase Levels

A significant interaction was observed between the training program and acute exercise for both groups (F(1,9)=58.50 for MICT, F(1,9)=49.85 for HIIT, p<0.001, η^2=0.867 and 0.847, respectively), indicating that the exercise-induced increase was greater after the program (Table 3). However, no significant interaction involving the group type was detected (F(1,18)=0.75, p=0.3979, η^2=0.040), suggesting that the effects of the program and exercise were similar between the MICT and HIIT groups.

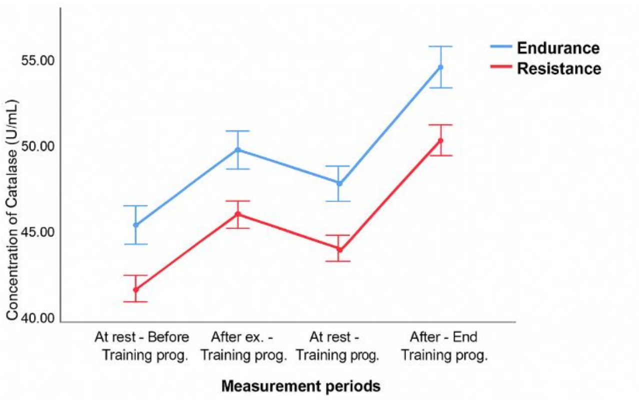
Table 2. Comparison of Catalase Means by Repeated-Measures ANOVA for Each Group

Effect	Group : MICT			Group: Resistance		
	F	p	η^2	F	p	η^2
Training Program	2053.50	<0.001	0.996	2166.00	<0.001	0.996
Exercise	5953.50	<0.001	0.998	5766.00	<0.001	0.998
Interaction Program× Exercise	58.50	<0.001	0.867	49.85	<0.001	0.847

Table 3. Post-Hoc Paired Comparisons

Effect	Test Bonferroni			Effect Size
	Group	F	p	(η^2)
Effect of Exercise at Beginning of Training Program	MICT	562.50	<0.001	0.984
	Resistance	562.50	<0.001	0.984
Effect of Exercise at End of Training Program	MICT	2166.00	<0.001	0.996
	Resistance	2053.50	<0.001	0.996
Effect of Training Program at Rest	MICT	216.00	<0.001	0.960
	Resistance	253.50	<0.001	0.966
Effect of Training Program Post-Exercise	MICT	562.50	<0.001	0.984
	Resistance	562.50	<0.001	0.984

Figure 1. Variation in Catalase Means ± Standard Deviations (U/mL) Before and After Exercise During the Training Program in MICT and HIIT Athletes



Superoxide Dismutase (SOD) Variation

Descriptive statistics for superoxide dismutase (SOD) levels (U/mL) are presented in Table 4. Data are expressed as means ± standard deviations, with 95% confidence intervals. Changes by athlete group are illustrated in Figure 2. Analyses of the effects of exercise, training program, and their interactions are presented in Tables 5 and 6

Table 4. Descriptive Statistics for Superoxide Dismutase (SOD) Before and After Exercise During the Training Program

Group	Beginning of the Training Program				End of the Training Program			
	At Rest		Post-Exercise		At Rest		Post-Exercise	
	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI
MICT	56.84 ± 1.50	[55.76, 57.91]	63.43 ± 2.88	[61.37, 65.50]	59.83 ± 1.50	[58.76, 60.91]	67.23 ± 3.75	[64.55, 69.92]
Resistance	54.80 ± 7.46	[49.46, 60.13]	59.80 ± 7.46	[54.46, 65.13]	54.40 ± 4.51	[51.17, 57.62]	64.80 ± 7.46	[59.46, 70.13]
Total	55.82 ± 5.34	[53.32, 58.32]	61.62 ± 5.81	[58.90, 64.33]	57.12 ± 4.30	[55.10, 59.13]	66.02 ± 5.88	[63.26, 68.77]

Effect of Exercise on SOD Levels and Differences Between the Two Groups

The evaluation of the overall and differential effects of physical exercise on SOD levels is presented in Tables 5 and 6.

Acute exercise significantly increased SOD levels in both groups, both at the start and end of the training program. At the start, the mean increase was 6.59 U/mL for the MICT group (from 56.84 ± 1.50, 95% CI [55.76, 57.91] to 63.43 ± 2.88, 95% CI [61.37, 65.50]; $F(1,9)=175.02$, $p<0.001$, $\eta^2=0.951$) and 5.00 U/mL for the HIIT group (from 54.80 ± 7.46, 95% CI [49.46, 60.13] to 59.80 ± 7.46, 95% CI [54.46, 65.13]; $F(1,9)=259.03$, $p<0.001$, $\eta^2=0.966$). At the end, the increase was 7.40 U/mL for the MICT group (from 59.83 ± 1.50, 95% CI [58.76, 60.91] to 67.23 ± 3.75, 95% CI [64.55, 69.92]; $F(1,9)=106.22$, $p<0.001$, $\eta^2=0.922$) and 10.40 U/mL for the HIIT group (from 54.40 ± 4.51, 95% CI [51.17, 57.62] to 64.80 ± 7.46, 95% CI [59.46, 70.13]; $F(1,9)=118.14$, $p<0.001$, $\eta^2=0.929$). No significant difference was observed between the groups in the magnitude of the exercise-induced increase at either time point ($F(1,18)=2.28$, $p=0.148$, $\eta^2=0.113$).

Effect of the Training Program on SOD Levels

The training program significantly increased SOD levels in the MICT group, both at rest (from 56.84 ± 1.50, 95% CI [55.76, 57.91] to 59.83 ± 1.50, 95% CI [58.76, 60.91]; +5.3%, $F(1,9)=67.50$, $p<0.001$, $\eta^2=0.882$) and post-exercise (from 63.43 ± 2.88, 95% CI [61.37, 65.50] to 67.23 ± 3.75, 95% CI [64.55, 69.92]; +6.0%, $F(1,9)=43.91$, $p<0.001$, $\eta^2=0.830$). In the HIIT group, no significant change was observed at rest (from 54.80 ± 7.46, 95% CI [49.46, 60.13] to 54.40 ± 4.51, 95% CI [51.17, 57.62]; -0.7%, $F(1,9)=0.17$, $p=1.000$, $\eta^2=0.019$), but a significant increase was noted post-exercise (from 59.80 ± 7.46, 95% CI [54.46, 65.13] to 64.80 ± 7.46, 95% CI [59.46, 70.13]; +8.4%, $F(1,9)=135.38$, $p<0.001$, $\eta^2=0.938$). The overall effect of the program was significant ($F(1,18)=68.51$, $p<0.001$, $\eta^2=0.783$).

Interaction Effects

A significant interaction was observed between the training program and acute exercise for both groups ($F(1,9)=6.00$ for MICT, $p=0.037$, $\eta^2=0.400$; $F(1,9)=31.85$ for HIIT, $p<0.001$, $\eta^2=0.780$), indicating that the exercise-induced increase was greater after the program, particularly in the HIIT group (Table 5). No significant interaction involving the group type was detected ($F(1,18)=2.79$, $p=0.112$, $\eta^2=0.134$), suggesting that the effects of the program and exercise were similar between the MICT and HIIT groups.

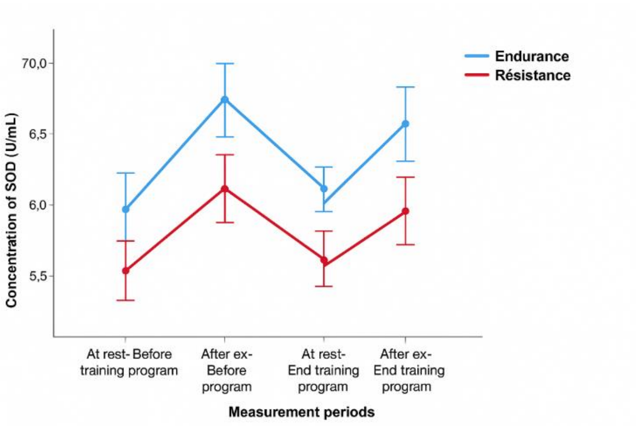
Table 5. Repeated-Measures ANOVA and Post-Hoc Paired Comparisons

Effect	Group: MICT			Group : Resistance		
	F	p	Eta squared	F	p	Eta squared
Training Program	56.54	<0.001	0.863	23.11	<0.001	0.720
Exercise	137.81	<0.001	0.939	259.03	<0.001	0.966
Interaction Program × Exercise	6.00	0.037	0.400	31.85	<0.001	0.780

Table 6. Post-Hoc Paired Comparisons

Effect	Test Bonferroni			Size Effect
	Group	F	p	**Eta** squared
Effect of Exercise at Beginning of Training Program	MICT	175.02	<0.001	0.951
	Resistance	259.03	<0.001	0.966
Effect of Exercise at End of Training Program	MICT	106.22	<0.001	0.922
	Resistance	118.14	<0.001	0.929
Effect of Training Program at Rest	MICT	67.50	<0.001	0.882
	Resistance	0.17	1.000	0.019
Effect of Training Program Post-Exercise	MICT	43.91	<0.001	0.830
	Resistance	135.38	<0.001	0.938

Figure 2. Variation in SOD Means ± Standard Deviations Before and After Exercise During the Training Program in MICT and HIIT Athletes



Malondialdehyde (MDA) Variation

Descriptive statistics for MDA levels (µmol/L) are presented in Table 7. Data are expressed as means ± standard deviations with 95% confidence intervals, and their changes by athlete group are presented in Figure 3. Analyses of the effects of exercise, training program, and their interactions are presented in Tables 8 and 9.

Table 7. Descriptive Statistics for MDA Before and After Exercise During the Training Program

Groups	Beginning of the Training Program				End of the Training Program			
	At Rest		Post-exercise		At Rest		Post-exercise	
	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI	Mean ± standard deviation	95% CI
MICT	0.79 ± 0.14	[0.69, 0.90]	0.88 ± 0.13	[0.78, 0.97]	0.67 ± 0.14	[0.56, 0.77]	0.93 ± 0.20	[0.78, 1.07]
Resistance	2.54 ± 0.98	[1.84, 3.25]	2.99 ± 1.00	[2.27, 3.70]	2.10 ± 0.87	[1.48, 2.73]	2.60 ± 1.01	[1.88, 3.33]
Total	1.67 ± 1.13	[1.14, 2.20]	1.93 ± 1.29	[1.33, 2.53]	1.38 ± 0.96	[0.94, 1.83]	1.77 ± 1.12	[1.24, 2.29]

Effect of Exercise on MDA Levels and Differences Between the Two Groups

The evaluation of the overall and differential effects of physical exercise on MDA levels is presented in Tables 8 and 9.

Acute exercise significantly increased MDA levels in both groups, both at the start and end of the training program. At the start, the mean increase was 0.09 µmol/L for the MICT group (from 0.79 ± 0.14, 95% CI [0.69, 0.90] to 0.88 ± 0.13, 95% CI [0.78, 0.97]; $F(1,9)=22.15$, $p=0.004$, $\eta^2=0.711$) and 0.45 µmol/L for the HIIT group (from 2.54 ± 0.98, 95% CI [1.84, 3.25] to 2.99 ± 1.00, 95% CI [2.27, 3.70]; $F(1,9)=272.25$, $p<0.001$, $\eta^2=0.968$). At the end, the increase was 0.26 µmol/L for the MICT group (from 0.67 ± 0.14, 95% CI [0.56, 0.77] to 0.93 ± 0.20, 95% CI [0.78, 1.07]; $F(1,9)=178.94$, $p<0.001$, $\eta^2=0.952$) and 0.50 µmol/L for the HIIT group (from 2.10 ± 0.87, 95% CI [1.48, 2.73] to 2.60 ± 1.01, 95% CI [1.88, 3.33]; $F(1,9)=62.50$, $p<0.001$, $\eta^2=0.874$). The increases were significantly greater in the HIIT group compared to the MICT group at both time points ($F(1,18)=31.91$, $p<0.001$, $\eta^2=0.639$).

Effect of the Training Program on MDA Levels

The training program significantly reduced MDA levels at rest in both groups: for the MICT group, from 0.79 ± 0.14, 95% CI [0.69, 0.90] to 0.67 ± 0.14, 95% CI [0.56, 0.77] (-15.2%, $F(1,9)=253.50$, $p<0.001$, $\eta^2=0.966$); for the HIIT group, from 2.54 ± 0.98, 95% CI [1.84, 3.25] to 2.10 ± 0.87, 95% CI [1.48, 2.73] (-17.3%, $F(1,9)=57.32$, $p<0.001$, $\eta^2=0.864$). Post-exercise, no significant change was observed in the MICT group (from 0.88 ± 0.13, 95% CI [0.78, 0.97] to 0.93 ± 0.20, 95% CI [0.78, 1.07]; +5.7%, $F(1,9)=2.81$, $p=0.511$, $\eta^2=0.238$), while a significant decrease was noted in the HIIT group (from 2.99 ± 1.00, 95% CI [2.27, 3.70] to 2.60 ± 1.01, 95% CI [1.88, 3.33]; -13.0%, $F(1,9)=135.38$, $p<0.001$, $\eta^2=0.938$). The overall effect of the program was significant ($F(1,18)=24.98$, $p<0.001$, $\eta^2=0.568$).

Interaction Effects

A significant interaction was observed between the training program and group type ($F(1,18)=144.11$, $p<0.001$, $\eta^2=0.889$), indicating that the program had a more pronounced effect on reducing MDA levels in the HIIT group, particularly post-exercise (Table 8). Additionally, a program × exercise interaction was noted in the MICT group ($F(1,9)=33.91$, $p<0.001$, $\eta^2=0.790$), but not in the HIIT group ($F(1,9)=0.60$, $p=0.460$, $\eta^2=0.062$), suggesting that the effect of exercise on MDA was modulated by the program only in the MICT group.

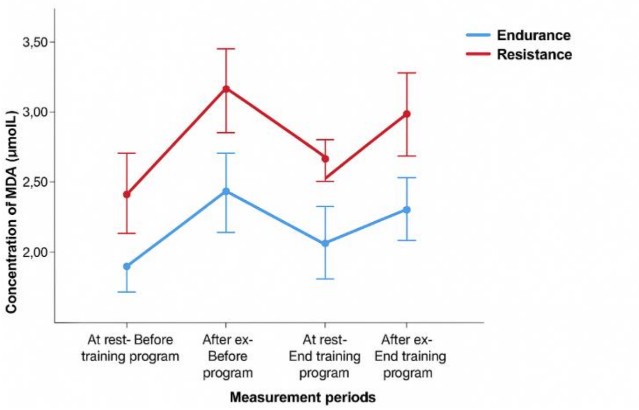
Table 8. Comparison of MDA Means by Repeated-Measures ANOVA for Each Group

Effect	Group : MICT			Group : Resistance		
	F	p	Eta carré	F	p	Eta squared
Training Program	6.70	0.029	0.427	236.39	<0.001	0.963
Exercise	306.00	<0.001	0.971	261.59	<0.001	0.967
Interaction Program× Exercise	33.91	<0.001	0.790	0.60	0.460	0.062

Table 9. Post-Hoc Paired Comparisons

Effect	Group	Test Bonferroni		Effect size
		F	p	Eta squared
Effect of Exercise at Beginning of Training Program	MICT	22.15	0.004	0.711
	Resistance	272.25	<0.001	0.968
Effect of Exercise at End of Training Program	MICT	178.94	<0.001	0.952
	Resistance	62.50	<0.001	0.874
Effect of Training Program at Rest	MICT	253.50	<0.001	0.966
	Resistance	57.32	<0.001	0.864
Effect of Training Program Post-Exercise	MICT	2.81	0.511	0.238
	Resistance	135.38	<0.001	0.938

Figure 3. Variation in MDA Means ± Standard Deviations Before and After Exercise During the Training Program in MICT and HIIT Athletes



Discussion

This study examined the effects of a one-month MICT and high-intensity interval training (HIIT) program on oxidative stress markers (CAT, SOD, MDA) in young male athletes. Both exercise modalities induced a significant increase in catalase at rest and post-exercise, confirming that chronic training enhances antioxidant defenses. This elevation reflects adaptation to increased H_2O_2 production and is likely mediated by Nrf2 pathway activation, which regulates antioxidant gene expression in response to ROS (Karimi et al., 2014; Radak et al., 2013; Thirupathi et al., 2021a). Unlike previous studies reporting more pronounced increases with MICT than high-intensity interval training (HIIT) (Karimi et al., 2014), our results showed similar effects between groups, suggesting that the short program duration may limit differentiation of adaptations (Gomes et al., 2021).

Superoxide dismutase activity increased consistently in the MICT group, whereas the HIIT group exhibited changes only post-exercise, reflecting the distinct ROS production profiles of each exercise type. MICT, by exposing muscles to prolonged oxidative stress, promotes sustained SOD elevation, whereas high-intensity interval training (HIIT) generates acute stress, requiring rapid post-exercise antioxidant responses (Bloomer et al., 2005; Amiri & Sheikholeslami-Vatani, 2023; Stojiljković et al., 2024).

Malondialdehyde levels decreased at rest in both groups and post-exercise in the HIIT group, indicating reduced lipid damage and improved redox homeostasis. The lack of post-exercise change in the MICT group suggests a more stable antioxidant adaptation, while the more pronounced post-exercise effect in the HIIT group reflects rapid mobilization of defenses against acute oxidative stress (Fisher-Wellman & Bloomer, 2009; Karimi et al., 2014; Thirupathi et al., 2021b; Ye et al., 2021).

These findings indicate that moderate-intensity continuous training (MICT) favors continuous regulation of antioxidant enzymes due to prolonged ROS production, while high-intensity interval training (HIIT) optimizes immediate post-exercise responses. This differential profile suggests that combined training could harness the benefits of both modalities, as reported by Medeiros et al. (2015) and Karimi et al. (2014). Underlying mechanisms include Nrf2 activation, improved mitochondrial function reducing electron leakage, and upregulation of antioxidant enzymes, thereby limiting lipid peroxidation (Gomes et al., 2021; Margaritelis et al., 2020; Radak et al., 2013).

These adaptations have practical implications for athletes, as they protect against cellular damage, reduce muscle fatigue, accelerate recovery, and may contribute to preventing chronic oxidative stress-related diseases (De Sousa et al., 2017; Thirupathi et al., 2021b). However, the small sample size and short program duration limit the generalizability of the findings, and the absence of a control group complicates isolation of the specific training effects. Additionally, the study was limited to young men, restricting applicability to other populations (Nikolaidis et al., 2012).

The literature presents similar limitations, including small sample sizes, heterogeneous protocols, and variable biomarker measurement methods, which complicate direct comparisons between studies (Thirupathi et al., 2021a; Ye et al., 2021). Future research should include larger samples, longer interventions, control groups, and diverse populations, including women and older adults. In-depth exploration of molecular mechanisms, particularly Nrf2 activation and interactions with antioxidant supplementation, remains essential to better understand exercise-induced antioxidant adaptations (Gomez-Cabrera et al., 2015; Radak et al., 2013; Nikolaidis et al., 2012).

Conclusions

In conclusion, both MICT and high-intensity interval training (HIIT) improve antioxidant defenses and reduce oxidative stress in young male athletes, with modality-specific effects. These findings highlight the importance of combined training to optimize health and performance. However, further studies are needed to deepen the understanding of mechanisms, include diverse populations, and evaluate long-term effects.

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Authors and translators' details:

Lotfi Nizar	nizarlotfi99@gmail.com	Author/ Translator
Madani Mohamed	madani.mohamed@gmail.com	Author