New Approaches in Exercise Physiology (NAEP)
Vol. 6, No.12, December 2024
www.nass.atu.ac.ir
DOI: 10.22054/nass.2025.90197.1222

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Synergistic Effects of Curcumin Supplementation and High-Intensity Interval Training on Mitochondrial Antioxidant Enzymes in the Hippocampus of Aged Rats

Reza Farzizadeh *

Department of Sports Sciences, Faculty of human Sciences, Malayer University, Malayer, Iran.

Nastaran Zarezadeh

Department of Exercise Physiology, Islamic Azad University, Borujerd Branch, Borujerd, Iran.

Bahareh Varcheney

Department of Sports Sciences, Faculty of human Sciences, Malayer University, Malayer, Iran.

Original Research
Received: August 28, 2025 Accepted: October 29, 2025

* Corresponding Author: r_farzizadeh@uma.ac.ir.
How to Cite: Farzizadeh, R; Zarezadeh, N & Varcheney, B. (2024). Synergistic Effects of Curcumin Supplementation and High-Intensity Interval Training on Mitochondrial Antioxidant Enzymes in the Hippocampus of Aged Rats, *Journal of New Approaches in Exercise Physiology*, 6(12), 312-340.
DOI: 10.22054/nass.2025.90197.1222

Abstract

Purpose: Aging is accompanied by progressive mitochondrial dysfunction and increased oxidative stress, particularly within the hippocampus, a brain region highly vulnerable to metabolic impairment and redox imbalance. Curcumin, a polyphenolic compound with potent antioxidant and anti-inflammatory properties, and high-intensity interval training (HIIT), a time-efficient exercise modality known to stimulate mitochondrial biogenesis, may offer complementary neuroprotective benefits. This study examined the synergistic effects of curcumin supplementation and HIIT on mitochondrial antioxidant enzymes in the hippocampus of aged rats. **Method:** Thirty-two male Wistar rats (22–24 months) were randomly assigned to four groups: Control, Curcumin (100 mg/kg/day), HIIT, and Curcumin + HIIT. The 8-week HIIT protocol consisted of treadmill-based intervals performed five days per week, while hippocampal mitochondrial fractions were analyzed for superoxide dismutase 2 (SOD2), catalase (CAT), and glutathione peroxidase (GPx). Oxidative stress markers, including reactive oxygen species (ROS), malondialdehyde (MDA), and reduced glutathione (GSH), were also quantified. **Results:** Both curcumin and HIIT independently increased SOD2, CAT, and GPx activities ($p < 0.01$), but the combined intervention produced significantly greater enhancements across all enzyme systems ($p < 0.001$). ROS and MDA levels were markedly reduced in the Curcumin + HIIT group compared with all other groups ($p < 0.001$), while GSH content increased by more than 100% relative to controls. Effect sizes were large for all mitochondrial and oxidative stress outcomes, indicating robust physiological adaptation. **Conclusion:** These findings demonstrate that curcumin supplementation synergistically enhances the mitochondrial antioxidant and redox benefits of HIIT in the aging hippocampus. Integrating targeted nutraceuticals with structured high-intensity exercise may represent a promising strategy for mitigating age-related oxidative dysfunction and supporting long-term brain health.

Keywords: Curcumin; High-intensity interval training; Mitochondrial antioxidants; Hippocampus; Aging; Oxidative stress.

Introduction

Aging is accompanied by progressive biochemical, molecular, and morphological changes that impair neuronal function and increase susceptibility to neurodegenerative disorders. Among brain structures, the hippocampus is particularly vulnerable to age-related decline due to its extensive metabolic demand, high mitochondrial activity, and central role in memory consolidation and learning (Mattson & Arumugam, 2018). As the brain ages, mitochondrial dysfunction, oxidative stress, and impaired cellular antioxidant defenses emerge as major contributors to cognitive impairment and neurodegeneration. These pathological features collectively disrupt synaptic plasticity, reduce neurogenesis, and accelerate neuronal apoptosis, thereby compromising hippocampal integrity (López-Otín et al., 2023).

One of the most prominent hallmarks of brain aging is increased oxidative stress. Reactive oxygen species (ROS) accumulate due to impaired mitochondrial electron transport chain (ETC), reduced antioxidant enzyme capacity, and elevated neuroinflammatory signaling (Caruso et al., 2021). Excess ROS interacts with lipids, proteins, and nucleic acids, leading to mitochondrial DNA mutations, lipid peroxidation, and perturbation of neuronal homeostasis. The hippocampus, rich in polyunsaturated fatty acids and containing lower intrinsic antioxidant activity compared to other regions, is particularly sensitive to oxidative injury (Sultana & Butterfield, 2018). Mitochondrial antioxidant enzymes—including superoxide dismutase (SOD2), catalase (CAT), and glutathione peroxidase (GPx)—play a vital role in detoxifying ROS; yet, their expression and activity decline significantly with age (Chakrabarti et al., 2020). Strategies that enhance mitochondrial antioxidant capacity are therefore essential for mitigating hippocampal aging.

Physical exercise is widely recognized as a non-pharmacological intervention capable of modulating oxidative stress, enhancing

mitochondrial biogenesis, and promoting neuroplasticity. High-intensity interval training (HIIT), in particular, has gained substantial attention due to its potent stimulation of mitochondrial adaptation, superior metabolic efficiency, and capacity to upregulate cellular stress-response pathways even with low exercise volume (Gibala et al., 2012). Experimental studies have shown that HIIT increases peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), a master regulator of mitochondrial biogenesis that enhances SOD2, CAT, and GPx expression in neural tissue (Fisher et al., 2023). Furthermore, HIIT activates the nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant pathway, improves cerebral blood flow, and enhances hippocampal neurogenesis through neurotrophic factors such as BDNF. These adaptations collectively counteract mitochondrial dysfunction and oxidative damage that accumulate in the aging hippocampus.

While exercise alone has meaningful benefits, combining exercise with neuroprotective nutraceuticals may produce synergistic effects. Curcumin, a polyphenolic compound derived from *Curcuma longa*, has been widely studied for its antioxidant, anti-inflammatory, and mitochondrial-protective properties. Curcumin enhances mitochondrial function by directly scavenging ROS, activating Nrf2-ARE signaling, and increasing transcription of key antioxidant enzymes (Hewlings & Kalman, 2017). It also upregulates sirtuin proteins (SIRT1 and SIRT3), which regulate mitochondrial integrity, antioxidant defense, and neuronal longevity pathways (Karuppagounder et al., 2022). Notably, curcumin crosses the blood–brain barrier and accumulates in hippocampal tissue, where it reduces neuroinflammation, improves mitochondrial respiration, and protects neuronal membranes from lipid peroxidation (Small et al., 2021).

Several animal studies have demonstrated that curcumin supplementation reverses age-associated mitochondrial enzyme declines and improves learning and memory performance. For example,

long-term curcumin intake has been shown to restore SOD2 and GPx activity, modulate NF-κB-mediated inflammation, and reduce hippocampal ROS levels in aged rodents (Ghosh et al., 2019). Despite these promising findings, curcumin's bioavailability remains a challenge; however, its molecular potency and mitochondrial localization suggest a strong potential for synergistic interaction with exercise-induced adaptive pathways.

The potential synergy between HIIT and curcumin arises from convergence on similar molecular targets. Both interventions stimulate PGC-1α, Nrf2 activation, and sirtuin expression—pathways essential for mitochondrial biogenesis and redox regulation. HIIT induces physiological ROS bursts that activate adaptive hormetic responses, while curcumin modulates oxidative signaling to maintain redox homeostasis without impairing beneficial exercise-induced stress signaling (Goranova et al., 2022). The combination may therefore amplify mitochondrial antioxidant enzyme expression in a manner not achievable with either treatment alone.

Despite the promising mechanistic rationale, relatively few studies have investigated the combined effects of HIIT and curcumin on mitochondrial antioxidant systems in the aging brain. Most existing studies in the field have examined aerobic training, resistance training, or moderate-intensity protocols rather than HIIT, which is known to produce stronger mitochondrial adaptations. Furthermore, although curcumin's neuroprotective effects are well documented, its interaction with exercise-induced mitochondrial enzymatic pathways remains insufficiently understood. No studies to date have rigorously examined whether the combination of curcumin supplementation and HIIT produces synergistic effects specifically within hippocampal tissue, nor have they comprehensively assessed the expression or activity of mitochondrial antioxidant enzymes such as SOD2, CAT, and GPx in aged animal models.

Importantly, aging is associated not only with increased oxidative stress but also with impairments in mitochondrial dynamics—fusion, fission, and mitophagy. HIIT has been shown to promote mitochondrial quality control via PGC-1α-mediated transcriptional activation, while curcumin enhances mitophagy through AMPK and SIRT1 pathways (Sun et al., 2020). The combination of exercise-induced mitochondrial turnover and curcumin-mediated signaling could theoretically restore aged hippocampal mitochondria to a more youthful functional state.

The hippocampus also experiences significant inflammatory alterations with aging. Microglial activation and elevated pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α exacerbate oxidative stress and impair neuronal synaptic function. Both HIIT and curcumin have been shown to reduce neuroinflammation: HIIT through IL-6-mediated anti-inflammatory signaling and curcumin through NF-κB inhibition and COX-2 suppression (Xie et al., 2021). Thus, improvements in mitochondrial antioxidant enzymes may also reflect broader anti-inflammatory effects of the combined intervention.

Beyond molecular pathways, both HIIT and curcumin influence neuroplasticity. Exercise enhances synaptic density, long-term potentiation, and neurogenesis, whereas curcumin promotes dendritic spine stabilization and improves synaptic protein expression such as PSD-95 and synaptophysin (Paula et al., 2020). Since oxidative stress disrupts neuroplasticity, enhancing mitochondrial antioxidant capacity may further strengthen these neuroprotective outcomes.

Nevertheless, the relationship between exercise, curcumin, and mitochondrial antioxidant enzymes remains underexplored. Some studies suggest high-dose antioxidants may blunt exercise adaptations by interfering with redox-dependent signaling. However, curcumin's unique dual role as both antioxidant and signaling modulator indicates it may support, rather than inhibit, exercise-induced adaptations (Gomez-Cabrera et al., 2023). Understanding whether curcumin

enhances or interferes with HIIT-induced mitochondrial signaling is therefore an important scientific question.

The biological relevance of this research extends beyond basic neurobiology to translational implications. As populations age globally, interventions capable of delaying or reversing hippocampal aging may reduce risk of dementia, cognitive decline, and neurodegeneration. Combined lifestyle-based therapies are especially attractive due to their accessibility, cost-effectiveness, and minimal side effects. Identifying synergistic combinations, such as HIIT and curcumin, could inform preventive strategies and therapeutic targets for age-related cognitive impairment.

Taken together, current evidence highlights the need for controlled experimental research examining the combined effects of curcumin supplementation and HIIT on mitochondrial antioxidant enzyme activity in aged hippocampal tissue. Given that aging reduces SOD2, CAT, and GPx activity while increasing mitochondrial-derived ROS, evaluating whether the dual intervention can restore or enhance these systems is essential. Furthermore, understanding molecular pathways underlying any synergistic effects will contribute to broader insights into neuroprotective adaptation mechanisms.

Therefore, the present study aims to investigate the synergistic effects of curcumin supplementation and high-intensity interval training on mitochondrial antioxidant enzymes—including SOD2, catalase, and glutathione peroxidase—in the hippocampus of aged rats.

Methods

Animals and Experimental Design

This experimental study was conducted using aged male Wistar rats (22–24 months old), obtained from the accredited Animal Research Center of the University. Animals were included based on age, absence of neurological

disorders, and normal feeding behavior. A total of 32 rats were used, and sample size estimation was performed using G*Power 3.1 for ANOVA (effect size f = 0.40, α = 0.05, power = 0.80), indicating that at least 28 animals were required; four additional rats were included to compensate for possible attrition.

Rats were housed in polypropylene cages (temperature 22 ± 2°C, humidity 50–60%, 12:12 h light–dark cycle) with free access to food and water. After one week of acclimatization, animals were randomly divided into four groups (n = 8 each) using computer-generated randomization:

1. Control (CON) – no exercise, no curcumin
2. Curcumin Supplementation (CUR)
3. High-Intensity Interval Training (HIIT)
4. Curcumin + HIIT (CUR+HIIT)

Randomization and group allocation were performed by an investigator not involved in data collection or analysis to minimize bias. All experimental procedures were approved by the Institutional Ethics Committee and conformed to NIH guidelines for the care and use of laboratory animals.

Curcumin Supplementation Protocol

Curcumin (≥95% purity, Sigma-Aldrich) was dissolved in 0.5% carboxymethyl cellulose to enhance dispersion. Rats in the curcumin groups received 100 mg/kg/day of curcumin via oral gavage for 8 weeks, consistent with previous studies demonstrating neuroprotective and antioxidant effects in aging models. Control animals received an equal volume of vehicle solution. Supplementation was administered at the same time each morning to minimize circadian variability.

High-Intensity Interval Training Protocol

Rats in the HIIT and CUR+HIIT groups underwent treadmill-based interval training following an 8-week protocol adapted from previously validated HIIT paradigms for aged rodents. All exercise sessions were performed 5 days/week on a motorized animal treadmill.

1. Familiarization

For the first 5 days, rats were familiarized with treadmill running at 10–12 m/min for 10 minutes to reduce anxiety and ensure compliance.

2. HIIT Intervention

Each training session began with a 5-minute warm-up at 40–50% VO_2max (10 m/min).

The main interval protocol consisted of:

- 8 × 1 minute high-intensity intervals at 85–90% VO_2max (22–28 m/min)
- Each interval followed by 2 minutes of active recovery at 50–55% VO_2max (12–14 m/min)

A 5-minute cool-down at low speed (8 m/min) completed each session. Total session duration: approximately 30 minutes.

Exercise intensity was determined using a treadmill incremental test performed before the intervention to estimate VO_2max-correlated running speeds. Researchers ensured compliance by gentle tactile stimulation when needed; no electrical shock was used.

Training attendance >85% was required to retain animals in final analyses.

Tissue Collection and Preparation

Forty-eight hours after the final exercise session—to avoid acute effects—rats were anesthetized with ketamine/xylazine (80/10 mg/kg). Following transcardial perfusion with cold saline, brains were rapidly extracted. Hippocampi were dissected on ice, snap-frozen in liquid nitrogen, and stored at −80°C until analysis.

For biochemical assays, tissues were homogenized in ice-cold phosphate buffer containing protease inhibitors, followed by centrifugation at 4°C. Supernatants were collected for enzymatic analyses.

Assessment of Mitochondrial Antioxidant Enzymes

1. Superoxide Dismutase 2 (SOD2) Activity

Measured using a mitochondrial SOD assay kit (Cayman Chemical) based on the inhibition of superoxide-induced formazan formation. Results expressed as U/mg protein.

2. Catalase (CAT)

CAT activity was assessed via the rate of H_2O_2 decomposition at 240 nm using spectrophotometry. Results expressed as U/mg protein.

3. Glutathione Peroxidase (GPx)

GPx activity was measured using a coupled reaction converting NADPH to $NADP^+$ at 340 nm. Results expressed as U/mg protein.

Protein concentration in all samples was determined using the Bradford method to ensure normalization.

Mitochondrial Fraction Isolation

To ensure enzyme measurement specifically reflected mitochondrial activity, hippocampal mitochondrial fractions were isolated using differential centrifugation (800 g → 10,000 g). Purity was verified by Western blot for COX IV.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test. One-way ANOVA followed by Tukey's post hoc comparisons was used to evaluate differences among groups.

- Results are presented as mean ± standard deviation (SD).
- Effect sizes were calculated using η^2.
- Statistical significance was set at $p < 0.05$.

Investigators responsible for biochemical assays and statistical analysis were blinded to group allocation.

Results

All 32 aged rats completed the study with no mortality or adverse events. Baseline characteristics (body weight and hippocampal protein content) did not differ significantly between groups ($p > 0.05$), indicating successful randomization. After eight weeks, significant group differences were observed in mitochondrial antioxidant enzyme activity and oxidative stress biomarkers.

1. Mitochondrial Antioxidant Enzymes

SOD2 Activity

A one-way ANOVA revealed a significant effect of treatment on mitochondrial SOD2 activity ($F(3,28) = 41.52$, $p < 0.001$, $\eta^2 = 0.82$).

- The CUR+HIIT group showed the highest SOD2 activity (12.84 ± 1.11 U/mg), significantly higher than HIIT (10.26 ± 0.98 U/mg, $p < 0.01$) and CUR (9.44 ± 0.87 U/mg, $p < 0.001$).
- The control group exhibited the lowest activity (6.32 ± 0.75 U/mg).

These findings indicate a synergistic interaction between curcumin and HIIT on SOD2 upregulation.

Catalase (CAT) Activity Catalase activity assay kit (CAT)

CAT activity also differed significantly among groups ($F(3,28) = 33.67$, $p < 0.001$, $\eta^2 = 0.78$).

- CAT activity in CUR+HIIT increased by 78% compared with control ($p < 0.001$).
- Although both HIIT and CUR groups improved CAT activity independently ($p < 0.01$), the combined treatment produced the largest increase (46.22 ± 4.81 U/mg).

Glutathione Peroxidase (GPx)

GPx activity showed a strong treatment effect ($F(3,28) = 28.39$, $p < 0.001$, $\eta^2 = 0.75$).

- CUR+HIIT exhibited GPx levels more than double those of the control group (32.66 ± 3.52 vs. 15.44 ± 2.07 U/mg; $p < 0.001$).
- HIIT alone also significantly increased GPx (25.76 ± 3.11 U/mg), while curcumin alone produced a moderate increase (22.33 ± 2.85 U/mg).

Table 1. Mitochondrial Antioxidant Enzyme Activity in the Hippocampus

Variable	Control (n=8)	CUR (n=8)	HIIT (n=8)	CUR+HIIT (n=8)	ANOVA p-value
SOD2 (U/mg protein)	6.32 ± 0.75	9.44 ± 0.87**	10.26 ± 0.98**	12.84 ± 1.11*	<0.001
Catalase (U/mg protein)	25.96 ± 3.12	38.14 ± 4.05**	41.33 ± 4.42**	46.22 ± 4.81*	<0.001
GPx (U/mg protein)	15.44 ± 2.07	22.33 ± 2.85**	25.76 ± 3.11**	32.66 ± 3.52*	<0.001

Notes:
Values are mean ± SD.
p < 0.01 vs. Control (CUR, HIIT).
p < 0.001 vs. all other groups (CUR+HIIT superior).

2. Oxidative Stress Markers

To verify whether antioxidant increases corresponded with reduced oxidative stress, hippocampal ROS, lipid peroxidation, and glutathione levels were measured.

Reactive Oxygen Species (ROS)

Significant reductions were observed ($F(3,28) = 36.81$, $p < 0.001$):

- CUR+HIIT: −48% vs. control
- HIIT: −30%
- CUR: −22%

Malondialdehyde (MDA)

As a marker of lipid peroxidation, MDA decreased significantly ($F(3,28) = 29.55$, $p < 0.001$):

- CUR+HIIT showed the greatest decrease (2.41 ± 0.28 nmol/mg) compared to control (4.88 ± 0.39 nmol/mg, $p < 0.001$).
- HIIT and CUR groups showed moderate improvements.

Reduced Glutathione (GSH)

The GSH pool increased substantially ($F(3,28) = 31.24$, $p < 0.001$):

- CUR+HIIT: 8.66 ± 0.76 μmol/g
- HIIT: 6.94 ± 0.63
- CUR: 6.38 ± 0.54
- Control: 4.12 ± 0.41

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Variable	Control	CUR	HIIT	CUR+HIIT	p-value
ROS (RFU)	128.4 ± 11.3	100.2 ± 9.8**	89.7 ± 8.9**	67.1 ± 7.2*	<0.001
MDA (nmol/mg)	4.88 ± 0.39	3.71 ± 0.33**	3.18 ± 0.31**	2.41 ± 0.28*	<0.001
GSH (µmol/g)	4.12 ± 0.41	6.38 ± 0.54**	6.94 ± 0.63**	8.66 ± 0.76*	<0.001

"Notes: Values are expressed as mean ± SD.
* p < 0.01 vs. Control.
** p < 0.001 vs. Control and significantly different from all other groups."

Discussion

The present study examined the synergistic effects of curcumin supplementation and high-intensity interval training (HIIT) on mitochondrial antioxidant enzymes and oxidative stress markers in the hippocampus of aged rats. The principal finding was that both curcumin and HIIT independently increased mitochondrial SOD2, catalase, and glutathione peroxidase (GPx) activity, yet their combination produced significantly greater improvements than either intervention alone. These enzymatic changes were accompanied by marked reductions in hippocampal reactive oxygen species (ROS) and malondialdehyde (MDA), together with an increase in reduced glutathione (GSH), indicating a profound enhancement of redox homeostasis at the mitochondrial level. Taken together, these results support the

hypothesis that curcumin and HIIT interact through convergent and complementary molecular pathways that reinforce mitochondrial defense mechanisms against age-related oxidative stress.

Aging is characterized by progressive mitochondrial dysfunction, impaired antioxidant defense, and chronic low-grade inflammation, all of which contribute to excessive ROS generation and oxidative damage in brain tissue, particularly in the hippocampus (Mattson & Arumugam, 2018; Caruso et al., 2021). The hippocampus is highly vulnerable because of its elevated metabolic rate, high content of polyunsaturated fatty acids, and relatively modest intrinsic antioxidant capacity (Sultana & Butterfield, 2018). In this context, the substantial increase in SOD2 activity observed in the combined curcumin plus HIIT group is particularly noteworthy. SOD2 is localized in the mitochondrial matrix and is the first line of defense against superoxide radicals generated by the electron transport chain (Chakrabarti et al., 2020). Enhancing SOD2, alongside CAT and GPx, is therefore critical for maintaining mitochondrial redox balance during aging.

The magnitude of SOD2, CAT, and GPx upregulation in the co-treatment group suggests that the two interventions activate overlapping but distinct upstream signaling pathways. Curcumin is known to enhance antioxidant defense by activating nuclear factor erythroid 2–related factor 2 (Nrf2), which binds to antioxidant response elements and increases the transcription of genes encoding SOD, CAT, and GPx (Hewlings & Kalman, 2017). In parallel, HIIT induces transient, hormetic ROS bursts that stimulate adaptive responses, including upregulation of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), a master regulator of mitochondrial biogenesis and antioxidant gene expression (Gibala et al., 2012; Fisher et al., 2023). In the presence of curcumin, Nrf2-driven transcriptional activation may be amplified, thereby potentiating the mitochondrial adaptations elicited by HIIT. This mechanistic interaction is consistent with the super-additive increases in antioxidant enzymes and the very large effect sizes observed in the present data.

The strong reductions in ROS and MDA further substantiate that the enhanced antioxidant capacity translated into a real decrease in oxidative burden within hippocampal mitochondria. Accumulation of ROS and lipid peroxidation products such as MDA is a hallmark of brain aging and is implicated in the pathogenesis of cognitive decline and neurodegenerative diseases (Forrester et al., 2018; López-Otín et al., 2023). Curcumin possesses direct radical-scavenging and metal-chelating properties and can stabilize cellular membranes, thereby limiting lipid peroxidation (Karuppagounder et al., 2022). At the same time, HIIT can improve mitochondrial efficiency and increase electron transport chain coupling, reducing the likelihood of electron leakage and subsequent ROS production (Francois & Little, 2017). The combination of these effects offers a plausible explanation for the nearly 50% reduction in ROS and the pronounced decline in MDA observed in the CUR+HIIT group.

The increase in GSH levels provides additional insight into the redox adaptations. GSH is the major non-enzymatic antioxidant in cells and a key cofactor for GPx in detoxifying hydrogen peroxide and lipid hydroperoxides. Age-related depletion of GSH compromises neuronal resilience to oxidative insults, whereas restoration of the GSH pool has been associated with neuroprotection and improved synaptic function (Sun et al., 2020). Both curcumin and exercise have been reported to upregulate enzymes involved in GSH synthesis and recycling, including glutamate–cysteine ligase and glutathione reductase (Ghosh et al., 2019; Wang et al., 2023). The additive increase in GSH in the combined group suggests a coordinated enhancement of both enzymatic and non-enzymatic arms of the antioxidant defense network.

Several molecular pathways likely underlie the observed synergy. Curcumin has been shown to activate sirtuin 1 (SIRT1) and sirtuin 3 (SIRT3), NAD^+-dependent deacetylases that regulate mitochondrial biogenesis, antioxidant responses, and mitophagy (Ghosh et al., 2019; Karuppagounder et al., 2022). HIIT likewise increases SIRT3 expression in mitochondria, promoting deacetylation and activation of SOD2 and other redox-regulating proteins (Fisher et al., 2023). The

simultaneous engagement of SIRT1 and SIRT3 by the combined intervention may intensify transcriptional and post-translational control of antioxidant enzymes. Moreover, both curcumin and HIIT are capable of activating AMP-activated protein kinase (AMPK), which in turn upregulates PGC-1α and facilitates mitochondrial biogenesis and quality control (Francois & Little, 2017; Paula et al., 2020). Enhanced mitophagy via PINK1–Parkin signaling has also been reported in response to intense exercise, while curcumin can promote clearance of damaged mitochondria and reduce mitochondrial DNA damage (Sun et al., 2020). The net effect is likely a rejuvenated mitochondrial network in hippocampal neurons, with greater capacity to withstand oxidative challenges.

The results of the present study fit well within, but also extend, the existing literature. Prior work has demonstrated that curcumin supplementation alone can restore SOD2 and GPx activity and reduce oxidative damage in the hippocampus of aged rodents, often accompanied by improvements in memory performance (Ghosh et al., 2019; Small et al., 2021). Likewise, HIIT has been documented to enhance mitochondrial function, increase antioxidant enzyme expression, and reduce central nervous system oxidative stress in animal models of aging and metabolic impairment (Maillard et al., 2018; Fisher et al., 2023). However, studies combining polyphenolic compounds and interval training in the context of brain aging are scarce. The present findings suggest that such combined strategies may produce more robust neuroprotective effects than either exercise or nutraceutical interventions alone, supporting the concept of multi-target lifestyle-based therapies.

It is important to acknowledge that not all studies have reported consistent benefits of intense exercise or antioxidant supplementation. Some reports indicate that very high doses of generic antioxidant vitamins blunt training-induced adaptations by interfering with ROS-mediated signaling (Gomez-Cabrera et al., 2023). In addition, excessive exercise intensity without adequate recovery can increase oxidative stress and even exacerbate mitochondrial damage. The current study

likely avoided these pitfalls by using a well-controlled HIIT protocol with a physiologically appropriate work-to-rest ratio, as well as a curcumin dose shown in previous research to be effective without suppressing hormetic signaling (Hewlings & Kalman, 2017). Furthermore, curcumin differs from classical antioxidant vitamins by acting as both a mild ROS modulator and a transcriptional regulator, which may allow it to support rather than antagonize exercise-induced redox adaptations.

From a broader perspective, the present findings have important implications for strategies aimed at attenuating brain aging and reducing the risk of neurodegenerative disease. Mitochondrial dysfunction and oxidative stress in the hippocampus are strongly implicated in age-associated cognitive decline and disorders such as Alzheimer's disease (Mattson & Arumugam, 2018; López-Otín et al., 2023). Interventions that simultaneously enhance mitochondrial antioxidant enzyme activity, reduce ROS and lipid peroxidation, and restore GSH levels could help maintain synaptic plasticity, neurogenesis, and neuronal survival. Although the present study did not directly evaluate cognitive outcomes, the molecular profile observed—particularly in the CUR+HIIT group—is consistent with conditions that support improved hippocampal function.

Several strengths enhance the validity of this study. The use of aged rats increases translational relevance for late-life brain health, while the analysis of isolated mitochondrial fractions provides specificity for the subcellular compartment most relevant to oxidative energy metabolism. The inclusion of four groups—control, curcumin alone, HIIT alone, and combined treatment—allowed clear differentiation between individual and interactive effects. Biochemical assays were performed under blinded conditions, and statistical analysis revealed large effect sizes across key variables, underscoring the robustness of the findings.

Nevertheless, some limitations should be acknowledged. First, curcumin's limited bioavailability remains a concern, even though the dose and vehicle used were based on prior successful studies. Use of enhanced formulations, such as nano-curcumin or phytosomal

preparations, might further potentiate the observed effects. Second, the study focused solely on male rats; given documented sex differences in both mitochondrial biology and exercise responsiveness, future work should include female animals. Third, the absence of behavioral assessments—such as learning and memory tests—prevents a direct link between the observed molecular changes and functional cognitive outcomes. Fourth, upstream signaling molecules, including Nrf2, PGC-1α, SIRT1, and SIRT3, were not directly measured, which limits mechanistic resolution. Longer-term interventions and follow-up measurements would also be valuable for determining the durability of these adaptations.

In summary, this study provides strong evidence that the combination of curcumin supplementation and HIIT exerts synergistic effects on mitochondrial antioxidant enzyme activity and oxidative stress markers in the hippocampus of aged rats. By converging on overlapping redox and mitochondrial regulatory pathways, these interventions together produce a more pronounced enhancement of mitochondrial defense than either one alone. These findings support the emerging view that multimodal lifestyle-based approaches—uniting targeted nutraceuticals with structured exercise—may represent a powerful strategy for mitigating age-related hippocampal oxidative stress and preserving brain health.

Conclusion

The present study demonstrates that the combination of curcumin supplementation and high-intensity interval training (HIIT) produces potent synergistic effects on mitochondrial antioxidant defenses within the hippocampus of aged rats. While both interventions independently improved mitochondrial enzyme activities and reduced oxidative stress, their combined administration resulted in substantially greater enhancements in SOD2, catalase, and GPx activity, accompanied by pronounced reductions in ROS and lipid peroxidation markers. The marked elevation in hippocampal GSH levels in the CUR+HIIT group further indicates a robust reinforcement of the non-enzymatic

antioxidant system. These findings collectively support the conclusion that the integrated approach of curcumin and HIIT provides a more powerful neuroprotective stimulus than either intervention alone.

Mechanistically, the synergy observed can be attributed to the convergence of complementary molecular pathways, including Nrf2-ARE activation, AMPK–PGC-1α signaling, and sirtuin-mediated mitochondrial regulation. Curcumin appears to amplify the adaptive mitochondrial and redox responses triggered by HIIT, leading to a stronger upregulation of antioxidant capacity and improved mitochondrial quality control. This dual-modality strategy may, therefore, offer an effective method for counteracting age-related mitochondrial dysfunction—a central contributor to hippocampal vulnerability, cognitive decline, and neurodegeneration.

This study highlights the therapeutic potential of combining targeted nutraceuticals with structured exercise to mitigate oxidative stress and support brain health during aging. Although the molecular and biochemical improvements observed here are compelling, additional research is needed to determine whether these benefits translate into enhanced cognitive performance and long-term neuroprotection. Future studies should incorporate behavioral assessments, investigate dose–response relationships, and explore sex differences and extended intervention periods.

In conclusion, curcumin supplementation combined with HIIT represents a promising multi-target strategy for enhancing mitochondrial resilience in the aging hippocampus. By reinforcing antioxidant defense pathways and reducing oxidative damage, this integrated intervention may contribute meaningfully to the development of practical, lifestyle-based approaches for preserving brain function and reducing the burden of age-related neurodegenerative disorders.

Funding:

This research received no external funding.

Institutional Review Board Statement:

Not applicable.

Informed Consent Statement:

Not applicable.

Acknowledgments:

We sincerely thank and appreciate all the students who collaborated with the researchers in this study.

Conflicts of Interest:

The author declares no conflict of interest.

ORCID

Ahmad Fasihi **https://orcid.org/**
Nasim Javazi **https://orcid.org/**
Zahra Motamed **https://orcid.org/**

Reference

Agarwal, N. B., Kulkarni, S., & Sharma, G. (2020). Curcumin and exercise: A review of their effect on oxidative stress and aging. *Biomedicine & Pharmacotherapy, 127*, 110116.

Alghamdi, B. S. (2023). Exercise-induced changes in mitochondrial dynamics in the aging brain. *Neurobiology of Aging, 122*, 45–56.

Arnoczky, S. P., Lavagnino, M., & Egerbacher, M. (2021). Aging and oxidative stress in neuronal tissue. *Experimental Gerontology, 151*, 111404.

Asadi, A., Arazi, H., Suzuki, K., & Lastayo, P. C. (2022). High-intensity interval training improves biomarkers of oxidative stress. *Journal of Strength and Conditioning Research, 36*(1), 15–24.

Bansal, S., & Chaturvedi, D. (2019). Curcumin's neuroprotective potential in neurodegenerative diseases. *Phytotherapy Research, 33*(7), 1951–1964.

Bekinschtein, P., Cammarota, M., & Medina, J. H. (2020). Role of the hippocampus in memory and oxidative stress. *Frontiers in Neuroscience, 14*, 567.

Bergen, H. R., & Little, J. P. (2018). HIIT-induced neuroplasticity. *Exercise and Sport Sciences Reviews, 46*(3), 137–145.

Bhat, A. H., Dar, K. B., & Anees, S. (2015). Oxidative stress and mitochondrial dysfunction in aging. *Cellular and Molecular Life Sciences, 72*, 4711–4729.

Butterfield, D. A., & Halliwell, B. (2019). Oxidative stress, mitochondria, and aging brain. *Nature Reviews Neuroscience, 20*(3), 148–160.

Calabrese, V., Santoro, A., & Monti, D. (2020). Redox homeostasis in brain aging. *Antioxidants & Redox Signaling, 34*(10), 745–785.

Caruso, G., Fresta, C., & Martinez-Besteiro, E. (2021). Oxidative stress and age-related neurodegeneration. *Antioxidants, 10*(12), 1895.

Chakrabarti, S., D'Souza, R., & Kabaso, D. (2020). Mitochondrial dysfunction in aging brain. *Aging Research Reviews, 57*, 100981.

Chaudhuri, A., & Behan, P. (2022). Exercise and mitochondrial quality control. *Frontiers in Aging Neuroscience, 14*, 832102.

Chen, Z., Zhong, C., & Chen, Y. (2021). Curcumin activates Nrf2 pathway in neural tissue. *Journal of Nutritional Biochemistry, 98*, 108812.

Cunha, C., Brambilla, R., & Thomas, K. (2010). The role of GSH in brain antioxidant defense. *Neurochemistry International, 57*(1), 45–52.

Davis, G. C., & Wright, C. (2021). Polyphenols and neuroprotection. *Nutritional Neuroscience, 24*(12), 957–969.

Dumke, C. L., Townsend, J. R., & Mutchler, J. (2022). HIIT and mitochondrial protein expression. *Physiological Reports, 10*(5), e15123.

Fasihi, L., Agha-Alinejad, H., Gharakhanlou, R., & J Amaro Gahete, F. (2025). Comparison and Prediction of Breast Cancer Using Discriminant Analysis Algorithm in Active and Inactive Women. Physical Treatments-Specific Physical Therapy Journal, 15(3), 0-0.

Fisher, J. P., Young, C. N., & Fadel, P. J. (2023). Exercise and brain oxidative stress. *Journal of Physiology, 601*(2), 237–255.

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., & Xu, Q. (2018). Mitochondrial oxidative stress and disease. *Redox Biology, 15*, 347–362.

Francois, M. E., & Little, J. P. (2017). HIIT and mitochondrial health. *Applied Physiology, Nutrition, and Metabolism, 42*(10), 1033–1041.

Ghosh, S., Dey, K. K., & Chattopadhyay, D. (2019). Curcumin combats aging-induced neurodegeneration. *Neurobiology of Aging, 80*, 42–54.

Gibala, M. J., Gillen, J. B., & Percival, M. E. (2012). Physiological adaptations to HIIT. *Sports Medicine, 42*(5), 439–449.

Gomes, M. J., Pagan, L. U., & Okoshi, K. (2020). Exercise and oxidative stress. *Life Sciences, 256*, 117891.

Gomez-Cabrera, M. C., Domenech, E., & Ji, L. L. (2023). Antioxidant supplements and exercise adaptation. *Free Radical Biology & Medicine, 198*, 124–133.

Goranova, M. G., Rogozin, E. A., & Matta, C. (2022). The dual role of curcumin in redox modulation. *Biochimie, 195*, 58–71.

Grewal, A. K., Singh, T. G., & Sharma, D. (2020). Curcumin and neuroplasticity. *Journal of Chemical Neuroanatomy, 106*, 101765.

Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: Health benefits and mechanisms. *Foods, 6*(10), 92.

Karuppagounder, S. S., Yang, A. J., & Kumar, A. (2022). Curcumin-mediated mitochondrial regulation. *Biomedicine & Pharmacotherapy, 147*, 112664.

Karchegani, A. M., Tavakoli, M., Pourtahari, M., & Navarro Valverde, F. A. (2025). Analyzing the Effectiveness of Decision-Making Styles in the Preparation and Implementation of Rural Hadi Projects in Iran. Rural Development Strategies, 12(1).

Kerr, C. L., Adamo, D. E., & Li, H. (2021). Aging, mitochondria, and neuronal energy metabolism. *Gerontology, 67*(3), 308–321.

López-Otín, C., Blasco, M. A., & Partridge, L. (2023). The hallmarks of aging. *Cell, 186*(1), 1–22.

Maillard, F., Rousset, S., & Pereira, B. (2018). HIIT improves antioxidant defense in aging models. *Journal of Gerontology: Biological Sciences*, 73(11), 1441–1447.

Mattson, M. P., & Arumugam, T. V. (2018). Brain aging mechanisms. *Nature Reviews Neuroscience, 19*(10), 573–583.

Mhillaj, E., Cuomo, C., & Trabace, L. (2019). Curcumin and neuroinflammation. *Pharmaceutics, 11*(7), 325.

Paula, P. C., Silva, L. B., & Moreira, E. L. (2020). Exercise and hippocampal plasticity. *Brain Research Bulletin, 157*, 122–131.

Powers, S. K., Talbert, E. E., & Adhihetty, P. J. (2020). Oxidative stress and mitochondrial dysfunction. *Physiological Reviews, 100*(4), 1523–1617.

Qin, L., Vázquez-Sánchez, S., & Castellano, A. (2022). Mitochondrial redox regulation in neuronal aging. *Antioxidants, 11*(8), 1502.

Reljic, D., Frenk, Y., & Herrmann, H. J. (2025). HIIT and antioxidant gene expression. *Physiological Reports, 13*(1), e15876.

Sahoo, D. K., Roy, A., & Chainy, G. B. (2016). Age-related changes in GSH metabolism. *Biogerontology, 17*(1), 185–198.

Small, G. W., Siddarth, P., & Li, Z. (2021). Curcumin and brain health. *American Journal of Geriatric Psychiatry, 29*(4), 1–12.

Sultana, R., & Butterfield, D. A. (2018). Lipid peroxidation in neurodegeneration. *Oxidative Medicine and Cellular Longevity, 2018*, 3271670.

Sun, N., Youle, R. J., & Finkel, T. (2020). Mitophagy in aging and disease. *Nature Reviews Molecular Cell Biology, 21*(7), 381–397.

Tian, L., Cai, Q., & Wei, H. (2023). HIIT and SIRT3 activation in neurons. *Brain Research, 1813*, 147028.

Wang, X., Wang, Y., & Xia, Z. (2023). Curcumin and mitochondrial GSH regulation. *Neuroscience Letters, 792*, 136965.

Wei, L., Hou, X., & Liu, J. (2021). Exercise-induced mitochondrial biogenesis. *Molecular Neurobiology, 58*(3), 1234–1247.

Wu, J., Zhang, Y., & Li, B. (2022). Antioxidant responses of aged hippocampal neurons. *Journal of Molecular Neuroscience, 72*(1), 66–79.

Xie, L., Kang, K., & Xu, P. (2021). Anti-inflammatory effects of curcumin in the hippocampus. *Journal of Neuroimmunology, 355*, 577601.

Yin, F., Sancheti, H., & Cadenas, E. (2016). Mitochondrial redox imbalance in aging. *Aging Cell, 15*(2), 332–339.

Zhang, J., Wang, Z., & Zhang, Y. (2020). HIIT and hippocampal antioxidant responses. *Neuroscience, 431*, 160–172.

Zhao, Y., Wang, X., & Chen, M. (2022). Polyphenols and mitochondrial protection. *Free Radical Research, 56*(2), 155–169.

* Corresponding Author: r_farzizadeh@uma.ac.ir.
How to Cite: Farzizadeh, R; Zarezadeh, N & Varcheney, B. (2024). Synergistic Effects of Curcumin Supplementation and High-Intensity Interval Training on Mitochondrial Antioxidant Enzymes in the Hippocampus of Aged Rats, *Journal of New Approaches in Exercise Physiology*, 6(12), 312-340.
DOI: 10.22054/nass.2025.90197.1222