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Original Article

The effect of eight weeks of high-intensity interval training and curcumin supplementation on salivary 8-hydroxydeoxyguanosine and glutathione peroxidase levels in overweight women

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Abstract

Background and aims: Oxidative stress, a significant contributor to numerous diseases, including obesity, is explicitly targeted by high-intensity interval training (HIIT) and curcumin supplementation due to its antioxidant properties. This study aimed to investigate the effects of eight weeks of HIIT and curcumin supplementation on the salivary levels of 8-hydroxydeoxyguanosine (8-OHdG) and glutathione peroxidase (GPx) in overweight women.

Methods: Forty-eight overweight women were randomly assigned to four groups: HIIT, HIIT + curcumin, curcumin, and placebo. The HIIT groups performed three sessions of HIIT per week for eight weeks, while the curcumin groups received 80 mg of curcumin per day for eight weeks. Salivary 8-OHdG and GPx levels were measured at baseline and after eight weeks. One-way analysis of variance with repeated measures was used to assess both interactive and between-group changes.

Results: The results indicated that the interaction effect of the time × group was statistically significant for salivary 8-OHdG ($P = 0.001$) but not for the salivary GPx ($P = 0.054$). Salivary 8-OHdG and GPx levels showed significant decreases and increases, respectively, in the supplement ($P = 0.001$), exercise + supplement ($P = 0.017$), and exercise + placebo ($P = 0.001$) groups.

Conclusion: HIIT, along with curcumin supplementation, can reduce oxidative stress levels and effectively reduce inflammatory status and weight loss in overweight women.

Keywords: High-intensity interval training, Curcumin, 8-hydroxydeoxyguanosine, Glutathione peroxidase

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Introduction

Obesity is characterized by a state of low-grade inflammation known as "inflammation" (1). Individuals with obesity have impaired regulation of fatty acid oxidation and lipid peroxidation (2), resulting in increased markers of oxidative stress, heightened inflammation, and a suppressed antioxidant defense system (3). Obese individuals are even more susceptible to oxidative damage due to a deficiency in crucial antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), vitamin A, vitamin E, vitamin C, and β-carotene (4). However, it is crucial to find ways to restore the balance of antioxidants to prevent and treat diseases linked to oxidative stress. Adopting appropriate lifestyle habits such as regular physical activity is essential for maintaining health and preventing chronic diseases. For instance, moderate-intensity aerobic exercise improves the body's biochemical adaptability and antioxidant defense system while reducing lipid peroxidation levels in adults (5).

However, evidence suggests that high-intensity exercise can increase the production of reactive oxygen species (ROS), deplete endogenous antioxidant resources, and cause oxidative damage to biological macromolecules such as lipids, proteins, and nucleic acids (6). This type of exercise can ultimately lead to severe oxidative damage to proteins, lipids, and genomic structures (7).

Guanine is more prone to oxidation than other nucleic acid bases because it has a lower oxidation-reduction potential and is exposed to hydroxyl radicals. One of the most common oxidative base lesions is 8-hydroxydeoxyguanosine (8-OHdG) (8). Levels of 8-OHdG are used to evaluate DNA damage and cytotoxicity resulting from oxidative stress. Numerous studies have demonstrated elevated levels of 8-OHdG in body fluids such as urine, plasma, and saliva in the presence of chronic inflammatory conditions (9). Several factors, including exercise training and changes in body mass index, can influence levels of 8-OHdG and 8-oxo guanine DNA glycosylase (10,11). Additionally, the combination of exercise training and the use of herbs and supplements has always attracted interest in obtaining additional benefits. It has been demonstrated that antioxidant supplements can reduce oxidative stress and the risk of obesity-related

complications while restoring adipokine expression (12). Many plants contain polyphenolic compounds known for their powerful antioxidant activity, which can help reduce complications associated with metabolic syndrome. Curcumin, derived from *Curcuma longa*, is one such compound. It exhibits strong antioxidant properties by scavenging reactive oxygen species such as superoxide anion radicals, hydroxyl radicals (13), and nitrogen dioxide radicals (14). These antioxidant effects contribute to the protective properties of curcumin. Research has indicated that curcumin plays a crucial role in combating diseases caused by oxidative stress, including diabetes, obesity, and cardiovascular diseases (15). However, the exact mechanism by which curcumin reduces oxidative stress is not yet fully understood.

The impact of exercise and drug supplementation on inflammatory status and oxidative stress, as well as their overall effectiveness, has been investigated. Nevertheless, existing studies have not provided comprehensive information on the most effective training conditions. To summarize, the available research on the effect of high-intensity interval training (HIIT) on salivary 8-OHdG and GPx levels in overweight women is limited and inconsistent. Given the importance of exercise training in improving oxidative stress indices in overweight women and the lack of studies exploring the combined effect of curcumin supplementation with HIIT in this population, this study sought to investigate the effect of HIIT and curcumin supplementation on salivary 8-OHdG and GPx levels in overweight women.

Materials and Methods

Study stages

This quasi-experimental research was conducted in Neyshabur in 2023, utilizing a pre-test and post-test design with four comparison groups. The statistical population consisted of overweight women who met specific inclusion criteria: no orthopedic or neurological restrictions, no tobacco use, no athletic background or regular physical activity, and no chronic diseases such as cardiovascular, kidney, or diabetes. The study included healthy overweight women with a body mass index (BMI) between 25 and 29.9 kg/m2. Exclusion criteria included failure to attend at least two training sessions, neuromuscular inability to perform exercise training, a history of smoking, and not taking the supplement for two consecutive meals. Participants voluntarily participated in the study and provided informed consent, fully understanding the study conditions. The sample size was determined using G.Power 3.1 software, based on the study by Sahaf et al (16). With an alpha level of 0.05, an effect size of 0.27, and mean changes for 5 units, a statistical power of 0.85 was achieved, yielding a recommended sample size of 48. To account for potential dropouts during the study, 50 participants were voluntarily selected. The participants were randomly assigned into one of four groups: HIIT + placebo (n = 12), HIIT + curcumin supplement (n = 12), curcumin supplement only (n = 12), and placebo only (n = 12). All training sessions were supervised by a certified sports physiologist.

Anthropometric measurements

Height: Height was measured using a stadiometer (Seca, Germany). To measure height, a tape measure was attached to the wall, and subjects stood barefoot with their backs to the wall and the tape, ensuring full posture extension. Then, a ruler, parallel to the ground and tangent to the subject's head, was used to record the height in centimeters.

Weight: Weight was measured using a Seca digital scale. Subjects were instructed to stand on the scale without shoes, wearing light and comfortable clothing. The weight was then recorded by the examiner. BMI was calculated by dividing weight (kg) by the square of the height (m) using equation 1.

$$BMI\ (kg/m^2) = Weight\ (kg) / Height\ (m)^2 \quad \text{Eq. (1)}$$

Waist and hip circumference: Waist and hip circumferences were measured using a non-stretchable tape measure. The waist circumference was measured at the narrowest part of the waist, and the hip circumference was measured at the widest part of the hip. No pressure was applied to the tape during the measurements. The waist-to-hip ratio was calculated by dividing the smallest waist circumference by the largest hip circumference.

Body fat percentage

Body fat percentage and bone density (BD) were measured using a Slim Guide Caliper (USA). The Jackson and Pollock three-point skinfold equation (triceps, supra-iliac, and thigh) and the Siri equation were used to calculate body fat percentage. Equation 2 was applied to calculate bone density, where Z represents the sum of the triceps, thigh, and supra-iliac skinfolds (in millimeters). Fat-free mass was calculated by multiplying body fat percentage by total mass and dividing by 100.

$$BD = 1.0994291 - 0.0009929\ (Z) + 0.0000023\ (Z2) - 0.0001392\ (age)$$

$$\text{Body fat percentage: } [(4.95/BD) - 4.5] \times 100 \quad \text{Eq. (2)}$$

Curcumin supplementation

Prior to the start of supplementation, the subjects received educational materials to control their dietary amount of curcumin to help standardize the diet. This was taken to ensure that the participants' curcumin consumption from their dietary sources remained consistent throughout the study period. During the initial training session, participants were instructed to maintain their daily consumption of turmeric, ginger, curry, and black pepper in meals the same as before. It was emphasized that these items should not be increased in any way. The curcumin

supplementation protocol, manufactured by Elixir Nano Sina Company, was administered to participants in both the exercise + supplement and supplement-only groups. Each participant received 80 mg of curcumin per day with a glass of water before lunch, for eight weeks (seven days per week). In the placebo group, participants received capsules containing powdered milk (17).

Saliva sampling

Saliva sampling was conducted in two stages, before the intervention and after eight weeks. First, 3 mL of unstimulated whole saliva was collected from each participant while seated in a resting position, one day before the start of the research protocol. The sampling conditions required participants to abstain from food and drink for at least 90 minutes prior to sampling and to rinse their mouths with water. Then, for 10 minutes, they emptied their saliva into a test tube every two minutes until the tube was full. The samples were then immediately frozen at -80 ˚C. At the end of the eight-week training program, resampling was performed 48 hours after the final training session, following the same protocol. To assess salivary levels of 8-OHdG and GPx, enzyme-linked immunosorbent assay (ELISA) kits were purchased from Padgin Teb Company, and analyses were conducted using ZellBio kits manufactured in Germany.

High-intensity interval training program

The HIIT program included a 10-minute warm-up and a 10-minute cool-down at the beginning and end of each session. Following the warm-up, participants performed stretching, rhythmic, and aerobic exercises. The program lasted for eight weeks, with three sessions per week. Each session lasted between 20 to 45 minutes and was performed at a constant intensity of 85%-90% of the participants' maximum heart rate. The training volume increased gradually throughout the intervention as follows: 10 sets of 2 minutes in weeks 1 and 2, 11 sets of 2 minutes in weeks 3 and 4, 12 sets of 2 minutes in weeks 5 and 6, and 13 sets of 2 minutes in weeks 7 and 8. Rest intervals between sets were set at 90 seconds (see Table 1). Maximum heart rate was estimated using the formula HRmax = 220 − age. Exercise intensity was continuously monitored using a Polar heart rate monitor (18).

Statistical analysis

All data were analyzed using SPSS version 26, and initial data processing involved raw data. The normality of the data distribution was evaluated using the Shapiro-Wilk test, and the homogeneity of variances between groups was assessed using Levene's test. One-way repeated measures analysis of variance (ANOVA) was then performed to evaluate the interaction effects of time and group. Within-group changes were analyzed using paired sample t-tests. Pairwise comparisons between groups were performed using the Bonferroni post hoc test. A significance level of $P < 0.05$ was used to determine statistical significance.

Results

The characteristics of the subjects in the experimental and placebo groups are presented in Table 2.

Table 3 summarizes the results, demonstrating that the interaction effect of time and group was statistically significant for body weight ($P = 0.001$), BMI ($P = 0.001$), and waist-to-hip ratio ($P = 0.011$). The Bonferroni post hoc test revealed a significant difference in the waist-to-hip ratio only between the placebo and exercise + placebo groups ($P = 0.039$). When comparing the mean values Within-group comparisons revealed significant reductions in body weight in the curcumin supplement, exercise + curcumin, and exercise + placebo groups ($P = 0.001$), with no significant change observed in the placebo group ($P = 0.879$). Similarly, BMI significantly decreased in the curcumin supplement, exercise + curcumin, and exercise + placebo groups ($P = 0.001$), but no significant changes were observed in the placebo group ($P = 0.824$). Waist-to-hip ratio significantly decreased only in the curcumin supplement group ($P = 0.001$), with no significant changes in the exercise + curcumin, exercise + placebo, or placebo groups. The body fat percentage significantly declined in the curcumin supplement, exercise + curcumin, and exercise + placebo groups ($P = 0.001$), whereas no significant change was observed in the placebo group ($P = 0.189$), as depicted in Figures 1-4.

The results presented in Table 4 indicate a statistically significant interaction between time and group for salivary 8-OHdG levels ($P = 0.001$). However, the interaction is not statistically significant for the salivary GPx levels ($P = 0.054$). The Bonferroni post hoc analysis revealed a significant difference in salivary 8-OHdG between the exercise + placebo and placebo groups ($P = 0.036$).

The within-group comparisons showed a significant decrease in salivary 8-OHdG in the supplement group ($P = 0.001$), exercise + supplement group ($P = 0.017$), and exercise + placebo group ($P = 0.001$), whereas the placebo group exhibited a significant increase ($P = 0.003$). In terms of salivary GPx, a significant increase was observed in

Table 1. Intermittent High-Intensity Exercise Protocol for Overweight Women in the Exercise Groups

Weeks	1st	2nd	3rd	4th	5th	6th	7th	8th
Session per week	3	3	3	3	3	3	3	3
Training intensity (HRmax)	85%-90%	85%-90%	85%-90%	85%-90%	85%-90%	85%-90%	85%-90%	85%-90%
Training period (min)	10 × 2	10 × 2	11 × 2	11 × 2	12 × 2	12 × 2	13 × 2	13 × 2

Note. * Active rest time between each set is 90 seconds; HRmax: Maximum heart rate.

Table 2. Characteristics of participants in the experimental and placebo groups (mean ± SD)

Groups	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m²)	WHR (cm)	BFP (%)
Supplement	33.50 ± 1.50	160.35 ± 6.39	70.85 ± 7.43	27.55 ± 2.31	0.86 ± 0.06	36.92 ± 3.38
Exercise + supplement	33.09 ± 2.34	161.72 ± 8.87	79.97 ± 16.28	30.77 ± 6.79	0.82 ± 0.02	37.32 ± 7.63
Exercise + placebo	32.66 ± 2.10	161.91 ± 7.76	76.11 ± 13.32	29.04 ± 4.64	0.85 ± 0.05	36.07 ± 5.22
Placebo	32.81 ± 2.04	164.18 ± 7.02	71.72 ± 8.92	26.75 ± 4.72	0.80 ± 0.05	34.48 ± 4.76
P value	0.793	0.710	0.288	0.240	0.139	0.640

Note. BFP: Body fat percentage; BMI: Body mass index; WHR: Waist–hip ratio.

Table 3. Comparison of within- and between-group mean changes in body composition in overweight women

Variables	Groups	Stages			Within and Between Group Mean Changes			
		Pre-test (Mean ± SD)	8th Week (Mean ± SD)	Changes (%)	*P* value**	Time *P*-value	Group *P* value	Time × Group *P* value
Weight (kg)	Supplement	70.85 ± 7.43	69.60 ± 7.17	-1.79	0.005†	0.001	0.404	0.003†
	Exercise + supplement	79.97 ± 16.28	76.95 ± 16.21	-3.92	0.005†			
	Exercise + placebo	76.11 ± 13.32	74.20 ± 12.26	-2.57	0.001†			
	Placebo	71.72 ± 8.92	71.79 ± 9.92	0.09	0.879			
BMI (kg/m²)	Supplement	27.55 ± 2.31	27.06 ± 2.11	-1.81	0.005†	0.001	0.357	0.002†
	Exercise + supplement	30.77 ± 6.79	29.58 ± 6.62	-4.02	0.004†			
	Exercise + placebo	29.04 ± 4.64	28.31 ± 4.22	-2.57	0.002†			
	Placebo	26.75 ± 4.72	26.79 ± 4.72	0.14	0.824			
WHR	Supplement	0.86 ± 0.06	0.83 ± 0.05	-3.61	0.024†	0.228	0.162	0.011†
	Exercise + supplement	0.82 ± 0.02	0.83 ± 0.03	1.20	0.212			
	Exercise + placebo	0.85 ± 0.05	0.84 ± 0.04	-1.19	0.081			
	Placebo	0.80 ± 0.05	0.81 ± 0.04	1.23	0.400			
Body fat (%)	Supplement	36.92 ± 3.38	34.78 ± 3.66	-6.15	0.001†	0.001	0.812	0.132
	Exercise + supplement	37.32 ± 7.63	33.71 ± 7.59	-10.70	0.004†			
	Exercise + placebo	36.07 ± 5.22	32.88 ± 4.77	-9.70	0.001†			
	Placebo	34.48 ± 4.76	33.17 ± 5.52	-3.94	0.189			

Note. BMI: Body mass index; WHR: Waist–hip ratio; SD: standard deviation. †A significant level $P<0.05$; ** *P* value within the group.

the supplement group ($P=0.005$), exercise + supplement group ($P=0.023$), and exercise + placebo group ($P=0.034$), but no significant changes were observed in the placebo group ($P=0.716$), as illustrated in Figures 5 and 6.

Discussion

Numerous studies have consistently demonstrated that regular physical activity is effective in reducing obesity. In line with these findings, the present study revealed a statistically significant interaction effect between time and group for body weight, BMI, and waist-to-hip ratio following eight weeks of HIIT combined with curcumin supplementation. Significant reductions in body weight, BMI, and body fat percentage were observed across all three groups (curcumin supplement only, exercise + supplement, and exercise + placebo). However, a significant decrease in waist-to-hip ratio was observed only in the curcumin supplement group. Regarding percentage changes in body weight, BMI, and body fat percentage, the exercise + supplement and exercise + placebo groups showed the highest reductions. These findings are consistent with the study by Saadati et al (19). On the other hand, our findings contradict those of Fakhri et al and Mohammadi et al which demonstrated that curcumin supplementation did not impact anthropometric indices (20,21). This disparity between our study and previous research may be due to variations in training duration, number of sessions, and training intensity. While the precise mechanism through which curcumin influences body weight and BMI remains unknown, it is known to regulate the activity of the Janus kinase (JNK) enzyme, which plays a role in the development of obesity (22). Curcumin also inhibits the enzyme 11βHSD1, which activates cortisol, a hormone associated with central obesity (23). Additionally, curcumin prevents adipocyte differentiation at early stages by suppressing the peroxisome proliferator-activated receptor-c (PPAR-c) transcription factor and enhancing AMP-activated protein kinase (AMPK)-mediated lipolysis (23). Furthermore, curcumin supplementation may reduce energy expenditure (22). Another potential mechanism by which curcumin impacts obesity is through its influence on hormonal activity. Meta-analyses suggest that curcumin can lower leptin levels and enhance adiponectin levels, thereby regulating appetite and energy balance. The activation of AMPK through adiponectin

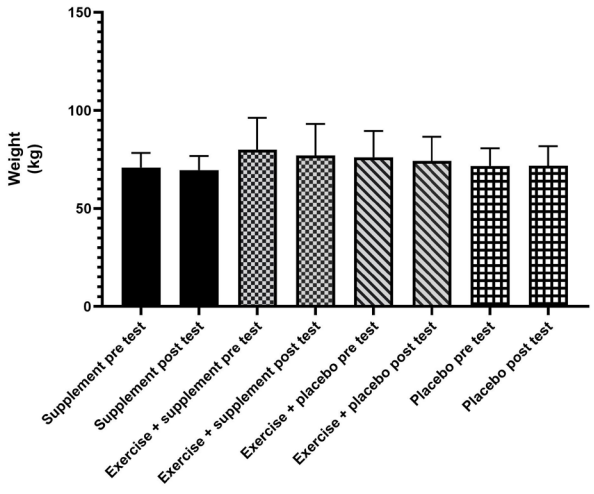

Figure 1. Body weight changes in curcumin, exercise+curcumin, exercise+placebo, and placebo groups

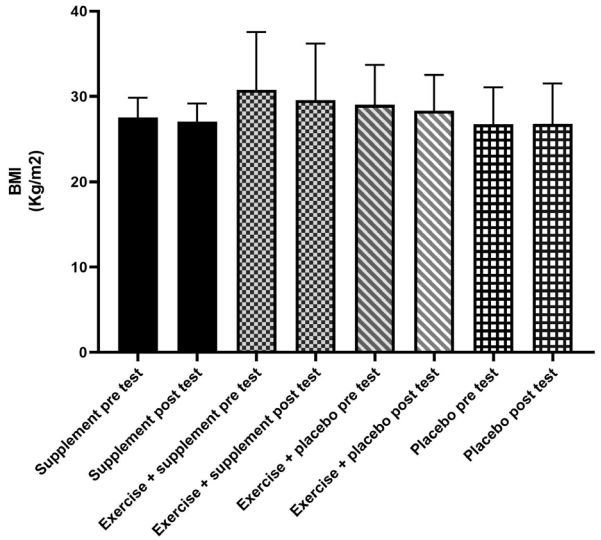

Figure 2. Body mass index changes in curcumin, exercise+curcumin, exercise+placebo, and placebo groups

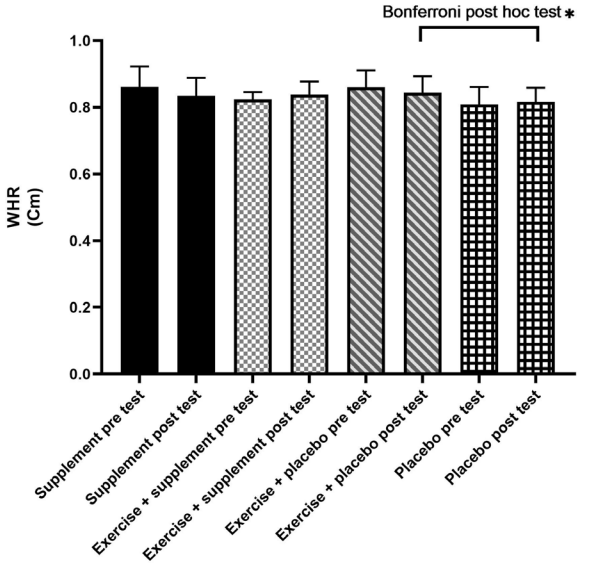

Figure 3. Waist-to-hip ratio changes in curcumin, exercise+curcumin, exercise+placebo, and placebo groups

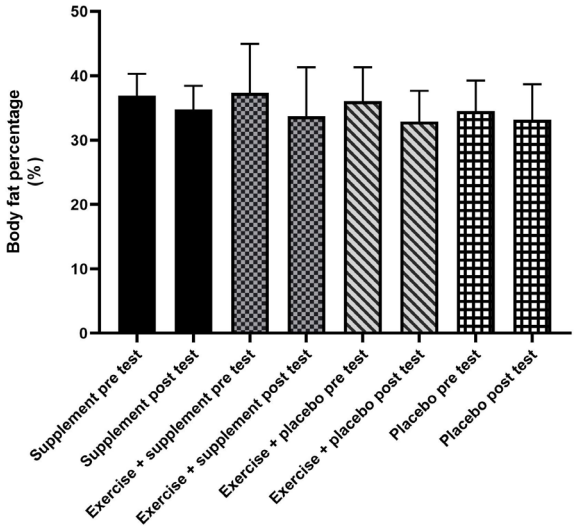

Figure 4. Body fat percentage changes in curcumin, exercise+curcumin, exercise+placebo, and placebo groups

overexpression may further promote weight loss (24).

In this study, significant statistical changes were observed in the interaction of time and group regarding the amount of salivary 8-OHdG after eight weeks of HIIT with curcumin supplementation. The levels of salivary 8-OHdG significantly reduced in all three groups: curcumin supplement, exercise+supplement, and exercise+placebo, after eight weeks of HIIT with curcumin supplementation. These results align with the findings of Hejazi et al (25) but contradict the findings of Afroozi-Gerow et al (10). A review of the literature suggests that exercise training can affect antioxidant enzyme activity, depending on the exercise protocol, volume, and inclusion of rest periods. There is a strong connection between reactive oxygen species generation and exercise intensity. High-intensity aerobic exercise, especially at maximal capacity, often leads to increased anaerobic metabolism and hypoxia. This intense exercise disrupts the balance between free radical production and the body's antioxidant defenses, leading to greater oxidative damage to lipids and proteins and inducing apoptosis. In contrast, moderate-intensity aerobic exercise strengthens antioxidant defenses and decreases oxidative stress (26). These adaptations help protect the body from the negative effects of oxidative stress. Additionally, several weeks of aerobic exercise can decrease markers of lipid and protein oxidative damage, likely due to increased activity of extracellular SOD activity, which enhances the body's antioxidant defenses (27).

The present study investigated the impact of HIIT combined with curcumin supplementation on GPx antioxidant activity. The results displayed a significant effect, with the curcumin supplement group experiencing

Table 4. Comparison of within- and between-group mean changes in oxidative stress in overweight women

Variables	Groups	Stages			Within- and between-group mean changes			
		Pre-test (Mean ± SD)	8th Week (Mean ± SD)	Changes (%)	*P* value**	Time *P* value	Group *P* value	Time × Group *P* value
8-OHdG (ng/mL)	Supplement	0.401 ± 0.074	0.261 ± 0.83	-53.63	0.001†	0.007	0.199	0.001†
	Exercise + supplement	0.372 ± 0.071	0.300 ± 0.055	-24	0.017†			
	Exercise + placebo	0.420 ± 0.054	0.287 ± 0.059	-46.34	0.001†			
	Placebo	0.214 ± 0.094	0.386 ± 0.105	44.55	0.003†			
GPx (ng/mL)	Supplement	0.189 ± 0.015	0.229 ± 0.026	17.46	0.005†	0.001	0.001	0.054
	Exercise + supplement	0.182 ± 0.020	0.200 ± 0.017	9	0.023†			
	Exercise + placebo	0.208 ± 0.018	0.267 ± 0.080	22.09	0.034†			
	Placebo	0.212 ± 0.020	0.215 ± 0.018	1.39	0.716			

Note. 8-OHdG: 8-hydroxydeoxyguanosine; GPx: Glutathione peroxidase; SD: standard deviation. †A significant level $P<0.05$; ** *P* value within the group.

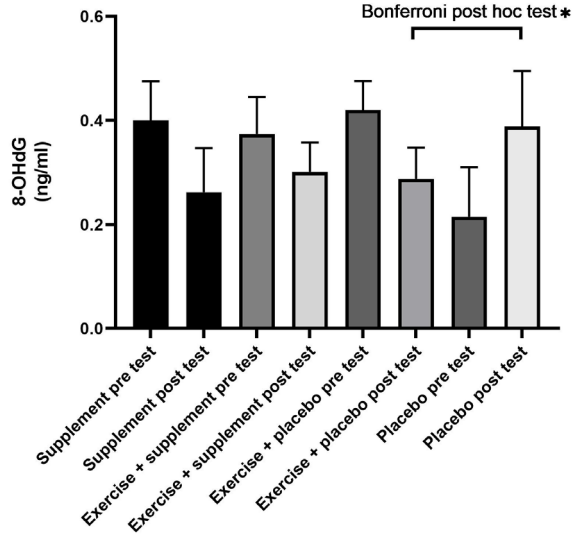

Figure 5. 8-OHdG changes in curcumin, exercise + curcumin, exercise + placebo, and placebo groups. *Note.* 8-OHdG: 8-hydroxydeoxyguanosine

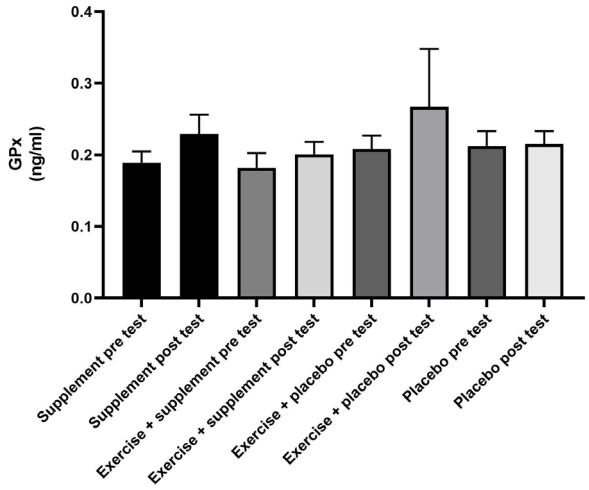

Figure 6. Glutathione peroxidase changes in curcumin, exercise + curcumin, exercise + placebo and placebo groups

a 17.46% increase in GPx activity, a 22.09% increase in the exercise + placebo group, and a 9% increase in the exercise + supplement group. The antioxidant properties of curcumin, including its ability to scavenge free radicals and inhibit oxidative enzymes, make it a valuable compound (28). These findings align with the results of Farzanegi et al (29) but differ from those of Rahimi et al (30).

GPx is a key enzyme involved in inhibiting lipid peroxidation and preventing DNA and RNA damage. Farzanegi et al reported an increase in GPx and enhanced antioxidant defense when combining endurance training with curcumin's antioxidant effect (29). Curcumin extract likely reduces oxidative damage by facilitating the elimination of toxic metabolites and quickly neutralizing lipid peroxide radicals before they can damage the lipid membrane. Similarly, Takahashi et al observed a significant increase in free radical levels in the placebo group compared to pre-exercise values, whereas these levels were lower in the experimental group receiving curcumin supplementation. Furthermore, serum antioxidant concentrations increased immediately after exercise in the curcumin supplementation group, indicating that curcumin can mitigate exercise-induced oxidative stress by enhancing the blood's antioxidant capacity (31). This scavenging effect is likely attributed to the increased activity of both enzymatic and non-enzymatic antioxidants. The study also demonstrated a significant increase in serum antioxidant capacity and glutathione (GSH) concentration following curcumin supplementation (32). The thiol groups in GSH play a crucial role in reducing oxidative stress through their antioxidant effects (33). Hydroxyl groups in curcumin enhance its antioxidant capacity by donating hydrogen to the thiol group of glutathione (34).

However, it is pertinent to acknowledge the limitations of this study, which include the varying nature of participants' diets, diverse responses to physical activity adaptations, a small sample size due to participant dropouts, and individual differences. Additionally, only salivary 8-OHdG and GPx levels were assessed, which means that other oxidative stress markers such as total antioxidant capacity and SOD. Consequently, caution should be exercised when interpreting these findings.

Conclusion

In conclusion, the results of this study suggest that the combined effects of HIIT and curcumin supplementation have a more substantial impact on improving the balance of the antioxidant/oxidant system and mitigating oxidative stress compared to either intervention alone. Consequently, it is recommended that overweight women consider incorporating curcumin supplementation alongside HIIT to mitigate oxidative stress and lipid peroxidation.

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Authors' Contributions

Conceptualization: Zahra Barzanooni.
Data curation: Zahra Barzanooni, Keyvan Hejazi.
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Funding acquisition: Keyvan Hejazi.
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Methodology: Zahra Barzanooni.
Project administration: Zahra Barzanooni, Keyvan Hejazi.
Resources: Zahra Barzanooni, Keyvan Hejazi, Amir Hossein Haghighi.
Software: Zahra Barzanooni, Keyvan Hejazi, Amir Hossein Haghighi.
Supervision: Keyvan Hejazi, Amir Hossein Haghighi.
Writing–original draft: Zahra Barzanooni.
Writing–review & editing: Keyvan Hejazi.

Competing Interests

The authors declare no conflict of interests.

Ethical Approval

The study adhered to the principles outlined in the Helsinki Declaration, and the opinions of the Research Ethics Committee were strictly followed throughout the research process. Additionally, the Ethics Committee of Hakim Sabzevari University approved the relevant stages of the experiments with the code IR.HSU.REC.1402.031.

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