

## Research Article

# Muscle and serum antioxidant cross talk following curcumin and light resistance training during strenuous endurance training in male Wistar rats

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## Abstract

It has been proven that strenuous endurance training increases oxidative stress in body. This study investigated the effects of curcumin supplementation and light resistance training during 8 weeks of endurance training on muscle and serum antioxidant capacity and lipid peroxidation of male wistar rats. 44 male Wistar rats (weight: 254.31±17.72 g and age: 8 weeks) were randomly divided to 6 groups; Control (n=6, sham), Curcumin (n=6), Endurance (n=8), Endurance-curcumin (n=8), Endurance-Resistance (n=8), and Endurance-curcumin-resistance (n=8). Endurance training performed on rodent treadmill for 8 weeks and 5 sessions a week. The speed and duration of running were 10 m/min and 30 min at first week. The intensity and duration reached to the 35 m/min and 70 min up to the last week. Resistance training (8 weeks, 2 sessions / week) performed on vertical ladder (with 30-70% BW). The animals received curcumin supplement by sub peritoneal injection (8 weeks, 3 sessions / week, 30 mg/kg.Bw). Superoxide dismutase (SOD) enzyme activity was measured by Elisa kit and Malondialdehyde (MDA) was measured by the thiobarbituric acid reactive substances (TBARS). The results of this study showed that strenuous endurance training ( $p < 0.05$ ) reduces the serum levels of SOD significantly, and caused a significant increase in the lipid peroxidation (MDA in muscle and serum). Curcumin supplementation and light resistance training could increase antioxidant enzymes activity (SOD) and decrease the MDA levels. The prolonged strenuous endurance training can induce oxidative stress and curcumin supplementation along with light resistance training could restore antioxidant enzymes activity and decrease the MDA levels.

**Key Words:** Resistance training, Endurance training, Curcumin, Antioxidant, Malondialdehyde, Muscle, Cross talk

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## Introduction

Exercise training can induce an imbalance between reactive oxygen species (ROS) and antioxidants, which is referred to as oxidative stress (Thirumalai, Therasa, Elumalai, & David, 2011). Reactive species and free radicals are molecules that, due to their molecular instability (e.g., unpaired electron), promote oxidation reactions with other molecules, such as proteins, lipids, and DNA, in order to become stabilized (Gomes, Silva, & Oliveira, 2012). Some studies show that high-volume endurance training usually used by elite athletes may reduce the efficiency of the antioxidant system, increases free radicals and finally induces oxidative stress (Teixeira, Valente, Casal, Marques, & Moreira, 2009). Energy requirements during exercise result in increased oxygen consumption and oxygen availability to active tissues and in turn increases the production of reactive oxygen species (Lee, 2000; Morillas-Ruiz et al., 2005). Previous research reported an increase of 10–15-fold in the rate of whole body oxygen consumption following strenuous exercise training (Sen, 1995). Uncontrolled generation of intracellular reactive oxygen species causes oxidative stress (Afzalpour, Gharakhanlou, Gaeini, Mohebi, & Hedayati, 2006; Powers & Jackson, 2008). The oxidative stress is an imbalance between the generation of ROS and the antioxidant defense capacity of the body (Goodarzi & KHosravi, 2013).

Malondialdehyde (MDA) is secondary product of lipid peroxidation that can be measured as an index of oxidative stress. Increased level of MDA indicates an increased lipid peroxidation level and cell membrane damage (Chang & Chuang, 2010). Lipid peroxidation of cell membranes changes membrane integrity, leads to increased swelling, and reduces the ability of the cell to maintain ion gradients (Goodarzi & KHosravi, 2013). Lipid peroxidation is commonly quantified in research studies by measuring the accumulation of the by-products resulting from this process. One of these by-products is MDA (Kerksick & Willoughby, 2005). High-intensity exercises induced more lipid peroxidation compared to moderate- or low-intensity exercises (Balci, Pepe, Güneş, Özer, & Revan, 2012).

Under normal conditions, there is also a natural defense system provided by several enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) which performs a vital role for detoxification of free radicals (Akpınar, Yargıoğlu, Derin, Alicigüzel, & Agar, 2008). SOD, CAT, and GPx provide the primary defense against ROS generated during exercise, and activities of these enzymes are known to increase in response to exercise in both animal and human studies (Thirumalai et al., 2011). Antioxidants counters oxidation reactions to stabilize and neutralize harmful effects of free radicals. Free radicals are unpaired electrons that have a strong tendency to react with other molecules (Lamina, Ezema, Theresa, & Anthonia, 2013). Antioxidant enzymes may be activated selectively during an acute bout of strenuous exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity (Thirumalai et al., 2011).

There is much evidence that the consumption of plant foods, such as fruits, vegetables and spices, provides protection against various diseases (Kettawan, Wongsansri, Chompoonong, & Rungruang, 2012). This protection can be explained by the free-radical scavenging capacity of antioxidants (Kettawan et al., 2012). Plant foods are a good source of polyphenols, which have been reported to decrease oxidative stress and inhibit lipid peroxidation (Sonwa & König, 2001).

Curcumin is a phenolic compound derived from root stocks of the rhizome *Curcuma longa*, the yellow pigment used in cooking. Curcumin has been used pharmacologically in traditional Chinese medicine for centuries (Liu et al., 2014). Curcumin has been shown to be a potent scavenger of a variety of ROS including O<sub>2</sub><sup>-</sup>, OH<sup>-</sup>, nitrogen dioxide radicals and non-free radical, such as H<sub>2</sub>O<sub>2</sub>. It has also been shown to enhance the activity of antioxidant enzymes and counteract the activity of ROS generating enzymes in different tissues (Ali Gorzi & Ekradi, 2020; Ali Gorzi, Ekradi, & Rahmani, 2018; Tofigi, Gorzi, & Amin, 2017).

It is believed that regular and moderate exercise improves the body's antioxidant status and decreases production of free radicals in the body, in this way, cell damage can be controlled (Halle, Berg, Baumstark, & Keul, 1999). Some studies have shown that both aerobic and anaerobic exercise of low to moderate intensity and duration can increase the activity of antioxidant enzymes, reduce oxidative stress and promote antioxidant capacity of organisms (Ali Gorzi, Ekradi, et al., 2018). Therefore, the purpose of this study was to investigate the effects of curcumin supplementation and light resistance training during 8 weeks of strenuous endurance training on antioxidant capacity and lipid peroxidation of male Wistar rats.

## Materials and Methods

## Animal

Forty-four male wistar rats (with 8 weeks old and 254.31±17.72 gr body weight) were provided from Pasteur Institute, Tehran, Iran. All animals were kept in polycarbonate cages (5 rats in each cage), and room temperature (22±1.4°C), light-dark cycle (12:12), humidity (40-50%), and ad libitum water and food were controlled. The animals became familiarized with training protocol for 1 week and then they were randomly divided to six groups; control (Con; n=6), Curcumin (Cur; n=6), endurance (End; n=8), endurance-curcumin (End-Cur; n=8), endurance-resistance (End-Res; n=8) and endurance-curcumin-resistance (End-Cur-Res; n=8) (Table 1).

## Exercise protocols

### Endurance training protocol

Endurance training protocol consisted of 8 weeks (5 session/week) of running on a motorized rodent treadmill (Pishro Andishe Sanat Comp, Iran) (Ali Gorzi & Asadi, 2020; Joo, Sone, Fukunaga, Lim, & Onodera, 2003). Exercise training program started with speed of 10 m/min for 30 min at the first week and increased incrementally (every week) to 35 m/min for 70 min (equal to 80-85% of Vo<sub>2</sub>max) at last week (Table 2). An under loading week was designed at week 5 for nonlinear loading to preventing from overtraining. Animals were weighed prior to each training session (Ali Gorzi & Ekradi, 2020). Also, before starting exercise training in each session, rats carried out a 5 minute of warm-up with speed of 8 m/min and a 5-minute, cool-down with speed 6 m/min at the end of the exercise session. Control group (Sham group) experienced the same experimental condition as the treatment groups, with the exception of running, resistance training and curcumin supplementation. Exercise training protocol was performed in the animal house of University of Zanjan, Iran.

**Table 1. The initial and final weight of animals in different groups of studies**

Groups	N	Initial weight	Final weight	Weight changes
Control	6	255.67±20.43	357.33±29.35	+101.66
Curcumin	6	253.83±29.30	322.17±51.13	+68.34
Endurance	8	252.22±13.07	279.67±21.14	+27.45
Endurance-Curcumin	8	256.11±19.30	282.89±28.58	+26.78
Endurance-Resistance	8	251.30±14.73	296.33±31.11	+45.03
Endurance-Curcumin-Resistance	8	256.78±9.54	300.33±19.46	+43.55
Total	44	254.31±17.72	306.45±30.12	+52.14

Table 2. Strenuous endurance training and light resistance training protocols

Type of training/Weeks		1	2	3	4	5	6	7	8
Endurance	Duration (min)	30	40	45	50	40	60	70	70
	Speed (m/min)	10	20	20	25	15	30	30	35
Resistance	Load (percent of body weight)	30	35-40	40-45	45-50	35-40	50-55	60-65	70

### Resistance training protocol

Resistance training protocol consisted of 8 weeks (2 session/week) climbing on a 26 stairs ladder (designed by the researcher) with load which was suspended from the tail. Load was equal to the 30% of the animal body weight at first week, which was incrementally increased and reached to the 70% of body weight at last week (Table 2). Resistance training included 3 sets of 4 reps with 3 min rest between sets and 10-20 second between repetition (Banaeifar et al., 2011).

### Curcumin supplementation

In order to prepare the solution of curcumin, 1 gram of curcumin powder provided from Merk Company (Germany) was mixed with 1 cc of pure alcohol and then diluted with ethylolate solvent (Merk Company-Germany) to reach to 100 cc solution and then was injected sub peritoneal (30 mg/kg of rat's body weight) (Gorzi, Asadi, Voltarelli, & Shamsi, 2021; Gorzi, Tofighi, & Amiri, 2018) for 3 days a week (Every alternate day- Sun, Tues and Thurs) in curcumin, endurance-curcumin and endurance-curcumin-resistance groups.

### Biochemical measurements

Forty-eight hours after the last training session, animals were anesthetized with ketamine (30-50 mg/kg of BW) and xylazine (3-5 mg/kg of BW). Then blood was taken from the heart of rats. Blood centrifuged at 3000 rpm for 10 min at 4°C and serum stored at -20°C until laboratory assessment. Serum was used for measuring the activity of serum antioxidant capacity (SOD) and Malondialdehyde (MDA). Moreover, soleus muscles of rat were immediately excised under the sterilized situation and stored at -80o C for lab analysis.

SOD activity was assayed by miller and et al. (1993) method using SOD Elisa kit (Rat SOD ELISA kit, randox made in UK) (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993) and the results were expressed as unit per mg protein and µl/ml in muscle and serum, respectively. MDA levels were assessed utilizing the thiobarbituric acid reactive substances (TBARS) method by Kaya et al. (2004). The concentration of thiobarbituric acid reactive substances was measured at 532 nm using a standard curve of malondialdehyde and the results were expressed as nmol MDA/mg protein (Kaya et al., 2004).

### Statistical analysis

After ensuring of the normality of data with Shapiro-Wilk test, means and standard deviation (SD) were determined for all data, and one-way analysis of variance (ANOVA) followed by Tukey's post hoc test were used for pairwise comparisons. SPSS version 20.00 was used for statistical analysis and statistical significance was set at P<0.05.

## Results

### SOD

The results of ANOVA demonstrated no significant effect of curcumin supplementation in conjunction with light resistance tra-

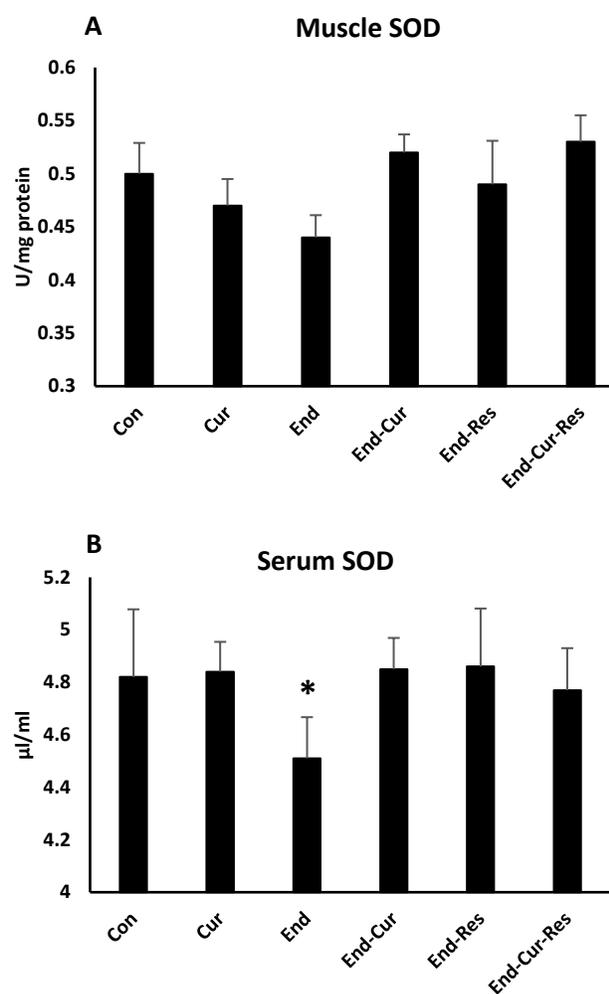


Figure 1. Muscle and serum levels of SOD enzyme in different groups of study (A & B). \* Significantly lower than all other groups (P<0.05).

-ining on SOD activity in the soleus muscles of rats ( $p>0.05$ ) (Figure 1- A).

However, the results demonstrated a significant effect of curcumin supplementation along with light resistance training on SOD activity in the serums of rats (Figure 1 B). After 8 weeks, compared with the control group ( $4.82\pm 0.258$ ), SOD activity of the endurance group ( $4.51\pm 0.157$ ) decreased significantly ( $p=0.027$ ). Also in comparison with endurance group SOD activity of the End-Cur ( $4.85\pm 0.119$ ;  $p=0.004$ ) and End-Res ( $4.77\pm 0.160$ ;  $P=0.048$ ) groups were significantly higher. There were no significant differences between End-Cur and End-Res ( $4.86\pm 0.221$ ) groups with End-Cur-Res group ( $p>0.05$ ).

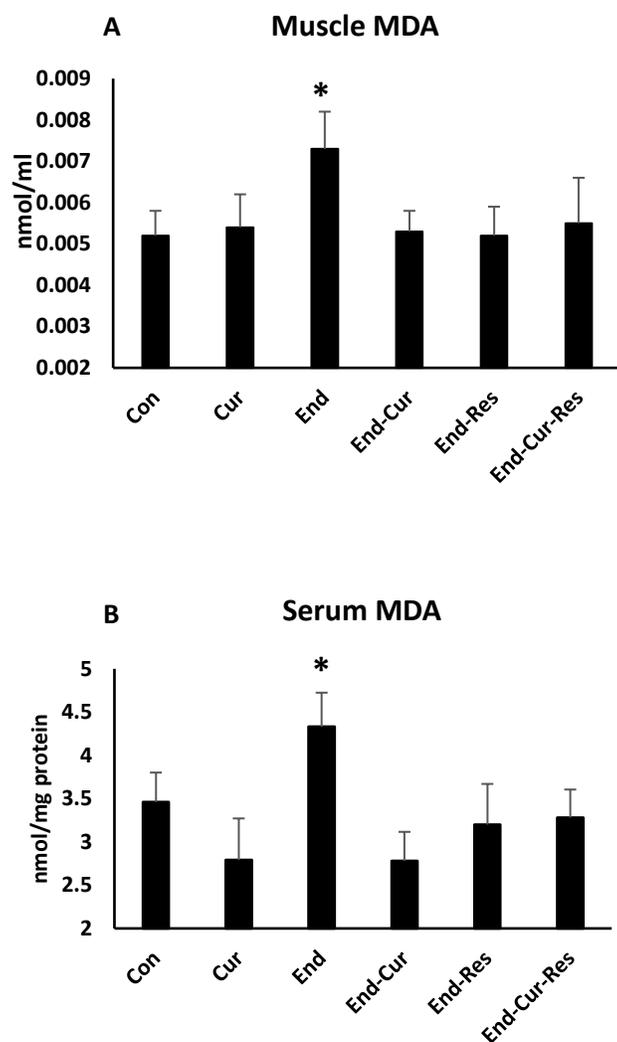


Figure 2. Muscle and serum levels of MDA enzyme in different groups of study (A & B). \* Significantly higher than all other groups ( $P<0.05$ ).

## MDA

Muscle and serum levels of MDA in endurance group were significantly higher than those in the control group ( $p<0.05$ ). The results demonstrated the negative effect of curcumin supplementation on MDA levels of muscle and serum (Figure 2 A & B). Muscle and serum levels of MDA in End-cur, End-Res and End-Cur-Res groups were significantly lower than those in the endurance group ( $p<0.05$ ). Moreover, there were no significant differences among End-Cur, End-Res and End-Cur-Res groups ( $p=0.255$ ), and between Cur group and control groups ( $p=0.240$ ).

## Discussion

The result of this study demonstrated that strenuous endurance training induces the oxidative stress and overwhelm the antioxidant capacity. Thirumalai et al. (2011) in their study on male Wistar rats showed that prolonged strenuous exercise program (swimming) reduces the activity of antioxidant enzymes SOD (-51.78 percent), CAT (-37.69 percent), GPX(-37.54 percent) and GST(-58.94 percent) (Thirumalai et al., 2011). Consistent with the results of this study, Olah et al. (2021) showed that balanced intense exercise training induces atrial oxidative stress counterbalanced by the antioxidant system and atrial hypertrophy that is not associated with pathological remodeling or Arrhythmogenicity. Therefore, strenuous endurance exercise reduces the efficiency of the antioxidant system and increases the production of free radicals, and finally, it creates oxidative stress, which causes oxidation of lipids, proteins and nucleic acids (Teixeira et al., 2009).

Silva et al. (2013) showed that SOD and CAT antioxidant enzymes activity in serum and muscle increased after aerobic exercise with a speed of 13-16 meters per minute (Silva et al., 2013). The difference observed between the results of Silva with our results is probably due to the higher intensity of training used in our study. Then, there is a delicate cut point for volume, intensity and loading in designing training programs for elite athletes (Ali Gorzi & Asadi, 2020).

Our results showed that curcumin with increasing SOD and decreasing MDA could reduce lipid peroxidation and prevent exercise-induced oxidative damage. In comparison with the endurance group, SOD activity of serum in endurance-curcumin and endurance-curcumin-resistance groups were significantly higher, and MDA levels of serum and muscle were significantly lower. It seems that there is a cross talk between local muscle and systemic serum levels of oxidant to antioxidant balance. It has been shown that curcumin supplementation could prevent oxidative stress. Dabidi et al. (2013) showed that curcumin decreased MDA in rat brain (Dabidi, Hosseinzadeh, Mahjoub, H-

-osseinzadeh, & Myers, 2013). Also, Samy et al. (2014) showed that curcumin increased the activity of the SOD enzyme (Samy Ali Hussein Aziza, 2014). As reported in other studies, curcumin has a protective and compensating effect by increasing the activity of antioxidant enzymes (Samy Ali Hussein Aziza, 2014).

Interestingly, the results of this study showed that curcumin supplementation could prevent from such extra oxidative stress and this effect was highlighted by adding 2 sessions per weeks of light resistance training as a beneficial mode of training. While, the general belief is that increasing the volume of training induces more physiological stress and leads to dysfunction of antioxidant defense system, based on our results, after 8 weeks, SOD activities of serum were significantly higher in the control, curcumin, endurance-curcumin and endurance-curcumin-resistance groups compared with the endurance group. On the other hand, MDA activities of serum in the control, curcumin, Endurance-curcumin, and Endurance-curcumin-Resistance groups were significantly lower compared with the endurance group. According to Figures 1 and 2, the lack of sharp increase in SOD activity and decrease in MDA level in endurance-curcumin-resistance group was probably due to the high load of resistance training (70% in last weeks). Therefore, SOD activity and MDA levels of serum didn't change in the endurance-curcumin-resistance group compared to the endurance-curcumin group but showed a significant difference with the endurance group. It seems that if instead of %70 of resistance training, we had used 50-60% of loading in last weeks, we could have obtained larger decrease of MDA and increase of SOD activity in End-Cur-Res group.

Güzel et al. (2007) examined the effect of two different programs of strength training with high and low intensity on oxidative stress and showed that MDA levels were lower in those who had low intensity resistance training (Güzel, Hazar, & Erbas, 2007). After 8 weeks, SOD enzyme activity and MDA levels of the End-Cur and End-Cur-Res groups were not significantly different compared with the control group. As a result, curcumin supplementation and curcumin combined with light resistance training can prevent the serum SOD activity reduction and lipid peroxidation and returns to normal. Serum SOD enzyme activity and serum MDA level showed no significant difference between the curcumin and control groups. Therefore, it can be concluded that regular curcumin supplementation is not useful for the general population. The results of this study indicated that use of curcumin supplement and curcumin combined with light resistance training can prevent the oxidative stress in highly endurance trained athletes.

## Conclusion

The prolonged strenuous endurance training can induce oxidative stress and curcumin supplementation and light resistance training could restore antioxidant enzymes capacity. Thus, using 2 sessions per week of light resistance training and curcumin supplementation (3 times a week) is recommended for endurance elite athletes.

## What is already known on this subject?

Endurance training induces oxidative stress.

## What this study adds?

Curcumin supplementation and light resistance training prevent oxidative stress.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** We followed ethical guidelines approved by the Institutional Animal Ethics Committee located in the University of Zanjan and laboratory conditions were provided according to the university guidelines for caring.

**Informed consent** Not applicable.

## Author contributions

Conceptualization: A.G., F.H.; Methodology: A.G., F.H.; Software: A.G.; Validation: F.H.; Formal analysis: A.G.; Investigation: A.G.; Resources: F.H.; Data curation: F.H.; Writing-original draft: A.G., F.H.; Writing - review & editing: A.G., F.H.; Visualization: A.G.; Supervision: A.G.; Project administration: A.G.; Funding acquisition: F.H.

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