High Intensity Interval Training Is More Beneficial to Regulate Free Radicals and mtDNA Oxidative Damage in Aged Rats.

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Research Article

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Abstract

Background: The aim of this study was to compare the effect of 12 weeks of high intensity interval training (HIIT) and continues training (CT) on SIRT3 protein level, FOXO3 gene expression, and mtDNA oxidative damage in gastrocnemius of female aged rats.

Methods and Results: Female wistar rats (18 months, 250-300 g, n=36) were divided into three groups (HIIT, CT, control). Based on aerobic capacity determined using the ramp protocol, HIIT intensity corresponded to 85-90% and CT was 65-70% of VO2max. Rats trained three times per week for 12 weeks. Forty-eight hours after the last training session, rats were sacrificed and gastrocnemius muscle excised. Our results showed that both exercise protocol increased SIRT3 protein content and FOXO3 gene expression compared to control. However, no significant difference was observed between HIIT and CT. In addition, our data showed that MnSOD activity was significantly higher in HIIT compared to both CT and control and CT versus control. Finally, the level of 8-OHdg was significantly lower in HIIT and CT compered to control and there was no significant difference between HIIT and CT.

Conclusion: Our results showed that 12 weeks of HIIT and CT increase SIRT3 protein content, improve antioxidant defense of aged female waster rats. Therfore, HIIT is a time efficient protocol for improving some aspects of mitochondrial function.

Introduction

Aging is associated with decline in adaptation to stress leading to malfunctioning of various organs. This is a multifactorial phenomenon regulated by diverse molecular and cellular events such as genomic instability, cell death, senescence, inflammation, and metabolic dysfunction[1]. Mitochondria are responsible for producing the majority of cellular ATP. Moreover, they are involved in several processes beyond energy production, including apoptosis and calcium and redox regulation[2]. Based on the mitochondrial theory of aging, mitochondrial abnormalities have been implicated in the pathogenesis of musculoskeletal ageing. As a primary source of intracellular ROS, mitochondria are particularly affected, leading to changes in structure and genetic information[3].

Sirtuins are NAD^+-dependent protein deacetylase that regulate adaptive responses to a variety of stresses, including calorie restriction, metabolic stress, and exercise. Sirtuin3 (SIRT3) is localized in the mitochondrial matrix, where it regulates the acetylation of different enzymes and influences almost every major aspect of mitochondrial biology [4, 5]. Decline in SIRT3 content is well-documented with aging[5, 6]. Recent studies have shown that Sirt3 plays a key role in mitochondrial ROS homeostasis. SIRT3 regulates ROS production by directly binding and deacetylating mitochondrial complex I and II[7, 8]. Sirt3 also regulates ROS clearance via changes in SOD2 acetylation-a major mitochondrial antioxidant enzyme [9, 10]. Qui and colleague (2010) reported that SIRT3 reduces cellular ROS via activation of SOD through deacetylation of lysine residues[11]. It is reported that SIRT3 deacetylates FOXO3 to up-regulate MnSOD

and catalase to scavenge ROS[12]. Therefore, MnSOD as a major cellular antioxidant is regulated at several levels by SIRT3 and FOXO3.

Exercise training is a successful strategy to delay age-related decline in various organs. While aging may impair the adaptive response of muscle mitochondria to stress and redox changes, many studies have reported that exercise has strong influence on mitochondrial biogenesis as well as all other mitochondrial aspects in animals and human including ROS production, ATP synthesis, and mitophogy [13]. Several studies have shown positive effects of exercise training on SIRT3 protein content in skeletal muscle in young and aged subjects [14, 15]. However, Edgett and colleagues (2016) showed that two and six weeks of sprint interval training did not alter SIRT3 and PGC-1α protein expression in skeletal muscle [16]. In the context of aging and different exercise training, SIRT3 and its downstream proteins have been less studied. There are numerous studies that show HIIT training is a time-efficient method compere to conventional continuous exercise. In some situations, it has been shown that HIIT is more beneficial. We hypothesized that increasing training intensity with reducing duration could have same positive effect in comparison to prolonged aerobic exercise on mitochondrial ROS regulation. In this study, we aimed to investigated the role of two different types of training (HIIT and continuous) on muscle SIRT3 content, FOXO3 mRNA and markers of oxidative stress aged female rats.

Material And Methods

Animals: All animal procedures were conducted in accordance to ethical guidelines approved by university of Tehran (IR.UT.SPORT.REC.1397.021). Female Wistar rats (n = 36, 18 months) were obtained from Razi Institute (Razi institute, Tehran, Iran). Animal were housed in cages (three/cage) and water and food were *ad libitum*. Animals were kept in 12/12 light-dark cycle at 25^{o}C. One week after acclimation, animals were randomly separated into high intensity interval (HIIT), continuous training (CT), and control (CON) groups. The animals were killed two days after the last exercise session in order to avoid metabolic effects of the final run. Then, gastrocnemius muscles were carefully excised, flash-frozen in liquid nitrogen, and kept in -80^{o}C freezer.

Maximum speed test: A modified ramp protocol in blinded situation was used to determine exercise capacity as previously described[17]. Briefly, after five minutes of warm up at 10m/min on eight-lane motorized treadmill, speed increased 2m/2min until exhaustion (unable to keep running even with mechanical stimuli). The last stage was considered as the individual maximum speed. The maximum speed test was performed every four weeks in order to design training protocols according to the load principle.

Training protocol

HIIT was performed on an eight-lane motorized treadmill using a custom program starting with a 3-minute warm-up at 10m/min followed by four bouts of 2-minutes work/rest intervals. Treadmill speed was adjusted every four weeks to ensure that intensity corresponded to 85–90% of maximum (Table 1).

Continuous training was performed on the same treadmill and inclination. Treadmill speed was adjusted every four weeks so that training intensity corresponded to 65–70% of maximum (Table 2). Each training sessions was followed by a 5-minute cool down.

Immunoblotting

Total protein was extracted from gastrocnemius using RIPA Lysis Buffer including protease inhibitor. Protein content was quantified by using BCA Protein Assay Kit (Kalazist, DB9684#). Equal amounts of protein (25 µg) were loaded onto 12% polyacrylamide gels and separated by SDS-PAGE then transferred onto a PVDF membrane. The blots were blocked with 5% non-fat milk in TBS and incubated overnight at $4^{o}C$ with the appropriate dilution of primary antibodies: anti-Sirt3 (1:1000, Abcam: ab189860). Then, membranes were washed and incubated with secondary antibody (Abcam: ab6721) for 1 hour. B-actin (Abcam: ab8226) was used as an internal control. Blot was imaged by using Bio-Rad ChemiDoc Imagers and images were analyzed by using ImageJ software.

Quantitative real-time PCR

Total RNA was extracted from gastrocnemius using QIAzol Reagent (Qiagen, Germany) according to manufacturer's instruction. cDNA was made using cDNA synthesis kit following manufacturer's instruction (Fermentas, USA). The following PCR steps were used for the experiment: 40 cycles at $94^{o}C$ for 20s, $60^{o}C$ for 30s, and $72^{o}C$ for 30s. Primer sequence of FOXO3 are as following:

Forward: GCAACATGGGCTTGAGTGACTCC

Reverse: TCCAACCCATCGGCATCCATGAG

Relative quantification was performed using SYBR Green, GAPDH as reference gene and $2^{-\Delta\Delta CT}$ method[18]. The following is the primer sequence for GAPDH:

Reverse: CATACTCAGCACCAGCATCACC

Forward: AAGTTCAACGGCACAGTCAAGG

ELISA assay

MnSOD activity and 8-OHdG concentration in gastrocnemius muscle were determined by ELISA according to manufacturer's instruction (Zellbio, Germany).

In order to measure mitochondrial levels of 8-OHdG, mitochondria were isolated as previously described [19].

Statistical analysis

Results are provided as Mean ± SD. One-way ANOVA and ANCOVA were used to analyze study variables and p value < 0.05 was considered statistically significant. All analyzes were done using SPSS software (version 21).

Table 1
12-week of high intensity interval training program

Week	Work Intensity*bout	Active recovery intensity* bout	Time per each bout(min)	Duration (min)
1.	85–90% * 4	45–50% * 4	2	16
2.	85–90% * 4	45–50% * 4	2	16
3.	85–90% * 5	45–50% * 5	2	20
4.	85–90% * 5	45–50% * 5	2	20
5.	85–90% * 5	45–50% * 5	2	20
6.	85–90% * 6	45–50% * 6	2	24
7.	85–90% * 6	45–50% * 6	2	24
8.	85–90% * 6	45–50% * 6	2	24
9.	85–90% * 7	45–50% * 7	2	28
10.	85–90% * 7	45–50% * 7	2	28
11.	85–90% * 7	45–50% * 7	2	28
12.	85–90% * 7	45–50% * 7	2	28

Table 2
12-weeks of continuous training program

Week	Intensity (% VO2max)	Duration (min)
1.	65–70%	30
2.	65–70%	30
3.	65–70%	40
4.	65–70%	40
5.	65–70%	50
6.	65–70%	50
7.	65–70%	50
8.	65–70%	60
9.	65–70%	60
10.	65–70%	60
11.	65–70%	60
12.	65–70%	60

Results

Mean and standard deviation of weight and maximum speed before and after study are provided in Table 3.

Table 3
Weight and maximum speed before and after 12 weeks training

Group	Pre-test Weight (g)	Post Test weight (g)	Pre-test maximum speed (m/min)	Post-test maximum speed (m/min)
HIIT	282.8 ± 17.31	309.3 ± 13.39*	19.2 ± 1.78	23.6 ± 2.60*
CT	277 ± 17.14	297.2 ± 13.65*	20.4 ± 1.67	24.4 ± 2.19*
CON	285.8 ± 20.22	280.42 ± 22.09	18.8 ± 1.09	20 ± 1.67
*Changes are statistically significant				

Exercise capacity (F (2,11) = 53.194, $p < .001$) (maximum speed test) and body weight (F(2,11) = 8.347 $p = 0.006$) were significantly increased following twelve weeks in both HIIT and CT in aged rats (Table 3). Maximum speed was significantly higher in HIIT and CT versus CON (both $p < .001$), with no difference between HIIT and CT ($p = 1.000$) (Fig. 1). Body weight was significantly higher in HIIT and CT versus CON ($p = .007$ and $p = 0.048$ respectively), with no difference between HIIT and CT ($p = .945$) (Fig. 2).

Exercise training increases SIRT3 protein content

SIRT3 protein content in gastrocnemius muscle was significantly higher in both HIIT ($p = .001$) and CT ($p = .006$) compared to CON (Fig. 3a.b). No significant difference was observed between HIIT and CT ($p = .641$).

HIIT increases FOXO3 gene expression

FOXO3 gene expression was significantly higher in HIIT versus Control ($p = .013$), with no difference between HIIT and CT ($p = 0.167$), and CT versus CON ($p = .348$, Fig. 4).

HIIT increases MnSOD activity

MnSOD activity in gastrocnemius was significantly higher in HIIT versus CON ($p = .031$) with no difference between HIIT and CT ($p = .461$), and CT and control ($p = .244$, Fig. 5).

8-OHdG levels in gastrocnemius are lower following HIIT

8-OHdG content in gastrocnemius was significantly lower in HIIT versus Control ($p = .002$), with no difference between HIIT and CT ($p = .137$), and CT and CON ($p = .075$, Fig. 6).

Discussion

In this study, we aimed to investigate effect of HIIT and continuous training on SIRT3, MnSOD activity and mtDNA oxidative damage of aged female wistar rats. Our results showed that 12 weeks of exercise training –both HIIT and CT- improve exercise capacity, SIRT3 protein content, FOXO3a mRNA and decrease 8-OHdG as an indicator of mtDNA oxidative damage.

To the best of our knowledge, this is the first study comparing HIIT versus continuous training on SIRT3, ROS regulation, and mtDNA oxidative damage. We showed that HIIT training -with almost half of the duration of CT- could enhance abovementioned factors even better than continuous training.

As an index of oxidative damage to mtDNA, 8-OHdG in isolated mitochondria of gastrocnemius muscle was significantly lower in HIIT compared to CT and CON. To the best of our knowledge there is no previous study comparing effect of two training protocols on mtDNA oxidative damage. However, Radak and colleagues demonstrated that acute exercise and exercise training both increased the activity of DNA repairing enzyme (OGG1) to a greater extent in young than elderly[20, 21]. SIRT3 deacetylates OGG1 and increases its activity. Hence, we can assume that lower 8-OHdG could be related to SIRT3-induced OGG1 activation. Another possible mechanism of lower 8-OHdG can be increasing mitochondrial quality and quantity following training. It is well-documented that HIIT and CT increase PGC-1α content which directly regulates mitochondrial biogenesis. In another part of our study, Bakhtiary showed that HIIT resulted to greater increase in PGC-1 and Tfam than CT[22].

No precise and clear mechanism is available to rationalize difference in MnSOD activity following HIIT and continuous training. However, a potential reason could be the greater production of ROS in HIIT than continuous during the same period of time. That means removing ROS produced in an intense bout of exercise needs more activity of antioxidant enzymes. Another reason could be FOXO3 mRNA expression that showed an elevation in our study. FOXO3 directly regulates antioxidant gene expression and we observed higher FOXO3 expression in HIIT than CT and control. Hence, the higher MnSOD activity in HIIT is not surprising. On the other hand, our results show a significant effect of HIIT on higher FOXO3 mRNA. Although FOXO3 expression was 30% higher in CT group than control, it was not statistically significant. Jacob et.al showed SIRT3 physically interacts with FOXO3 and forms a complex within the mitochondria which promotes SOD transcription[1]. On the other hand, Tseng et.al showed that SIRT3 regulated expression of FOXO3[12]. The results of our study demonstrate that 12 weeks of HIIT and CT have beneficial effects on SIRT3 and Antioxidant defense. Improvements in mitochondrial function and gene expression in skeletal muscle are a typical response to aerobic training due to increases in PGC1-α and SIRT3, both protein abundance and gene expression.

It is well established that SIRT3 protein is elevated following aerobic exercise training in healthy young humans[14]. However, to the best of our knowledge, this is the first study that compared two types of exercise training in terms of SIRT3 protein level, Foxo3 expression, MnSOD activity, and 8-OHdG among aged rats. However, our results are consistent with several previous studies that have shown that exercise training ameliorates some age-related cellular damage and dysfunction [14, 20, 21, 23, 24]. Aging is an inevitable process accompanied with reduction in SIRT3 protein. As the key regulator of metabolic processes within the mitochondria, SIRT3 is decreasing in aging and it is correlated with many age-related diseases[5, 7]. Our data support our hypothesis suggesting that exercise training leads to higher content of SIRT3 in healthy aged rats or at least prevents from decreasing compared to sedentary control rats. We speculate that the observed changes in SIRT3 content are related to exercise-induced metabolic stress in which increases NAD+/NADH content lead to rising of SIRT3 protein, or mitochondrial biogenesis.

Conclusion

We observed a significant improvement in exercise capacity, increased body weight, and some markers of mitochondrial function in response to 12 weeks of exercise training with the most pronounced effects in HIIT. Since HIIT induced greater adaptations and was almost less than 50% of the time commitment required for CT, we propose that HIIT is a feasible approach to training in old age to improve mitochondrial ROS regulation and exercise capacity. However, generalization of these results to elderly people requires more investigation.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by **Ali Askarian**, Mohammadreza Kordi, and Siroos choobineh. The first draft of the manuscript was written by AbbasAli Gaeini and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

The approval was granted by the ethic committee of faculty of sport sciences at university of Tehran. The reference number for this approval is (IR.UT.SPORT.REC.1397.021).

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Figures

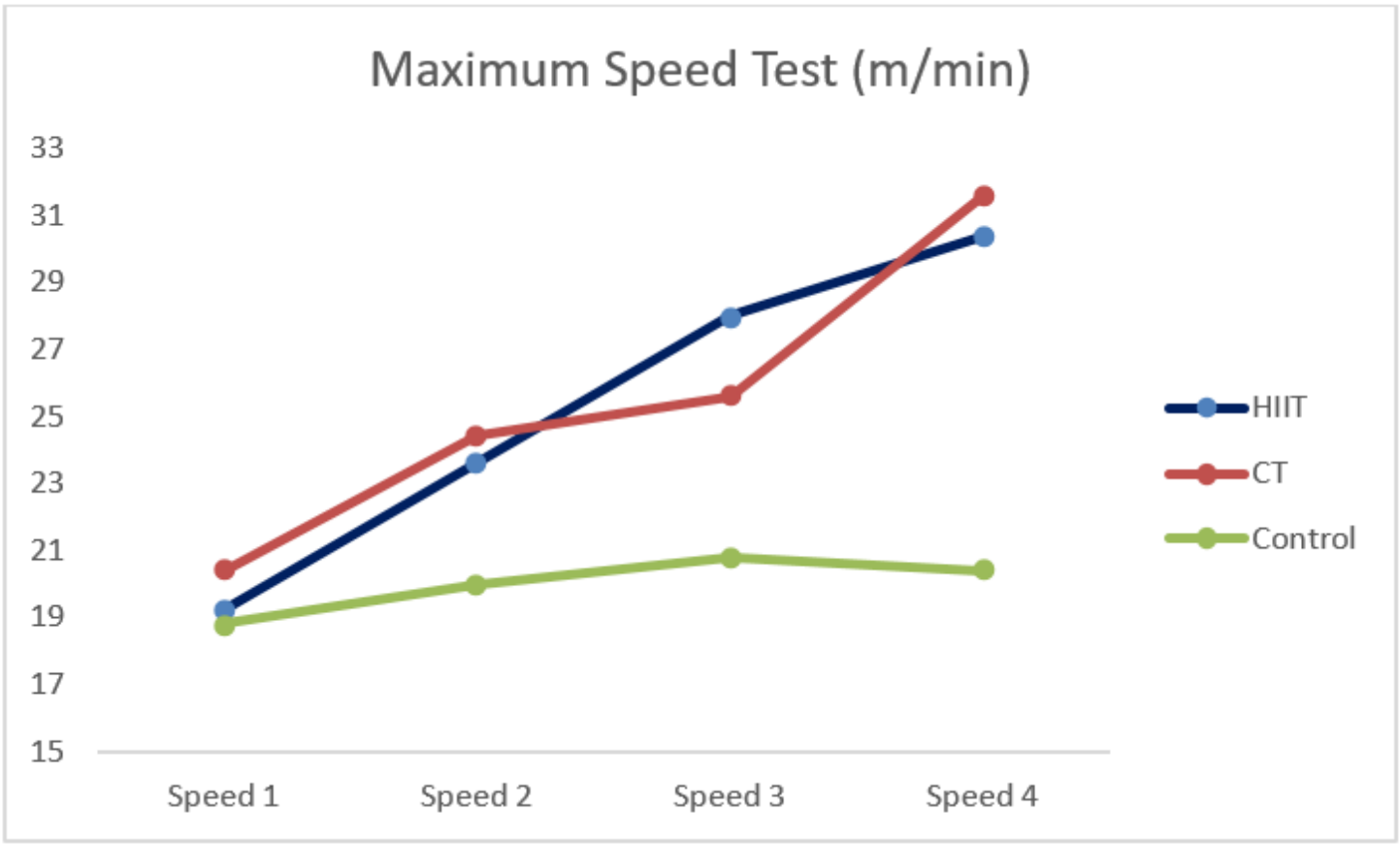


Figure 1

Changes in maximum speed of running obtained from incremental test during 12 weeks of study.

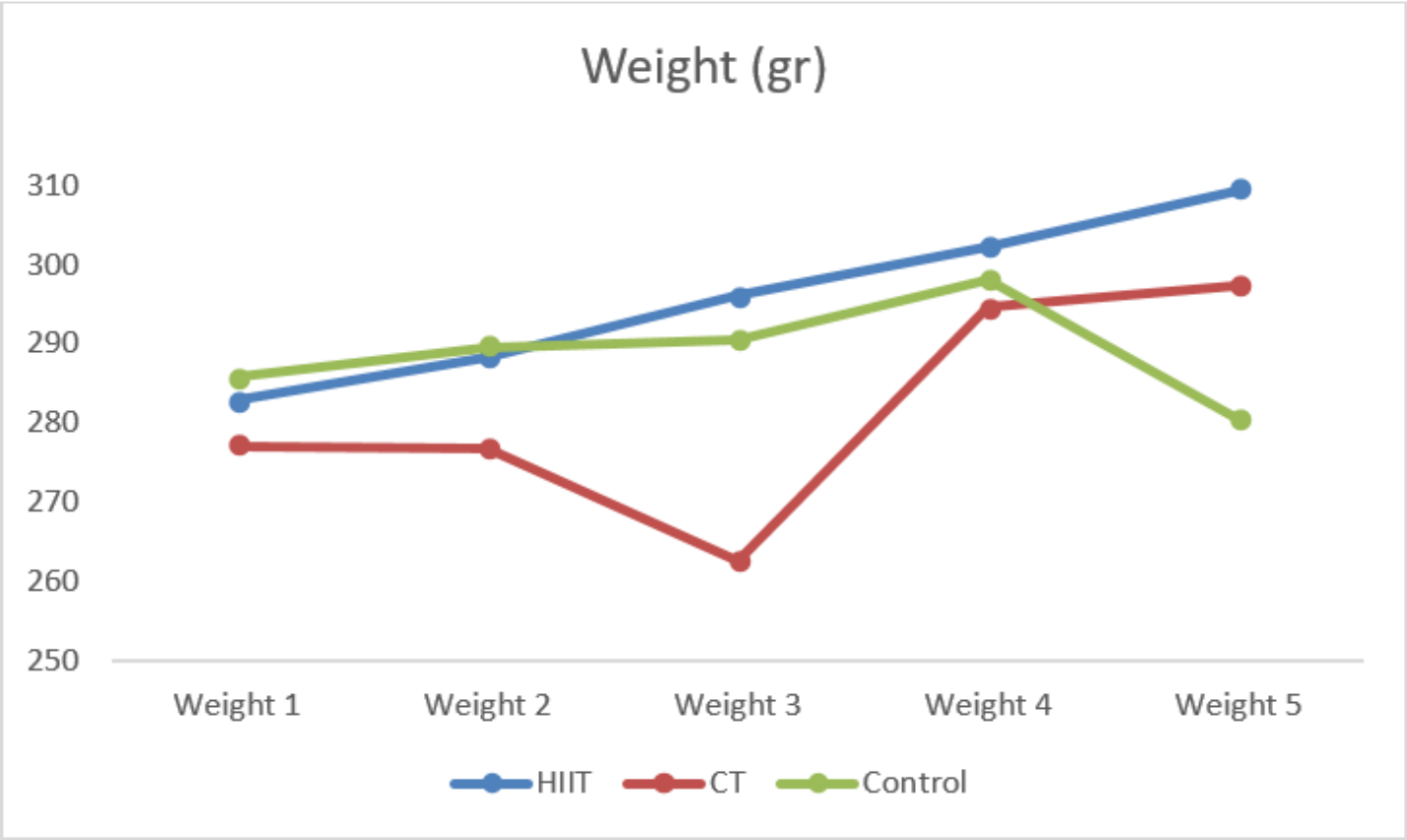
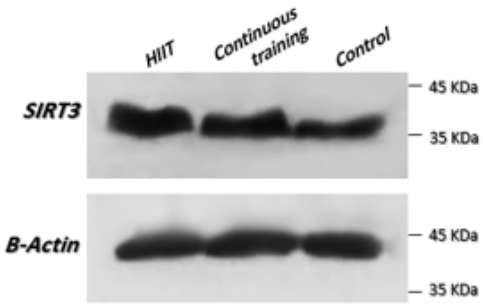


Figure 2

change in body weight of aged rats during 12 weeks of study.



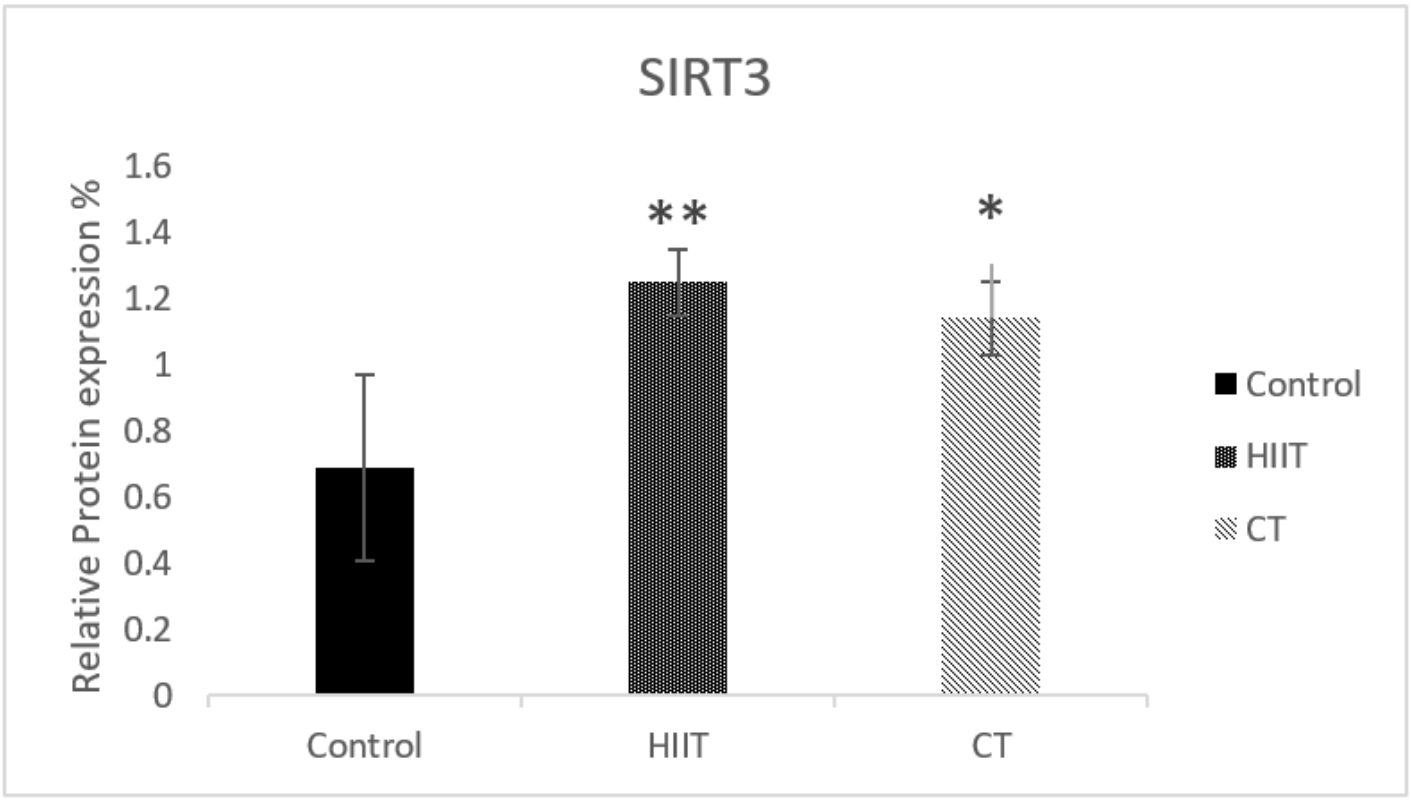


Figure 3

A: Representative blot for SIRT3. B: SIRT3 Protein expression after 12 weeks of exercise training (n=5).

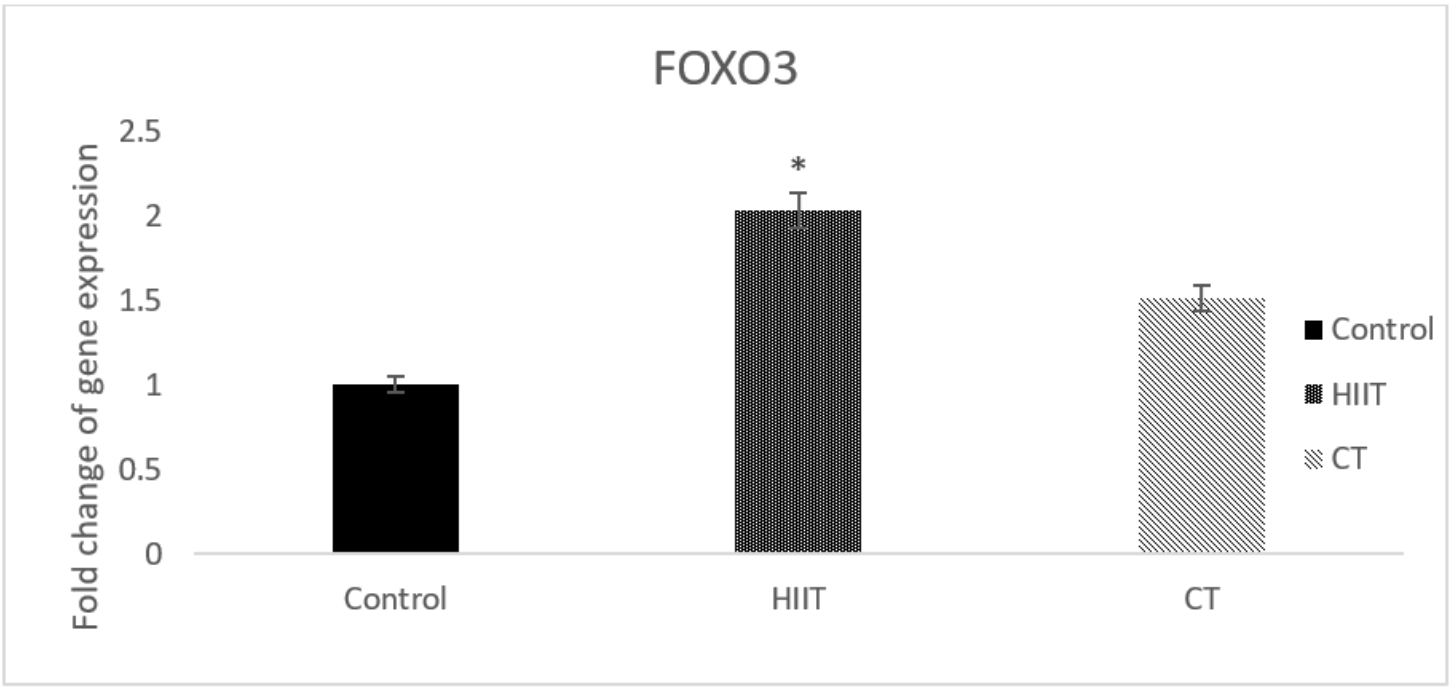


Figure 4

mRNA expression of FOXO3 in Gastrocnemius muscle of study groups (n=5). The obtained values are displayed as the means and SD of the mean (mean ± SD).

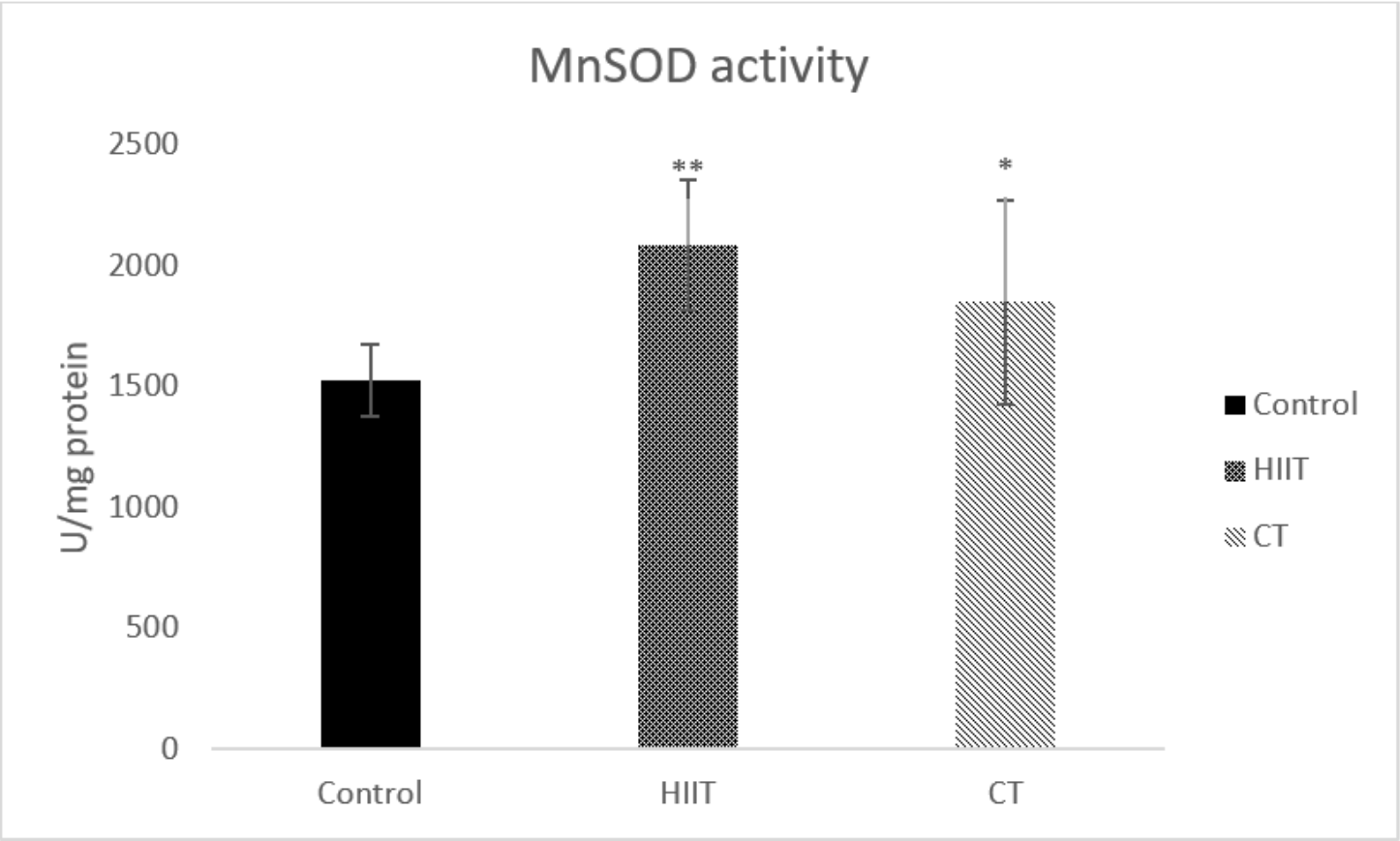


Figure 5

MnSOD activity in Gastrocnemius muscle of study groups (n=5). The obtained values are displayed as the means and SD of the mean (mean ± SD).

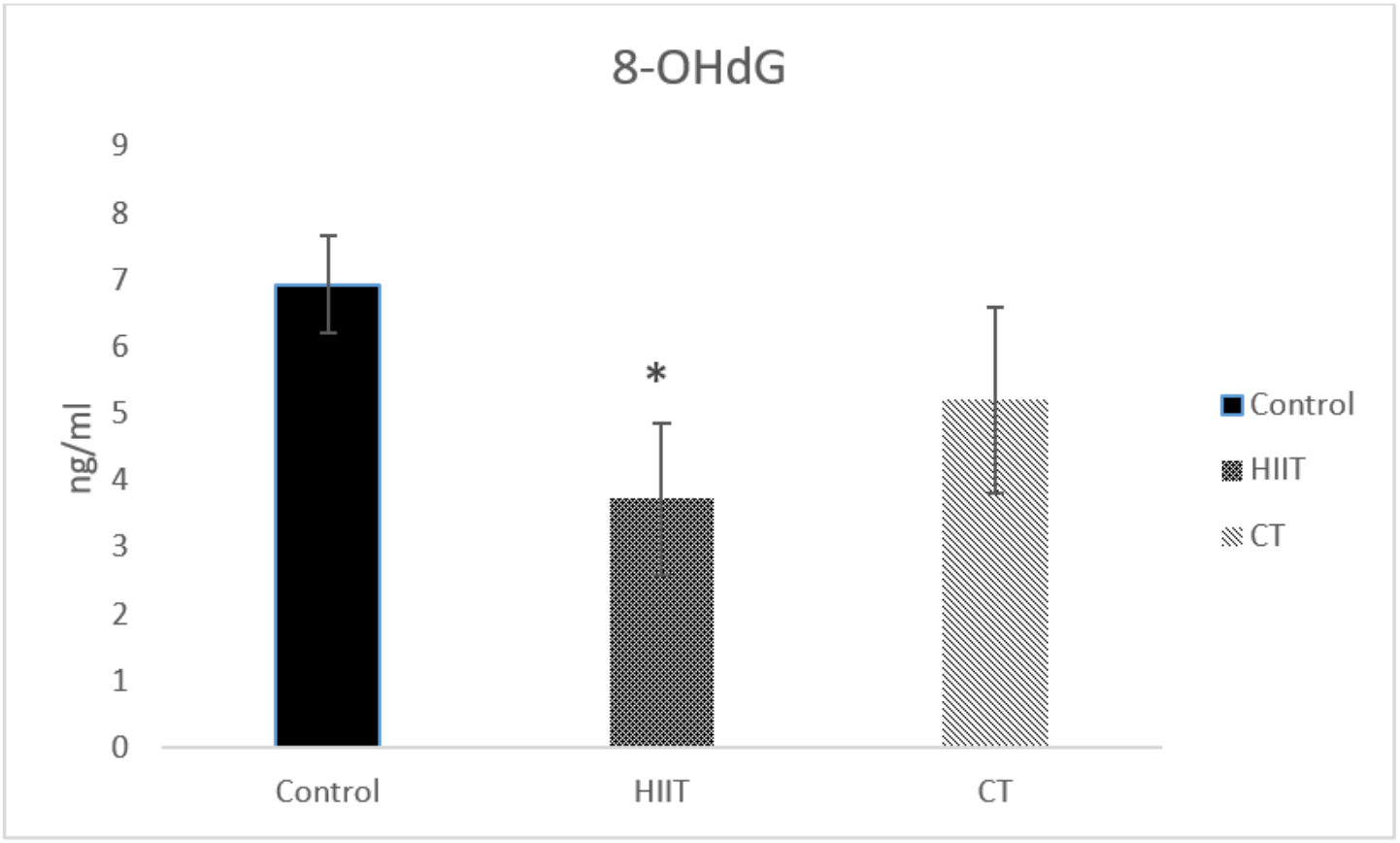


Figure 6

8-OHdG in Gastrocnemius muscle of study groups (n=5). The obtained values are displayed as the means and SD of the mean (mean ± SD).