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The effect of two different intense training protocols on oxidative stress of liver tissue during puberty in male rats

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Abstract

Background: Intense physical activity increases the production of reactive oxygen species in vital tissues such as the liver and causes oxidative stress.

Aim: This study investigates the effect of high-intensity interval training and intense endurance training on oxidative stress of liver tissue in immature male rats during puberty.

Materials and Methods: A total of 24 male Wistar rats (aged= 22 days, weight= 60±0.63 g), after one week of acclimatization, were divided randomly into three groups: control, IET, and HIIT. Rats were subjected to a four-week training on an animal treadmill. The effects of training treatment in rat liver were investigated by assaying oxidative stress biomarkers.

Results: Comparing to control group, in both training groups significantly lower Malondialdehyde (MDA) was seen ((*P*(IET)= 0.016/ *P*(HIIT)= 0.020). However, there were no statistical differences in Glutathione Peroxidase (GPX) (*P*= 0.463) and total antioxidant capacity (TAC) activity levels (*P*= 0.194) among groups. HIIT training significantly increased superoxide dismutase (SOD) (*P*= 0.040) and catalase enzyme (CAT) levels (*P*= 0.007). IET and HIIT had significantly increased endurance performance (both: *P*= 0.001).

Conclusion: Both training intensities did not lead to an increase in oxidative stress and can be used during puberty.

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1. Introduction

Despite widespread participation in both intense and light exercise, there is a limited awareness among coaches and trainers regarding the impact of different exercise types on oxidation and anti-oxidation responses in immature people. It has been declared that young people who participate in continuous intense exercise might be at risk of exercise-induced oxidative damage due to lower antioxidant supplies such as reduced glutathione (GSH) [1, 2].

There is, however, a growing body of evidence that chronic exercise may also benefit children. Likewise, empirical evidence supports the notion that oxidative stress-induced perturbations in anti-oxidant defense system can exert notable influences on the overall growth and development of children, while exercise-induced stress during the pubertal transition may elicit favorable adaptations within this system [3, 4].

Increasing oxygen consumption in working muscles during strenuous exercise and age, specific differences in oxidative metabolism in children are connected to higher exercise, induced oxidative stress in this population [1, 3].

The concept that children exhibit more rapid VO_2 kinetics during the developmental stages and are more aerobic with a greater mitochondria capacity for oxidative phosphorylation than adults during training, suggests that they're more dependent on mitochondrial metabolism than adults [2, 3]. These traits in children increase the chance to generate more reactive oxygen species (ROS) in response to exercise than in adults. Moreover, during pre-adolescence and childhood, with lower manufacture of growth hormone and testosterone, children may be less resistant to free radical generation and be disposed to reduced antioxidant capacity in response to exercise [5].

Still, the development of oxidative stress might do and alter the work of a vital organ like liver. The liver is a crucial body organ that plays a central role in the adjustment of carbohydrate and lipid stores, and generating a sufficient supply of metabolites for both intense physical activity and the synthesis of muscle and brain tissue [2, 6-11]. It has been demonstrated that regular moderate physical activity improves liver health and adverse functional changes can develop if perpetual activity is insufficient. Also, extra prolonged competitive exercise and very heavy training may be harmful and have adverse effects upon oxidant status [11].

In this regard, various studies have shown that the clinical signs of oxidative stress (lipid peroxidation markers like malondialdehyde (MDA)) and the status of antioxidant markers (enzymatic e.g., catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD)) and non-enzymatic (e.g., vitamin E, C, A; glutathione; uric acid; etc.) modification depending on the intensity, duration, repetition, and type of training [12-17].

Even though the modifications of pro-oxidant and anti-oxidant systems in children and young adults have been evaluated in some studies, there has not been any document on the effects of high-intensity training on the status of liver's oxidative stress markers and anti-oxidants during puberty. Furthermore, the application of information derived from adult research in sports programs for children may not always yield accurate results. However, due to ethical constraints in conducting studies on children and humans, rats were employed as subjects in the current investigation, necessitating

caution when extrapolating the findings.

Therefore, in this study, we aim to inspect the effect of intense interval training (HIIT) and intense endurance training (IET) on liver tissue of immature male rats.

2. Materials and Methods

2.1. Animals

Wistar immature male rats (n= 24, 3 weeks old, and weighting 60.16±7.35 g) [18] were obtained from Pasture Institute (Tehran, Iran). The animals were housed in polycarbonate cages (four per cage), and kept in a controlled room (humidity= 45±5%, temperature= 22±1.4°C, and light/dark cycle= 12/12 hours). During the training period, the animals were fed with free access to sterilized feed and distilled water. After a 7-days acclimation, all the rats were randomly stored into three groups and stratifed based on their body weight: Control (n= 8), Intense Endurance Training (n= 8), HIIT (n= 8) (Table 1).

Table 1 The initial and final weights of groups

Groups	N	Initial weight (g)*	Secondary weight (g)#	Final weight (g)$	Weight Changes 1 (g)	Weight Changes 2 (g)	Δ1 (%)	Δ2 (%)
Control	8	60.12	72.37	233.12	173.50	160.75	294	**224**
Endurance	8	60.50	72.50	214.75	157.87	142.25	269	**205**
HIIT	8	59.87	72.50	226	165.37	153.50	280	**217**
Total	24	60.16	72.45	224.62	165.58	152.16	281	**215**

* Weight at the beginning, # Weight after one week of acclimatization, $ Final weight before sample collection, Δ Percentage of weight changes

G*Power analysis was performed to estimate the required sample size (G*Power 3.1.9.6). A sample size of 24 subjects was calculated by a priori power analysis in G*power, using the following standard assumptions: rejection criterion= 0.05 and power (1-β) = 0.8 and efect size (ES)= 0.7 [19, 20]. The experiments approved by The Institutional Animal Ethics Committee at the University of Zanjan (Zanjan, Iran; Ethics Approval Code: IR.ZNU.REC.1400.009).

2.2. Training protocol

All rodents were 32 days old at the beginning of the training period (25 days plus a week of familiarization). In the pre-pubertal phase, one human year equals 3.3 rat days. Thus, all rats were 10.5 years old (pre-pubertal child) at the beginning of the training period [20]. After a week of familiarization, four weeks HIIT and IET protocol with progressive duration and intensity and following the principle of exercise overload was implemented on a motorized rodent treadmill (Pishro Andishe Sana't co). HIIT includes six days of training with one-day for rest per week.

Training protocol comprises 1 min intervals with the specified speed and 2-3 min of active rest between the intervals [20]. Based on the principle of overload, training was applied in such a way that the training speed in the familiarization week was equal to 10-16 m/min, and in the last week the training speed reached 36-40 m/min (equivalent to about 85% of VO_2max) [21, 22, 23].

Endurance training includes five days of training with two-days for rest per week. In this training protocol, following the principle of exercise overload, the speed

and duration of running were 10-18 m/min and 10-30 min in the first week and reached 30 m/min and 55-60 min in the last week (equivalent to about 85% of VO_2max) [24].

Before and after each training session, the rats run at a speed of 6 m/min for 2 min to warm up and cool down.

The control group had the same conditions, except they lacked running (Table 2).

Table 2. HIIT and IET protocols

Training type	Training parameters	Acclimatization	Week 1	Week 2	Week 3	Week 4
HIIT	Speed (m/min)	4-16	16-22	23-28	29-34	**35-40**
	Active duration (min)	1	1	1	1	**1**
	Rest duration (min)	2	2	2	3	**3**
	Repetitions (per session)	10	10	10	10	**10**
IET	Speed (m/min)	4-10	10-18	20-28	28-30	**30**
	Active duration (min)	4-10	10-30	30-50	50-54	**55-60**

2.3. Running performance test

Animals performed a running performance test 48 hours after the last training session in which, after 5-min running at 12 m/min for warm up, the speed increased by 1m/mi every 2 min till reaching 20 m/min. Then the pace was increased by 2m/min every 3 min until exhaustion, where animals stood upright on their feet or touched a shock gird 5 times in a min. Performance was calculated using the formula described below and was expressed in kg multiplied by m (kg×m).

$$Rp = \Sigma Rps = \Sigma mSsSrt \rightarrow \Sigma mTd$$

Rp: Rat's performance, Rps: Rat's performance each stage, m: body mass, Ss: Stage speed, Srt: stage running time, and Td: the total distance run.

At the end of the test, the rats had cooled down at a speed of 6 m/min. The running performance test were done at the same time as each training sessions [25]. The animals were weighed before the exhaustion test. The mass-dependent model was used to measure the rats' performance [26].

2.4. Sample collection and tissue homogenization for liver tissue

The animals were anesthetized with xylazine (3–5 mg/kg body weight) and ketamine (30–50 mg/kg body weight) after 48 hours of the running performance test. All the animals' Liver tissues were collected in a sterilized animal facility. The tissues were immediately frozen in liquid nitrogen (−196°C) and stored at −80°C. A piece of liver tissue (approximately 1 g) was homogenized by a homogenizer (10 ml phosphate buffer, pH 7.2) on the ice at 10000×g for 4 min at the first speed level. Then, the homogenate was centrifuged at 4500×g for 10 min at 4°C. Activities of the antioxidant enzymes GPx, SOD, CAT, TCA, and MDA levels were spectrophotometrically determined from these supernatants.

2.5. MDA assay

Tissue MDA levels were assayed by a spectrophotometric method based on the thiobarbituric acid reactive substances (TBARS) method [27] at 532 nm in a spectrophotometer.

2.6. SOD, GPx, CAT, TAC assay

The activity of antioxidants was measured

using the diagnostic kit RANSOD produced by RANDOX (Randox Laboratories Ltd., Crumlin, UK).

The activity of SOD was estimated based on the method used by Arthur and Boyne (1985) [28]. In this method, xanthine and xanthine oxidase (XOD) is used to produce superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan color. SOD activity is then measured at 37°C by the degree of prevention of this reaction spectrophotometrically at 505 nm (catalog number: sd125).

GPx activity was measured according to Paglia and Valentine (1967) [29]. This method requires the oxidation of glutathione (GSH) by cumene hydroperoxide catalyzed by GPx. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is instantly changed to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance is then measured spectrophotometrically at 340 nm (catalog number: 504).

Catalase activity was measured in hemolysates at 25°C according to the method of Aebi [30], by monitoring the initial rate of the disappearance of hydrogen peroxide at 240 nm in a spectrophotometer. TAC was determined using Miller et al. (1993) spectrophotometry method at 600 nm, 37°C [31] (catalog number: nx2332).

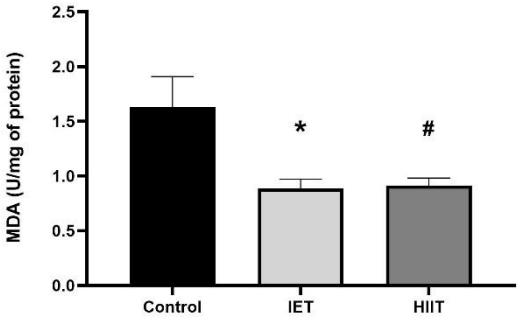
2.6. Statistic

SPSS version 26.0 (IBM, Armonk, NY, USA) was used for analyzing data. One-way analysis of variance (ANOVA) was used to analyze the differences in the evaluated outcome measures across the three experimental groups after ensuring the normality of the data using the Shapiro–Wilk test. The equality of variance was measured using the Levene test. Tukey's post hoc tests completed pairwise comparisons if the ANOVA test identified where significant differences arose. Partial eta-squared was analyzed for ANOVA. The statistical significance was set at $P<0.05$.

3. Results

3.1. MDA

Statistical differences were observed in liver MDA ($F(2, 21)= 6.03$, $P= 0.009$, $\eta^2= 0.36$; Figure 1). According to post hoc pairwise comparisons, MDA in the IET and HIIT training groups were statistically lower than the control group (respectively, $P= 0.016$ and $P= 0.020$). However, there was no significant difference between the two training groups ($P= 0.994$).

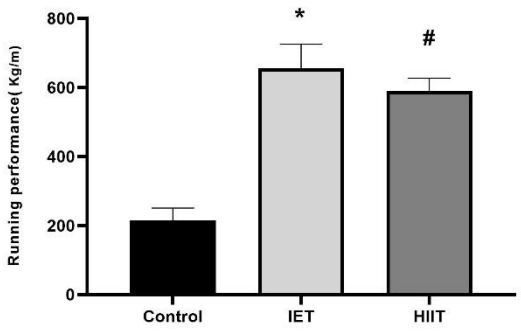


*, # statistically different from the control group

Figure 1. Liver Malondialdehyde (MDA)

3.1.1. Running performance test

According to The ANOVA test, statistical differences were seen across the three groups ($F(2, 21)= 22.64$, $P= 0.001$, $\eta^2= 0.68$; Figure 2). The Tukey's post hoc test showed higher running performance in training groups when compared to the control group (both: $P= 0.001$). However, there was no significant difference between the two training groups ($P= 0.671$).

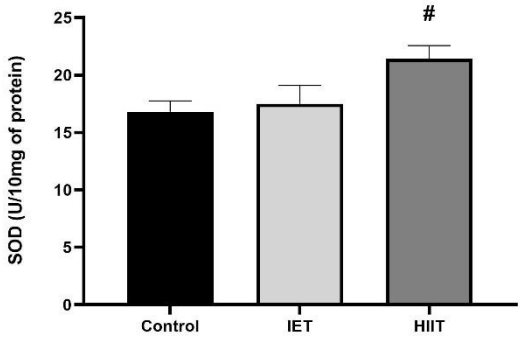


*, # statistically different from the control group

Figure 2. Running performance

3.1.2. SOD, CAT, GPX, TAC

Statistical differences were evident across the groups in liver SOD levels (F(2, 21)= 4.00, *P*= 0.034, η^2= 0.27; Figure 3); according to Tukey's post hoc test, SOD level in the HIIT group compared to the control group was significantly higher (*P*= 0.040).



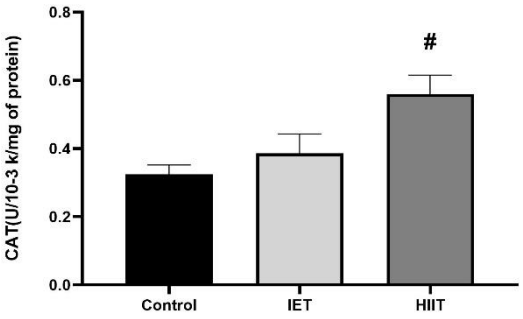
statistically different from the control group

Figure 3. Liver superoxide dismutase (SOD)

Statistical differences were observed in liver CAT levels (F(2, 21)= 6.33, *P*= 0.007, η^2= 0.37; Figure 4); Tukey's post hoc pairwise comparisons showed that CAT in the HIIT training group was statistically higher than the control (*P*= 0.007) and IET groups (*P*= 0.049).

The ANOVA test showed no statistical differences in liver GPX levels between the three groups (F(2, 21)= 0.79, *P*= 0.463, η^2= 0.07; Figure 5).

There were no statistical differences observed in liver TAC levels between the three groups (F(2, 21)= 1.77, *P*= 0.194, η^2= 0.14; Figure 6).



statistically different from the control and endurance training groups

Figure 4. Liver Catalase (CAT)

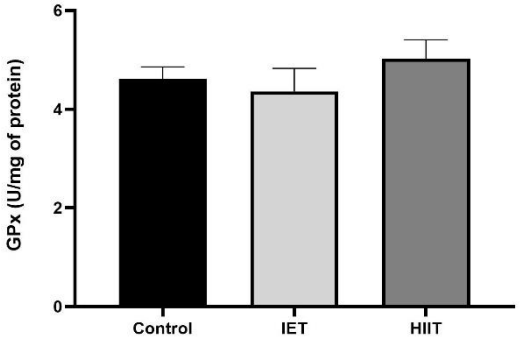


Figure 5. Liver Glutathione Peroxida (GPx)

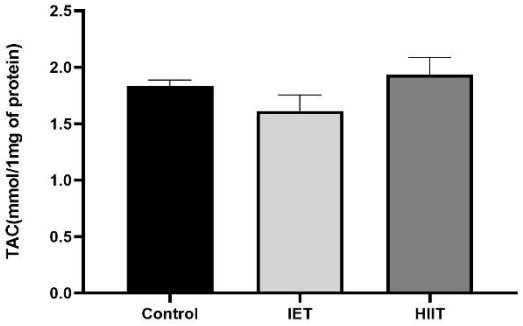


Figure 6. Liver Total Antioxidant Capacity (TAC)

4. Discussion

The purpose of study was investigating the effect of intense interval training (HIIT) and intense endurance training (IET) on oxidative stress markers and anti-oxidants of liver tissue of immature male rats. In this regard, the results showed that both training protocols reduced MDA levels in both

group but did not have a significant effect on the activity of the antioxidant enzymes GPX and TAC. On the other hand, SOD and CAT increased by HIIT also endurance performance improved in immature male rats.

Evidence showed that aerobic training can reduce MDA levels after 8 weeks, while antioxidant activity can be increased after 6 weeks. Also, it is believed that this time can be longer depending on the intensity, load, and repetition of the exercise [32]. According to our observations, this also seems to be different at younger ages.

The findings show that there is a significant decrease in MDA levels and an increase in CAT and SOD activity in the HIIT group. These findings suggested that during adolescence, a shorter HIIT period (4 weeks) reduces the amount of MDA levels and improves SOD and CAT antioxidant activity. In case of IET, despite the significant decrease in MDA levels, HIIT seems to be more effective at adjusting to oxidation stress with a positive effect on pro-oxidants and anti-oxidants, so it seems that a longer training period is needed for antioxidant activity to be observed to improve [33]. Further, in several studies continuous HIIT has demonstrated extensive compatibility, one of which is the increase in antioxidant capacity [34].

The increase in the CAT and SOD enzymes following HIIT may be associated with the fact that these enzymes are one of the first enzymes to enter the oxidation reactions in mammals' tissues [35].

Our finding was in agreement with the previous study, which suggested that CAT and SOD activity were responsive to exercise stress and increased significantly. Also, suggested that attainment of puberty might improve the SOD response to exercise and such response increased as a function of age [36].

Regarding tissue's TAC and its non-significant change in our study, it should be noted that unlike plasma's TAC, which is the result of the body's antioxidant enzymes, tissue's TAC is an index that expresses the antioxidant properties of a set of compounds in tissue. These intracellular antioxidants are different from extracellular and plasma compounds. Considering that the activity of TAC in the liver increases when the oxidizing agent is OH- [37], we attribute the non-significant change observed to the decrease in MDA in the liver after training, which could reduce the production of OH- oxidizing agent in the liver.

Our finding was in agreement with a previous study which showed that out of 3 intermediate endurance training protocols with different periods of 6, 9, and 12 weeks of training, only 12 weeks of training significantly reduced the amount of SOD and CAT in the liver of experimental groups. They concluded that moderate endurance training for up to 9 weeks could not adapt to the liver anti-oxidation enzyme system, but more weeks of the training results in anti-oxidation enzyme activity reduction [38].

Another study in agreement with the present study indicated that following an annual period of endurance training in track and field athletes in tow age groups (children and adults), GSH, TAC activity, and lipid peroxidation has been reduced in children. They stated that continuous increasing endurance training is likely to improve antioxidant performance and

create exercise-induced oxidative adaptations over time [39].

In contrast to the present study, Liu et al. (2000) reported that performing an exhaustive test at the end of 2 weeks of endurance training significantly increased liver MDA [40]. The increase in the MDA in their study may be related to training period, lack of load reduction during a training program, and lack of compliance with the overload principle. Gradually, increasing the training load results in less stress at each stage of the load increase, and existing a reduced load stage in the training protocol provides an opportunity for the muscles and tissues to achieve oxidative adaptations. Therefore, the practical factors for the decrease in MDA in our study include the type and method of training on oxidation indicators in different body systems, period, and frequency of stress exposure. Therefore, our observations allow us to theorize that by observing the overload principle, there was a decrease in the amount of MDA in training groups after the exhausting test, despite the shorter training period (4 weeks).

Similarly, Lima et al. (2013) [41] and Navarro et al. (2004) [42] reported a significant decrease in mitochondrial MDA of liver tissue after the exhausting test following 6 and 4 weeks of moderate endurance training in adolescents. The absence of an anaerobic system exercise group and the absence of a training group with different training pressures are some of the limitations of our study in regarding the conclusion of the effects of different types of intense training on oxidative indicators and the antioxidant system in liver tissue.

As a result, it can be said that intense training, depending on its type, causes different changes in liver oxidative and antioxidative markers in children and adolescents. However, these changes seem to be influenced by factors such as training phase, training load, level of physical fitness, exercise style, etc. Both training protocols improved endurance performance in immature male rats also reduced MDA levels in both group and did not lead to an increase in oxidative stress and did not have a significant effect on the activity of the antioxidant enzymes GPX and TAC. On the other hand, due to the increase of SOD and CAT by HIIT, this type of training can be used in the exercise planning during puberty.

5. Conclusion

Intense exercise, depending on its type, causes different changes in oxidative and anti-oxidant markers in children and adolescents. It is recommended that athletes in the pre-puberty period use this type of training, especially HIIT training, in their training program. Considering that the exercises investigated in this study are aerobic exercises, it is suggested to conduct a research in order to investigate the effects of anaerobic exercises such as strength and resistance exercises on the oxidative status and liver antioxidants in the pre-puberty period. Also, considering the lack of information on the effects of exercise in pre-puberty, it is suggested to compare the effects of intense exercise in pre-puberty and adulthood.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The author has completely considered

ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. The ethical code is IR.ZNU.REC.1400.009.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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