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Effect of intensive endurance training on rat brain and hepatic 8-oxoguanine DNA glycosylase and 8-hydroxy-2'-deoxyguanosine levels

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ABSTRACT

The purpose of this study was to investigate the effect of intensive endurance training on 8-oxoguanine DNA glycosylase (OGG1) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in liver and brain of rats. 16 adult and male albino wistar rats were randomly divided into sedentary control and endurance exercise training groups. Animals ran on treadmill for 6 weeks, 6 days a week, at a speed of 10 m/min (85 percent of maximal oxygen consumption). The content of OGG1 and 8-OHdG were measured using sandwich ELISA assay. Data analyzed using Student's T-test at $P \leq 0.05$ level. Our results showed that intensive endurance training has no significant effect on 8-OHdG contents in liver (t_{14}=1.09, p=0.29) and brain (t_{14}=0.93, p=0.36) of rats. However, contents of OGG1 in liver (t_{14}=5.84, p=0.001) and brain (t_{14}=4.09, p=0.001) of rats significant increases following intensive endurance training. Finally, there were no significantly differences between changes in contents of 8-OHdG (t_{14}=0.44, p=0.66) and OGG1 (t_{14}=1.72, p=0.10) in liver and brain of rats following endurance training. Intensive endurance training maintains 8-OHdG genomic damage in baseline level in liver and brain of rats by increasing contents of OGG1.

INTRODUCTION

Regular exercise training has many health benefits and act as protective approach against diseases (Ogonovszky *et al.*, 2005a, 2005b). Paradoxically, it is also obvious that free radical induced by intense exercise training can result in oxidative damage to cellular constituents (Radak *et al.*, 2006, 2008, Nikolaidis *et al.*, 2009). Free radical attacks to protein and lipids and yield carbonyl protein and malondialdehyde, respectively (Radak *et al.*, 2006, 2008, Nikolaidis *et al.*, 2009). In addition, free radical generated by contracting skeletal muscles penetrates into the mitochondria and the nucleus which result in oxidative damage to genomic constituents (Ogonovszky *et al.*, 2005a, 2005b). In fact, the production of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and subsequently cell apoptosis occurs following free radical attack (Ogonovszky *et al.*, 2005a, Radak *et al.*, 2007, 2008). Due to its lower redox potential compare to other nucleic acid bases, guanine is prone to undergo further oxidation upon exposure to hydroxyl radicals. Therefore, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is frequently generated oxidative base lesion (Radak *et al.*, 2003). Transversion of G:C to T:A and mutation occurs if unrepaired 8-OHdG is not repair. 8-OHdG level increases during many diseases such as cancer, atherosclerosis, diabetes and Alzheimer's disease (Ogonovszky *et al.*, 2005a, Radak *et al.*, 2007, 2008). Cells are equipped with DNA repair system to reduce the effects of these oxidative DNA damages. 8-oxoguanine DNA glycosylase (OGG1) recognizes and cleaves of oxidized guanine from DNA (Radak *et al.*, 2007, 2011). Maximum oxygen consumption (VO_2max) (Loft *et al.*, 1994), body mass index (BMI) (Kasai *et al.*, 2001) and exercise training (Ogonovszky *et al.*, 2005a, Koltai *et al.*, 2011) influence on OGG1 and 8-OHdG levels.

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Although, exercise training at low intensity had no significant effect on 8-OhdG and acetylated OGG1 levels, but overtraining increased 8-OhdG and OGG1 levels in liver tissue of rats (Ogonovszky *et al.,* 2005a). In contrast, a reduction in 8-OHdG levels in liver of old rats has been reported following 8 weeks running on treadmill (Nakamoto *et al.,* 2007). Furthermore, both of running on treadmill (Radak *et al.,* 2007, Nakamoto *et al.,* 2007) and swimming (Radak *et al.,* 2009) with moderate intensity increase OGG1 activity in the red type of skeletal muscle (Radak *et al.,* 2007) and liver (Nakamoto *et al.,* 2007) and subcellular compartments i.e nucleus and mitochondria (Radak *et al.,* 2009). Increases in OGG1 activity is higher in slow twich than fast twich muscle fiber following exercise training with moderate intensity (Radak *et al.,* 2007). Also, marathon running alters the DNA base excision repair in human skeletal muscle (Radak *et al.,* 2003). In contrast, these increases return to baseline level after detraining (Radak *et al.,* 2009).

In the context of other indicators of oxidative damage, Fauzi and colleague has been reported an increases in malondialdehyde and superoxide dismutase (Fauzi *et al.,* 207). Also, Kayatekin and co-workers have been shown that 6 weeks intensive interval training increases glutathione peroxidase levels, while has no effect on superoxide dismutase (Kayatekin et al., 2002). Moreover, Fisher and co-workers has been demonstrated increase in catalase, superoxide dismutase, and glutathione peroxidase level in lymphocytes (Fisher et al., 2010). Due to insufficient information regarding the health effects of intense endurance training on OGG1 and 8-OHdG, the results of present study will give us a new insight about intensity of training. Especially, due to activation of NADPH oxidase (Haram *et al.,* 2009) and xanthine oxidase (Kostaropoulos *et al.,* 2006) more free radical produces during intense training. In addition, liver and brain are considered as redox sensitive organs in body because of large number of mitochondria, ischemia/reperfusion of blood (Cooper *et al.,* 2002, Lamprecht *et al.,* 2004) and large amounts of iron and copper ions (Cooper *et al.,* 2002, Urso *et al.,* 2003). Finally, preserving the structure of DNA is critical for cell metabolism and the correct transmission of information. Collectively, the purpose of this study was to investigate the effect of intensive endurance training on OGG1 and 8-OHdG levels in the liver and brain of rats.

MATERIALS AND METHODS

Animals

All animal experiments conformed to the guidelines for the use and care of laboratory animals ("Principles of laboratory animal care", NIH publication No. 86-23. Revised 1996), and the study was approved by the ethics committee of Birjand University of Medical Sciences in Iran. Sixteen adult and male albino wistar (12 weeks of age, weighing 280 g) were randomly assigned to two groups of control and intensive endurance training. The animals were kept under controlled conditions with 25 ± 2°C and a 12-h light/12-h dark cycle. The rats had free access to tap water and food. The animals were accustomed to laboratory conditions for 2 weeks prior to the experiment.

Intensive endurance training

Animals were familiarized with running on a motor-driven treadmill (12-lane) for 5 days, 10 min/day at a speed of 10 m/min (Afzalpour *et al.,* 2015). Intensive endurance training was performed on the basis of overload principle for 6 weeks, 6 sessions per week (Table 1) (Afzalpour *et al.,* 2015). Overload was exerted by increasing the training time. At the beginning and end of intensive endurance training, warm-up and cool-down were performed at 16 m/min (corresponds to 68% VO_2max). Besides, intensities of intensive endurance training correspond to 80% VO_2max. The rats were motivated to run by a mild electrical current on the treadmill (0.5 mA, 1 Hz) (Afzalpour *et al.,* 2015). The rats of the control group were transported daily to the training room, exposed to the same environment as the exercising groups, and placed on the treadmill without running for as long as the exercising groups were on the treadmill (Afzalpour *et al.,* 2015).

Table 1: Intensive endurance trainings protocols.

Week	Day	Intensive endurance training
Week 1	1	20 min, 27 m/min
	2	22 min, 27 m/min
	3	24 min, 27 m/min
	4	26 min, 27 m/min
	5	28 min, 27 m/min
	6	30 min, 27 m/min
Weak 2	1	32 min, 27 m/min
	2	34 min, 27 m/min
	3	36 min, 27 m/min
	4	38 min, 27 m/min
	5	40 min, 27 m/min
	6	42 min, 27 m/min
Weak 3	1	44 min, 27 m/min
	2	46 min, 27 m/min
	3	48 min, 27 m/min
	4	50 min, 27 m/min
	5	52 min, 27 m/min
	6	54 min, 27 m/min
Weak 4	1	56 min, 27 m/min
	2	58 min, 27 m/min
	3	60 min, 27 m/min
	4	60 min, 27 m/min
	5	60 min, 27 m/min
	6	60 min, 27 m/min
Weak 5-6	1-12	60 min, 27 m/min, to end of 6th week

Tissue preparation and Biochemical assays

Rats were euthanized under deep anesthesia (Ketamine, 60–80 mg/kg and Xylazine, 8 mg/kg; IP) 48 h after last exercise session, between 10:00 and 11:00 am. The whole brain and liver rat was removed, washed by normal saline, and finally stored at -80 °C. Brain and liver were smashed into a fine powder by liquid nitrogen (22). Then, 1 ml 1× phosphate buffered saline and protease inhibitor cocktail (#GB-326-1, ProBlock™-50, Goldbio technology CO, USA) added to the microtubes. Commercially 96-well ELISA kits were used to measure the content of OGG1 (#CSB-EL016313RA, Cusabio Biotech CO., LTD. Sino-American) and 8-OHdG levels (#CSB-E10526r, Cusabio Biotech

CO., LTD. Sino-American). The sensitivities of OGG1 and 8-OHdG were less than 6.25 pg/ml and 0.078 ng/ml, respectively. The assays were carried out according to the manufacturer's instructions. Contents were expressed in mg tissue weight.

Statistical analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS Inc., Chicago, USA) version 16.0 and expressed as mean ± standatd deviation (SD). After determination of normality BY Shapiro-Wilk's test, the data were statistically analyzed by Student's t-test. Significance level was set at $p < 0.05$.

RESULTS

Our finding showed that intensive endurance training significantly increase contents of OGG1 in liver (t_{14}=**5**.84, p=0.001) and brain (t_{14}=4.09, p=0.001) of rats (Table 2). However, intensive endurance training has no significant effect on 8-OHdG contents in liver (t_{14}=1.09, p=0.29) and brain (t_{14}=0.93, p=0.36) of rats (Table 2). Also, there were no significantly differences between changes in contents of OGG1 (t_{14}=1.72, p=0.10) and 8-OHdG (t_{14}=0.44, p=0.66) of rats following intensive endurance training (Table 2).

Table 2: Effect of intense endurance training on OGG1 and 8-OHdG levels in liver and brain.

Dependent variables	Groups	
	Control	Intensive endurance training
Liver OGG1 (pg/mg tissue)	40.33±3.69	50±3.01*
Brain OGG1 (pg/mg tissue)	11.40±2.89	17.14±2.70*
Liver 8-OHdG (ng/mg tissue)	0.88±0.11	0.80±0.17
Brain 8-OHdG (ng/mg tissue)	0.29±0.08	0.25±0.08

* Indicate significant difference than control (P<0.05).

DISCUSSION

It has been shown that physical labor, BMI, inter-individual variation, nutrient (Kasai *et al.*, 2001) age (Radak *et al.*, 2011), smoking (Park *et al.*, 2011), VO_2max (Loft *et al.*, 1994), and mental state, especially clinical depression (Forlenza *et al.*, 2006), all affect OGG1 levels. Therefore, animal models were used in the present study to control the variables mentioned. Therefore, observed changes in OGG1 and 8-OHdG are simply due to intensive endurance training. While it have been reported that running (Koltai *et al.*, 2011) and swimming (Ogonovszky *et al.*, 2005b) with low to moderate intensity have no significant influence on OGG1 and 8-OHdG levels in rat's hippocampus, our findings show an increase in OGG1 contents of brain and liver following intensive endurance training. Our findings are supported by a study by Ogonovszky who reported an increase in OGG1 activity and 8-OHdG levels of rat liver following strenuous and overtraining (Ogonovszky *et al.*, 2005a). This increasing is largely attributed to high number of mitochondria, higher metabolism of liver cells, and no significantly changes in SOD, GPX and catalase activity of liver cells following exercise (Ogonovszky *et al.*, 2005a). In contrast, the level of DNA damage and OGG1 activity in brain did not significantly alter with increasing in exercise intensity (Ogonovszky *et al.*, 2005b) due to increasing of antioxidant enzymes activity in different region of brain (Ogonovszky *et al.*, 2005b).

Liver and brain, as two redox sensitive organs, react differently to changes in oxygen supply during exercise; however, adaptive processes related to oxidative challenges are very similar (Cooper *et al.*, 2002, Urso *et al.*, 2003, Lamprecht *et al.*, 2004). Higher levels of iron and copper ions in the brain tissue increase the possibility of Fenton reaction (Cooper *et al.*, 2002, Urso *et al.*, 2003). Furthermore, higher production of ROS in the liver cells is associated to high density of mitochondria (Lamprecht *et al.*, 2004, Cooper *et al.*, 2002).

In this regard, it is reported that 8-OHdG levels in mitochondria of liver is 10 times higher than 8-OHdG levels in nucleus because of close proximity to electron transport chain (Nakamoto *et al.*, 2007). Furthermore, ischemia/blood reperfusion which occurs at the beginning and end of each set of intense exercise training increases the xanthine oxidase enzyme activity and subsequently damage to genomic structures (Lamprecht *et al.*, 2004). However, in our study, HIIT had no significant effect upon the 8-OHdG levels of brain and liver. Studies have been shown that OGG1 activity increases after 8 weeks of running on treadmill exercise (Nakamoto *et al.*, 2007) and swimming training (Radak *et al.*, 2009). In addition, it has been reported that voluntary wheel running increases the activity of antioxidant enzymes in different region of brain (Ogonovszky *et al.*, 2005b, Jolitha *et al.*, 2006). Collectively, no change in 8-OHdG levels in brain and liver following intensive endurance training may be attributed to higher levels of OGG1 activity (Nakamoto *et al.*, 2007, Radak *et al.*, 2009), higher levels of antioxidant enzyme activity (Ogonovszky *et al.*, 2005a, Jolitha *et al.*, 2006) and higher contents of OGG1 as shown in the present study.

Interestingly, our results did not reveal any significant difference in changes of OGG1 and 8-OHdG between brain and liver. This suggests same response of OGG1 and 8-OHdG levels in two mentioned organs following intensive endurance training.

CONCLUSION

In general, intensive endurance training increases the OGG1 content in liver and brain and it seems that this adjustment is critical in control and modifies the oxidative damage following intensive endurance training.

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