

Effect of high intensity interval training on 8-oxoguanine DNA glycosylase and 8-hydroxy-2'-deoxyguanosine contents in the brain and liver of rats

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ABSTRACT

Production of reactive oxygen species (ROS) yields serious damage oxidation of proteins, lipids and genomic structures. Studies have shown that production of ROS increases during intensive exercise training. This study aimed to investigate the effect of high intensity interval training on 8-oxoguanine DNA glycosylase (OGG1) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in the brain and liver of rats. 16 adult Albino Wistar rats were randomly divided into sedentary control and high intensity interval training groups. Animals ran on treadmill for 6 weeks, 6 days per week, at 95 to 100 percent of maximal oxygen consumption. Using commercial kits, 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. High intensity interval training resulted in significant increases in contents of OGG1 in brain ($t_{14}=7.22$, $P=0.001$) and liver ($t_{14}=2.55$, $P=0.02$) of rats. However, high intensity interval training had no significant influence on 8-OHdG levels in brain ($t_{14}=1.60$, $P=0.13$) and liver ($t_{14}=1.28$, $P=0.22$) of rats. Also, there were no significant differences between changes in the brain and liver contents of OGG1 ($t_{14}=0.97$, $P=0.34$) and 8-OHdG ($t_{14}=0.42$, $P=0.68$) of rats following high intensity interval training. Taken together, separation of the training sessions to various bouts of exercise with maximum effort, through increase in OGG1 contents, will lead to modify of 8-OHdG levels in brain and liver.

INTRODUCTION

The positive effects of exercise training on various organelles of body have been documented. However, it appears that exercise, especially intensive exercise training, has potential to increase production of reactive oxygen species (ROS) through increases in oxygen consumption (Ogonovszky *et al.*, 2005a, 2005b). This occurrence subsequently yields serious damage oxidation of proteins, lipids and genomic structures (Radak *et al.*, 2006, 2008, Nikolaidis *et al.*, 2009). ROS increases oxidative base lesions within DNA structure, and eventually cell arrest, apoptosis, and necrosis (Radak *et al.*, 2003). Guanine is prone to undergo further oxidation upon exposure to hydroxyl radicals

due to its lower redox potential compare to other nucleic acid bases. Therefore, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most frequently generated oxidative base lesions (Radak *et al.*, 2011). Unrepaired 8-OHdG can lead to transversion of G : C to T:A and cause mutation. 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). To cope with such detrimental consequences, cells are equipped with DNA repair system to reduce the effects of these oxidative DNA damages. Major mammalian DNA glycosylase for recognizes and cleaves of oxidized guanine from DNA is 8-oxoguanine DNA glycosylase (OGG1) (Radak *et al.*, 2007, 2011). Many factors influence on OGG1 and 8-OHdG levels including 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).

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In context of exercise training, it has been shown that 2 months of regular treadmill running reduces 8-OHdG in the nuclear and mitochondrial DNA and increases OGG1 activity in the liver of old rats (Nakamoto *et al.*, 2007). Another study has demonstrated a significantly higher OGG1 activity in the red type of skeletal muscle compare with white fibers in old rats following 8 weeks running on treadmill (Radak *et al.*, 2007). Interestingly, 8 weeks swimming has been increased OGG1 levels/activity in the nucleus and mitochondria, whereas 8 weeks detraining reverses the up-regulating effect of training (Radak *et al.*, 2009). Besides, elevation of OGG1 and 8-OHdG levels in liver of rats has been reported following overtraining (Ogonovszky *et al.*, 2005a). In contrast, neither insulin-like growth factor-1 (IGF-1) supplementation and nor exercise training with 60% of VO_2 max had not any effect on OGG1 and 8-OHdG levels. However, combination of exercise and IGF-1 Supplementation increased acetylated OGG1 levels in hippocampus of old rats (Koltai *et al.*, 2011).

Evidences suggest that high-intensity exercise training (HIIT) increases superoxide dismutase (SOD), glutathione peroxidase (GPx) activity in soleus muscle (Criswell *et al.*, 1993) and reduces malondialdehyde (MDA) levels in fast-twitch extensor digitorum longus muscle (Cunningham *et al.*, 2005). Because of insufficient information regarding the health effects of HIIT on OGG1 and 8-OHdG, the results of present study will give new insights about HIIT. Especially, greater stress oxidative occurs during HIIT due to activation of NADPH oxidase (Haram *et al.*, 2009), xanthine oxidase (Kostaropoulos *et al.*, 2006), and hypoxic conditions (Rasmussen *et al.*, 2009). In addition, liver and brain are considered as redox sensitive organs in body due to 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). Collectively, the purpose of this study was to investigate the effect of HIIT on OGG1 and 8-OHdG levels in the liver and brain of rats.

MATERIALS AND METHODS

Animals and HIIT protocol

Sixteen pathogen-free adult (3 months old) male albino Wistar rats (purchased from the laboratory of bearing and multiplying at the Mashhad University of Medical Sciences, Iran) were randomly divided into either control (C) or high intensity interval training (HIIT) groups. 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). Rats had free access to water and food and kept in room with 25 ± 2 °C and a 12 h light/12 h dark cycle. After familiarization with walking on motor-driven treadmill (5 days, 10 min/day at 10 m/min), rats were performed intensive interval training based on overload principle for 6 weeks, 6 session per weeks at 95-100 % VO_2 max (Afzalpour *et al.*, 2015). In even days, rats were submitted to running at 40 m/min (3 min, 2 interval in first session and progressively increased to 6 repetitions in the 6th

week). In odd days, rats were submitted to running at 54 m/min (30 s, 3 intervals in first session and progressively increased to 20 repetitions in the 6th week), respectively (Afzalpour *et al.*, 2015). Warm-up and cool-down were performed at 16 m/min for 3 minutes.

Also, Active rest was performed between intervals in HIIT group for 60 s at 16 m/min. Intensities of active rest and interval training correspond to 68 and 95–100% VO_2 max, respectively (Afzalpour *et al.*, 2015). Mild electric shock (0.5 mA, 1 HZ) and soft sponge were used to stimulate the animals to run (Radak *et al.*, 2007). The rats of the C group were exposed to the same environment as HIIT groups without running (Ogonovszky *et al.*, 2005b).

Tissue preparation and Biochemical assays

Rats were sacrificed under deep anesthesia (Ketamine, 60–80 mg/kg and Xylazine, 8 mg/kg; IP) 48 h after last exercise session. 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 (Afzalpour *et al.*, 2015). Then, 1 ml $1 \times$ 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 in SPSS software (version 16.0), expressed as means \pm standard deviation. Initially, normality of distribution of dependent variables was approved by Shapiro-Wilk's test. Then, statistical significance was calculated using Student's t-test. Significance level was set at $p < 0.05$.

RESULTS

The results indicated a significant increases in OGG1 contents of brain (22.62 ± 3.29 vs. 11.40 ± 2.89 pg/mg for HIIT and C groups respectively; $t_{14} = 7.22$, $P = 0.001$) (Fig. 1A) and liver (48 ± 7.66 vs. 40.33 ± 3.69 pg/mg for HIIT and C groups respectively; $t_{14} = 2.55$, $P = 0.02$) (Fig. 1B).

In contrast, intensive interval training had no significant effect on 8-OHdG levels of brain (0.23 ± 0.07 vs. 0.29 ± 0.08 ng/mg for HIIT and C groups respectively; $t_{14} = 1.60$, $P = 0.13$) (Fig. 2A) and liver (0.77 ± 0.15 vs. 0.88 ± 0.11 ng/mg for HIIT and C groups respectively; $t_{14} = 1.28$, $P = 0.22$) (Fig. 2B).

Also, in comparison of two tissues, our results did not show any significant differences in change of protein content of OGG1 ($t_{14} = 0.97$, $P = 0.34$) and 8-OHdG ($t_{14} = 0.42$, $P = 0.68$) between brain and liver following HIIT.

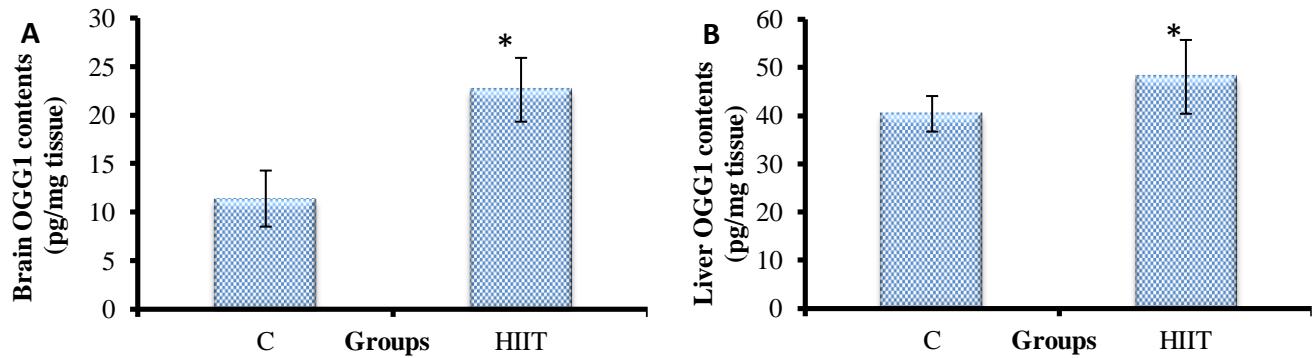


Fig. 1: Intensive interval training significantly increased OGG1 contents of brain (A) and liver (B). C; Control, HIIT; High intensive interval training.

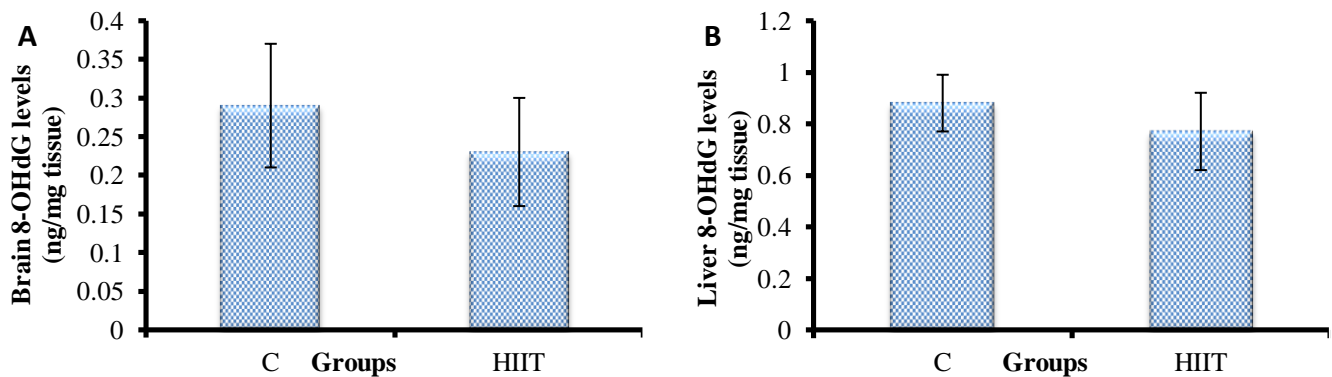


Fig. 2: Intensive interval training had no significant effect upon the 8-OHdG levels of brain (A) and liver (B). Abbreviations as donated in fig.1.

DISCUSSION

ROS generates by different sources in the cells including: mitochondrial electron transport chain, NADPH oxidase and xanthine oxidize (Ogonovszky *et al.*, 2005a, 2005b). Although, ROS at low doses plays an important role at physiological processes (Radak *et al.*, 2008), but ROS at large concentrations can modify oxidized lipids, proteins and DNA (Urso *et al.*, 2003, Radak *et al.*, 2008). Hence, cells are equipped with antioxidant and repairing enzymes to overcome stress oxidative conditions (Ogonovszky *et al.*, 2005a, 2005b). OGG1, as main repair enzyme, expresses in all kinds of tissues with significantly different levels, recognizes and cleaves 8-OHdG from DNA (Ogonovszky *et al.*, 2005a). There is now evidence that age (Radak *et al.*, 2011), smoking (Park *et al.*, 2011), VO_2 max (Loft *et al.*, 1994), physical labor, BMI, inter-individual variation, nutrient (Kasai *et al.*, 2001) 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. In addition, it seems that observed changes in OGG1 and 8-OHdG are simply due to HIIT protocol. While two previous studies 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 HIIT. Our findings are supported by a study by Ogonovszky *et al.* (2005), 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 HIIT 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 HIIT.

CONCLUSION

Separation of the training sessions to various bouts of exercise with maximum effort, through increase in OGG1 contents, will lead to modify of 8-OHdG levels in brain and liver.

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