

## Effect of Oxidative Injury and DNA Damage on Blood Cells Post Exhaustive Exercise

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The DNA damage and the level of anti-oxidative status on human peripheral blood cell has been investigated in different time after Marathon running, and the mechanism of DNA damage was discussed induced by extensive endurance exercise, which was aimed to provide some data for evaluating the training status and extent of fatigue on athletes. Twelve healthy male athletes were selected, who had volunteered to participate in International Marathon running in 2011 in Xiamen. The subjects were required to have a test at the time of twenty-four hours pre-exercise, also at 6 hours, 24 hours and 48 hours post exercise. The DNA damage were investigated on human peripheral blood cell by using the single cell gel electrophoresis (SCGE) and the level of anti-oxidative status were detected including the SOD, GSH and MDA in plasma. The results showed that the DNA damage on peripheral blood cell significantly increased after exercise in 6h and 24h ( $P>0.05$ ), and the DNA damage at 6h is significant higher than the 24h, then after 48 hours post exercise, it reduced to the level of rest pre-exercise ( $P>0.05$ ). The contents of SOD, GSH and MDA in plasma increased significantly at 6h post exercise ( $P<0.001$ ) and the activity of SOD and MDA were still significant higher than the Rest ( $P<0.001$ ), at 48h, the changes of free radical indexes in plasma had no significant difference compared with the rest pre-exercise.

**Key words:** exercise fatigue, blood cells, DNA damage, oxidative stress

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It is <sup>1</sup> well known that regular and appropriate exercise is good for our health, because it could reduce risk of a number of pathological disorders and extending the life-span of humans and laboratory animals (Davies, *et al.*, 1982). However, it has been reported that some types of prolonged physical exertion are detrimental to health, because exhaustive exercise is usually said to increase the generation of reactive oxygen species that are potentially harmful (Orhan, *et al.*, 1982). Strenuous exercise may increase the production of reactive oxygen species (ROS) by

enhanced oxygen consumption, leading to a situation of oxidative stress that has been discerned by detection of oxidatively damaged DNA bases and lipid peroxidation products (Aldred, *et al.*, 2007). The increase in oxygen consumption has been shown to be closely related to oxidative damage of DNA in humans, and Data have been presented suggesting that oxidative stress plays a role in the muscle fatigue and indirectly induce the DNA damage of body cells (Ji LL, 1993). Induction of oxidative damage in the body seems to be the result of an oxidative stress that exceeds the antioxidant capacity, which is composed of antioxidants and antioxidative enzyme. Some studies have observed exercise-induced DNA damage, most involved vigorous exercise conditions accompanied by muscle

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damage (Robertson, *et al.*, 1991). Some studies report that an acute bout of exercise increases the activities of superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase in skeletal muscle of rats (Fatouros, *et al.*, 2004). There are some studies have shown that exercise-induced lipid peroxidation in response to various modes of exercise, including endurance running (Mastaloudis, *et al.*, 2001). Generally, muscle damage and the subsequent inflammation are involved in exercise-induced DNA damage, particularly in the case of blood cells. Tissue injuries and phagocyte activation during exercise all contribute to increased reactive oxygen species (ROS) productions (Rada'k, *et al.*, 1999). These processes are expected to be more prominent in strenuous exercise such as running a marathon. Recently, there has been growing interest in exercise-induced DNA damage due to its potential involvement in various disease states, because when the body is repeatedly exposed to situations that could damage the DNA, the likelihood that some of these changes will not be repaired or will be incorrectly repaired (Sharbel, *et al.*, 2004). The relationship between oxidative stress due to vigorous exercise and induction of DNA modifications is unknown and their long term effects on health have yet to be elucidated (Hartmann, *et al.*, 1998 and Mastaloudis, *et al.*, 2004). The purpose of this study just once again to clarify what extend does the DNA damage occurred in exhaustive exercise such as marathon running and how long can it be repaired by our body's repair system. What the relationship is between exhaustive exercise, DNA damage, and antioxidant defense mechanisms in the human body.

## MATERIALS AND METHODS

### Subjects

We select twelve healthy male athletes (median age 21, range 20–24), who volunteer to participate in International Marathon race in 2011 in Xiamen (median running time 2:50 [h: min], range 2:45–3:10). Questionnaires were used to exclude the presence of acute or chronic infectious, inflammatory, or immune disorders during the study period. The participants did not take anti-inflammatory agents, steroid hormones,

antioxidants, or vitamin supplements. Informed consents were obtained from all the subjects. The participants were also asked to refrain from any form of exercise during the postrun recovery period. The subjects were required to have a test at the time of twenty-four hours before exercise, also 6 hours, 24 hours and 48 hours after exercise. We investigated the DNA damage on human peripheral blood cell by using the single cell gel electrophoresis (SCGE) and detected the level of anti-oxidative status including the SOD, GSH and MDA in plasma.

### Materials

The solutions and chemicals used in this study were purchased from the following companies. Dimethyl sulfoxide (DMSO), normal melting agarose (NMA), low melting agarose (LMA), sodium sarcosinate, Triton X-100, ethidium bromide (EB) and trypan blue were purchased from Sigma Chemicals Company (St. Louis, USA).

### Blood draws

Blood samples were obtained 24 h before the race (A: prerace (0 h)), 6 h after race end (B: postrace (6h)), 24 h after the race (C: postrace (24 h)), and 48 h after the race (D: postrace (48h)) for a total of four time points.

### Blood samples

Blood was drawn into two 5 cc green-top Vacutainer tubes (containing 143 USP units sodium heparin) and one 5 cc purple-top Vacutainer tube (containing 1 mg/ml EDTA). Aliquots of whole blood were taken immediately for the comet assay analysis. Remaining blood was centrifuged at 2500×g for 10 min; Plasma was then aliquoted to cryotubes for various assays. Samples were flash frozen in liquid nitrogen and stored at -80°C until time of analysis (within 6 months of collection).

### Alkaline comet assay

The alkaline comet assay was done as previously described methods (Godard, *et al.*, 1999). After automatic delimitation of nucleus head and tail as well as elimination of background fluorescence and touching cells, different parameters are calculated, describing nucleus geometry (length, areas . . .) and intensity (% of DNA in the head of the comet, tail moment . . .). Tail moment was defined by the product of the distance between the two barycentres of the head and the tail by the proportion of fluorescence in the tail of the comet.

### Activity of antioxidant systems

The livers were removed and frozen in liquid nitrogen, and subsequently homogenized in ice-cold Tris-HCl buffer (25 mmol/L Tris, 1 mmol/L EDTA, 10% glycerol, and 1 mmol/L DTT, pH 7.4) with a glass homogenizer. The homogenate was centrifuged at 10 000  $\times$ g for 20 minutes at 4°C. The supernatant and sediment fractions were separated, and the supernatant was aliquoted and stored at -80°C. The activity of SOD, GSH and MDA was determined spectrophotometrically according to the method of the Nanjing Jiancheng Bioengineering Institute(China) with a spectrometer.

### Statistical analysis

Data were expressed as the mean  $\pm$  S.D. for the number of experiments indicated. Statistical analyses of the data were analyzed by one-way analysis variance (ANOVA). Significance level was set at  $p < 0.05$ .

## RESULTS

### DNA damage of blood cells induced by marathon running

DNA damage is visualized at the individual cell level as an increased migration of genetic material (“comet tail”) from the nucleus (“comet head”). Tail moment is the product of tail length and percentage DNA in tail; thus tail moment

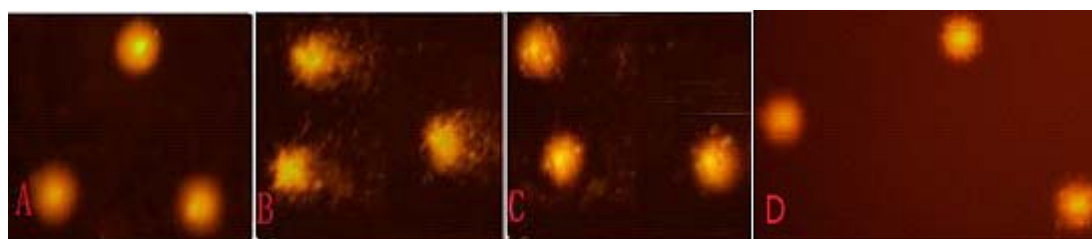
represents both the amount of DNA migrated into the tail and the distance migrated (Fig. 1). The tail moment is commonly reported (Godard, *et al.*, 1999) as a valid marker of single-strand DNA breakage. As presented in Figure 1, the control group(A: prerace (0 h)) did not have obvious “comet tail” (A); 6 h after race end ( B: postrace (6h)) has obvious “comet tail” (B); the 24 h after the race (C: postrace (24 h)) look like has a little “comet tail” (C); the post exercise 48 hours group(D: postrace (48h)) return back as the rest level and the “comet tail” disappear (D).

In Fig2, we also use the comet assay parameter tail moment to express the DNA damage of blood cell induced by repeated exhaustive exercise. We found that the higher of this value, the greater the damage that has occurred to the nuclear DNA. Statistically significant differences ( $p < 0.01$ ) in DNA damage were found in the blood cells of human body in 6 h after race ( B: postrace (6h)) as compared to rest level(prerace (0 h)), the DNA damage of blood cells in postrace (24 h) were significant higher than rest level(prerace (0 h)) ( $p < 0.05$ ). There were no significant differences between prerace (0 h) and postrace (48h). The control blood cells sustained the least background damage. Figure 1 and 2 summarize the different levels of DNA damage in both prerace and the post marathon running.

**Table 1.** The changing of SOD and MDA on human plasma after Marathon running

Group	n	SOD (U/ml)	GSH(mg/L)	MDA (nmol/ml)
rest	12	14.64 $\pm$ 3.11	126.3 $\pm$ 27.28	5.17 $\pm$ 1.11
6h	12	63.14 $\pm$ 9.62****#	302.61 $\pm$ 47.52****#	10.65 $\pm$ 1.14****#
24h	12	34.69 $\pm$ 4.67***	169.71 $\pm$ 32.1	8.5 $\pm$ 0.9***
48h	12	15.8 $\pm$ 3.6	145.95 $\pm$ 28.6	6.2 $\pm$ 1.12

Compared with the rest, \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; compared with the 24h, #  $P < 0.05$ , ##  $P < 0.01$ .



A: prerace (0 h)      B: postrace (6h)      C: postrace (24h)      D: postrace (48h)

**Fig. 1.** DNA comet image of peripheral blood cell in different time after exercise fatigue ( $\times 200$ )

### SOD activity, GSH level and MDA content in human plasma after marathon running

From Table 1, we can see that after marathon running at 6h and 24h, SOD activity level in human plasma were significantly higher ( $P < 0.001$ ,  $P < 0.01$ ) compared with rest level, however, postrace at 48h decreased significantly compared with rest level ( $P > 0.05$ ) than the pre-exercise, but 48h after exercise than before exercise significantly decreased the level of quiet ( $P < 0.05$ ). GSH levels in human plasma at 24h post-exercise was

significantly higher than pre-exercise level, and after exercise at 24h and 48h showed out downward trend, compared with the pre-exercise level, there was no significant difference ( $P > 0.05$ ). MDA content in human plasma were significantly increased ( $P < 0.001$ ) after marathon running at 6 and 24h than pre-exercise, after running one of 6h was significantly higher than that after running 24h ( $P < 0.01$ ), and after running exercise 48h, there is no significant difference ( $P > 0.05$ ).

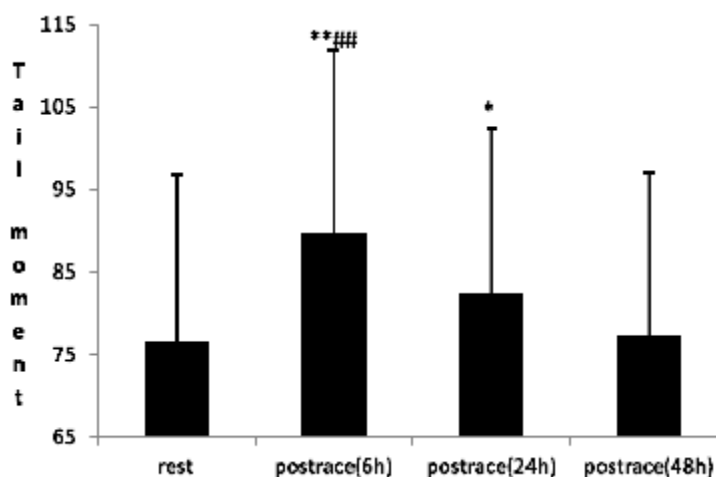


Fig. 2. Levels of DNA damage in blood cells of different time on marathon athletes. Compared with the rest, \*  $P < 0.05$ , \*\*  $P < 0.01$ ; compared with the 24h, #  $P < 0.05$ , ##  $P < 0.01$ .

### DISCUSSION

The alkaline single cell gel electrophoresis assay (SCGE), which also called comet assay, has been used in many in vitro applications to assess DNA damage in individualized mammalian cells. This assay sensitively detects DNA single- and double-strand breaks induced by chemical compounds (Roy, *et al.*, 2007). In the present study, the 21 km marathon race was long-duration and massive exercise mode. It showed that DNA damage occurred post exercise 6 hours and 24 hours, the extend of DNA damage ranged differently, the post exercise 6 hours reached highest, DNA damage returned back as normal control post exercise 48 hours. It is in a good agreement with the following published results. Niess(Niess, *et al.*, 1996) and Hartmann(Hartmann, *et al.*, 1995) reported the DNA damage increase in

comet tail moment 24 h after a maximal oxygen consumption test on a treadmill. Although there were some studies showed that the longer duration of the exercise, the shift in the peak of DNA damage may range differently (Hartmann, *et al.*, 1998 and Mastaloudis, *et al.*, 2004). All of these findings indicated that the extent of DNA damage and the DNA repair time can be an index to evaluate the situation of exercise fatigue on athletes. However, exercise-induced DNA damage may vary according to the mode, intensity, and duration of the exercise protocol and prior training status of the participants. So in our results showed that marathon race induced DNA damage heavily in first day, and after two days the DNA damage would recovery as rest level. It suggested that our human body has repair system to repair DNA damage from away further injury, but our body may need time, rest and energy to rebuild it.

In the current study, we investigated the hypothesis that athletes exposed to exhaustion exercise (Aldred, *et al.*, 2007) would be subjected to oxidative stress, and that this would be manifested as either alteration in the status of oxidative defense mechanisms, or in the levels of DNA damage. It is possible that ROS produced during exhaustive exercise is more likely to cause DNA damage. Antioxidant status was assessed by measurement of two key antioxidants (GSH and SOD). The present study also investigates the status of lipid peroxidation. Malondialdehyde level is a marker of lipid oxidation. Reactive oxygen species (ROS) cause lipid peroxidation and oxidation of some specific proteins, thus affecting many intra- and intercellular systems (Child, *et al.*, 1998). Since the protective action of GSH and SOD against oxidant injury is known to be due to its oxygen and other radical scavenging capacity (Demirbag, *et al.*, 2006), the level of GSH in our human plasma responded to be increased to fight against ROS to keep away from insult. Some studies report that an acute bout of exercise increases the activities of superoxide dismutase (SOD) (Ji, 1995). So in our research, the data showed that GSH and SOD level increased significantly post exercise at 6h, and then they turned out to be decreased at 24h and 48h. It may be due to the massive production of ROS in the first few hours post race, so antioxidants were stimulated to increase to protect our body. The fact that, in the present study, a significant level of DNA damage was detected after exhaustive running probably shows that DNA might be a weak link in a cell's ability to tolerate oxygen free-radical attack. It is conceivable that the levels of exercise attained in our experiments could be associated with oxidative stress, and perhaps the deleterious effects associated with such stress. It is possible that a depression in the running performance of athletes could be attributed to disruption of the oxidant/antioxidant balance consequently resulting in oxidative stress. According to our data, the blood cells were susceptible to the influence of elevated free radicals, because the SOD activities of 6h and 24h both increased significantly compared with rest level. We investigated that MDA Levels were significantly elevated in 6h and 24h, and 6h was the most highest among the four groups. This could be explained by the blood's prominent role

to carry increasing oxygen for body's need in exhaustive exercise; thus they are likely to act as the initial site of oxyradical formation due to the high levels of oxygen encountered and also the high rate of oxidative phosphorylation to support energy-dependent transport systems (Hartmann, *et al.*, 1994). Our findings indicate that the mechanism of lipid metabolism is impaired in exhaustive exercise. The balance between oxidative stress and antioxidant defense mechanism may be impaired by depletion of enzymatic antioxidants and increased blood levels of MDA in exhaustive athletes. The significant increase in DNA strand breaks in this study could partly be attributed to oxidative stress (Selman, *et al.*, 2002). Aside from direct attack of cell DNA by ROS, the various products of lipid peroxidation can also interact with DNA and cause oxidation of nucleotides (Blaisdell, *et al.*, 2001). Some authors have suggested that DNA repair enzymes respond rapidly to any oxidative insult to DNA (Bombail, *et al.*, 2001).

## CONCLUSION

In conclusion, the current data indicate that strenuous exercise caused oxidative stress and this was associated with elevated DNA damage, but in our human body DNA damage generally can be repaired by its own repair system, although the recovery time distinct from each other and it may depend on the exercise intensity and training status of athletes (Tsai, *et al.*, 2001). So, the more the repair activity, the less DNA damage that are generated (Emmanouil, *et al.*, 2006). The extent of blood cell DNA damage may be an index to evaluate the body's situation.

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