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# Protective effect of vitamin e on dna damage in liver cell during exercise

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## ABSTRACT

To observe the effect of vitamin E(VE) supplements on DNA damage in mice liver cell induced by acute exercise. 40 male Kunming mice were averagely randomized into four groups: Control group(CG), exercise group without VE supplements(EG),VE supplements group without exercise(VEG) and VE supplements group with exercise(E+VEG). We built up the exercise fatigue model for mice through the protocol of repeated exhaustive treadmill running and use the single cell gel electrophoresis(SCGE) to detect the DNA damage of liver cells in different groups. We also measured the changes of SOD, GSH and MDA on liver tissue. Our studies showed that the DNA damage of liver cell in E+VEG group was significantly lower than that of EG group($P<0.001$)and there was no statical difference between CG and VEG groups. And the level of SOD and GSH in E+VEG group were significantly lower than that of EG group($P<0.001$). MDA content in EG group was highest,the second was E+VEG group,and the last were CG and VEG groups. Those results leaded to conclusion that acute exercise induced more free radical to increase the level of SOD, GSH and MDA, and also occurred more DNA damage, however the VE supplements has obvious protective effect. Exercise induced oxidative stress is one of the mechanism of DNA damage on liver cells. 

## KEYWORDS

Vitamin E supplements;
Liver cell;
DNA damage;
Single cell gel electrophoresis(SCGE);
Oxidative stress.

## INTRODUCTION

It was reported that tissue injury could be occurred during the extremely exercise, and exercise oxidative stress induced lipid peroxidation may be one of the mechanisms causing DNA damage[1]. Antioxidant supplements to prevent or alleviate the damage of DNA has been experimentally confirmed[2]. Vitamin E (VE) is well known and effective lipid soluble antioxidant, and widely used in many fields[3]. VE can prevent the DNA damage of toxic effect to the body, and prevent or reduce the form of free radical during human exposure to ionizing radiation, smoking, food additives in the daily environment[3]. However, the current studies about the effect of VE on preventing DNA damage after exercise fatigue are still rarely reported. This experiment used single cell gel electrophoresis (single cell gel electrophoresis, DNA SCGE)[4] to detect the DNA damage induced by exercise fatigue in mice liver cells, and the protective effect of VE on liver injury in mice DNA cells were observed, so as to provide a reference basis on how to supplement VE in human body,

and also provides a new method for the prevention and elimination of exercise fatigue. Furthermore, it aimed to observe the effect of vitamin E(VE) to reduce the oxidative stress, through which the DNA damage would be repaired or not.

## MATERIALS AND METHODS

### Animals and exercise protocol

Healthy male mice (KM, supplied by animal center of medicine department of Peking University), 7-8 weeks, the mean weight were 28.68±2.56g. The animal room was kept at 20–24!and 40–60% humidity with a 12/12 hr dark/light cycle. 40 mice were randomized into four groups: Control group(CG), exercise group without Vitamin E supplements(EG),VE supplements group without exercise(VEG)and VE supplements group with exercise (E+VEG), each group had 10 mice.

The exercise protocol was done as Marra et al. [5], but it was repeated for seven days on the exercised group, once per day. It consisted of an acute exhaustive exercise bout of treadmill running. Mice were run at 28m/min on a 28 slope for 90 min following a brief (10min) warm-up. Exhaustion was determined by failure to run after continued prodding and splaying within the treadmill lane. Control group mice were exposed to the noise and vibration of the treadmill for the same duration as the exercised mice.

### Supplementation of vitamin E (VE)

The mice of VEG and S+ VEG group were fed with Vitamin E (VE) post exercise 1hour every day during the training period. VE was purchased from Beijing double crane pharmaceutical company, and its batch number was H11021397.

### Alkaline comet assay

The alkaline comet assay was done as described by Godard et al.[4]. Fully-frosted clean microscope slides were covered with 1% normal melting point (NMP) agarose and allowed to polymerize at room temperature to allow agarose to dry. After solidification, the gel was scraped off from the slide. The slides were further coated with 0.6% NMP agarose. When this layer had solidified a second layer containing the whole chicken blood sample (0.5μL) mixed with 0.5% low melting point (LMP) agarose was placed on the slides. After 10 min of solidification on ice, slides were covered with 0.5% of LMP agarose. An amount of 100 μL of this agarose cell suspension was layered on the top of the second layer. Finally, the fourth layer of 0.5% low melting point (LMP) agarose was added to cover the third layer and allowed to solidify for 10 min at 4°c.

Afterwards the slides were immersed for one hour in ice-cold freshly prepared lysis solution (2,5M NaCl, 100 mM $Na_2EDTA$, 1% Na-sarcosine, 1% 10 mM Tris–HCl, pH 10 with Triton X-100 and 10% DMSO added fresh to lyse cells and allow DNA unfolding). After lysis, slides were placed in the freshly prepared electrophoresis buffer (300 mM NaOH, 1 mM $Na_2EDTA$, pH 13.0) to remove salts. The slides were set in this alkaline buffer for 10 min to allow DNA unwinding and expression to alkali labile sites. Denaturation and electrophoresis was performed at 4°c under dim light at 25 V (300 mA). After electrophoresis, the slides were washed three times at 5 min intervals with buffer (1% Triton X-100, 10%DMSO) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 μg/ml) for 10 min and covered with a cover slip. Slides were stored at 4°c in humidified sealed containers until analysis. To prevent additional DNA damage, handling with blood samples and steps included in the preparation of slides for the comet analysis were conducted under yellow light or in the dark.

Slides were examined at 200× magnification on an Olympus fluorescence microscope (Olympus Optical Co, Ltd, Tokyo) with excitation at 520 nm green barrier filter. Twenty five randomly selected cells were submitted to image analysis system. After automatic delimitation of nucleus head and tail as well as elimination of background fluorescence and touching cells, different parameters are calculated. 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 liver tissue 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%

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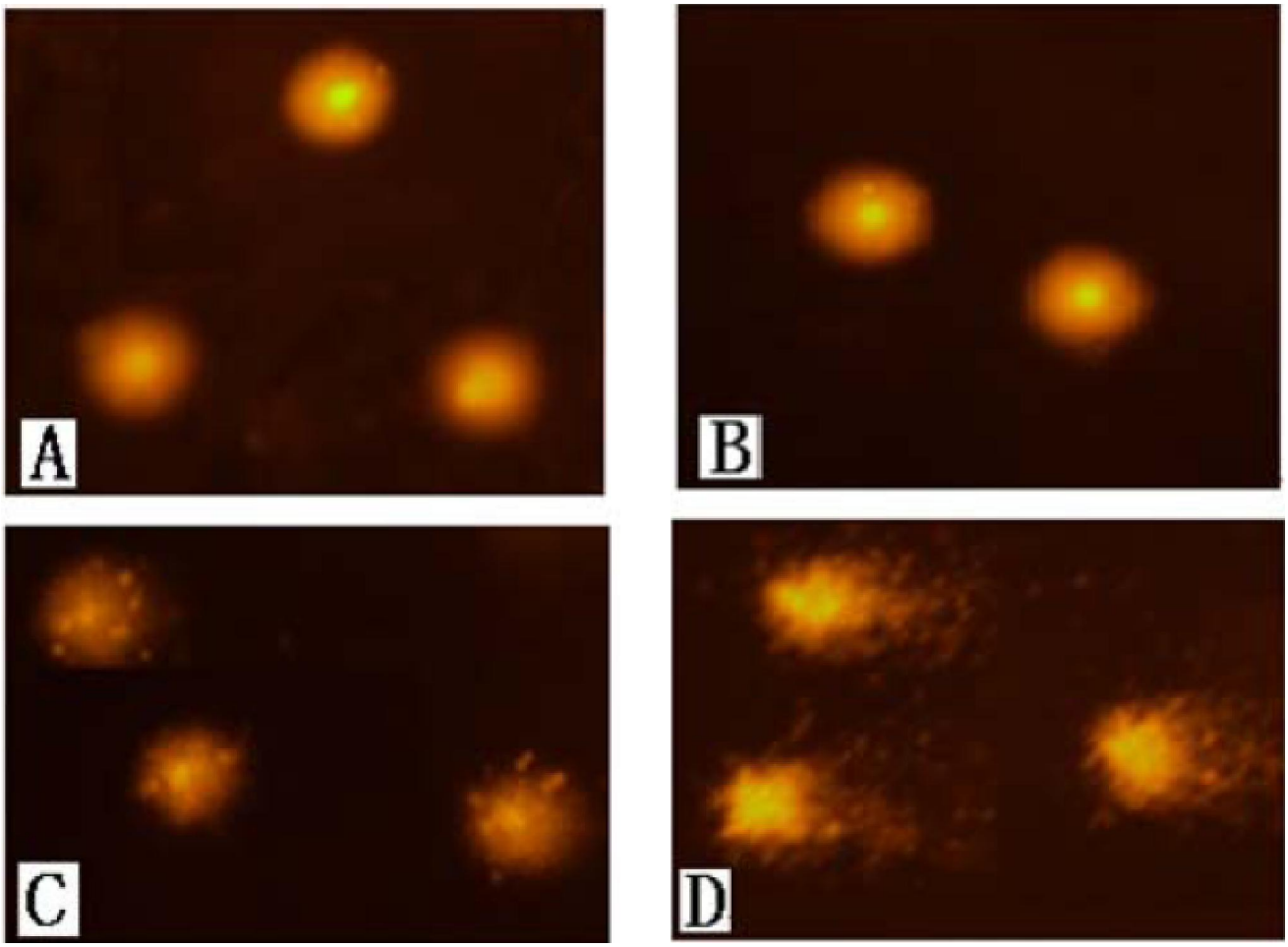
glycerol, and 1 mmol/L DTT, pH 7.4) with a glass homogenizer. The homogenate was centrifuged at 10 000 ×g for 20 minutes at 4°C. The supernatant and sediment fractions were separated, and the supernatant was aliquoted and stored at -80°C. The activity of SOD ÿGSH and MDA was determined spectrophotometrically according to the method of the Nanjing Jiancheng Bioengineering Institute(China) with a spectrometer.

## RESULTS

### DNA damage o of mice liver cells in different groups induced by exhaustive exercise

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 (Figure 1). The tail moment is commonly reported[4] as a valid marker of single-strand DNA breakage. As presented in Figure1, the control group(CG) and the VE supplementation group without exercise(VEG)did not have vivid “comet tail” (A and B respectively); the exercise group with VE supplements (E+VEG) showed out a little “comet tail” (C); the exercise group (EG) has obvious “comet tail”(D).

In TABLE 1, we also use the comet assay parameter to express the DNA damage of liver cell induced by repeated exhaustive exercise. We found that the higher this values, the greater the damage that has occurred to the nuclear DNA. Statistically significant differences ($P<0.001$) in DNA damage were found in the liver cells of mice in EG as compared to CG and VEG, the DNA damage of blood cells in E+VEG did not show significant higher than CG and VEG($P>0.05$). There were no significant differences between CG and VEG($P>0.05$). The CG and VEG cells sustained the




**Finger 1 : DNA comet image of mice liver cell in different group(×200).**

least background damage. Figure 1 and TABLE 1 summarize the different levels of DNA damage in different group of mice.

**TABLE 1 : The comet assay parameter of liver cell in different group**

| Group | Tail inertia | Olive tail moment | Tail Moment |
|---|---|---|---|
| CG | 3821.11±1231.21 | 3.21±0.86 | 53.12±15.31 |
| EG | 4572.62±1785.47* | 13.16±4.63*** | 57.64±16.21* |
| VEG | 3648.97±1023.65* | 2.7±0.75 | 50.35±13.86* |
| E+VEG | 3982.45±1461.34$^{\#}$ | 4.96±1.21$^{\#\#\#}$ | 52.73±14.26$^{\#}$ |

**All the data was expressed by x ±s , compared with CG, *P<0.05ÿ**P<0.01ÿ***P<0.001; compared with EG, $^{\#}$P<0.05, $^{\#\#}$P<0.01,$^{\#\#\#}$P<0.001.**

## Measurement of SOD, GSH and MDA

From the TABLE 2, it showed that the SOD activities in liver tissue of mice were largely altered in all groups. The SOD activities of EG group were significantly higher than the other three groups, it were the same with the level of GSH and MDA. The SOD activities and the level of GSH in VEG group increased obviously than CG group(P<0.001), however, the level of MDA reduced significantly(P<0.001). Compared with EG, the SOD activities and GSH level in E+VEG group reduced in varying degree(P<0.001,P<0.05), through the level of MDA reduced significantly(P<0.001).

**TABLE 2 : The changing of SOD, GSH and MDA on liver tissue in different group**

| Group | SOD(U/mgprot) | GSH(mg/gprot) | MDA(nmol/ mgprot) |
|---|---|---|---|
| CG | 62.67±7.31 | 52.51±4.16 | 6.86±1.21 |
| EG | 143.72±13.28*** | 68.78±7.41** | 15.48±1.46*** |
| VEG | 75.86±12.62*** | 57.98±3.63*** | 5.03±0.94*** |
| E+VEG | 114.78±8.17$^{\#\#\#}$ | 63.12±4.81$^{\#}$ | 7.12±1.51$^{\#\#\#}$ |

**All the data was expressed by x ±s ,compared with CG, *P<0.05, **P<0.01, ***P<0.001; compared with EG, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001**

## DISCUSSION

DNA damage are physical abnormalities in the DNA, such as single- and double-strand breaks. DNA damages can be recognized by enzymes, and, thus, they can be correctly repaired if redundant information, such as the undamaged sequence in the complementary DNA strand or in a homologous chromosome, is available for copying[6-7]. Moreover, DNA damaging agents can damage other biomolecules such as proteins, carbohydrates, lipids, and RNA. Generally, DNA damage is the leading basis in the carcinogenic process, however DNA can be repaired, for the body has some repair mechanism after injury, so as to avoid the occurrence of tumor[8-9]. The cause of damage of DNA has a lot of kinds, at present, some research has confirmed the high intensity exercise will lead to damage of the genetic material DNA[10]. Blood mononuclear cell DNA oxidative damage is found to increase in human body after intense exercise[11]. It was also found that the DNA of skeletal muscle cells, lymphocytes and liver cells occurred damage to some extent[12]. This study with the SCGE technique for the detection of DNA damage in mice liver cell found positive index tail inertia, olive tail moment, tail moments were significantly increased post exhaustive exercise, suggesting that DNA of mice liver cells appeared serious injury, this result consistent with other studies[13].

Antioxidant status was assessed by measurement of two key antioxidants (GSH and SOD). The present study also investigates the status of lipid peroxidation. Antioxidants work together in animal cells against toxic reactive oxygen species[14]. Reactive oxygen species (ROS) cause lipid peroxidation and oxidation of some specific proteins, thus affecting many intra- and intercellular systems[15]. SOD plays a key antioxidant role, which protects against oxidative damage especially mediated by free radicals and lipid perioxidation. GSH plays an important role in antagonizing exogenous poison,oxygen free radical injury, adjusting the immune function, maintaining cell protein and inhibiting cell apoptosis, which is he main material cells against reactive oxygen damage. And it is sensitive and can comprehensively reflect the capacity of cells against damage[16]. Malondialdehyde(MDA ) is the product of lipid peroxidation and its level is a marker of lipid oxidation, also its content can reflect the degree of lipid peroxidation in vivo and indirectly reflect the extent of the damage of cells[17]. The results of the experiments showed that SOD activity and GSH content increased as well as DNA damage in in mice liver tissue after acute exercise, and the VE supplements reduce the MDA level and DNA damage, which was concluded

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that DNA damage was related with tissue lipid peroxidation, and the high intensity exercise caused oxidative damage in the liver and eventually lead to DNA damage of liver cells. The fact that, in the present study, a significant level of DNA damage was detected after exhaustive running in the mice 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 the mice could be attributed to disruption of the oxidant/antioxidant balance consequently resulting in oxidative stress.

Vitamin E(VE ) as an antioxidant one hand can inhibit the oxidase enzymes system, activate and protect anti-oxidase system, on the other hand, it can directly react with lively free radical, change lipid peroxides into hydroxyl resin, and it is a strong free radical scavenger[18]. The study found that DNA damage of E+VEG group were significantly lower than that of EG in mice liver cell, indicating that VE can effectively alleviate and eliminate DNA damage of liver cell caused by high intensity exercise, the reason may be related to antioxidant effect in vivo on Vitamin E(VE). The results also showed that GSH and SOD activity and MDA content in E+VEG group were significantly lower than those of EG in liver tissue of mice, suggesting the supplement of VE can alleviate the oxidative damage effect on the liver induced by high intensity exercise, which reduce the free radicals attacking the liver nuclei and nuclear genetic material. Therefore DNA damage in E+VEG mice was significantly lower than that of EG mice. However, the activity of SOD and GSH and MDA content in E+VEG mice liver tissue were significantly higher than that of CG, the results may be related with VE supplementation dose or time. Therefore, the best effect of VE supplementation on the protection of DNA damage remains to be further studied.

## CONCLUSION

In conclusion, the current data indicated that strenuous exercise caused oxidative stress in these animals and this was associated with elevated DNA damage. In exhaustive exercise, Vitamin E supplements could reduce the DNA damage on mice liver cell during exercise.

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