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Original Article

Impact of different running distances on muscle and lymphocyte DNA damage in amateur marathon runners

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Abstract. [Purpose] The aim of this study was to investigate the impact of different marathon running distances (10 km, 21 km, and 42.195 km) on muscle and lymphocyte DNA damage in amateur marathon runners. [Subjects and Methods] Thirty male amateur runners were randomly assigned to 10 km, 21 km, and 42 km groups, with 10 subjects in each group. Blood samples were collected before and after the races and on the 3rd day of recovery to examine levels of muscle damage (creatine kinase and lactate dehydrogenase) and lymphocyte DNA damage (DNA in the tail, tail length, and tail moment). [Results] Serum creatine kinase, serum lactate dehydrogenase, and tail moment were significantly higher after the races compared with before the races in all groups. In addition, the 42 km group showed significantly higher levels of creatine kinase, lactate dehydrogenase, and tail moment than the 10 km and 21 km groups after the races. [Conclusion] Strenuous endurance exercise can cause muscle and lymphocyte DNA damage, and the extent of such damage can increase as running distance increases.

Key words: Marathon, Muscle damage, Lymphocyte DNA damage

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INTRODUCTION

Recently, there has been a growing worldwide interest in marathon running due to recognition of the positive effects of endurance exercise on health and physical strength. In particular, an increasing number of amateur runners are participating in recreational 10- and/or 21-km marathons, because the physiological demand is lower than for full or ultramarathons[1)]. However, while regular moderate exercise has beneficial effects on health, it has been reported that irregular strenuous exercise could exert a negative effect on health[2–4)].

Oxygen intake through respiration is involved in various metabolic processes as an essential factor for producing ATP, an energy source. However, incomplete reduction of oxygen during metabolic processes results in partial changes in the structure of the oxygen molecules in the body, to produce reactive oxygen species (ROS), which can show toxicity in the body by inducing excessive oxidative stress[5)]. Oxidative stress is generated by the disruption of homeostasis when oxidation reactions become dominant in the redox reaction, through which balance is maintained between oxidants and antioxidants[6)]. Regular moderate exercise can elevate antioxidant enzyme activities and reduce oxidant production. Thanks to the antioxidant defense mechanisms of the body, resting ROS production in a healthy person is within the capability of the antioxidant defense system. However, in a situation that demands several to dozens of times greater oxygen supply, as during strenuous

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Table 1. Characteristics of the subjects

Variables / group	10 km (n=10)	21 km (n=10)	42 km (n=10)
Age (years)	36.5±10.9	45.0±7.8	37.9±13.6
Height (cm)	173.1±4.3	167.3±5.2	175.9±9.2
Weight (kg)	68.9±7.2	66.6±6.1	69.4±8.8
Body fat (%)	15.5±3.8	17.7±3.8	13.9±2.1
Volume of training (hours/week)	5.0±1.5	5.6±3.6	6.1±1.2
Marathon careers (years)	5.9±4.0	6.6±2.2	7.0±4.2

Values are means ± SD

exercise, ROS production increases drastically[7, 8]. In particular, prolonged endurance exercise, such as marathon running, increases oxygen intake by the human body by more than 10–15 times compared with resting oxygen intake, and such increased use of oxygen can result in excessive production of ROS at a ratio that exceeds the human body's ability to recover the normal ratio[8–10]. Excessively produced ROS in turn can cause DNA damage and alterations. If there is failure to repair damaged DNA, consequent genomic and chromosomal aberrations can alter the activities of genes/proteins and thereby can lead to aging, cancer, arteriosclerosis, neuropathy, and inflammation[11].

On the other hand, endurance exercise without a break can cause damage due to repetitive muscle contractions[12]. Previous studies[13–15] suggested that competitive exercise resulted in an increase in creatine kinase (CK) and lactate dehydrogenase (LDH), which are important indices of the extent of skeletal muscle cell damage, and that running distance is associated with muscle damage.

Despite the fact that strenuous endurance exercise can have a negative effect on health as described above, long-distance running is increasing in popularity. The effect of long-distance running on health can vary, depending not only on personal physical strength and health but also on exercise intensity including running distance and duration. To reduce the adverse effect of exercise and maximize its positive effect on health, it is essential to understand the physiological response according to exercise intensity and to select a proper exercise method according to personal physical status.

Therefore, the purpose of the present study was to investigate the impact of different marathon running distances (10 km, 21 km, and 42.195 km) on muscle and oxidative DNA damage by analyzing lymphocyte DNA, serum CK, and serum LDH in amateur marathon runners.

SUBJECTS AND METHODS

The subjects were recruited from the participants in the Hangang Marathon race in Seoul, Republic of Korea. Of those initially recruited, 30 male amateur runners who were nonsmokers and nondrinkers, had no particular medical disease, and who had completed at least 3 full marathons and participated in regular running exercise at least 3 times a week were ultimately selected. The 30 subjects were randomly assigned to 10 km, 21 km, and 42 km groups, with 10 subjects assigned to each group. Physical characteristics are presented in Table 1. The study protocol was approved by an institutional ethics review board of the Department of Physical Education at Yonsei University. All participants provided written informed consent, and the study conformed to the standards set by the latest revision of the Declaration of Helsinki.

The day before the marathon race, height, weight, and body composition were measured using an impedance analyzer (InBody 4.0, Biospace, Seoul, Republic of Korea), and prerace blood samples were collected. The subjects were directed to avoid excessive physical activity from the day before blood collection and to maintain an empty stomach for at least 12 hours after the last meal. Blood samples were collected from a forearm vein before (Pre) and after the races (Post), and on the 3rd day of recovery (Recovery). Postrace blood collection was carried out at the finish line, immediately after completion of the individual marathon courses (10 km, 21 km, and 42.195 km). Blood collection on the 3rd day of recovery was carried out by the same method as used for the prerace blood samples. Subjects were directed not to take vitamin compounds or nutritional supplements from immediately after the marathon race until the 3rd day of recovery. Blood samples were centrifuged at 3,000 rpm for 20 min, stored at −80 °C, and directly analyzed.

Serum CK and LDH levels were determined using a clinical chemistry analyzer (Ektachem DTSCII, Kodak, USA).

Lymphocyte DNA damage was determined by using a comet assay, which showed single- or double-stranded DNA breaks. For the comet assay, 130 µl of whole blood was mixed with 900 µl of phosphate buffered saline (PBS) and poured gently over a 150 µl lymphocyte separation solution. After centrifugation at 1,450 rpm (4 min), lymphocytes were pipetted out and transferred to another tube. Seventy-five microliters of low melting temperature agarose (LMA) was put into the tube and mixed with a pipette. After removing the cover glass from the slide, the mixture was poured over the slide horizontally, covered with a cover glass, put on a freezing plate, and refrigerated for 5 min. Electrophoresis was conducted for 20 min at 25 V and 300 mA. When electrophoresis was finished, the nuclei were treated with fluorescence-based staining and observed under a fluorescence microscope (Leica, Germany). An image of each nucleus was captured with a CCD camera (Nikon,

Table 2. Comparison of serum CK and LDH levels according to the different running distances

Variables	Time	Group		
		10 km	21 km	42 km
CK (U/L)	Pre	136.1±40.7	143.2±39.2	140.6±56.4
	CV	0.30	0.27	0.40
	Post	175.0±52.8$^{*,\#}$	177.3±38.1$^{*,\#}$	312.6±146.5*
	CV	0.30	0.21	0.47
	Recovery	179.0±56.7$^{*,\#}$	176.5±51.6$^{*,\#}$	320.1±179.5*
	CV	0.32	0.29	0.56
LDH (U/L)	Pre	289.3±33.4	290.0±31.5	302.2±37.4
	CV	0.12	0.11	0.12
	Post	336.3±30.5$^{*,\#}$	343.8±32.0$^{*,\#}$	427.0±42.6$^{*,\#}$
	CV	0.09	0.09	0.10
	Recovery	302.8±28.4	282.5±28.3	327.9±65.2
	CV	0.09	0.10	0.20

Values are means ± SD. $^{*}p < 0.05$ vs. Pre; $^{\#}p < 0.05$ vs. 42 km

Table 3. Comparison of lymphocyte DNA damage according to the different running distances

Variables	Time	Group		
		10 km	21 km	42 km
DNA in tail (%)	Pre	7.99±1.33	8.23±1.21	8.32±2.21
	CV	0.17	0.15	0.27
	Post	12.35±1.91	12.20±1.52	13.22±2.11
	CV	0.15	0.12	0.16
	Recovery	9.04±1.60	8.68±0.89	8.86±0.89
	CV	0.18	0.10	0.10
Tail length (μm)	Pre	51.45±5.60	52.28±4.50	52.50±10.88
	CV	0.11	0.09	0.21
	Post	66.13±5.08	64.16±7.57	68.73±9.87
	CV	0.08	0.12	0.14
	Recovery	53.61±6.74	50.97±3.47	52.79±5.79
	CV	0.13	0.07	0.11
Tail moment	Pre	5.89±1.41	6.29±1.34	6.52±2.18
	CV	0.24	0.21	0.33
	Post	9.55±1.88$^{*,\#}$	9.49±1.93$^{*,\#}$	13.19±4.55*
	CV	0.20	0.20	0.34
	Recovery	5.92±1.87	5.77±1.16	6.62±1.50
	CV	0.32	0.20	0.23

Values are means ± SD. $^{*}p < 0.05$ vs. Pre; $^{\#}p < 0.05$ vs. 42 km

Japan) and was analyzed with the Komet 4.0 comet image analyzing system (Andor Technology, Belfast, UK).

Statistical analyses were performed with SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation (SD) unless otherwise stated. To identify differences in normally distributed results, two-way repeated analysis of variance (ANOVA) was employed. When a significant interaction was apparent, the simple main effects on measured variables were determined using one-way ANOVA. Tukey's post hoc test was subsequently used to conservatively locate significant differences. Statistical significance was set at $p < 0.05$.

RESULTS

Table 2 shows a comparison of serum CK and LDH levels at rest and in response to different marathon running distances. Serum CK levels were significantly higher at Post and Recovery than at Pre in all groups ($p < 0.05$). In addition, the 42 km

group showed significantly higher serum CK levels than the 10 km and 21 km groups at Post and Recovery ($p < 0.05$). Serum LDH levels were significantly higher at Post than at Pre and Recovery in all groups ($p < 0.05$). In addition, the 42 km group showed significantly higher LDH levels than the 10 km and 21 km groups at Post ($p < 0.05$).

Table 3 shows a comparison of lymphocyte DNA damage (DNA in the tail, tail length, and tail moment) at rest and in response to the different marathon running distances. Tail moment was significantly higher at Post than at Pre and Recovery ($p < 0.05$). In addition, the 42 km group showed a significantly higher tail moment level than the 10 km and 21 km groups at Post ($p < 0.05$). However, DNA in the tail and tail length were not significantly different among the groups and time points.

DISCUSSION

CK and LDH levels in the blood can be increased by prolonged physical exercise and are generally used as indices of skeletal muscle damage[16, 17]. The present study analyzed serum CK and LDH to determine the extent of skeletal muscle damage according to marathon running distance. During prolonged competitive exercise, such as a marathon, membrane permeability increases and larger amounts of the enzymes that reflect muscle damage, such as CK and LDH, are discharged into the blood[18]. Short-distance or less-intensive running can achieve muscle tissue exercise without a large change in membrane permeability[13]. Thus, many previous studies[2, 13, 14] have demonstrated that the duration and intensity of exercise are the main factors affecting the activities of such enzymes. The results of the present study also showed that the 42 km marathon group showed greater muscle damage than the other groups, which ran shorter distances. However, the 10 km and 21 km marathon groups also showed increases in CK and LDH after marathon races compared with the prerace levels. Kim et al.[3] reported that the CK concentration began to increase steadily after running 10 km in a 42.195 km marathon race, and Jastrzębski[14] reported that the CK and LDH concentrations began to increase after running 25 km in a 100 km ultramarathon. Such studies showed that muscle damage could be caused not only by full and ultramarathons but also by marathons of shorter distances, such as 10 km and 21 km half marathons. In addition, the present study showed that the CK levels of all groups were higher even 3 days after finishing a marathon. Kim et al.[3] reported that the CK concentration was high until the 4th day of recovery after a 42 km marathon, and until the 5th day of recovery after a 200 km marathon. Tsai et al.[19] reported that blood CK activity was high until the 7th day after a 42 km marathon. Therefore, it can be inferred that recovery from muscle damage induced by competitive endurance exercise takes at least 5 days, depending on various factors such as running distance and physical strength.

The human body has a mechanism for minimizing oxidative damage caused by ROS. However, if oxidative stress in a tissue exceeds the antioxidant capacity of the cells, damage to biological molecules, such as DNA[20], can occur. In the present study, the extent of oxidative DNA damage according to marathon distance was analyzed using the comet assay, which is considered a sensitive and fast method for detecting DNA damage[21]. The results were presented as the values of DNA in the tail, tail length, and tail moment.

It is well known that many kinds of exhaustive high-intensity endurance exercises cause DNA damage but that those exercises that do not cause a high level of stress in the body, such as habitual exercise or low- or moderate-intensity exercise, do not cause DNA damage[8, 22]. In the present study, although the 42 km marathon group showed a higher value for tail moment than the other groups, relatively short distance (10 km and 21 km) marathons were also shown to cause DNA damage. This result supports the results of previous studies[23, 24], showing that DNA damage had a positive correlation with a variety of exercises, such as treadmill running to exhaustion, half marathons, marathons, and triathlons. Increased oxygen intake and oxygen supply to active tissue during strenuous exercise, such as a marathon, result in increased ROS, which in turn can cause oxidative DNA damage. ROS can also be produced during the process of recovery from tissue damage after exhaustive exercise. That is, exhaustive exercise increases ROS production in the vascular system through inflammatory cell infiltration in myofibril injuries and through circulating phagocytes, and increased ROS can permeate into peripheral leukocytes, resulting in the modification of nucleic acids[9, 25, 26]. In addition, ROS may also be produced during the reparative process of tissue damage caused by exhaustive exercise[26, 27]. Tsai et al.[19] reported that there was a correlation between plasma CK and oxidative DNA damage caused by 42 km marathons, and the results of the present study also showed the same pattern of correlation between CK and DNA damage after marathons.

On the other hand, the present study showed that all 3 groups recovered from a significantly high level of DNA damage to a resting level 3 days after a marathon race. Hartmann et al.[28] reported that DNA migration increased to the maximum level 1 day after multiple step tests on a treadmill and then returned to a resting level 3 days after the test. However, a follow-up study on DNA effects of a short-distance triathlon event reported that DNA migration began to increase 1 day after the event, reaching the maximum level 3 days after the event, and that such an increase continued until 5 days after the event. Tsai et al.[19] reported that DNA damage began to increase 24 hours after a 42 km marathon and that such an increase continued until 7 days after the marathon. However, Mastaloudis et al.[8] reported that a high level of DNA damage caused by a 50 km ultramarathon race returned to the resting level 2 hours after the race. It has been reported that the extent and duration of DNA damage can vary according to the type, duration, running distance, and intensity of exercise, as well as training conditions and physical strength[29–33]. In the present study, the relatively fast recovery from DNA damage is presumed to be due to all subjects in the 3 groups, who were all amateur runners, getting regular aerobic exercise and being familiar with long-distance running, as they had participated in marathon races. However, there has been insufficient study of the effect of exercise

distance and other factors on the extent and time course of DNA damage. Accordingly, future studies should assess DNA damage responses according to health, physical strength, training condition, and various exercise distances and intensities.

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