

EFFECTS OF LONG-TERM AEROBIC EXERCISE ON THE ANTIOXIDANT SYSTEM AND LYMPHOCYTE DNA DAMAGE BY TRIATHLON DISTANCE

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ABSTRACT

Background and Objective

This study aimed to investigate the effects of long-term aerobic exercise on muscle damage markers, lymphocyte DNA damage, and antioxidant system in amateur athletes.

Material and Methods

Eleven healthy men in their 30s and 40s without any medical illness, who did not smoke or drink, and had completed at least two amateur triathlon races (O2 and Olympic courses) were enrolled. They underwent physical examination and four blood sampling sessions: at rest, immediately after a race, during recovery (3 and 6 days after the race), and after completing an Olympic course. Blood sampling was performed using the same method one month later. Weight (kg) and saturation of peripheral oxygen (SpO₂) were measured. Tail intensity, tail moment, and tail length, and levels of superoxide dismutase (SOD), creatine kinase (CK), and lactate dehydrogenase (LDH) were analyzed.

Results

First, the study found significant changes between the body weight at rest and immediately after the race ($p < .001$) and between those immediately after the race and 3 and 6 days after the race ($p < .001$) for both courses. Second, for both courses, SpO₂ declined immediately after the race and tended to rise again during recovery, but the difference was not significant. Third, in the Olympic course, significant differences were found between lymphocyte tail momentTM at rest and that immediately after the race ($p < .01$) and between those immediately after the race and 3 and 6 days after the race ($p < .05$, $p < .01$). In the O2 course, significant differences were found between lymphocyte TM at rest and that immediately after the race ($p < .01$), between those at rest and 3 days of recovery ($p < .001$), between those immediately after the race and 3 days of recovery ($p < .001$), between those at rest and 6 days of recovery ($p < .01$), and between those at 3 and 6 days after the

race ($p < .01$). Both courses significantly differed in lymphocyte TM immediately after the race ($p < .05$). Fourth, significant differences were observed between serum SOD at rest and that immediately after the race ($p < .05$), between those at rest and 3 days after the race ($p < .01$) and in serum SOD between that immediately after the race and 6 days after the race ($p < .05$) in the Olympic course. In the O2 course, serum SOD at rest and those at 3 and 6 days after the race significantly differed ($p < .05$). The two courses differed in serum SOD at 3 days after the race ($p < .05$). Fifth, in both courses, compared with the levels at rest, serum CK concentrations immediately after the race ($p < .001$) and 3 and 6 days after the race significantly differed ($p < .01$, $p < .001$). In both courses, significant differences were observed between serum CK concentrations immediately after the race and those at 3 and 6 days after the race ($p < .01$, $p < .001$) and between those at 3 and 6 days after the race ($p < .001$). Both courses significantly differed in serum CK concentrations immediately after the race ($p < .001$) and those at 3 and 6 days after the race ($p < .05$). In the Olympic course, serum LDH concentrations between those at rest and immediately after the race ($p < .001$), between those at rest and 3 days of recovery ($p < .01$), and between those immediately after the race and 3 and 6 days after the race showed significant differences ($p < .001$). In the O2 course, significant differences were found between serum LDH at rest and that immediately after the race ($p < .001$), between those at rest and 3 and 6 days after the race ($p < .01$, $p < .001$), between those immediately after the race and 3 and 6 days after the race ($p < .001$), and between those at 3 and 6 days after the race ($p < .001$). The two courses significantly differed in serum LDH levels immediately after the race ($p < .001$) and those at 3 and 6 days after the race ($p < .05$).

Conclusion

Triathlon, which involves long-term high-intensity aerobic exercise, leads to temporary weight loss, DNA damage, and muscle damage after the race, and such changes are affected by exercise duration and intensity. During this change, defense mechanisms, including the antioxidant defense mechanism, are thought to protect the body from DNA and muscle damage.

Personal leisure time has increased in modern society; hence, the interest and participation of amateurs in sports that were previously difficult to train for and participate in have increased. Specifically, participation in extreme sports, such as marathon, has increased. In the past few years, triathlon has quickly gained much popularity. Triathlon is an athletic contest in which three major aerobic sports, namely, swimming, cycling, and running, are performed in a row, and it requires self-control and patience, with emphasis on harmony and balance.¹

Exercise has been known to offer various health benefits and reduce risk of various diseases, such as diabetes, cancer, and cardiovascular diseases.^{2,3} Nevertheless, when a person engages in an exercise inappropriate for his or her own health status, cell damage and aging can occur from the free radicals generated,⁴ while long-term strenuous exercise can overproduce reactive nitrogen species and reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^{\bullet}), hypochlorous acid, and

nitric oxide (NO^{\bullet}), and oxidative stress increases when the balance between body's oxidants and antioxidants is disrupted by oxidation reaction.^{5,6}

The human body is constantly exposed to oxidative stress, not only during exercise but also at rest; as a result, ROS are produced continuously. However, given that healthy people possess antioxidant defense mechanism, ROS production at rest takes place within the capacity of the antioxidant defense mechanism of the body. However, under situations when active tissues require supply of oxygen at levels that are several to several dozen times higher, such as during exercise, ROS production increases even further.⁷

Studies have reported that overproduction of ROS damages cellular components, such as lipids, proteins, and nucleic acids,^{5,8} and oxidative stress can cause muscle damage, fatigue, and reduced exercise capacity,⁹ while deoxyribonucleic acid (DNA) damage and insufficient DNA repair could lead to chronic diseases, cancers, diabetes, and homonymous sclerosis.¹⁰ In particular, endurance exercise can increase oxygen

uptake by approximately 10–20 times in the body and 100–200 times in skeletal muscles.¹¹ Oxidative stress (malondialdehyde) was reported to increase after a match than that before the match in endurance exercise events.^{11,12}

Such oxidative stress appears differently based on exercise type, duration, and intensity.¹³ Previous studies mostly used 8-hydroxydeoxyguanosine (8-OHdG) to assess DNA damage. However, such measurement method cannot accurately measure DNA sensitivity.¹⁴ Recently, single-cell gel electrophoresis (SCGE) was used to measure oxidative stress-induced lymphocyte DNA damage. Ostling and Johanson (1984) introduced the microgel electrophoresis method to directly identify the degree of DNA damage in each cell,¹⁵ and it was upgraded by Singh et al. to detect DNA damage with greater sensitivity.¹⁶ SCGE, which can directly measure DNA damage at cellular level,¹⁶ is methodologically simple and allows thorough analysis; thus, the degree of such damage can be easily viewed with the naked eye.¹⁷

Study results on marathon, which involves long-distance high-intensity aerobic exercise similar to triathlon, showed that exercise distance and time were proportional to oxidative stress and DNA damage from exercise.¹⁸ However, studies on the effects of oxidative stress on DNA damage and those of the event and distance in triathlon on the antioxidant system are still lacking.

Accordingly, the present study used the SCGE method to investigate muscle damage markers (creatinase kinase [CK] and lactate dehydrogenase [LDH]), antioxidant enzyme (superoxide dismutase [SOD]), and lymphocyte DNA damage before a match, immediately after a match, and during the recovery period (here, the recovery period covers 3 and 6 days after the match) based on match events in an Olympic course (1.5 km of swimming, 40 km of cycling, and 10 km of running) and O2 course (3 km of swimming, 80 km of cycling, and 20 km of running) for amateur triathletes. The objective of the present study was to investigate the effects of long-term aerobic exercise on muscle damage markers, lymphocyte DNA damage, and antioxidant system based on triathlon distance and to provide the basic data for amateur athletes to set safe goals.

METHODS

Participants

The participants were 12 men aged 30–49 years who met the following criteria: (1) no medical illness, (2) neither smoke nor drink, and (3) completed an amateur triathlon O2 course at least twice. The participants fully understood the study and provided informed consent. Overall, one participant dropped out because of personal reasons; subsequently, the study was completed with 11 participants.

Experimental Methods

The experiment involved participant selection, basic tests, and blood collection. For the Olympic course event, blood was collected at four time points: before the event (at rest), immediately after the event, and at 3 and 6 days after the event. Blood collection was repeated 1 month later for the O2 course event, which utilized the same method as that for the Olympic course. The study protocol was approved by the Institutional Ethics Review Board of Yonsei University (1040917-201607-HRBR-203-02).

Procedures

Basic test and blood collection time points.

Participant's height and weight were measured (Table 1). For blood collection at rest, the participants were instructed to avoid excessive physical activities and dinner on the day before the experiment and to maintain an empty stomach from 22:00 onward. On the day before the match and during the recovery period, the participants visited the hospital to have their resting heart rate and oxygen saturation measured with an oximeter (Pulse Oximeter 8500A, Nonin Medical, MN, USA). In accordance with the analysis items, 5 mL of blood samples were collected at rest using an anticoagulant-treated Vacutainer tube. For blood collection immediately after the match, the research team waited on site and collected blood samples in the order in which the participants arrived. The participants were seated in a chair, and blood samples were collected by the same method used in the at-rest blood collection.

Blood analysis methods. For SOD, CK, and LDH, serum-separating tubes were used to collect 1.0 mL of serum samples. After clotting for more than 30 min at room temperature, the samples were centrifuged

(Microspin, Hanil, Korea) at 3000 rpm for 10 min, and the separated supernatant was transferred to a microtube. SOD was analyzed via colorimetry method using an SOD assay kit and a microplate reader (VERSA Max, Molecular device, USA). CK and LDH were analyzed via enzymatic kinetic assay using CK and LDH kits, respectively, and Modular Analytics (PE, Roche, Germany).

Analysis of degree of DNA damage (SCGE).

Slide preparation involved heating 50 mL of agarose used for electrophoresis with a microwave and placing it in a constant-temperature water bath at 40–50°C (45°C), while agarose was prepared in advance and stored in a 100-mL reagent bottle. After heating the slides with an alcohol lamp, 35 µL of 0.7% NMA was dispensed with a pipette in vertical direction and spread evenly with another slide.

A pipette was used to dispense 75 µL of NMA in horizontal direction onto a slide heated with an alcohol lamp. A cover glass was placed on top, making sure

there were no bubbles, and after allowing it to harden for 5 min on a freeze plate, the slide was stored in a slide box lined with wet tissue until the samples were dispensed.

Phosphate buffered saline (PBS, 900 µL) and whole blood contained in EDTA 2K bottle (130 µL) were mixed with 1,030 µL pipette, and 150 µL of histopaque was added slowly to prevent mixing. Then, the solution was centrifuged (3,000 rpm×5 min) at room temperature without vortexing. Preparation was made by dispensing 1,000 µL of PBS in a 1.5 microtube, and after removing the supernatant with 1,000 µL pipette and until it reaches the 0.5 line of the PBS, the pink layer (lymphocytes) was collected with 200 µL pipette and dispensed into 1,000 µL of PBS. Then, 1,000 µL of the PBS and pink layer were centrifuged (3,000 rpm×5 min).

By using a 1,000 µL pipette, the supernatant was discarded up to the 0.5 line, and along the wall at about 0.35–0.4, 15 µL was collected and placed on the bottom wall of a new microtube. After mixing 75 µL of low-melting agarose (LMA) with a pipette, the cover glass that was placed on the slide was removed. After dispensing in horizontal direction, was placed on the slide, making sure there were no bubbles. After dispensing 75 µL of LMA in horizontal direction, the slide was covered with a cover glass, making sure there were no bubbles. The slide was placed on a freeze plate in 5 min for cold storage.

The reagent below was mixed using a 25-mL pipette aid to prepare the lysis buffer, which was then stored in an aluminum foil container. Here, lysis buffer preparation was performed in a dark room. The slides from the same participant were placed back against each other. After removing the cover glass, the slides were inserted vertically into the aluminum foil container with the lysis buffer and left in a refrigerator at 4°C for lysis. During this time, electrophoresis buffer was prepared.

The electrophoresis buffer was prepared in advance on the day of the experiment and stored in a refrigerator. Ice packs were placed inside the electrophoresis system, and the slides were aligned without any gaps. The electrophoresis buffer was filled, starting from the left side, and after filling the right side with the buffer, the lid was closed. After letting it sit for

TABLE 1 Physical Characteristics and Match Records of Participants

Variables	Olympic, O2 (n=11)
Age (years)	37.91±6.19
Height (cm)	173.47±5.24
Weight (kg)	73.75±5.18
Saturation of peripheral oxygen (SpO ₂)	97.55±0.74
Resting heart rate (bpm)	52.36±3.98
Olympic course	
Swim 1.5 km (min)	28.13±5.52
Cycle 40 km (min)	74.91±5.53
Run 10 km (min)	51.79±6.18
Total (min)	159.83±14.21
O2 course	
Swim 3 km (min)	62.72±10.01
Cycle 80 km (min)	158.61±14.92
Run 20 km (min)	125.50±16.36
Total (min)	346.84±39.27

All values are means±standard deviation

40 min without any electricity, the cord was plugged in, and electrophoresis was performed for 20 min at 25 V and 300 mA. The slides were removed from the electrophoresis system and spread out, to which the neutralization buffer (50 µL tube) was evenly dispensed from above using a syringe with a thick, large needle.

After electrophoresis, the slides were immersed in 0.4M Tris buffer solution (pH 7.4) for 10 min. The washing process was repeated three times, and then the slides were dried. The nucleus was fluorescence stained with 20 µL/mL concentration of ethidium bromide; after covering the slides with a cover glass, the slides were observed with a fluorescence microscope (Leica, Germany). Each cell nucleus image that was sent from the CCD camera (Nikon, Japan) was analyzed using Komet 4.0 comet image analyzing system (Kinetic Image, UK) installed on a computer.

The degree of lymphocyte DNA damage was expressed as measurement of tail length (TL), which is the distance that the DNA fragment moved from the nucleus, and tail moment (TM), which is the value calculated by multiplying the TL with %DNA contained in the tail. For each blood sample, two slides were prepared. The degree of DNA damage was measured as the mean value derived from 100 lymphocytes, with 50 lymphocytes in each slide.

Statistical Analysis

The present results were analyzed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). All dependent variables measured before and after the race and during recovery for each course are presented with descriptive statistics (mean±standard deviation).

To examine the changes in lymphocyte DNA damage and activities of plasma CK, LDH, and SOD based on match events, two-way repeated measures analysis of variance (ANOVA, course (2) × blood collection time points (4), was performed. If interactions appeared, one-way ANOVA and independent samples t-test were used to analyze the main effect. Statistical significance level was set to p<.05.

RESULTS

The present study investigated the effects of Olympic and O2 courses on changes in body weight, heart rate, saturation of peripheral oxygen (SpO₂), lymphocyte DNA damage (tail intensity (TI), TL, and TM), and plasma SOD, CK, and LDH concentrations in 11 amateur triathletes.

Changes in Body Weight

Changes in body weight are shown in Table 2. Body weight changes at the four time points based on match events in both courses showed a decreasing pattern immediately after the match and increasing pattern during the recovery period in both courses. Two-way repeated measures ANOVA results on differences in body weight based on courses and time points showed a significant interaction effect (p<.001). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that both courses showed significant difference between at rest and immediately after the match (p<.001) and between immediately after the match and during the recovery period (p<.001).

TABLE 2 Changes in Body Weight at Rest, Immediately after the Match and During the Recovery Period (After 3 And 6 Days)

Courses	Rest (kg)	After match (kg)	Recovery-3 days (kg)	Recovery-6 days (kg)
Olympic course	73.74±5.33	72.52±5.28 ^{a***}	73.89±5.29 ^{b***}	73.92±5.13 ^{b***}
O2 course	73.76±5.27	69.60±4.59 ^{d***}	73.87±5.09 ^{e***}	73.97±5.11 ^{e***}

All values are means±standard deviation.

†The superscript letters (a-f) indicate:

***p<.001

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

Changes in SpO₂

Changes in SpO₂ are shown in Table 3. SpO₂ changes at the four time points based on match events in both courses showed a decreasing pattern immediately after the match and an increasing pattern during the recovery period in both courses. Two-way repeated measures ANOVA results on differences in SpO₂ based on courses and time points showed no interaction effect between courses and time points (p<.001).

Changes in Heart Rate

Heart rate changes are shown in Table 4. Heart rate changes in the four time points based on match events in both courses showed an increasing pattern immediately after the match and an increasing pattern during the recovery period in both courses. Two-way repeated measures ANOVA results on differences in heart rate based on courses and time points showed a significant interaction effect (p<.001). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that both courses showed significant difference between at rest and immediately

after the match (p<.001) and between immediately after the match and during the recovery period. A significant difference immediately after the match and after 3 days of recovery was found between courses (p<.05).

Changes in Lymphocyte TI (%DNA in the Tail)

TI changes are shown in Table 5. TI changes at four time points based on match events in both courses increased immediately after the match and decreased during the recovery period in the Olympic course and decreased immediately after the match, increased after 3 days of recovery, and decreased after 6 days of recovery in the O2 course. Two-way repeated measures ANOVA results on differences in TI based on courses and time points showed a significant interaction effect (p<.001). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that Olympic course showed significant differences between at rest and immediately after the match (p<.01) and between immediately after the match and after 3 and 6 days of recovery (p<.01 and p<.001, respectively). O2 course showed significant differences between at

TABLE 3 SpO₂ Changes at Rest, Immediately after the Match and During Recovery Period (After 3 And 6 Days)

Courses	Rest(%)	After match(%)	Recovery-3 days(%)	Recovery-6 days(%)
Olympic course	97.73±0.78	91.55±1.36	97.45±0.52	97.55±0.52
O2 course	97.36±0.67	90.73±0.90	97.64±0.50	97.76±0.60

All values are means±standard deviation.

TABLE 4 Heart Rate Changes at Rest, Immediately after the Match and During Recovery Period (After 3 And 6 Days)

Courses	Rest(bpm)	After match(bpm)	Recovery-3 days(bpm)	Recovery-6 days(bpm)
Olympic course	51.91±4.11	158.55±6.51 ^{a***}	52.82±3.97 ^{b***}	52.27±4.07 ^{b***}
O2 course	52.82±3.99	150.45±7.87 ^{d***}	50.64±4.56 ^{e***}	51.82±3.99 ^{e***}

All values are means±standard deviation.

†The superscript letters(a-f) indicate:

***p<.001

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

rest and immediately after the match ($p < .001$) and after 3 days of recovery ($p < .01$), between immediately after the match and after 3 days of recovery ($p < .001$) and after 6 days of recovery ($p < .01$), and between 3 days of recovery and after 6 days of recovery ($p < .01$). A significant difference was found immediately after the match between courses ($p < .01$).

Changes in Lymphocyte TM

TM changes are shown in Table 6. TM changes at the four time points based on match events in both courses increased immediately after the match and decreased during the recovery period in the Olympic course and decrease immediately after the match and increase during the recovery period in the O2 course. Two-way repeated measures ANOVA results on differences in TM based on courses and time points showed a significant interaction effect ($p < .001$). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that the Olympic

course showed significant differences between at rest and immediately after the match ($p < .01$) and between immediately after the match and after 3 and 6 days of recovery period ($p < .05$ and $p < .01$, respectively). The O2 course showed significant differences between at rest and immediately after the match ($p < .01$) and after 3 days of recovery ($p < .001$), between immediately after the match and after 3 days of recovery ($p < .001$) and after 6 days of recovery ($p < .01$), and between 3 days of recovery and after 6 days of recovery ($p < .01$). A significant difference was found immediately after the match between courses ($p < .05$).

Changes in Lymphocyte TL

TL changes are shown in Table 7. TL changes at the four time points based on match events in both courses increased immediately after the match and decreased during the recovery period in the Olympic course and decreased immediately after the match, increased after 3 days of recovery, and decreased after 6 days of recovery in the O2 course. Two-way repeated measures

TABLE 5 Tail Intensity Changes at Rest, Immediately after the Match, and During Recovery Period (After 3 And 6 Days)

Courses	Rest(%)	After match(%)	Recovery-3 days(%)	Recovery-6 days(%)
Olympic course	17.25±1.91	18.33±1.561 ^{a**}	17.63±1.35 ^{b**}	17.16±1.64 ^{b***}
O2 course	17.20±1.23	15.75±1.60 ^{d***}	17.80±1.15 ^{d** e***}	17.20±0.91 ^{e** f**}

All values are means±standard deviation

†The superscript letters(a-f) indicate:

*** $p < .001$, ** $p < .01$

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

TABLE 6 Tail Moment Changes at Rest, Immediately after the Match, and During Recovery Period (After 3 And 6 Days)

Courses	Rest(%)	After match(%)	Recovery-3 days(%)	Recovery-6 days(%)
Olympic course	17.47±2.79	18.68±3.08 ^{a**}	17.95±2.34 ^{b*}	17.59±2.52 ^{b**}
O2 course	17.99±0.83	16.41±1.62 ^{d**}	18.73±0.91 ^{d*** e***}	17.84±0.85 ^{e** f**}

All values are means±standard deviation

†The superscript letters(a-f) indicate;

*** $p < .001$, ** $p < .01$, * $p < .05$

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

ANOVA results on differences in TL based on courses and time points showed a significant interaction effect ($p < .001$). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that both courses showed significant differences between at rest and immediately after the match ($p < .001$) and after 3 days of recovery ($p < .01$), between immediately after the match and after 3 days of recovery ($p < .001$) and after 6 days of recovery ($p < .01$), and between 3 days of recovery and after 6 days of recovery ($p < .01$). A significant difference was found immediately after the match between courses ($p < .01$).

Changes in Serum SOD

Serum SOD changes are shown in Table 8. Serum SOD changes at the four time points based on match events in both courses increased immediately after the match and decreased during the recovery period in the Olympic course and increased immediately after the match and after 3 days of recovery and decreased

after 6 days of recovery in the O2 course. Two-way repeated measures ANOVA results on differences in SOD based on courses and time points showed a significant interaction effect ($p < .001$). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that the Olympic course showed significant differences between at rest and immediately after the match ($p < .05$) and after 3 days of recovery ($p < .01$) and between immediately after the match and after 6 days of recovery ($p < .05$). The O2 course showed significant differences between at rest and during the recovery period ($p < .05$). A significant difference was found after 3 days of recovery between courses ($p < .05$).

Changes in Serum CK

Serum CK changes are shown in Table 9. Serum CK changes at the four time points based on match events in both courses increased immediately after the match and decreased during the recovery period in both courses. Two-way repeated measures ANOVA results

TABLE 7 Tail Length Changes at Rest, Immediately after the Match, and During Recovery Period (After 3 And 6 Days)

Courses	Rest(µm)	After match(µm)	Recovery-3 days(µm)	Recovery-6 days(µm)
Olympic course	100.09±5.78	102.87±6.28 ^{a***}	101.48±5.93 ^{a** b***}	100.28±5.52 ^{b*** c**}
O2 course	99.65±3.10	95.48±4.36 ^{d***}	101.32±3.66 ^{d** e***}	99.70±3.08 ^{e*** f**}

All values are means±standard deviation

†The superscript letters(a-f) indicate;

*** $p < .001$, ** $p < .01$

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

TABLE 8 Serum Superoxide Dismutase Changes at Rest, Immediately after the Match, and During Recovery Period (After 3 And 6 Days)

Courses	Rest(mg/dL)	After match(mg/dL)	Recovery-3 days(mg/dL)	Recovery-6 days(mg/dL)
Olympic course	1.24±0.11	1.38±0.18 ^{a*}	1.29±0.12 ^{a**}	1.27±0.10 ^{b*}
O2 course	1.19±0.14	1.32±0.36	1.77±0.65 ^{d*}	1.42±0.43 ^{d*}

All values are means±standard deviation

†The superscript letters(a-f) indicate;

** $p < .01$, * $p < .05$

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

on differences in CK based on courses and time points showed a significant interaction effect ($p < .001$). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that both courses showed significant differences between at rest and immediately after the match ($p < .001$) and after 3 and 6 days of recovery ($p < .01$ and $p < .001$, respectively), between immediately after the match and after 3 and 6 days of recovery ($p < .01$ and $p < .001$, respectively), and between after 3 days of recovery and after 6 days of recovery ($p < .001$). A significant difference was found immediately after the match ($p < .001$) and during the recovery period between courses.

Changes in Serum LDH

Serum LDH changes are shown in Table 10. Serum LDH changes at the four time points based on match events in both courses increased immediately after the match and decreased during the recovery

period in both courses. Two-way repeated measures ANOVA results on differences in LDH based on courses and time points showed a significant interaction effect ($p < .001$). One-way ANOVA and independent samples t-test results on the main effect on the interaction effect between courses and time points indicated that the Olympic course showed significant differences between at rest and immediately after the match ($p < .001$) and after 3 days of recovery ($p < .01$) and between immediately after the match and during the recovery period ($p < .001$). The O2 course showed significant differences between at rest and immediately after the match ($p < .001$) and after 3 and 6 days of recovery ($p < .01$ and $p < .001$, respectively), between immediately after the match and during the recovery period ($p < .001$), and between after 3 days of recovery and after 6 days of recovery ($p < .001$). A significant difference was found immediately after the match ($p < .001$) and during the recovery period ($p < .05$) between courses.

TABLE 9 Serum Creatine Kinase Changes at Rest, Immediately after the Match, and During Recovery Period (After 3 And 6 Days)

Courses	Rest(U/L)	After match(U/L)	Recovery-3 days(U/L)	Recovery-6 days(UL)
Olympic course	124.09±9.35	294.63±62.14 ^{a***}	263.54±54.04 ^{a***b*}	136.18±11.47 ^{a***b***c***}
O2 course	132.63±20.80	573.18±93.97 ^{d***}	271.00±60.01 ^{d***e***}	165.63±32.79 ^{d***e***f***}

All values are means±standard deviation

†The superscript letters(a-f) indicate;

*** $p < .001$, ** $p < .01$

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

TABLE 10 Serum Lactate Dehydrogenase Changes at Rest, Immediately after the Match, and During Recovery Period (After 3 And 6 Days)

Courses	Rest (U/L)	After match(U/L)	Recovery-3 days(U/L)	Recovery-6 days(U/L)
Olympic course	323.72±20.58	404.18±17.65 ^{a***}	349.45±30.22 ^{a**b***}	326.36±19.63 ^{b***c***}
O2 course	331.36±25.24	470.63±37.15 ^{d***}	381.81±34.57 ^{d***e***}	354.54±32.18 ^{d***e***f***}

All values are means±standard deviation

†The superscript letters(a-f) indicate;

*** $p < .001$, ** $p < .01$

Olympic course

^a vs. Rest ^b vs. After match; ^c vs. Recovery at 3 days;

O2 course

^d vs. Rest; ^e vs. After match; ^f vs. Recovery at 3 days

DISCUSSION

We measured body weight before and after both courses and during recovery period to examine the relationship between changes in body weight and the type of triathlon course. For both courses, body weight was significantly decreased after the race than that at rest and significantly increased during recovery than that immediately after the race. These patterns in body weight changes agreed with those described by previous studies of triathlon athletes.¹⁹ Generally, the results also agreed with previous studies of various marathon courses and triathlon races that showed weight loss after prolonged (at least several hours) aerobic exercise.^{20,21}

Furthermore, in the present study, an average of 1.2 kg of weight loss occurred after finishing the Olympic course, while an average of 4.2 kg of weight loss occurred after the O2 course, showing that the O2 course led to greater weight loss (3 kg more than the Olympic course). This agrees with previous findings that weight loss after a triathlon race was generally proportional to exercise intensity and distance.²² Pinho et al. measured an average weight loss of 2 kg after the Ironman course (3.8 km swimming, 180 km cycling, and 42.2 km running).²³ A previous study found an average weight loss of 2.3 and 2.5 kg after the Ironman race.¹⁹ These values were lower than the 4.2 kg weight loss after the O2 course found in our study, presumably because although the Ironman course is longer than the O2 course, athletes perform the Ironman course with lower exercise intensity than the O2 course, in which the goal is to finish the race. Moreover, these differences may also be related to differences in the extent of participants' training. We determined that the extent of training may affect the degree of weight loss; previous studies on Ironman athletes studied participants with extensive training,^{19,23} whereas our participants were amateurs who have never finished an Ironman course. However, Del Coso et al. found an average weight loss of 3.8 kg in well-trained triathletes after finishing a half-Ironman course.²⁴ Thus, the extent of weight loss between our study (O2 course) and other studies (Ironman course) differed because the exercise was performed with a higher intensity in a shorter period. Overall, temporary weight loss occurs after prolonged,

high-intensity aerobic exercise, and such changes are affected by exercise duration and intensity.

In the present study, we measured the SpO₂, an indicator of saturation of arterial oxygen (SaO₂), in subjects after completing the two courses. The results showed that the SpO₂ significantly decreased after the race than at rest for both courses, but the SpO₂ was significantly higher during recovery than that immediately after the race. For specific courses, the average reductions in SpO₂ after the Olympic and O2 courses were 6.18% and 6.63%, respectively. This agrees with previous findings that SpO₂ decreases after prolonged high-intensity exercise.²⁵ The marked reduction in SpO₂ after both courses may be a compensatory action resulting from prolonged high-intensity exercise to increase the body's oxygen dissociation capacity to ensure adequate oxygenation.²⁶ By contrast, SaO₂ was reported to remain unchanged after moderate exercise.²⁷

SpO₂ significantly decreased immediately after exercise than at rest, and the reported degree of reduction appears to be a decrease in the width of the elite athlete than the general population.²⁸ Kim et al.²⁵ studied long-distance athletes who showed a significant decrease immediately after exercise, because the cardiopulmonary function was improved by endurance training. The present study showed that the decrease in SpO₂ was remarkable in participants with improved cardiopulmonary function despite being amateur players.

The human body uses an antioxidant defense mechanism to minimize oxidative damage caused by ROS.²⁹ However, excessive oxidative stress that surpasses the cell's antioxidant capacity may not only inflict damage on biochemical molecules such as DNA but also result in adverse metabolic consequences, such as a failure to repair the damaged DNA.³⁰ DNA damage may cause genomic and chromosomal mutations, which alter gene and protein activation and potentially induce carcinogenesis.³⁰ Several methods have been employed to observe DNA damage in humans, including DNA adduct, chromosomal aberration, sister chromatid exchanges, micronuclei, and 8-OHdG assays.³¹ However, the SCGE has recently gained popularity for providing more visual, sensitive, and clear outcomes.³²

In the present study, the SCGE was conducted to examine changes in lymphocyte DNA damage in triathlon athletes and revealed varying trends in athletes who completed both courses. TM, an index of DNA damage, increased from 17.47% at rest to 18.68% after the race for the Olympic course, and the damage generally returned to at-rest levels during recovery, from 17.95% 3 days after the race to 17.59% 6 days after the race. These patterns support the findings of Vezzoli et al. who found that DNA damage,³³ as determined by 8-OHdG levels, was increased after 50-km and 100-km ultra-marathon races. For instance, Cho and Roh found that DNA damage after 85% VO₂max all-out exercise in 40 men and women had returned to at-rest levels 24 h later.³⁴ By contrast, for the O2 course in this study, the TM decreased immediately after the race (16.41%) than at rest (17.99%), and DNA damage continued to occur 3 days after the race (18.73%), but returned to at-rest levels 6 days after the race (17.84%). This pattern differs from that after the Olympic course, where oxidative DNA damage increased immediately after the race and then decreased throughout recovery.

Exercise duration may play a role in these outcomes. The mean duration of the Olympic course in this study was less than 3 h (159.83 min), with 28.13 min for swimming, 74.91 min for cycling, and 51.79 min for running. The duration of the O2 course, which is two times longer than the Olympic course, was more than double that of the Olympic course (346.84 min), with 62.72 min for swimming, 158.61 min for cycling, and 125.5 min for running. Our findings differ from those of previous studies of the Olympic course and relatively short aerobic exercises (<3 h). However, when Reichhold et al. analyzed DNA damage at 20 min after the Ironman course in 28 well-trained triathletes, DNA damage was lower after the race than that at rest, but increased 24 h after the race, which was observed for the O2 course in our study. DNA damage returned to at-rest levels 5 days later and decreased to a level lower than that at rest by 19 days later.³⁵ Notably, DNA damage decreased immediately after the race contrary to predictions, suggesting activation of a temporary defense mechanism after a prolonged race (>5 h), although the exact mechanism is unclear. DNA damage was increased at 3 days after the race

than that at rest, which may be caused by a buffering action of the defense mechanism that occurred over time during the race.

DNA stability was reported to influence the extent of the athlete's prior training.³⁵ This is supported by the concept of adaptive responses,³⁶ which was found to affect gene expression of antioxidant adaptation and antioxidant factors.³⁷ However, considering that the same participants participated in both courses analyzed in our study, the differences in the outcomes between both courses are likely the result of exercise duration or intensity as opposed to the difference in the extent of prior training. Additional studies are needed to identify the potential defense mechanism that may have been activated, as inferred from the reduction in DNA damage immediately after the race and increased DNA damage 3 days after the O2 course.

Healthy people have antioxidant defense mechanisms.²⁹ However, prolonged vigorous aerobic exercise leads to continuous ROS production, which induces oxidative stress.³³ The main antioxidant enzymes that inhibit ROS and prevent oxidative damage are superoxide dismutase (SOD), superoxide oxidoreductase, glutathione peroxidase, and hydrogen peroxide oxidoreductase (CAT).³⁸

This study examined the changes in serum SOD (mg/dL) to investigate the effects of SOD activity in triathlon athletes (Olympic and O2) on changes in the antioxidant system. Our findings showed the Olympic course has higher SOD immediately after the race (1.38 mg/dL) than that at rest (1.24 mg/dL), which progressively decreased at 3 days (1.29 mg/dL) and 6 days (1.27 mg/dL) of recovery. In the O2 course, SOD was higher immediately after the race (1.32 mg/dL) than that at rest (1.19 mg/dL), which further increased at 3 days (1.77 mg/dL) of recovery, and SOD remained higher at 6 days of recovery (1.42 mg/dL) than that at rest and immediately after the race. These results are supported by previous studies showing that SOD activity is increased after prolonged exercise. Shin et al. found a higher SOD concentration after moderate exercise (60% or 80% of maximum oxygen consumption) consuming 400 kcal of energy than that before exercise.³⁹ Pinho et al. reported that SOD concentration was increased after finishing an Ironman course.²³ This increase is related to SOD function, as it responds to

oxidative stress and effectively defends against free radicals produced during exercise. In our study, SOD activity was highest 3 days after the O2 race. Considering that DNA damage from the O2 course was also the highest 3 days after the race, the increase in SOD resulted from higher oxidative stress caused by the ultra-marathon and Ironman course in the triathlon,¹² which feature longer distances than the Olympic course. The increase in SOD activity immediately after the O2 course may not be noteworthy because it confirms previous findings; however, we found that SOD activity was increased while DNA damage was decreased after the O2 course, strongly supporting the existence of another defense mechanism in addition to the antioxidant defense mechanism. In addition, the change in SOD concentration according to the game performance is increased at various time points such as immediately after or after the performance, which is considered to be different depending on the exercise intensity and time and training level. However, it cannot be denied that the action of the defense system is affected by the exercise.

Here, we examined the changes in serum CK and LDH to investigate the relationship between CK and LDH concentrations and muscle damage in triathletes. The results showed that for the Olympic course, serum CK was higher immediately after the race (294.63 U/L) than that at rest (124.09 U/L), which gradually decreased during recovery with 263.54 U/L at 3 days and 136.18 U/L at 6 days after the race. For the O2 course, serum CK was increased immediately after the race (573.18 U/L) compared with that at rest (132.63 U/L), but gradually decreased throughout recovery, with 271 U/L at 3 days and 165.63 U/L at 6 days after the race. Furthermore, for the Olympic course, serum LDH was higher immediately after the race (404.18 U/L) than that at rest (323.72 U/L), which gradually decreased throughout recovery, with 349.45 U/L at 3 days and 326.36 U/L at 6 days after the race. For the O2 course, serum LDH was higher immediately after the race (470.63 U/L) than at rest (331.36 U/L), which gradually decreased throughout recovery, with 381.81 U/L at 3 days and 354.54 U/L at 6 days after the race. For both courses, elevated CK and LDH concentrations indicated muscle damage immediately after the race; although these concentrations were decreased at

3 and 6 days after the race, they remained higher than those at rest. These patterns of muscle damage indicators agree with those of previous studies examining high-intensity aerobic exercise.^{18,20} Areces et al. reported that muscle damage indicators were higher immediately after a half-Ironman race (808 U/L) than that at rest (173 U/L) in 10 male triathletes.⁴⁰ In addition, Pinho et al. suggested that CK concentration was increased after an Ironman course. In addition, muscle damage was higher after the longer O2 course than after the shorter Olympic course.²³ This may be because increased CK and LDH concentrations are associated with exercise duration and intensity. Ryu et al. found that CK and LDH concentrations were increased with increasing marathon distance (10, 20, and 42.2 km).¹⁸ Our findings that CK and LDH concentrations did not recover to at-rest levels by 6 days of recovery after a triathlon race indicates that recovery is delayed following longer exercise. Thus, these athletes require more than 6 days for recovery after a triathlon race.

CONCLUSION

In conclusion, the high-intensity long-term aerobic exercise triathlon leads to temporary weight loss, DNA damage, and muscle damage after the race, and such changes are affected by exercise duration and intensity. During these changes, defense mechanisms to protect against DNA damage and muscle damage such as antioxidant defenses may also occur during recovery and race.

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