Training Induced Oxidative Stress-Derived DNA and Muscle Damage in Triathletes

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ABSTRACT

Objective: Regular moderate-intensity exercise has beneficial health effects, whereas regular strenuous exercise increases the production of oxidants that may lead to DNA, skeletal, and cardiac muscle damages. Triathletes experience strenuous muscular activity both during competition and training, being at risk of developing these tissue damages. The objective of the present study was to estimate DNA, skeletal, and cardiac muscle damages using blood biomarkers, 8-hydroxy-2′-deoxyguanosine (8-OHdG), myoglobin, and cardiac troponin I (cTnI) among young triathletes.

Materials and Methods: Age-matched seven male and seven female triathletes were recruited for the study. They were on a standardized training regimen and on average competed in at least one endurance event every month for the past 3-4 years. Serum biomarkers were measured using enzyme-linked immunosorbent assay at the start and at end of the racing season.

Results: Both male and female triathletes showed a statistically significant increase in 8-OHdG. A similar pattern of increase was seen with serum myoglobin, which was not statistically significant in both male and female triathletes. cTnI levels did not show any change in both sexes.

Conclusion: Our study shows that there could be an increased evidence of DNA damage among triathletes. However, similar effects were not observed with skeletal and cardiac muscle biomarkers.

Keywords: DNA damage, triathlete, endurance training, myoglobin, cardiac troponin I

Introduction

Exercise and health share a "U"-shaped relationship [1]. Regular moderate exercise has beneficial health effects, whereas acute strenuous exercise causes increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that may accumulate and cause DNA damage [2]. Since a triathlon race mimics acute strenuous exercise over a period of 6-8 h, questions were raised as to whether triathletes suffered DNA damage after a race. Niess et al. [3] investigated DNA damage in 12 triathletes taking part in a half-marathon and found that 80% of the competitors show increased DNA migration 24 h after the race. Similar results were obtained by Borghini et al. [4], Briviba et al. [5], and Ryu et al. [6]. Thus, it became clear that endurance competitions, such as triathlons, may have a DNA-damaging effect. However, most of the studies monitored DNA damage up to 14 days after the race.

The DNA-damaging effect of a competition is also dependent on its duration. Studies that investigated the DNA-damaging effect of competitive endurance exercise were based on single competitions, which varied in distances/duration, and had different experimental designs and different methods of assessing DNA damage. Though these studies do not provide consistent information regarding the impact of exercise duration on DNA damage [7], it may well be concluded that DNA damage after competitive ultra-endurance exercise does not appear to be persistent [8].

Triathlon involves endurance training and requires the athlete to divide time equally among the three disciplines of swimming, cycling, and running. During training, athletes may achieve performance levels that are similar to those maintained during a race, but on a day-to-day basis. This may add to the DNA damage. For a competitive triathlete, it would mean periods of "high endurance training" and "high endurance competitions" throughout one's career. Though nor-

mal DNA repair mechanisms would be able to reverse DNA-damaging effect, it is possible that over a long period of "training and competition" involving high endurance, increased DNA damage could occur, and the effect of DNA repair enzymes might not be sufficient to reverse the accumulated damage.

Similarly, strenuous training and competition involved in triathlon could also lead to skeletal and cardiac muscle damages. Exercise-induced skeletal muscle damage is a common complication of triathlon competition due the sheer strenuous nature of the sport. Many studies have shown that immediately after a triathlon race, skeletal muscle damage biomarkers, such as myoglobin [9-10], lactate dehydrogenase [11], and creatinine kinase levels [12], are increased significantly, often >5–100 fold. Studies that have followed up athletes for a longer duration after the race have shown that these skeletal muscle damage biomarkers return to normal after 7–10 days with proper post-race recovery [12]. Strenuous exercise has been associated with increase in cardiac troponins in healthy individuals who have no signs of myocardial disease [12]. It has also been shown that this increase in cardiac troponins after strenuous exercise is dependent on the intensity and duration of the exercise [13]. However, there are no studies that have examined the effect of long-term training and competition on skeletal muscle and cardiac muscle damages.

Table 1. A typical yearly racing calendar

Date	Beginning of season
February 8	MetaSprint Aquathlon, Singapore
February 15	Oral Cancer Run 10 km, Malaysia
March 8	Nestle Fitnesse Run, Malaysia
March 15	Penang Triathlon, Malaysia
March 28	Melawati 10k run, Malaysia
April 5	Putrajaya Triathlon, Malaysia
April 12	Kapas Marang Swimathlon, Malaysia
April 19	MetaSprint Triathlon, Singapore
May 2	Kenyir Lake Triathlon, Malaysia
May 3	Xterra, Malaysia
June 13	Asian Championships, Taipei
July 12	Osaka ASTC, Japan
July 25	Singapore ASTC Cup, Singapore
August 1	Port Dickson Triathlon, Malaysia
September 6	Singapore National Aquathlon Champs, Singapore
October 4	Singapore National Duathlon Champs, Singapore
October 18	ITU World Duathlon, Adelaide, Australia
October 31	ASTC Cup Sprint, Hong Kong
November 15	Powerman Philippines Duathlon, Philippines
	End of season

ASTC: Asian Triathlon Confederation; ITU: Internation Triathlon Union

The aim of the present study was to examine the effects of long-term training and competition on specific DNA, skeletal, and cardiac damage biomarkers in elite triathletes.

Materials and Methods

Triathletes from a professional triathlon team based in Kuala Lumpur, Malaysia were recruited for the study. The study included seven male and seven female triathletes. The average ages of male and female triathletes were 17.7±3.6 and 16.4±4.28 years, respectively. These triathletes were coached by a dedicated and trained triathlon coach and went through a systematic training schedule that lasted for approximately 10 months in a year. During this 10-month triathlon season, triathletes underwent regular training to be able to participate in at least six triathlon events in addition to duathlon and marathon events. A typical yearly racing calendar is shown in Table 1. Male and female triathletes have been training and competing for 4.14±1.57 and 3.29±2.14 years, respectively. The training schedule was periodized into 4×4 training blocks with week 4 being a low-volume "recovery" week. Both male and female triathletes trained for approximately 9-15 h/week, and the average distance/week (including swimming, biking, and running) was 144.8 km/week. The triathletes were recruited for the study after a thorough discussion with and cooperation of the coach. They were informed of the risks and benefits of the study, as well as the study protocol. Informed consent was obtained from the triathletes. The study was conducted in accordance with the Declaration of Helsinki and the guidelines of Resolution on 198/96 of the National Health Council. Ethical approval for the present study (SG/2014/SP/UPNM/1) was obtained from the Faculty Research Committee of the university (FPKP/RC/2016/BIL.1).

Table 2. Anthropometry and body composition profile of the athletes

Variables	Male triathletes	Female triathletes
Height (cm)	166.09±10.83	157.26±5.13
Weight (kg)	56.13±10.65	50.29±3.24
BMI (kg/m2)	19.80±2.21	20.33±1.11
Body fat (%)	16.71±3.88	25.67±3.82
Fat-free mass (kg)	45.96±9.23	37.31±2.97
Fat mass (kg)	9.13±2.24	12.91±2.19

Triathletes were invited to the Human Performance Laboratory at two time points (phases 1 and 2); phase 1, 2 weeks before the beginning of the triathlon season (week 1 of February) and phase 2, at the end of the triathlon season (last week of November). The phase 2 sample was collected 2 weeks after the last triathlon event. At both time points, the athletes were advised to avoid strenuous exercise or physical activity for 48 h prior to their appointment to the laboratory. They were also asked to avoid alcohol and caffeinated drinks for 24 h. On their arrival to the laboratory, basic anthropometry and body composition measurements using a N_2O segmental body composition analyzer (U. Healthcare System, Singapore) were made. Table 2 provides the basic anthropometry and body composition profile of the athletes. Then, blood samples were collected and were immediately centrifuged, and the separated serum was stored at -80 °C until analysis.

Serum 8-hydroxy-2′-deoxyguanosine (8-OHdG), myoglobin, and cardiac troponin I (cTnI) levels were measured using kits based on the enzyme-linked immunosorbent assay (ELISA) principle. Serum myoglobin was measured using Sigma-Aldrich Myoglobin ELISA Kit (Sigma Aldrich, USA), whereas serum cTnI and serum 8-OHdG were measured using Cloud-Clone Corp ELISA Kit (Cloud-Clone, USA). Protocols of the respective kits were followed, and absorbance was measured using SpectraMax 5M analyzer (Molecular Devices, USA). The intra-assay coefficient of variation was calculated for all the three ELISA kits and was determined to be within 5%. A calibration curve was generated by a four-parameter logistic regression analysis using the SpectraMax software (Molecular Devices,USA).

Statistical Analysis

The results were expressed as mean±standard deviation. Student's paired t-test was used to compare between phase 1 and phase 2 of serum myoglobin, cTnI, and 8-OHdG of male and female triathletes. A p value of <0.05 was considered statistically significant. All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) package version 16.1 (SPSS Inc., Chicago, IL, USA).

Results

The results of our study showed a statistically significant increase in serum 8-OHdG levels in both male and female triathletes between phase

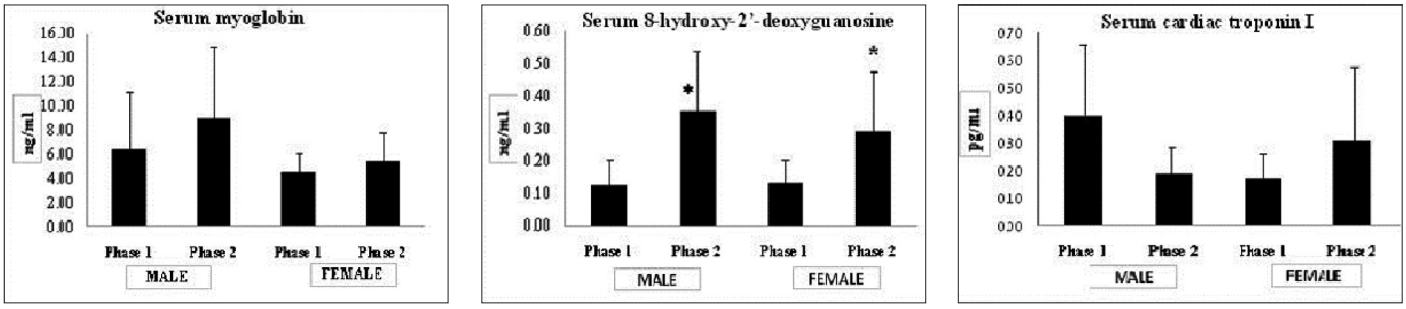

Figure 1. Serum myoglobin (ng/mL), serum 8-hydroxy-2′-deoxyguanosine (ng/mL), and serum cardiac troponin I (pg/mL) in male and female triathletes at phase 1 and phase 2 *p<0.05

1 and phase 2. There was a 3-fold increase in male triathletes, whereas there was a 2-fold increase in female triathletes in serum 8-OHdG levels. We also found an increase in serum myoglobin levels in both male and female triathletes at phase 2. However, this increase was not statistically significant. We found a decrease in serum cTnI levels in male triathletes at phase 2, whereas there was an increase in cTnI levels in female triathletes at phase 2. However, these changes were also not statistically significant (Figure 1).

Discussion

In the present study, we hypothesized that high endurance "training and competition" cycles over time could have an increased effect on DNA damage and could compromise the body's defense mechanisms. We used serum 8-OHdG as a marker of oxidative stress-derived DNA damage to study the effect of one season of triathlon "training and competition" on DNA damage. Paulsen et al. [14] and Tsai et al. [15] showed an increase in 8-OHdG following 30 days of hard exercise and marathon race, respectively. Similar results were also obtained from studies on animals. Pozziet et al. [16] showed significant DNA damage after acute strenuous exercise in rats. However, there are several studies with contrasting findings [17-19]. These studies found no increase in 8-OHdG after maximal and submaximal aerobic exercises. This was attributed to the effect of exercise on DNA repair enzyme upregulation or related to the level of training. Asami et al. [17] found that in untrained athletes, the DNA repair activity increases significantly after exercise, whereas no such increase is noted in trained athletes. Taken collectively, though strenuous exercise causes an increase in DNA damage markers in humans, it appears to depend on the type and intensity of the exercise, as well as the adaptation/training of the individual.

Studies on DNA damage examined the effect of an individual triathlon race and its effect on DNA damage and found that immediately after the race, there is an increase in DNA damage markers [14, 15, 20]. In contrast to these above findings, some studies did not document an increase in DNA damage after the race [21-23]. However, even with an increase in DNA damage after the race, it was noted that after a period of 7–14 days, DNA damage marker levels were back to pre-race levels. This was attributed to the effective DNA repair mechanisms. However, it is noteworthy that these studies considered the effect of a single race and not a long-term effect of DNA damage over time. In the study by Okamura et al. [24], the cumulative effect of three consecutive races on DNA damage was studied, and no significant cumulative effect was observed. The absence of any cumulative effect was attributed to the adaptive responses of the body due to long-term regular training [20-22]. Thus, the key to the prevention of accumulated DNA damage is an adequate washout period after a race, as well as a regularized training. In the absence of these prerequisites, one would expect failing of the body's adaptive defense mechanism and an accumulation of DNA damage. In our study, we found a significant increase in serum 8-OHdG in both male and female triathletes. This result indicates that over a long period of training and competition (1 year), of a typical triathlete calendar, there appears to be an evidence of increase in DNA damage. This could be due to the insufficient recovery time between competition and training that reduces the effect of DNA repair enzyme mechanisms. We also found that male triathletes had a 3-fold increase, whereas female triathletes had a 2-fold increase. In a study on the effect of smoking on lymphocyte DNA damage, Betti et al. [25] found that men have more significant damage than women. Similarly, in a study on the effects of chronic low-dose irradiation on exposed workers, Wojewódzka et al. [26] found that men have higher damage and women. Though no specific reasons were attributed for these differences, animal models indicate that it could be due to the gender differences in the antioxidant pathways that are involved in the removal of ROS. Female rats exposed to ultraviolet B had significantly higher total antioxidant capacity than male rats [27]. Higher and efficient removal of ROS will lead to the prevention of DNA damage. This could be one of the reasons for the lower fold increase in female triathletes than in male triathletes in our study.

Similar to DNA damage in triathletes, it has been hypothesized that prolonged high endurance exercise can lead to cardiac myocyte necrosis. This finding is supported by evidence from many studies on the elevation of cardiac troponin levels in blood after marathons, ultra-marathons, triathlons, and long-distance cycling events [28]. These participants sustain elevated cardiac outputs for several hours, leading to increased work stress on the myocardium. The elevated production of ROS and RNS may possibly damage cardiomyocytes. Though this is a plausible explanation, studies have not shown any long-term effects of cardiac damage to the athletes. Thus, it was suggested that damage to the cardiomyocytes following endurance exercise could be transient and reversible, and the release of cardiac troponins represents a cardiac remodeling process. However, if and whether these transient and reversible changes over a lifetime of endurance and ultra-endurance exercise could lead to irreversible myocardial damage is not known. In the present study, we followed up triathletes over a period of 1 year and found that there was no significant difference in cardiac troponin levels between the baseline and at the end of a season of endurance and ultra-endurance training and competition. This could mean that in the long-term, there is no effect of endurance training and competition on cardiac troponins, in contrast to DNA damage. In our study, we also examined the long-term effect of endurance training and competition on skeletal muscle damage using serum myoglobin as biomarker. Similar to cardiac troponin, our results showed no statistically significant difference in serum myoglobin level between baseline values and the values at the end of 1 year of endurance training and competition. However, though not significant, we did observe an increase in serum myoglobin levels at the end of the training and competition period. Increase in serum myoglobin is likely due to damage to the skeletal muscle

following endurance exercise causing disruption of skeletal muscle ultrastructurally, resulting in "leakage of myoglobin" and other enzymes and proteins into the bloodstream [29]. Though not conclusive, skeletal muscle damage, in contrast to cardiac troponin, appears to have a long-term effect due to endurance training and competition.

The main limitation of the present study is that we did not measure the biomarkers before and after the races and during the training. Since the objective of our study was to explore the possibility of the long-term effect of DNA, cardiac, and skeletal muscle damages, we did not measure these biomarkers during and after every race. It is also a well-established fact that these markers would be expected to be elevated after a triathlon race. The other limitation was the two-point sample collection—at the beginning and at the end of the study, which was used in the present study. This protocol would not be able to control for the variability that could occur during the study period. We were aware of this limitation; however, owing to the lack of compliance from the triathletes, we were not able to increase sample collection points. However, we made sure that all the athletes had similar training and participated in similar triathlon events. In the present study, we were also not able to examine the gene expression of DNA repair enzymes to conclusively prove the long-term effect of DNA damage.

In conclusion, the results of the present study show that strenuous endurance "training and competition" over a period of 9 months has an increased effect on DNA damage as shown by an increase in 8-OHdG in male and female triathletes. We also noticed a similar pattern with skeletal muscle damage though it was not significant statistically. Our study did not find similar effect on cardiac muscle damage. The underlying mechanisms causing this increased effect of DNA and skeletal muscle damages remain to be studied, and further investigation is warranted. However, the findings of the present study would be useful for establishing a perfect recovery time so as to reduce the effect of DNA damage on triathletes. This would minimize health risk, as well as improve performance.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Faculty of Medicine Defence Health, National Defence University of Malaysia/29th February 2016/No.1/2016.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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