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

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Abstract

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

Methods: Seven age-matched 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-hydroxy-2'-deoxyguanosine. A similar pattern of increase was seen with serum myoglobin, which was not statistically significant in both males and females. Cardiac troponin I levels did not show any change in both sexes.

Conclusions: 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

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Exercise and health share a “U”-shaped relationship [1]. Regular moderate exercise has beneficial health effects, while acute strenuous exercise causes increased production of reactive oxygen (ROS) and nitrogen species (RNS) that may accumulate and cause DNA damage [2]. Since a triathlon race mimics acute strenuous exercise over a time period of 6-8 hours, 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 showed increased DNA migration 24 hours 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 the 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), 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 normal DNA repair mechanisms would be able to reverse DNA damaging effect, it is possible that over a long period of “training and competition”

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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 damage. 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 more than 5-100 fold. Studies which 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 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 which have looked into the effect of long term training and competition on skeletal muscle damage and cardiac muscle damage.

Accordingly, the aim of this 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 for the study were recruited from a professional triathlon team based in Kuala Lumpur, Malaysia. They included 7 male and 7 female triathletes. The average ages of male and female triathletes

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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 which lasted for approximately 10 months in a year. During this 10-months triathlon season, triathletes underwent regular training in order to be able to participate in at least 6 triathlon events in addition to duathlon and marathon events. A typical yearly racing calendar is shown in Table 1. The 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 periodised into 4 x 4 training blocks with the 4th week being a low volume 'recovery' week. Both male and female triathletes trained for approximately 9-15 hours/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 co-operation of the coach. They were informed of the risks and benefits of the study as well as the study protocol. Informed consent was obtained and 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 this study (SG/2014/SP/UPNM/1) was also approved by the Faculty Research Committee of university (FPKP/RC/2016/BIL.1).

Triathletes were invited to the Human Performance Lab at two time points (Phase 1 and 2); phase 1, two weeks before the beginning of the triathlon season (1st week of February) and phase 2 at the end of the triathlon season (last week of November). The phase 2 sample was collected two weeks after the last triathlon event. At both time points, the athletes were advised to avoid strenuous exercise or physical activity for 48 hours prior to their appointment to the lab. They were also asked to avoid alcohol and caffeinated drinks for 24 hours. On their arrival to the lab, basic anthropometry and body composition measurements using a N2O segmental body composition analyser (U. Healthcare System, Singapore)

were made. Table 2 provides the basic anthropometry and body composition profile of the athletes. Later

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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 Corp., Missouri, USA), while serum cTnI and serum 8-OHdG was measured using Cloud-Clone Corp ELISA Kit (Cloud-Clone Corp., Texas, USA). Protocols of the respective kits were followed and absorbance was measured using SpectraMax 5M (Molecular Devices, CA, USA) analyzer. The intra assay CV was calculated for all the three ELISA kits and were determined to be within 5%. A calibration curve was generated by a four-parameter logistic regression analysis using the SpectraMax software (Molecular Devices, CA, USA).

Statistical Analysis

The results were expressed as means \pm 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 carried out using SPSS package Version 16.1 (SPSS, Chicago, IL, USA)

Results

Results of our study showed a statistically significant increase in serum 8-OHdG levels in both male and female triathletes between phase 1 and phase 2. In male triathletes there was a 3-fold increase while in female triathletes there was a 2-fold increase 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

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statistically significant. We found a decrease in serum cTnI levels in male triathletes at phase 2, while in female triathletes there was an increase in cTnI levels at phase 2. However these changes were also not statistically significant (Fig.1).

Discussion

In the present study we hypothesized that high endurance “training and competition” cycles over a period of time could have an increased effect on DNA damage and could compromise the body’s defence 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. [15] and Tsai et al. [16] showed 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. [17] showed significant DNA damage after acute strenuous exercise in rats. However there are several studies with contrasting findings [18-20]. These studies found no increase in 8-OHdG after maximal and sub-maximal aerobic exercise. This was attributed to the effect of exercise on DNA repair enzyme up-regulation or related to the level of training. Asami et al. [18] found that in untrained athletes, the DNA repair activity increased significantly after exercise, while no such increase was noted in trained athletes. Taken collectively, though strenuous exercise causes increase in DNA damage markers in humans, it appears to depend on the type, intensity of the exercise as well as the adaptation /training of the individual.

Studies on DNA damage looked at 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 [15, 16, 21]. In contrast to these above findings, some studies did not document an increase in DNA damage after the race [22-24]. However, even with an increase in DNA damage after the race, it was noted that after a period of

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7-14 days, DNA damage marker levels were back to pre-race levels. This was attributed to the effective DNA repair mechanisms. But a point to note is that these studies considered the effect of a single race and not a long term effect of DNA damage over a period of time. In a study by Okamura et al., [25] 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 [21-23]. 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 defence 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 (one year), of a typical triathlete calendar, there seems to be an evidence of increase in DNA damage. This could be due to the insufficient recovery time between competition and training which reduces the effect of DNA repair enzyme mechanisms. We also found that male triathletes had a 3 fold increase while the female triathletes had a 2 fold increase. In a study on effect of smoking on lymphocyte DNA damage, Betti et al., [26] found that men had more significant damage than women. Similarly, in a study on effects of chronic low-dose irradiation on exposed workers Wojewódzka et al., [27] found that men had 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 is involved in the removal of reactive oxygen species. Female rats exposed UVB had significantly higher total antioxidant capacity than the male UVB exposed rats [28]. Higher and efficient removal of reactive oxygen species will lead to prevention of DNA damage. This could be one of the reason for the lower fold increase in our female triathletes when compared to male triathletes in our study.

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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 elevation of cardiac troponin levels in blood after marathons, ultra-marathons, triathlons and long distance cycling events [29]. These participants sustain elevated cardiac outputs for several hours which, leads to increased work stress on the myocardium. 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 a 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 life time 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 one year and found that there was no significant difference in the 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 long term there is no effect of endurance training and competition on cardiac troponins, unlike DNA damage. In our study we also looked into the long term effect of endurance training and competition on skeletal muscle damage using serum myoglobin as bio-marker. 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 one 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

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bloodstream [30]. Though not conclusive, skeletal muscle damage, unlike cardiac troponin seems to have a long term effect due to endurance training and competition.

The main limitation of this 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 the DNA, cardiac and skeletal muscle damage, 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 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 this 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 due to 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 took part in similar triathlon events. In this study we were also not able to look into the gene expression of the DNA repair enzymes in order to conclusively prove the long term effect of the DNA damage.

In conclusion, results of this study point to the conclusion that strenuous endurance ‘training and competition’ over a period of 9 months has an increased effect on the DNA damage as shown by 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 the DNA and skeletal muscle damage remain to be studied and further investigation is warranted. However, the findings of this study would be useful for establishing a perfect recovery time so as to reduce the effect of DNA damage on triathletes. This would minimise health risk as well as improve performance.

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Table 1**A typical yearly racing calendar.**

Date	BEGINNING OF SEASON
8th Feb	Metasprint Aquathlon, Singapore
15th Feb	Oral Cancer Run 10km, Malaysia
8th March	Nestle Fitness Run, Malaysia
15th March	Penang Triathlon, Malaysia
28th March	Melawati 10k run, Malaysia
5th April	Putrajaya triathlon, Malaysia
12th April	Kapas Marang swim, Malaysia
19th April	Metasprint triathlon, Singapore
2nd May	Kenyir Lake Triathlon, Malaysia
3rd May	Xterra, Malaysia
13th June	Asian Championships, Tapei
12th July	Osaka ASTC , Japan

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25th July	Singapore ASTC Cup, Singapore
1st August	Port Dickson Triathlon, Malaysia
6th September	Singapore National Aquathlon Champs, Singapore
4th October	Singapore National Duathlon Champs, Singapore
18th October	ITU World Duathlon, Adelaide, Australia
31st October	HK ASTC Cup Sprint, Hong Kong
15th November	Powerman Philippines Duathlon, Philippines
	END OF SEASON

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/m ²)	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

Values are mean ± standard deviation

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Figure 1:

Fig. 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

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