No Acute and Persistent DNA Damage after an Ironman Triathlon

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Abstract

During acute and strenuous exercise, the enhanced formation of reactive oxygen species can induce damage to lipids, proteins, and nucleic acids. The aim of this study was to investigate the effect of an Ironman triathlon (3.8 km swim, 180 km cycle, 42 km run), as a prototype of ultra-endurance exercise, on DNA stability. As biomarkers of genomic instability, the number of micronuclei, nucleoplasmic bridges, and nuclear buds were measured within the cytokinesis-block micronucleus cytome assay in once-divided peripheral lymphocytes of 20 male triathletes. Blood samples were taken 2 days before, within 20 min after the race, and 5 and 19 days post-race. Overall, the number of micronuclei decreased (P < 0.05) after the race, remained at a low level until 5 days post-race, and declined further to 19 days post-race (P < 0.01). The frequency of nucleoplasmic bridges and nuclear buds did not change immediately after the triathlon. The number of nucleoplasmic bridge declined from 2 days pre-race to 19 days post-exercise (P < 0.05). The frequency of nuclear buds increased after the triathlon, peaking 5 days post-race (P < 0.01) and decreased to basic levels 19 days after the race (P < 0.01). The results suggest that an Ironman triathlon does not cause long-lasting DNA damage in well-trained athletes. (Cancer Epidemiol Biomarkers Prev 2008;17(8):1913–9)

Introduction

Regular moderate physical activity is associated with various health benefits such as decreased risk of cardiovascular diseases, diabetes, cancer, and other lifestyle-dependent diseases (1-5). In a recent review, Rundle (6) showed several possibilities how exercise positively influences different phases of carcinogenesis including enhanced detoxification of reactive oxygen species, increased DNA repair activity, and improved immune functions. However, it is known that acute and strenuous exercise also induces oxidative stress through the enhanced formation of reactive oxygen species (7, 8), which in turn may result in the damage of lipids, proteins, and nucleic acids (9-12). Oxidative stress-induced DNA damage as well as insufficient DNA repair may play an important role in the etiology of cancer, diabetes, and arteriosclerosis (10). Potential pathways for exercise-induced oxidative stress include increased oxygen consumption, autooxidation of catecholamines, activation of inflammatory cells due to tissue damage and ischemia, or hypoxia (13, 14).

Thus far, only a small number of studies have been conducted to investigate the influence of physical activity on DNA damage and the findings are inconsistent due to the use of different protocols and different endpoints. The majority of the studies were based on single-cell gel electrophoresis (SCGE) assays and on the determination of urinary excretion of 8-hydroxy-2'-deoxyguanosine (15-21). Investigations concerning the effect of physical activity on the micronuclei frequency, which are formed as a consequence of chromosome breakage and chromosome loss (22), are still limited and the data are controversial. Although no alterations of micronuclei were found after treadmill running (23) and a short-distance triathlon (16), elevated levels of micronuclei were observed after two exhaustive sprints (24). It is important to point out that the duration of exercise in the latter studies are not comparable to an Ironman triathlon race, where the athletes are extraordinary in their level of training and in the endurance and intensity of exercise done. Because the number of nonprofessional athletes training for and competing in ultra-endurance events continually increases, it is of particular importance to investigate this group.

The cytokinesis-block micronucleus cytome (CBMN Cyt) assay is a test that enables the detection of genomic instability, including chromosome breakage, chromosome loss, chromosome rearrangements, and gene amplification and nondisjunction (25). Furthermore, this endpoint has been reported to detect DNA damage caused by dietary, environmental, and lifestyle factors (26), and a causal link between micronuclei and the risk of cancer has been described in a recent cohort study (27).

The major aims of the present study were to examine for the first time (a) the effect of an Ironman triathlon race, as a prototype of ultra-endurance exercise, on DNA damage in lymphocytes, (b) to find out whether an association exists between DNA damage and training level, and (c) to study the influence of ultra-endurance exercise on the formation of nucleoplasmic bridges as...
well as nuclear buds. To verify the complete recovery period, the variable were monitored over a longer time (19 days).

Materials and Methods

Study Group. Of the entire study group (n = 48), 24 subjects were randomized for the CBMN Cyt assay. Statistical analysis was done for 20 subjects. The experimental design is summarized in Fig. 1. The study was reviewed and approved by the local ethics committee of the Medical University of Vienna.

All participants were healthy nonsmokers and were asked to document their training 6 months pre-race and thereafter until 19 days post-race including the weekly training (km), the total weekly exercise time (h), and the weekly net endurance exercise time (h). At each blood collection, a 24-h recall was completed to record nutritional information. All participants were physically fit, free of acute or chronic diseases, within normal range of body mass index, and not taking any medication. They were also asked to abstain from the consumption of supplements in excess of 100% of the Recommended Dietary Allowance threshold level per day, in addition to their normal dietary intake of antioxidants, vitamins, and minerals including vitamin C, vitamin E, β-carotene, selenium, and zinc in tablet or capsule form 6 weeks before the triathlon until the last blood sampling 19 days after. Only on race day and 1 day post-race, the athletes were allowed to eat and drink ad libitum; however, data regarding their intake were documented. Only subjects who finished the race were kept within the study group.

Before each blood sampling (except the sampling immediately after the race) and also 2 days before the spiroergometry, the subjects were told to refrain from intense exercise. After the race, the training of the subjects had a regenerative character and was only of moderate intensity until the end of the study.

To assess the physiological characteristics, the subjects were tested on a cycle ergometer (Sensormedics, Ergometrics 900) 3 weeks before the triathlon. The maximal test protocol started at an initial intensity of 50 W followed by 50 W increments every 3 min until exhaustion. Oxygen and carbon dioxide fractions (both via Sensormedics 2900 Metabolic measurement cart), power output, heart rate, and ventilation were recorded continuously and earlobe blood samples for the measurement of the lactate concentrations were taken at the beginning and end of each step.

VO₂ peak values were used to divide the total group of participants into two subgroups regarding their training levels. There is good evidence that endurance training leads to adaptations of the endogenous antioxidant defense system (2), and some studies have also shown that the enhancement in these protective mechanisms can be correlated with the maximum or peak oxygen consumption (28). A VO₂ peak value of 60 mL/kg/min was considered as the cutoff point. Subjects with a VO₂ peak <60 mL/kg/min formed the trained (T) group (n = 10) and participants with VO₂ peak >60 mL/kg/min formed the very trained (VT) group (n = 10).

Race Conditions. The Ironman triathlon was held in Klagenfurt, Austria on July 16, 2006. The event comprises a 3.8 km swim, a 180 km cycle, and a 42 km run. The race started at 7:00 a.m., when the air temperature was 15°C, lake temperature was 25°C, and relative humidity was 77%. By finishing time (median time for participants 5:43 p.m.), air temperature and relative humidity were 27.2°C and 36%, respectively (data provided by the Carinthian Center of the Austrian Central Institute for Meteorology and Geodynamics).

Reagents. Dulbecco’s PBS, RPMI 1640, cytochalasin B, trypan blue, DMSO, sodium pyruvate, l-glutamine, FCS, penicillin, streptomycin, and Histopaque-1077 were obtained from Sigma-Aldrich. Phytohemagglutinin (M form) was purchased from Invitrogen. DiffQuik was procured from Dade Behring. Other reagents were obtained from Merck.

Blood Sampling. Blood samples were collected by venipuncture in heparinized and EDTA tubes (Vacuette) 2 days before, within 20 min after the race, and 5 and 19 days post-race. The blood samples were processed immediately, as described below, or stored below 6°C for no longer than 7 h before processing.

CBMN Cyt Assay. The CBMN Cyt assay was carried out according to the method of Fenech (29). Briefly, lymphocytes were isolated using Histopaque-1077 as a density gradient and resuspended in RPMI 1640, which was supplemented with 11% heat-inactivated FCS, 2.0 mmol/L l-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and sodium pyruvate. Phytohemagglutinin (30 μg/mL) was added to stimulate cell

Figure 1. Experimental design showing when the CBMN Cyt assay was done and spiroergometry and the determination of vitamin B₁₂ and folate were done.
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Table 1. Baseline characteristics of subjects (mean ± SD)

<table>
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<th>Total group (n = 20)</th>
<th>T (n = 10)</th>
<th>VT (n = 10)</th>
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<tr>
<td>Age (y)</td>
<td>31.7 ± 6.1</td>
<td>33.1 ± 7.6</td>
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<td>Weight (kg)</td>
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<td>Height (cm)</td>
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<td>Body mass index (kg/m²)*</td>
<td>22.9 ± 1.5</td>
<td>23.0 ± 1.8</td>
<td>22.8 ± 1.3</td>
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<tr>
<td>VO₂ peak (mL/kg/min)</td>
<td>60.8 ± 8.8</td>
<td>54.3 ± 3.5</td>
<td>67.4 ± 7.4</td>
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<td>Race time (h)</td>
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<td>Weekly net endurance exercise time (h)</td>
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<tr>
<td>Total weekly exercise time (h)</td>
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<td>Cycle training/wk (km)</td>
<td>180.4 ± 22.1</td>
<td>193.6 ± 49.5</td>
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<td>Run training/wk (km)</td>
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<td>Folate (µg/L)</td>
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<td>Body mass index (µg/L)</td>
<td>347.2 ± 147.8</td>
<td>364.2 ± 149.7</td>
<td>326.0 ± 152.8</td>
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</tbody>
</table>

*Weight in kilograms divided by squared height in meters.

1 P < 0.01 (T versus VT).

The one-sample Kolmogorov-Smirnov test was used to test all data for their normal distribution.

The paired t test (for normally distributed data) was implemented to assess statistically significant differences between the four time points of blood sampling for each group. The unpaired t test was used to analyze the differences between the T and the VT subjects. As the number of nucleosomes in binucleated cells, as well as the number of nucleoplasmic bridges 20 min post-race, were not normally distributed, the data were tested using the nonparametric Wilcoxon matched-pairs test and the Mann-Whitney U test, respectively. P < 0.05 was regarded as statistically significant.

Results

As three participants did not finish the race and one triathlete could not participate in the entire study, the CBMN Cyt assay was done with peripheral lymphocytes from 20 subjects at four different blood sampling points. This high number of subjects has not been investigated in previous studies. Therefore, the collective was further divided into two subgroups (T and VT; n = 10 each) to investigate whether changes are based on different training levels (cutoff point: VO₂ peak value of 60 mL/kg/min). The baseline characteristics of the total group (n = 20) as well as the subgroups T and VT subjects are summarized in Table 1.

The overall plasma vitamin B₁₂ and folate levels 2 days before the race (347.2 ± 147.8 ng/L and 8.3 ± 4.3 µg/L, respectively) were similar to those 5 days post-race (409.9 ± 237.0 ng/L and 7.5 ± 4.8 µg/L, respectively).

The overall number of binucleated cells with micronuclei decreased significantly (P < 0.05) after the race, remained at a low level until 5 days post-exercise, and declined further until 19 days post-race (P < 0.01; Fig. 2A). Only in the VT subgroup, the number of binucleated cells with micronuclei decreased significantly (P < 0.05) from 2 days before the triathlon to 20 min post-race (Fig. 2A). However, in both subgroups, the number of binucleated cells containing micronuclei showed a highly significant decrease from 2 days pre-race to 19 days post-race (T: P < 0.05; VT: P < 0.01) as well as 5 to 19 days post-race (T: P < 0.05; VT: P < 0.01).
In addition, a highly significant ($P < 0.01$) decrease from 2 days pre-race to 5 days post-race was seen in the VT group (Fig. 2A), whereas no significant change in the number of binucleated cells with micronuclei was observed from 20 min to 5 days post-race. Similar results were obtained with regard to the number of micronuclei in binucleated cells (Fig. 2B). This marker also decreased significantly ($P < 0.05$) after the race and declined again between days 5 and 19 after the race ($P < 0.01$) in the total group. The VT subjects showed a significant reduction in the number of micronuclei in binucleated cells immediately after the race ($P < 0.05$), which prolonged until 5 days post-exercise and then further declined ($P < 0.01$). The lowest value was reached 19 days after the triathlon (Fig. 2B). In the T subgroup, no significant change was found 20 min post-race. However, this marker declined significantly ($P < 0.05$) 5 days post-race compared with pre-race values. The decrease in the number of micronuclei in binucleated cells from 5 to 19 days post-race was also seen in the T group ($P < 0.05$; Fig. 2B).

Immediately after the triathlon, the frequency of nucleoplasmic bridges did not change significantly (Fig. 2C). Overall, the marker declined significantly from 2 days pre-race to 19 days post-exercise ($P < 0.05$), but in the two subgroups the frequency of nucleoplasmic bridges did not change significantly (Fig. 2C).

The number of nuclear buds did not change immediately after the triathlon, neither in the total collective nor in the subgroups (Fig. 2D), but it increased after the triathlon, reached a maximum 5 days post-race (T: nonsignificant; total group and VT: $P < 0.01$; comparing 20 min post-race with 5 days post-race), and then decreased significantly 19 days after the race to basic levels (T: $P < 0.05$; total group and VT: $P < 0.01$).

Data of the nuclear division index are shown in Table 2. The nuclear division index increased significantly ($P < 0.01$) after the race and remained at a high level until 19 days post-race in all subjects. In addition, only the VT subjects showed a significant decrease from 20 min to 5 and 19 days post-race ($P < 0.05$).

Discussion

Physical activity is reported to play an important role in the reduction of the susceptibility to cancerous diseases and their primary prevention (3, 37, 38). On the other
hand, oxidative DNA damage induced by the formation of reactive oxygen species, which is also linked to acute and strenuous exercise, has been suggested to be involved in aging as well as various diseases such as cancer (12, 39). The present study was conducted to assess for the first time the influence of an Ironman triathlon race, as a model of ultra-endurance exercise, on the DNA stability of athletes with different training levels. The participants of our study were nonprofessionals, but all at a high training level. The CBMN Cyt assay was applied to examine the effect of intensive endurance exercise on the formation of micronuclei, nuclear buds, and nucleoplasmic bridges, because the association between micronuclear frequency and cancer incidence was shown recently (27). The CBMN Cyt assay was proposed by the authors to be a sound biomarker for identifying genetic, nutritional, and environmental factors, which may be carcinogenic (27).

Thus far, only a few studies concerning the frequency of micronuclei after exhaustive exercise have been conducted and the data are conflicting. Although Schiffl et al. (24) found significantly elevated levels of micronuclei in six subjects after two sprints until exhaustion, no alterations were observed after treadmill running at 85% of maximal oxygen uptake for 30 min (23) or a short-distance triathlon of 2.5 h duration (16). The present study was the first investigating micronuclei after an ultra-endurance exercise with a duration between 9 and 14 h. Interestingly, a significant change in the number of binucleated cells with micronuclei was observed. The significant decrease and the low level of binucleated cells with micronuclei, even after 19 days, show that ultra-endurance exercise does not induce chromosome breaks and/or chromosome loss, immediately after or within 3 weeks post-exercise. DNA stability is impaired by deficiencies of vitamin B12 and folate, which in turn can lead to the formation of micronuclei (40, 41). However, a deficiency of these micronutrients in the present study collective can be excluded.

In a review, Moller et al. (42) emphasize that besides a small number of studies that examined the influence of strenuous exercise on the formation of micronuclei, the majority of the investigations focused on the effect of both moderate and excessive exercise on oxidative DNA damage as detected by the SCGE assay as well as 8-hydroxy-2’-deoxyguanosine. In contrast to our investigation, where the number of micronuclei decreased after the Ironman triathlon race and remained at a low level until 19 days post-race, elevated levels of DNA migration were found in some studies with different exercise protocols in which the duration was <3 h. Hartmann et al. (15) observed increased DNA migration in SCGE assays 6 h after treadmill running at maximal oxygen consumption, which peaked 24 h after the exercise. Although the experiment was conducted only with three subjects, the authors concluded that physical activity higher than the aerobic-anaerobic threshold leads to altered levels of DNA migration. Previous studies with different models of massive aerobic exercise, such as a marathon (20) or a short-distance triathlon (16), detected increased levels of DNA migration 24 h post-exercise in the SCGE assay. In the latter study, DNA migration reached a maximum 72 h post-exercise. However, urinary 8-hydroxy-2’-deoxyguanosine remained unchanged. Therefore, the authors concluded that the DNA migration after the short-distance triathlon does not lead to oxidized DNA bases and does not result in DNA damage. On the contrary, increased urinary 8-hydroxy-2’-deoxyguanosine levels were detected 1 day after the start of a supra-marathon (4-day race), which declined on the fourth day of running. The authors suggested that repeated extreme exercise leads to an adaptation and normalization of oxidative DNA damage (21). Due to the significantly elevated level of DNA damage 1 day following a half marathon, Niess et al. (19) proposed that intense endurance exercise induced DNA damage is caused by reactive oxygen species. Similar results were observed after a 42-km marathon run (20). The latter investigation detected elevated DNA single-strand breaks in the standard SCGE assay 24 h after the race, which persisted through 7 days. Furthermore, oxidative effects on nucleotides were perceived using lesion-specific endonuclease immediately after the marathon and they lasted for >1 week. The same course was observed for urinary 8-hydroxy-2’-deoxyguanosine. Immediately after a half-marathon (running time ≤2.6 h) and a marathon (running time ≤4.8 h), Briviba et al. (43) found no increased levels of endogenous DNA strand breaks and formamidopyrimidine glycolase-sensitive sites, but oxidative DNA damage, assessed as endonuclease III sites, was significantly increased and the ex vivo resistance to DNA damage induced by hydrogen peroxide was decreased after the half-marathon and marathon.

Several authors detected DNA damages at times ranging from immediately until 24 h after strenuous exercise; however, Mastaloudis et al. (17) observed a significantly increased proportion of damaged cells (10%) at midrace in subjects attending an ultra-marathon with an average duration of 7.1 h, but 2 h after the event the values declined to baseline. Six days after the ultra-marathon, the proportion of damaged cells was even lower than before the race. Based on this observation, the authors proposed that the change is not persistent. This assumption is in agreement with our results, where no prolonged DNA damage was detected after a mean of 10.4 h of exercise. However, it is important to point out that the different test systems detect different types of DNA alterations. Whereas the CBMN Cyt assay detects mutations that persist at least for one mitotic cycle, repairable DNA lesions or alkali-labile sites are detected by the SCGE assay (44). Thus, it can be hypothesized that the endurance and intensity of exercise done during an Ironman triathlon race do not lead to fixed mutations probably due to the up-regulation of repair mechanisms and enhanced endogenous antioxidative systems.
Regarding the influence of different training levels on DNA damage, Umegaki et al. (23) found that intensive exercise caused no increased chromosomal damage in trained and untrained subjects after 30 min treadmill running at 85% of maximal oxygen uptake. However, in the untrained group, X-ray-induced chromosomal damage was significantly altered. Thus, the authors concluded that the difference in chromosomal damage between trained and untrained subjects was due to an enhanced DNA repair system and an increased capacity of endogenous antioxidant systems. In addition, Niess et al. (18) investigated in another study the effect of a treadmill test until exhaustion on DNA migration as detected by the SCGE on six trained and five untrained subjects. They found higher DNA migration levels in the untrained study group compared with trained subjects 24 h post-exercise. Comparisons between subjects of different training levels showed that athletes had higher levels of spontaneous chromosomal damage in lymphocytes at rest than the untrained subjects, yet the basal value appeared to be unchanged after a cycle-ergometer exhaustive test (45). The authors hypothesized that this phenomenon may be due to chronic stress in the athlete group caused by their habitual intensive training. These findings are supported by the results of the present study, as chromosomal damage tended to be higher (nonsignificant) in the T subjects than in the VT group. The differences between T and VT subjects could be due to adaptive responses of regular training, such as a more efficient electron chain in muscle mitochondria (46, 47) and up-regulation of repairing systems such as the 8-oxoguanine repair enzyme (48). Furthermore, an extended capability of endogenous antioxidant systems (in the VT subjects) might lead to the reduction of oxidative stress–induced effects and thus improved oxidative balance during exercise (2, 11, 18, 49).

According to our knowledge, the present study is the first dealing with the influence of strenuous exercise on the formation of nucleoplasmic bridges as well as nuclear buds. A recent investigation conducted by Gisselsson et al. (50), with primary cultures of solid tumors, showed that nucleoplasmic bridge and micronuclei as well as nuclear blebs are found in different cancer cells. The authors postulated that these abnormal nuclear morphologies are characteristic for genomic instability. In the current investigation, we found no significant change in the frequency of nucleoplasmic bridges immediately after the race, which was mainly due to the high individual variation. However, the significant decline of this marker 19 days after the triathlon may suggest that strenuous exercise either does not lead to the formation of dicentric chromosomes and telomere end-fusions or enhances DNA repair mechanisms to prevent DNA misrepair and thus the formation of nucleoplasmic bridges.

Based on the number of nuclear buds, a similar trend for both groups was observed, but again it was more distinct in the VT group. Lindberg et al. (51) suggested when using 9-day cultures of human lymphocytes that nuclear buds and micronuclei have partly different mechanistic origins. However, in vitro experiments with mammalian cells (33, 52) showed that during S phase of the cell cycle amplified DNA is removed via nuclear budding to generate micronuclei. Thus, it could be hypothesized that nuclear buds formed 5 days after the exercise bout may be eliminated by forming micronuclei, which in turn may be extruded from the cytoplasm (53) before the last time point of blood sampling (19 days post-race). However, the exact duration of the nuclear budding process and the extrusion of the resulting micronuclei from the cell have not been clarified thus far (54).

In conclusion, the present investigation shows that an Ironman triathlon race, as a model of massive physical exercise, does not cause DNA damage in endpoints detected by the CBMN Cyt assay. It is likely that regular training leads to adaptive mechanisms including the up-regulation of repair mechanisms as well as an increase in the activity of the endogenous antioxidant system, which may prevent severe oxidative stress and DNA damage even after strenuous exercise. To clarify the influence of strenuous exercise on the formation of nuclear buds and also nucleoplasmic bridges further detailed studies will be needed.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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