Sportive activity reduces genomic damage

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Research Article

Keywords: MNi Micronuclei, RONS Reactive oxygen and nitrogen species, AT Athletes, SC Sedentary controls

Posted Date: December 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2333411/v1

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Abstract

Purpose - The influence of training on the levels of genomic damage are not univocal, and to date not completely understood. Indeed, studies in this area remain scant and do not draw definitive conclusions. Our aim was to evaluate the influence of training on the levels of DNA damage in young athletes. We also wanted to explore the possibility of genomic damage to be driven by single-gene polymorphisms on metabolic and DNA repair genes in our sample.

Method - We compared the levels of Micronuclei and other nuclear aberrations in buccal mucosa cells of a group of 35 healthy, young martial artists with the ones of a group of 35 healthy, young sedentary controls. We also genotyped all subjects for three metabolic and two DNA repair genes in order to investigate the influence of the related single-gene polymorphisms on the frequency of genomic damage.

Results - We observed a decrease in the levels of genomic damage in athletes relatively to sedentary controls; this decrease was significant in both Micronuclei and Total Aberrations. Instead, single-gene polymorphisms did not alter significantly the levels of nuclear aberrations.

Conclusion - The decrease in genomic damage observed in athletes suggests a protective effect of training and corroborates the beneficial effects of sportive activity on human health. Moreover, even other factors, such as the nature of the sport and training length, could influence the results.

1. Introduction

Sportive activity (both aerobic and anaerobic) is well known to elicit the production of reactive oxygen and nitrogen species (RONS), whose overproduction can disrupt the intracellular redox equilibrium, thus causing oxidative stress [1]. However, RONS' overproduction caused by chronically-repeated physical exercise is thought to be a signal for the upregulation of the antioxidant system's activity [1], and sportive activity has also been reported to stimulate it [2, 3, 4]. It was also found that, whereas an excessive frequency of training is associated with a constant oxidative insult, a moderate one is able to stimulate the antioxidant repair systems without implying a severe oxidative stress [3]. Other authors [5] found no influence of a resistance training period on the oxidative activity (as a measure of the production of reactive oxygen species) of neutrophils and monocytes in elderly women.

Among the many tests available to assess genotoxicity and study DNA damage, the micronucleus test is one of the most versatile and important ones [6]. Micronuclei (MNi) are small nucleus-like bodies which consist of a membrane containing chromosomes or chromosome fragments that were not included in the main nucleus after a cell division [7]. MNi have been identified as a source of genome rearrangements such as chromothripsis [7], as well as many case-control studies have demonstrated an increase of MNi frequency in most types of cancers [8, 9]. High MNi frequencies have also been correlated with many other pathological conditions and/or diseases, such as infertility, diabetes, chronic kidney disease, cardiovascular and neurological diseases [8, 9].

Oxidative stress can lead to DNA damage [10] and thus to increased levels of MNi [11, 12]. To date, few literature investigated the correlation between sportive activity and MNi frequency, and most of published
studies were referred to the variation in MNi frequency in peripheral blood lymphocytes following physical activity [13]. However, studies investigating the role of physical activity in modulating the basal frequency of MNi in buccal mucosa cells remain scant. The correlation between MNi frequency and training, besides, appears to be anything but univocal.

Indeed, for example, no influence of a six-month resistance-training period on the frequency of MNi in buccal cells was found among institutionalized elderly [14], whereas an increase in chromosomal damage in buccal cells of professional hockey and baseball-softball players, was observed [15].

This discrepancy may be due to the different age and physical request of the two trainings. In fact, other authors compared MNi frequency in lymphocytes of professional road-racing cyclists with non-agonists (subjects with a lower weekly training frequency), and found higher values in the former ones [3].

The aim of this study was to evaluate whether physical activity plays a role in the modulation of the MNi frequency levels in buccal mucosa cells of young martial artists. On the basis of result published in literature [3, 5], we will expect an increase in MNi frequency in athletes (AT), due to an overproduction of RONS. This increase should be particularly important in AT which train more, while in AT training less the upregulation of the antioxidant system may compensate the production of RONS [3]: we will also discuss this possibility.

Moreover, it has been demonstrated that the level of the genomic damage also depends on the different individual susceptibility, and single-gene polymorphisms can influence the susceptibility to MNi formation [16, 17]. This susceptibility is in large part due to polymorphisms in metabolic genes, such as cytochrome P450 (CYP) and glutathione-S-transferase (GST) family genes or DNA repair genes as XRCC and XPC genes [17, 18, 19, 20].

GSTs genes in humans are polymorphic due to a deletion which causes the inability to synthesize the protein; this deletion has been correlated to an increase in DNA damage susceptibility [19] and to the development of some types of cancer [21, 22].

CYP1A1 gene is fundamental for the metabolism of polycyclic aromatic hydrocarbons, present in cigarette smoke. One of the known polymorphisms at CYP1A1 gene consists of a point mutation (A > G) at codon 462 of exon 7, with the subsequent replacement of an isoleucine with a valine: this polymorphism has been associated with an increased risk of lung cancer [22, 23].

XRCC1 is a molecular scaffold protein involved in the repair of many DNA single-strand breaks, and its presence has been revealed to play a role in preventing neurodegenerative diseases [24]. Among the many XRCC1 gene polymorphisms, one of the most studied is Arg194Trp on exon 6 [25]; regarding this polymorphism, higher levels of MNi have been found in subject carrying the XRCC1 194 Arg/Trp and Trp/Trp genotypes with respect to those carrying the Arg/Arg genotype in workers exposed to 1,3-butadiene [25]. Moreover, the Arg194Trp polymorphism has been associated with increased bladder cancer risk [26].
XPC (i.e. Xeroderma Pigmentosum group C) gene belongs to the Nucleotide Excision Repair (NER) pathway; this gene encodes a protein binding damaged DNA in apoptosis and in the first phases of DNA repair [27]. The Lys939Gln polymorphism of this gene consists in an A > C transition at codon 939 of exon 15; this polymorphism has been studied in relation to the probability of developing many cancer types, with inconsistent results [27, 28, 29].

Due to the variation of metabolism and the oxidative stress resulting from physical activity, we expect metabolic and DNA repair genes to be particularly important in athletes.

For these reasons we also decided to genotype all subjects involved in this study for the following polymorphisms: GSTT1, GSTM1, CYP1A1 (Ile/Val), XRCC1 (Trp194Arg) and XPC (Lys939Gln). We analyzed the possible correlation between those polymorphisms and the occurrence frequency of MNi in order to evaluate their role in predisposing carriers to DNA damage.

Lastly, dietary supplementation is known to be able to reduce the levels of DNA damage [30, 31]. Given 13 of the 35 sampled AT made use of dietary supplements, we compared the levels of cytogenetic damage of the two AT subgroups to exclude a possible role of this factor in reducing aberrations frequencies.

2. Methods

2.1. Subjects

The sample of this study was composed of 35 healthy agonists practicing martial arts (both striking and wrestling disciplines) and 35 control healthy sedentary subjects. Both the athlete group and the control group were composed of 28 males and 7 females. Athletes (AT) trained from a minimum of 3 to a maximum of 11 times per week (5.657 ± 2.802), while sedentary controls (SC) did not train at all. Each training session lasted at least 90 min; during most of them AT alternated phases of aerobic training, anaerobic training and rest; few trainings were instead merely based on aerobic (run) or anaerobic (weightlifting) training. The age of the subjects ranged from 18 to 36 years (mean age 22.686 ± 4.276 and 22.857 ± 4.188 for AT and SC, respectively) (Table 1).

Table 1 – Demographic and training characteristics of the studied groups
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Athletes</th>
<th>Sedentary Controls</th>
</tr>
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<tbody>
<tr>
<td><strong>Subjects</strong></td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
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<tr>
<td>Males</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Females</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sample</td>
<td></td>
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<tr>
<td>Mean years ± S.D.</td>
<td>22.686±4.276</td>
<td>22.857±4.188</td>
</tr>
<tr>
<td>Range (years)</td>
<td>18-34</td>
<td>18-36</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean years ± S.D.</td>
<td>23.179±4.473</td>
<td>23.143±4.584</td>
</tr>
<tr>
<td>Range (years)</td>
<td>18-34</td>
<td>18-36</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean years ± S.D.</td>
<td>20.714±2.812</td>
<td>21.714±1.704</td>
</tr>
<tr>
<td>Range (years)</td>
<td>18-25</td>
<td>20-25</td>
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<tr>
<td><strong>Trainings per week</strong></td>
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<tr>
<td>Total sample</td>
<td></td>
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<tr>
<td>Mean ± S.D.</td>
<td>5.657±2.802</td>
<td>/</td>
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<tr>
<td>Range</td>
<td>3-11</td>
<td>/</td>
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<tr>
<td>Males</td>
<td></td>
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</tr>
<tr>
<td>Mean ± S.D.</td>
<td>5.268±2.566</td>
<td>/</td>
</tr>
<tr>
<td>Range</td>
<td>3-11</td>
<td>/</td>
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<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>7.214±3.365</td>
<td>/</td>
</tr>
<tr>
<td>Range</td>
<td>3-11</td>
<td>/</td>
</tr>
</tbody>
</table>

All participants were informed volunteers and were asked to compile a questionnaire where data regarding age, sex, training frequency, radiation exposure, diet, supplements, alcohol and drug consumption were collected. All subjects were non-smokers, non-alcoholics, were not under any medication and have not been exposed to X-rays on the cranial region in the last year. Apart from the training activity, all lifestyle traits of the two sampled groups (AT and SC) were comparable. This work
has been approved by the local committee responsible for human experimentation (protocol n° 0609375 of 10/28/2021), and all procedures were carried out according to the 2013 Helsinki declaration.

2.1 Buccal MNi assay
We made buccal epithelial smears to gather buccal mucosa cells by scraping for 1 minute a toothbrush in the inner side of one cheek of each participant. The tip of the toothbrush was then immediately immersed in a fixative solution composed of methanol/acetic acid 3:1. Test tubes were thus brought to the laboratory, where they were centrifuged and the supernatant was sucked away. Pellet remained in a small amount of fixative, which was aspirated with pasteur pipettes and dripped on slides. Finally, dried slides were stained with 5% Giemsa (pH 6.8) prepared in Sörensen buffer to proceed with the MN assay. We performed the microscopic analysis using a light microscope at 1,000x magnification. For each subject we evaluated 1,000 cells with preserved cytoplasm; among these cells, we looked for micronuclei (fig. 1A), nuclear buds (fig. 1B), notched nuclei (indentation, fig. 1C) and broken egg nuclei (fig. 1D). These nuclear anomalies were photographed, and photos were inspected on the computer screen to validate their presence.

2.2. DNA extraction and polymorphism analysis
After the toothbrush smear, another smear was performed with a buccal swab. The swab was scraped for 1 minute in the inner side of the cheek of each participant, and then immediately immersed in an eppendorf with 400 μl of lysis solution (10 mM Tris pH 7.6; 10 mM EDTA pH 8; 50 mM NaCl). DNA was extracted according to the following protocol: 7.5 μl of SDS 20% and 15 μl of 10 mg/mL proteinase K were added to the solution, which then was heated at 56°C for 1 hour. 400 μl of chloroform were added, everything was shaken and then centrifuged 10 min at 640 x g. Supernatant was aspirated and added to a new eppendorf, where an equal volume of isopropanol was added; the eppendorf was gently shaken for 1 min, and then centrifuged at 640 x g for 20 min. Supernatant (isopropanol) was aspirated, and 400 μl of 75% ethanol were added. Everything was centrifuged for 15 min, and then ethanol was inspired and the pellet was left to completely dry at room temperature. Finally, the pellet was suspended in 50 μl of Tris-EDTA and, thus, was ready for the PCR analysis. We genotyped all subjects for CYP1A1, GSTT1, GSTM1, XRCC1 Trp194Arg and XPC. Primers and methodologies were described in Scarfò et al. 2021 [32].

For PCR reactions, we used 2 μL of the solution containing DNA. PCR reactions were performed in a final reaction volume of 25 μL, containing 1× reaction buffer, 1.5 mM MgCl2, 5% DMSO, 250 μM dNTPs, 0.5 μM of each primer, 1 U/sample of Taq DNA polymerase (Fischer Scientific, Milan, Italy), and sterile water until reaching the final volume of 25 μL. The PCR cycles were set as follows: 95°C for 5 min, followed by 40 cycles of 1 min at 95°C, 1 min at 60°C, 1 min at 72°C, and a final extension step of 10 min at 72°C. The amplification products were electrophoresed using 2% agarose gel and detected by ethidium bromide staining. Negative PCRs were repeated at least once to validate their negativity.

2.3. Statistical analysis
Statistical analysis was performed with R Studio interface for R software, version 4.0.2. We performed the Shapiro test in order to verify the normality of the distribution of nuclear aberrations in the two groups. Since the distribution was never found to be normal, we carried on the Kruskal-Wallis test to search for differences in the frequencies of the above-mentioned aberrations between AT and SC. We applied the Kruskal-Wallis test also to evaluate whether genetic polymorphisms could influence the frequency of nuclear damage and to exclude, within the AT group, a possible influence of dietary supplements. Spearman test was performed in order to investigate a correlation between nuclear aberrations and age or weekly training sessions.

3. Results

3.1. Sport influence on nuclear aberrations

We analyzed 35,000 buccal cells for each studied category, for a total of 70,000 cells observed. Frequencies of the analyzed aberrations in the two groups are shown in Fig. 2. MNi were found to be significantly higher in SC group with respect to AT group (p < 0.001); however, the frequency of the other 3 analyzed nuclear aberrations (nuclear buds, notched nuclei and broken egg nuclei) was not found to be statistically different between the two groups. The total number of aberrations was significantly higher in the SC group (p = 0.010, see Fig. 2). We report in Supplementary Materials 2 the frequencies of aberrations in the two groups and in the Males/Females subgroups.

3.2. Genetic polymorphisms and nuclear aberrations

Figure 3 shows correlations between the studied polymorphisms and the incidence of cytogenetic damage. Overall, the genetic polymorphisms object of this study did not induce any significant difference in the aberrations frequencies (see figg. 3A-3C, 3E), except for XRCC1 and broken egg nuclei: subjects carrying the mutated allele of this gene showed a slightly but significantly higher frequency of broken egg nuclei (p = 0.045, see Fig. 3D).

3.3 Age, sex, weight, training sessions per week and dietary supplements

Spearman test did not reveal any significant correlation between the levels of cytogenetic damage and age, sex or weight. We didn’t find any correlation between the number of trainings per week and the frequency of analyzed aberrations (Supplementary Materials 1). Dietary supplementations did not imply a reduction in the levels of aberrations; conversely, AT undertaking dietary supplements showed a slight but significant increase in notched nuclei relative to the other, non-supplementing, AT subgroup (p = 0.040, see Fig. 3F).

4. Discussion
It is well known that physical activity can lead to many benefits on the human health; these benefits include the prevention of obesity, cardiovascular and tumor diseases [33]. However, the molecular relationship between training and the subsequently gained health benefits are not fully understood [34]. In this context, the production of RONS has ambivalent effects: on the one hand, RONS' production is important for signaling pathways and for the upregulation of the antioxidant system [1, 35]; on the other hand, an excessive production of RONS can lead to protein, lipid and DNA damage [36]. It follows that albeit the activity of the antioxidant system can be enhanced in subjects practicing sports [2, 3, 4], an excessive training load can bring to unbearable levels of RONS and subsequent genomic damage [2, 11, 12]. In this scenario, to date it is yet not fully understood how RONS' production (or overproduction) induced by training can influence the levels of genomic damage in terms of MNi, and the correlation between training and genomic damage remains not univocal. In fact, our results are (at least partially) in disagreement with the ones obtained by other authors [3, 15], who found higher levels of cytogenetic aberrations in hockey and baseball-soft ball players relatively to sedentary controls; however, these authors carried out the test on professional players who trained 4–6 hours a day, whereas athletes in our AT group didn't maintain such a high training frequency. Hence, it is probable that sampling martial artists with a higher training frequency could lead to different results. On the other hand, one of the above mentioned studies [3] did not include a sedentary control group in their study, but found an increase in the basal levels of MNi in professional athletes with respect to non-agonists; instead, we were not able to find a significant relationship between training frequency and cytogenetic damage within the AT group at the Spearman test. Nevertheless, the relationship between weekly training sessions of the AT group and MNi frequency, albeit not significant, was negative (rho = -0.295 and p = 0.086, see Supplementary Materials 1). These differences could be explained by the training regime required by the nature of each discipline, as even the nature of the sport (aerobic/anaerobic/mixed) and the training length may influence the obtainable results. In fact, different levels of oxidative stress biomarkers have been found in athletes practicing different disciplines [37, 38]. The variable biochemical and cellular responses to the different sport types are thus anything but univocal, and could explain our results.

Besides, our results could be exacerbated by the fact that sampling occurred in the first two years of Covid-19 pandemic, during which lockdowns sometimes led people to adopt a series of unhealthy behaviours (as reducing the hours of sleep, see [39]) which are typically correlated to an increase in genomic damage [40, 41], but not easily detectable by a questionnaire. In this context, practicing sportive activity could have represented an outlet for people who were authorized to keep on doing it, and may have prevented them from such unhealthy behaviours. Albeit these possible explanations, the relationship between sportive activity and genomic damage is far from being clear, and more studies are needed to better understand the cellular benefits and damages induced by a certain training regime.

Regarding polymorphisms’ analysis, despite analyzed polymorphisms are well known to influence the levels of genomic damage [17, 20], their influence in our sample was not relevant. The absence of any correlations (except for XRCC1 and Broken eggs, see Fig. 3D) between a particular allele and an increase/decrease of aberrations helps to better understand the great magnitude of influence of the training status on the observed differences in genomic damage between SC and AT. In fact, despite our
sample may have been too small to detect specific allele-mediated differences in the levels of genomic damage, the differences in Micronuclei and Total Aberrations between SC and AT were significant.

5. Conclusions

We compared the levels of nuclear aberrations between a group of 35 sedentary controls and a group of 35 martial artists. We suggest that practicing martial arts can lead to a reduction of genomic damage, at least in terms of MNi and Total Aberrations; this reduction was particularly relevant in our sample, given that (expectable) influences of single-gene polymorphisms on nuclear aberrations were instead not found. Our results are partially in disagreement with the ones obtained by other authors [3, 15], which evaluated the frequencies of MNi in athletes of other disciplines. These differences may be due to the different training frequencies of the sampled subjects, as well as to the variable training regime required by each sport and to the positive effects which sport may have provided to athletes’ habits and behaviours during Covid-19 lockdowns. However, other studies on subjects practicing different disciplines are necessary to draw general conclusions about the influence of training on the levels of genomic damage.

Declarations

Acknowledgements

We are deeply grateful to the trainers and gyms which have been involved in this study. In particular, we are grateful to Andrea Mariotti (Invictus Borgone), Angelo Gasparini (Fight Club Pianezza), Davide Bellagarda (Judo Club 2001), Dennis Marsala, Massimiliano Pasca (Judo Kumiai).

Funding information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no potential conflict of interest.

Ethics approval

This work has been approved by the local committee responsible for human experimentation (protocol n° 0609375 of 10/28/2021), and all procedures were carried out according to the 2013 Helsinki declaration.

Informed consent

Subjects received detailed information about aims and experimental procedures of the study and gave their informed consent.
References


**Figures**

![Figure 1](Image)
analyzed nuclear aberrations. (A): micronucleated cell; (B) cell with a nuclear bud; (C) nuclear indentation; (D) broken egg nucleus.

Figure 2

The frequency of nuclear aberrations in the two study groups. * = p < 0.05; ** = p < 0.001.
Figure 3

(A-E): frequency of nuclear aberrations in subjects carrying the different single-gene polymorphisms. (F): frequency of nuclear aberrations compared in the two AT subgroups: subjects undertaking dietary supplements or not. * = p < 0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• Supplementarymaterials1.xlsx
• Supplementarymaterials2.xlsx