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Assessment of DNA damage in a group of professional dancers during a 10-month dancing season

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ABSTRACT

Despite the numerous health benefits of physical activity, some studies reported that increased intensity and duration may induce oxidative stress in several cellular components including DNA. The aim of this study was to assess the level of basal DNA damage as well as oxidative DNA damage in a group of professional dancers before and after a 10-month dancing season. A group of individuals from general population was also assessed as a control. The alkaline version of the comet assay was the method selected to measure both basal DNA damage and oxidative stress, since this method quantifies both endpoints. In order to measure oxidative stress, the comet assay was coupled with a lesion-specific endonuclease (formamidopyrimidine glycosylase) to detect oxidized purines. The levels of oxidative DNA damage in dancers were significantly increased after the dancing season. Pre-season levels of oxidative DNA damage were lower in dancers than those obtained from the general population, suggesting an adaptation of antioxidant system in dancers. Results of the present biomonitoring study indicate the need for more effective measures to protect ballet dancers from potentially occupational health risks related to regular intensive physical exercise.

Introduction

Professional dancing is an activity with high artistic and physical demand and only few apprentices attain professional status. Recent data from Portugal indicate that only 17% of the students registered in dance degree concluded their graduation (INE (Statistics Portugal), 2015). Dancers are expected to display a repertoire of attributes such as motor coordination, agility, speed, psychological capacity, and strength to perform different types of physical exercises (Russell, 2013). Several studies reported a high incidence of injuries among professional dancers (Allen et al., 2012) most occurring as a result of intense training and lack of appropriate resting periods (Khan et al., 1995; Strassel et al., 2011). Musculoskeletal injuries are the most common health issue noted in professional dancers (Hincapie et al., 2008). The high frequency of injuries and subsequent diminished ability to perform may be related to the severity of

muscle damage and oxidative stress (Finaud et al., 2006; Niess & Simon, 2007).

Despite all benefits associated with regular physical activity such as reduction in health risks including cardiovascular diseases, diabetes, cancer, and osteoporosis as well as promotion of well-being (Hills et al., 2015; Powers & Jackson, 2008), this process requires increased oxygen uptake to generate byproducts to maintain the physiological responses needed for dancing. However, elevated oxygen uptake may also generate excess production of reactive oxygen species (ROS) (Kurucu, 2010). Subsequently, the ROS may react with different biomolecules, namely DNA, lipids, and proteins (Birben et al., 2012) inducing oxidative damage that may compromise cell genomic integrity altering many physiological and pathophysiological conditions leading to atherosclerosis (Young & McEneny, 2001), pulmonary damage (Ghio et al., 2012) cancer (Brieger et al., 2012), and aging (Beckman & Ames, 1998).

Although oxidative responses among different sport athletes were reported in several studies (Hadžović-Džuvo et al., 2014 ; Marin et al., 2013; Rowlands et al., 2012), only few studies measured DNA damage (Borges et al., 2014; Orhan et al., 2004). Borges et al. (2014) investigated the effects of a ballet class in 13 ballerinas on DNA fragmentation by flow cytometry and found a significant increase 18 hr after ballet class. Orhan et al. (2004) evaluated the effect of 60 min on a cycle ergometer in 18 trained males. As a biomarker of oxidative DNA damage, urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was shown to be significantly increased after exercise.

Genotoxicity evaluation is an important tool in human biomonitoring, especially in occupational health enabling identification of potential risk factors. The comet assay or single cell gel electrophoresis (SCGE) is a simple, quick, and sensitive method for quantitatively measuring DNA strand breaks in individual cells. For the last two decades, this method has been extensively employed in biomonitoring studies and in a range of *in vivo* and *in vitro* systems (Cemeli & Anderson, 2011; Dusinska & Collins, 2008; Ferreira et al., 2016; Trindade et al., 2016). Cells with increased DNA damage display enhanced DNA migration from the nucleus toward the anode (Dusinska & Collins, 2008). DNA repair glycosylases may also be added to the classic version of comet assay to measure specific DNA lesions, such as oxidized bases. The most commonly used enzyme is formamidopyrimidine glycosylase (FPG) detects oxidized purines, including 8-oxoguanine one of the most common DNA lesions resulting from ROS and an important biomarker of oxidative stress (Danielsen et al., 2008). Thus, incubation with this specific enzyme enables an estimation of the levels of oxidative damage (Azqueta et al., 2009).

Ballet dancers often go beyond their limits due severe competition in Ballet Companies to maintain their positions (Byhring & Bo, 2002), and consequently, these individuals often overlook injuries (Bowling, 1989). Since there is some evidence that professional dancers are subject to oxidative stress with accompanied DNA damage under acute conditions (Borges et al., 2014; Orhan et al., 2004), it was of interest to determine the consequences of prolonged physical exercise on several genotoxicity

biomarkers in a group of ballet dancers. In order to assess the effect of exercise on DNA damage among professional dancers, the alkaline version of the comet assay was employed in a group of professional dancers from a Portuguese Ballet Company to quantify DNA damage and oxidized purines (FPG sensitive-sites) at individual cell level, before and after a 10-month dancing season.

Material and methods

Study population

The general characteristics of the study population are summarized in Table 1. Study population consisted on a total of 28 subjects: 14 professional dancers, which agreed to participate in the study representing 1/3 of the dancers of a Portuguese Ballet Company, performing during the 2014/2015 dancing season; and 14 controls, healthy subjects selected from general population with sedentary lifestyle, that is, no regular physical activity. Ballet dancers worked about 10 months having a rest period of only 2 months (mid-July to mid-September). For each subject, relevant individual information such as gender, age, smoking habits, health conditions, and medication was assessed by self-administrated questionnaires. Smoking habits groups were established as non-smokers and smokers, and 24 were nonsmokers, four (dancers) having stop smoking for at least 2 years, and therefore considered nonsmokers. Professional dancers were also asked regarding the number of years of practice as professional dancers and their training schedule. Dancers had

Table 1. Characteristics of the study population.

	N	Controls	N	Dancers
Gender	14		14	
Females		11 (78.6%)		11 (78.6%)
Males		3 (21.4%)		3 (21.4%)
Age (years) ^a	14	39.36 ± 8.58 (24 – 57)	14	32.50 ± 6.38* (22 – 42)
Body Mass Index (kg/m ²) ^a		25.23 ± 4.37 (19 – 33)		19.30 ± 2.84 * (15–26)
Underweight		-		5 (35.7%)
Normal weight		9 (64.3%)		8 (57.1%)
Overweight		3 (21.4%)		1 (7.1%)
Obese		2 (14.3%)		-
Years of practice ^a		-		21.21 ± 8.21 (2 – 33)

^aMean ± SD (standard deviation), (range)

**p* < 0.05, significantly different from control group (*Student's t-test*).

8-hr daily training, at least 5 days per week with an average of about 12 performances per month (2 to 3 hours). All subjects were fully informed regarding the procedures and objectives of the study and signed an informed consent form prior to the study. Ethical approval was obtained from the Ethical Board of the National Institute of Health Doutor Ricardo Jorge.

Sample collection

Venous blood samples (6ml) were collected in 6-ml Vacutainer® EDTA tubes (Becton, Dickinson and Company) by venipuncture from an antecubital vein before and after dancing season. Blood was immediately stored at 4°C, transported to the lab, and immediately processed for further analysis. All samples were coded and analyzed under blinded conditions and cryopreserved. For cryopreservation, an equal amount of 1:4 (V/V) mixture of DMSO and RPMI 1640 was added to blood samples. Samples were then stored in aliquots (200µl each) at -80°C.

Chemicals (solution composition)

Dimethyl sulfoxide (DMSO) solution, sodium chloride (NaCl), potassium chloride (KCl), ethanol, and Tris-base were purchased from Merck KGaA, MSD. Fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), normal melting point agarose (NMP), and disodium ethylenediaminetetraacetate dehydrate (NA₂EDTA) were obtained from Thermo Fisher Scientific, Lonza Group AG, VWR Prolabo, and Bioline, respectively. RPMI 1640 solution, phosphate buffered saline (PBS) solution, low-melting point agarose (LMA), tetramethylbutylphenylpolyethylene glycol solution (Triton X-100), hydroxyethylpiperazine ethane sulfonic acid (HEPES), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Company. SYBER® Gold manufactured by Invitrogen™ was supplied by Thermo Fisher Scientific.

Alkaline comet assay

The alkaline comet assay was performed as described by (Singh et al., 1988) with minor

modifications (Al-Salmani et al., 2011; Costa et al., 2008). Before the assay whole blood samples were rapidly thawed on ice and washed twice (223g for 10 min) with 5 ml DMEM, supplemented with 2% FBS, 5 µl sample was dispersed in 0.6% LMP and dropped into a frosted slide pre-coated with 1% NMP. Samples were assessed in duplicate that is two mini-gels per subject. Slides were immersed in cold and freshly prepared lysis solution (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris-base, 0.25 M NaOH, pH 10; 1% Triton X100) for at least 1hr at 4°C in the dark. After this period, slides were submerged in electrophoresis solution (1 mM Na₂EDTA, 300 mM NaOH) for 20 min at 4°C, to enable DNA unwinding. Electrophoresis was carried out for 20 min at 1.2V/cm. For neutralization, slides were washed for 10 min in cold PBS and rinsed for another 10 min in cold deionized water. Slides were then fixed in 70% ethanol for 15 min and in absolute ethanol for further 15 min. Dried slides were stained with SYBER® Gold at the dilution recommended by the manufacturer in a bath with agitation for 30 min, rinsed twice with water and left to dry at room temperature. A total of 150 cells per subject (75 cells per mini-gel) were scored using the semi-automated image analysis system Comet Assay IV (Perceptive Instruments, UK). Microscopic analyses were performed on a Nikon Eclipse E400 Epi-fluorescence microscope. The percentage of DNA in the comet tail (%TDNA) was the parameter used to evaluate DNA damage.

Enzyme-comet assay (FPG)

The modified comet assay was performed as described by Azqueta and Collins (2013). For measurement of oxidized purines, after lysis, slides were washed 3 times (5 min each time) with cold buffer (0.1 M KCL, 0.5 mM Na₂EDTA, 40 mM HEPES, 0.2 mg/mL BSA, pH 8.0) and incubated for 30 min at 37°C with FPG (kindly donated by Prof Andrew R. Collins, University of Oslo, Oslo, Norway) or with buffer alone. The following steps were performed according to the comet assay classical version described above. The percentage of DNA in the comet tail (%TDNA) was the DNA damage parameter evaluated to describe comet

formation. Net FPG-sensitive sites, for each subject, were calculated by subtracting the % TDNA value obtained for buffer and from the value obtained for FPG.

Statistical analysis

A general description of the study population was performed through univariate analysis. The distribution within the study groups of socio-demographic and lifestyle factors was evaluated with the Student's t-test for continuous variables and the Pearson's chi-square test for categorical variables. Normality was assessed by Shapiro–Wilk test considering the size of the study population ($n = 28$). Both oxidative damage and basal DNA damage departed significantly from normality, and therefore, the analysis of this variable was performed using nonparametric test. To compare the results within each group, a Wilcoxon signed ranks test was used. To compare the variables between groups (comparison between the variation pre- and post-season), a Mann–Whitney test was employed. The influence of factors such as age and years of practice on the endpoints examined was evaluated by nonparametric (Kruskal–Wallis). To test the possible association between variables, a Spearman's rank correlation test was performed. Since four subjects were ex-smokers, the influence of previous smoking habits was also analyzed through Spearman's rank correlation test; however, no marked effect was found. The level of statistical significance was set at 0.05. All analyses were performed using the software Statistical Program for Social Sciences—SPSS version 20.0.

Results

Table 1 describes the general characteristics of the study population. Dancers and control group were matched for gender; however, no match was found regarding age and BMI. The difference of BMI values between dancers and controls may be justified by the high physical demand and ability to perform certain movements (Koutedakis & Jamurtas, 2004), and this

result is in accordance with several studies on dance nutrition reporting low BMI for dancers compared to general population (Mihajlovic & Mijatov, 2002; Ribeiro & Da Veiga, 2010). The low body weight among dancers, particularly ballet professionals, is a concern because it might result in nutritional disorders and other health problems (Abraham, 1996). In our investigation, five dancers were underweight and one was overweight; it should be noted that muscle mass is not included in BMI calculation which may lead to the misclassification of an athlete as overweight (Jonnalagada et al., 2004).

Data from genotoxicity biomarkers in professional dancers before and after the 10 month-season dancing season are reported in Table 2. Both endpoints were increased after dancing season; however, this difference was only statistically significant with respect to the level of oxidative damage (Net-FPG). A significant inverse association was found for age and DNA damage after season, where the level of DNA damage post-season was higher in younger dancers compared to older dancers. Regarding the years of ballet practice, it was no marked correlation with endpoints which was observed.

Univariate comparisons between professional dancers (pre-season) and control group (general population) are presented in Table 3. No marked differences were found in the endpoints studied, associated with no significant influence of age, BMI, and gender.

Table 2. Genotoxicity biomarkers measured before and after the season in professional dancers.

	Dancers			
	pre-season		post-season	
	N	mean \pm SE	N	mean \pm SE
DNA damage	14	6.10 \pm 0.52	14	6.55 \pm 0.64
Net-FPG	14	1.06 \pm 0.16	14	4.67 \pm 0.68*

SE—Standard Error Deviation.

* $p < 0.05$, significantly different from control group.

Table 3. Genotoxicity biomarkers in both studied groups.

	Controls		Dancers (pre-season)	
	N	mean \pm SE	N	mean \pm SE
DNA strand breaks	14	5.16 \pm 0.34	14	6.10 \pm 0.52
Net-FPG	14	2.21 \pm 0.50	14	1.06 \pm 0.16

SE—Standard Error Deviation.

Discussion

Regular intensive physical activity is associated with an increased production of free radicals that may overcome the antioxidant system capacity to maintain homeostasis and lead to cell damage (Fisher-Wellman & Bloomer, 2009). Dancers are considered both artists and athletes (Rodrigues-Krause et al., 2014) as a consequence of complex artistic performances that require a high level of athletic skills (Allen et al., 2012).

Our results demonstrated an increase on both levels of genotoxic endpoints after the 10-month dancing season, but only oxidative damage reached statistical significance. The available literature on biomonitoring studies comprising professional dancers is limited, and the evaluation of genotoxic effects encompassing these professionals is even scarcer. Borges et al. (2014) investigated the effect of chronic inflammation and neutrophil activation including neutrophils DNA fragmentation (an apoptosis biomarker) in 13 ballerinas from a ballet class. Blood samples were taken from dancers before, immediately after and 18 hr after class, a significant elevation was found in neutrophils DNA fragmentation 18 hr after class compared to pre-class and immediately after class, illustrating an impact of intense physical exercise on cytogenetic markers after a ballet class.

Dancers are also seen as athletes due to their intense training, some studies investigated the oxidative stress among athletes of different sports, and in accordance with our results, data demonstrated a rise in oxidative stress biomarkers after a period of physical exercise (Chatzinikolaou et al., 2014; Marin et al., 2013; Rowlands et al., 2012). Silva et al. (2013) analyzed biomarkers of oxidative stress (urinary malondialdehyde (MDA) levels) and antioxidant status (activities of glutathione reductase (GR) and superoxide dismutase (SOD) in 14 soccer players during the sport season. A significant increase of oxidative stress biomarkers was noted in the middle and end of the season. However, values return to normal during the off-season. Several studies showed an increased activity of antioxidant system during periods of exercise (Kyparos et al., 2009; Marin et al., 2011). Similar results were also found for athletes (Cash et al., 2014). Marin et al. (2013) examined the effect of a 6-month handball season

on oxidative stress (lipidic and amino acid oxidation) and antioxidant system activity biomarkers (SOD, catalase (CAT), glutathione peroxidase (GPx), and (GR) among 10 handball athletes. Results revealed an elevation of oxidative stress parameters during most intense periods but also a significant rise in erythrocyte antioxidant enzyme activity suggesting a training-induced antioxidant adaptation. Data found in the present study also suggest an adaptation of antioxidant system, the pre-season levels of oxidative damage were significantly lower than post-season levels, and although not significant, the pre-season levels were also lower than the level obtained for controls with no regular physical activity.

In the present study, DNA damage seemed to decrease significantly with age among professional dancers, which may be related to lower production of oxidants, up-regulation of antioxidant capacity, or even higher DNA repair activity (Cash et al., 2014; Marin et al., 2013; Soares et al., 2015).

No marked differences were found in the levels of genotoxicity biomarkers between control and pre-season professional dancers. Niess et al., (1996) used comet assay to assess the occurrence of DNA damage before and after exhaustive exercise in whole blood of 11 subjects, six well-trained men runners, and five untrained men, physically active but without regular exercise. A significant elevation in DNA migration was noted in both groups only after exercise.

The major confounders, gender and age, did not significantly influence the levels of the endpoints studied. Although the influence of gender on the level of DNA damage remains controversial (Giovannelli et al., 2002; Kasai et al., 2001), Angerer et al. (2007) showed that this is not a factor that affects the damage evaluated by the comet assay. Some investigators examined the influence of age on DNA damage assessed by the comet assay, but with conflicting results (Heuser et al., 2007; Möller, 2006).

The assessment of effect biomarkers such as oxidative and DNA damage in human biomonitoring studies is of paramount importance because of its predictive value and identification of potential genotoxic risk factors (Andersen et al., 2010). Comet assay is a rapid, simple, and relatively economical method that detects DNA damage in individual

cells (Azqueta et al., 2011), and the possibility of using a specific restriction endonuclease in the comet assay expands the flexibility of the method. Enzymatic version of comet is an important sensitive method for monitoring oxidized DNA bases which may serve as useful markers in multiple diseases including diabetes, rheumatoid, or vascular diseases (Wentzel et al., 2010).

The action of ROS has been widely studied among athletes although there are few investigators that examined the genotoxic effects of sports practice (Niess et al., 1996; Soares et al., 2015). Our findings indicate that the comet assay is a sensitive technique to measure the DNA damage effects resulting from intense physical activity in professional dancers, namely ballet dancers.

To the best of our knowledge, no apparent reports have described the influence of a seasonal period on genotoxicity biomarkers, nor assessed both DNA damage and oxidative damage in professional dancers. Thus, our study constitutes the first examining the genotoxic effects of an entire dancing season in a group of ballet dancers.

However, our study has some limitations including the number of subjects, disproportion number of genders, self-administered questionnaires, and lack of other information regarding lifestyle factors namely diet that may have influenced the endpoints studied. Further studies with larger populations are needed to confirm these results. A study design that combines the measurements of DNA damage, gene expression, and DNA repair mechanisms before, during and after the season, would clarify the mechanisms that maintain DNA stability in response to regular and intensive physical exercise.

Conclusions

Understanding the role that genotoxic effects play in occupational diseases might improve risk assessment and provide for a better worker health protection through development of prevention methods. Data from our study indicate a significant increase in oxidative damage as a result of a 10-month period of intense physical activity in professional dancers from a Portuguese Ballet Company, suggesting a potential health risk situation. Moreover, our results demonstrated that

comet assay is a useful tool to assess genotoxic effects related to regular intensive exercise. However, these findings need to be carefully interpreted due to the limited population studied. Nevertheless, data found clearly highlight the need of further investigations to elucidate the occupational-health risks related to intense regular physical exercise in this particular group of professionals.

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