ISSN 2237-048X
REVISTA JOVENS Pesquisadores
v.12, N.2 (2022)
EDUNISC

DOI: 10.17058/rjp.v12i2.17487 Recebido: 22/03/2022 Aceito: 06/04/2022

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DNA DAMAGE AND OTHER NUCLEAR ANOMALIES AMONG GYM USERS: A COMPARATIVE STUDY BETWEEN BRAZIL AND SPAIN

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KEYWORDS: DNA Damage. Exercise. Fitness Centers.

ABSTRACT

Populations from different countries may present different cellular modifications among themselves, and the Buccal Micronucleus Cytome (BMCyt) assay in human buccal mucosal tissue may be a marker to evaluate these modifications. This study evaluated and compared DNA damage and other nuclear anomalies between Brazilian and Spanish gym users. This is a cross-sectional study carried out with gym users of Santa Cruz do Sul/Brazil and Madrid/Spain. The BMCyt assay was performed for biomarkers of DNA damage (micronuclei and/or nuclear buds), cytokinetic defects (binucleated cells), proliferative potential (basal cell frequency) and/or cell death (condensed chromatin, karyorrhexis, pyknotic and karyolytic cells) in human buccal mucosal. Of the 228 individuals evaluated, 163 were Brazilian, and 65 were Spanish. Gym users of both countries differed between weight, body mass index, body fat, and muscle mass. The Brazilians presented a significantly higher frequency of micronuclei, nuclear buds, cells with condensed chromatin and karyorrhexis. Spaniards, however presented a significantly higher frequency of karyolytic cells. In conclusion, Brazilian gym users presented significantly higher rates of DNA damage and cell death, while the Spanish presented a higher frequency of advanced stage cell death.

DANO NO DNA E OUTRAS ANOMALIAS NUCLEARES ENTRE PRATICANTES DE ACADEMIA: UM ESTUDO COMPARATIVO ENTRE BRASIL E ESPANHA

PALAVRAS-CHAVE: Dano DNA. Exercício Físico. Academia de Ginástica.

RESUMO

Populações de diferentes países podem apresentar diferentes modificações celulares entre si e o ensaio de micronúcleos de células bucais esfoliadas (BMCyt) pode ser um marcador para avaliar essas modificações. Este estudo avaliou e comparou o dano no DNA e outras anomalias nucleares entre brasileiros e espanhóis praticantes de academia. Trata-se de um estudo transversal, realizado com praticantes de academia de Santa Cruz do Sul/Brasil e Madrid/Espanha. O ensaio BMCyt foi realizado para avaliar os biomarcadores de danos no DNA (micronúcleos e/ou brotos nucleares), defeitos citocinéticos (células binucleadas), potencial proliferativo (frequência de células basais) e/ou morte celular (cromatina condensada, cariorréxica, células picnóticas e cariolíticas), na mucosa bucal humana. Dos 228 indivíduos avaliados, 163 eram brasileiros e 65 espanhóis. Os praticantes de academia brasileiros e espanhóis diferiram entre peso, índice de massa corporal, gordura corporal e massa muscular. Ademais, os brasileiros apresentaram significativamente maior frequência de micronúcleos, brotos nucleares, células com cromatina condensada e cariorréxicas. Além disso, os espanhóis apresentaram significativamente maior frequência de células cariolíticas. Em conclusão, os praticantes de academia brasileiros apresentaram significativamente maiores índices de danos no DNA e morte celular, enquanto os espanhóis apresentaram maior frequência de morte celular em estágio avançado.

1 INTRODUCTION

Cellular modifications can be influenced by different factors, including environmental exposures, age, gender, body composition, dietary habits, and exercise habits (GAJSKI et al., 2018). A few tests evaluate DNA damage caused by these factors. One of these biomonitoring tests is the BMCyt assay (BOLOGNESI; FENECH, 2019; KNASMUELLER et al., 2011; NERSESYAN et al., 2014; SETAYESH et al., 2020), which is a minimally invasive method and has been used to assess biomarkers of DNA damage, defects in cytokinesis, and cell death (KASHYAP et al., 2012; THOMAS et al., 2009).

Although the benefits of physical exercise are already known (RUEGSEGGER; BOOTH, 2018; WORLD HEALTH ORGANIZATION, 2019), few studies evaluate DNA damage in physically active individuals. The results of studies that have specifically evaluated DNA damage and physical exercise are divergent, being observed from increases to decreases in post-exercise DNA damage frequency (HARTMANN et al., 1998; HUANG et al., 2009; PITTALUGA et al., 2006; REICHHOLD et al., 2008; SCHIFFL; ZIERES; ZANKL, 1997; UMEGAKI et al., 1998). Furthermore, among exercisers, some evidence suggests that there are no differences in the frequency of DNA damage among resistance exercisers, aerobic exercisers, and sedentary individuals (REICHHOLD et al., 2009; SHAFI et al., 2020).

The population of Brazil and Spain are culturally distinct and seem to differ in habits related to physical exercise (MATIAS; ROCHA; MASCARENHAS, 2020). In addition, the literature is scarce regarding the evaluation of DNA damage in individuals who practice physical exercise. We have not found studies comparing cellular modifications between gym users in different countries. Although, some recent studies are associating DNA damage frequency with the onset of certain types of diseases (BOLOGNESI et al., 2015; NERSESYAN et al., 2022), it is becoming increasingly important to evaluate these cellular modifications. Therefore, this study aimed to evaluate and compare DNA damage and other nuclear anomalies between Brazilian and Spanish gym users.

2 THEORETICAL FUNDAMENTALS

The scientific community has shown a great interest in investigating lifestyle habits to monitor cellular alterations and DNA damage to avoid deleterious consequences to the population's health (FENECH; FERGUSON, 2001; FENECH et al., 1999; ROHR et al., 2020). Several factors may contribute to cellular alterations and DNA damage in different populations. Cultural, economic, and environmental factors, as well as eating habits and physical activity levels, are examples of such. (HUANG et al., 2009).

The micronucleus test is the standardized test to assess DNA damage and to determine genomic instability (HEDDLE et al., 2011) to detect health risks and possible diagnosis of diseases (NERSESYAN et al., 2022). One of the most widely used techniques is the BMCyt assay, which the HUMNxl group has protocoled (THOMAS et al., 2009; THOMAS; FENECH, 2011). Specifically, micronucleus assays in humans can be performed from peripheral blood lymphocytes or with exfoliated epithelial cells.

The BMCyt assay, which is minimally invasive and low-cost, consists in collecting cells from the oral mucosa, later analyzed under a microscope, where normal cells can be distinguished from abnormal ones, based on cell morphology and nucleus texture, being possible to quantify the number of cell damages (THOMAS et al., 2009). This assay offers further information about the origin of DNA damage, cytokinesis, cytotoxicity, and cell death (BOLOGNESI et al., 2013; THOMAS et al., 2009). In this manner, the following cellular abnormalities can be

found: micronuclei (a biomarker of DNA damage, characterized by the presence of a main nucleus and one or more smaller nuclear structures, with the same staining intensity as the main nucleus), nuclear buds (biomarker of DNA damage, characterized by the connection between the nucleus and a nuclear "bud", of the same staining and smaller than a main nucleus), binucleated cells (defect in cytokinesis, the cell containing two nuclei), cells with condensed chromatin (indicative of cell death, striated cell nucleus), karyorrhexis cells (indicative of cell death, dotted nuclear pattern), pyknotic cells (indicative of cell death, shrunken nucleus, with a high density of nuclear material) and karyolytic cells (very advanced stage of cell death, where the nucleus is completely depleted of DNA, without staining) (BOLOGNESI et al., 2013; THOMAS et al., 2009).

Studies evaluating DNA damage in individuals who exercise are scarce in the literature. Some studies reported associations between physical exercise and micronuclei. However, these studies evaluated the frequency of micronuclei in different types of assays (peripheral blood lymphocytes and buccal mucosal tissue). Two studies observed a reduction in the frequency of micronuclei associated with regular exercise (>2 times/week) or after a triathlon race (HUANG et al., 2009; REICHHOLD et al., 2008). Other studies have reported no effect of exercise on micronucleus frequency, after an exhaustive session on the cycle ergometer or after a short-distance triathlon (PITTALUGA et al., 2006; HARTMANN et al., 1998). As in the study of Shafi et al. (2020), no differences were observed in the micronuclei frequency of oral cells among bodybuilders, soccer players, and sedentary individuals. However, the study of Schiffl, Zieres and Zankl (1997), after running sessions (sprint) until exhaustion, an increase in the frequency of micronuclei in individuals was observed. Similar data were found by Umegaki et al. (1998), where an increase in micronuclei frequency was observed in healthy subjects after a treadmill running session. Considering the growing demand for physical exercise in gyms around the world, researches that evaluate DNA damage and other nuclear anomalies are important to early detect risk factors associated with genomic instability in different populations.

3 MATERIALS AND METHODS

3.1 Population, methodological design, and ethical considerations

This is a cross-sectional, quantitative, descriptive, observational, and comparative study carried out with gym users of Santa Cruz do Sul/Brazil and Madrid/Spain. The sample was selected by convenience, which included gym users of both sexes, over 18 years old, and excluded pregnant women and individuals with incomplete data.

The present study was approved by the Ethics and Research Committee of the University of Santa Cruz do Sul (number 20.20.170), following all the guidelines established in the Declaration of Helsinki and resolution 466/2012 of the National Health Council of Brazil. The Informed Consent Form was obtained from all participants.

For the characterization of the sample, the weight, body mass index (BMI), body fat percentage, and skeletal muscle mass percentage of each participant were measured using a bioimpedance scale (OMRON HBF-514C, OMRON Healthcare Brazil, Brazil). Data on weekly exercise frequency and sociodemographic data were self-reported by the participants through an online questionnaire.

3.2 Evaluation of a DNA damage and other nuclear anomalies by the BMCyt assay

The preparation of slides for analysis of DNA damage and other nuclear anomalies was performed in the Laboratories of Experimental Nutrition of UNISC/Brazil and Laboratorio de Fisiología del Esfuerzo UPM/Spain. The

BMCyt assay was performed according to the protocol adapted from Thomas et al. (2009). The procedure in more detail is described in the study by Borba et al. (2019).

The slides were analyzed in duplicate by a single experienced examiner, under blinded conditions, in a conventional optical microscope at 400x magnification (Leica DMLB®, Wetzlar, Germany). One thousand cells per individual were evaluated, and the following cells and cellular abnormalities were analyzed according to their cellular and nuclear morphology: normal cells (cells without abnormalities), micronuclei and nuclear bud (indicative of DNA damage), binucleated cells (indicative of a defect in cytokinesis), cells with condensed chromatin karyorrhectic cells pyknotic cells (indicatives of cell death) and karyolytic cells (advanced stage of cell death) (BOLOGNESI et al., 2013; THOMAS et al., 2009).

3.3 Statistical Analysis

The analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 23.0 IBM, Armonk, NY). Descriptive statistics were expressed as mean values and standard deviations for continuous variables or absolute and relative frequencies for categorical variables. The Bootstrapping procedure for independent Student's t-test was used to check for differences between Brazilians and Spaniards on continuous variables. Cohen's d values were used to check the size of the standardized mean differences for continuous variables. Values of $d<0.39$ indicated small differences; $0.40<d<0.79$ indicated medium differences; and $d>0.80$ indicated large differences (COHEN, 1988). The chi-square test was used to check for differences between Brazilians and Spaniards on the categorical variables. In the case of the 2x2 table (sex x country of origin), the chi-square value was corrected by Yates' continuity correction test. Effect size measures (Phi or Cramer's V) for the chi-square test were also presented. The significance level adopted was $p<0.05$.

4 RESULTS AND DISCUSSION

We evaluated 228 gym users (163 Brazilians and 65 Spaniards), with a prevalence of males (51.3%) and a mean age of 41.04±14.78 years. Data on the sample characterization are presented in Table 1. No significant differences were found regarding sex ($p=0.154$) and age ($p=0.198$) between gym users in Brazil and Spain. However, individuals from Spain had lower mean weight ($p=0.022$) (small differences, $d>0.34$), lower mean BMI ($p=0.036$) (small differences, $d>0.031$), lower mean body fat percentage ($p=0.015$) (small differences, $d>0.38$), and higher mean muscle mass percentage ($p=0.041$) (small differences, $d>0.31$). There was no difference in weekly exercise frequency at the gym between the two countries ($p=0.228$).

Table 1. Descriptive characteristics of gym users. Values expressed as mean and standard deviation (SD) for continuous variables and absolute (n) and relative (%) frequencies for categorical variables; Differences between Brazil and Spain were verified by the Bootstrapping procedure for Student's t test for independent samples for continuous variables and by chi-square test for categorical variables (p<0.05); Effect size of the chi-square or Fisher exact calculated by Phi* and Cramer's V** measure, and of Student's t test calculated by Cohen's d.

	Brazil	*Spain*	*All*		*Standardized*
	n = 163	*n = 65*	*n = 228*	*p*	*Difference*
	Mean (SD)				*Cohen's d*

Age (years)	*41.45 (14.85)*	*38.66 (14.45)*	*41.04 (14.78)*	*0.198*	*0.19*
Body Weight (Kg)	*73.71 (14.52)*	*69.03 (12.08)*	*72.37 (14.00)*	*0.022*	*0.34*
BMI (weight/height²)	*25.66 (3.83)*	*24.47 (3.81)*	*25.32 (3.86)*	*0.036*	*0.31*
Body fat (%)	*30.12 (9.14)*	*26.69 (8.78)*	*29.24 (9.24)*	*0.015*	*0.38*
Muscle mass (%)	*31.02 (6.29)*	*32.95 (6.26)*	*31.52 (6.34)*	*0.041*	*0.31*
		n (%)		*p*	*Effect Size Phi or Cramer's V*
Sex					
Male	*74 (45.4)*	*37 (56.9)*	*117 (51.3)*	*0.154*	*0.10**
Female	*89 (54.6)*	*28 (43.1)*	*111 (48.7)*		
Gym Frequency					
Up to 2 times per week	*14 (8.6)*	*7 (10.8)*	*21 (9.2)*		
3 times per week	*72 (44.2)*	*19 (29.2)*	*91 (39.9)*	*0.228*	*0.14***
4 to 5 times per week	*63 (38.7)*	*32 (49.2)*	*95 (41.7)*		
More than 5 times per week	*14 (8.6)*	*7 (10.8)*	*21 (9.2)*		

Our results showed that the Brazilians presented a higher micronuclei (p=0.001) (mean differences, d>0.66) and nuclear buds frequency (p=0.001) (mean differences, d>0.79), indicative of DNA damage; cells with condensed chromatin (p=0.001) (small differences, d>045) and karyorrhectic cells (p=0.001) (mean differences, d>0.73), indicative of cell death, compared to the Spanish. Spanish gym users had a higher karyolytic cells frequency (p=0.001) (large differences, d>0.88), a nuclear anomaly indicative of advanced stage cell death. Binucleated and pyknotic cells frequencies showed no significant differences between Brazilians and Spaniards (Table 2).

Table 2. DNA damage and other nuclear anomalies in gym users. *Values per 1,000 cells analyzed. Values expressed as mean and standard deviation (SD) for continuous variables; Differences between countries were verified by the Bootstrapping procedure for Student's t test for independent samples. The effect size of the Student's t test was calculated by Cohen's d. significance level of p<0.05.

	Brazil	*Spain*	*All*	*p*	*Standardized Difference*
	n = 163	*n = 65*	*n = 228*		
		Mean (SD)			*Cohen's d*
Basal cells per 1,000 cells	*967.71 (15.02)*	*962.58 (19.60)*	*966.32 (16.72)*	*0.069*	*0.31*
Micronuclei per 1,000 cells	*1.77 (2.16)*	*0.49 (1.23)*	*1.61 (4.24)*	*0.001*	*0.66*
Nuclear buds per 1,000 cells	*1.84 (1.54)*	*0.71 (1.11)*	*1.49 (1.50)*	*0.001*	*0.79*
Binucleated cells per 1,000 cells	*2.68 (2.48)*	*2.54 (2.31)*	*2.62 (2.39)*	*0.668*	*0.06*
Condensed chromatin cells per 1,000 cells	*4.62 (4.44)*	*2.85 (2.24)*	*4.10 (3.99)*	*0.001*	*0.45*
Karyorrhectic cells per 1,000 cells	*5.59 (6.70)*	*1.25 (3.26)*	*4.29 (6.09)*	*0.001*	*0.73*
Pyknotic cells per 1,000 cells	*10.17 (10.20)*	*12.85 (9.41)*	*10.78 (9.90)*	*0.082*	*0.27*
Karyolytic cells per 1,000 cells	*7.83 (7.09)*	*17.12 (16.42)*	*10.57 (11.57)*	*0.001*	*0.88*

Currently, micronuclei are the biomarkers with the best validation regarding genomic instability caused by environmental, nutritional, and lifestyle factors (FENECH et al., 2011). Our results showed that the individuals in the present study had an average of 1.61 micronuclei cells per 1,000 cells analyzed. Similar data to the present

was reported by Shafi et al. (2020), who found a frequency of 1.6 and 2.0 micronuclei per 2,000 cells analyzed from bodybuilders supplement users and non-supplement users, respectively.

When comparing the micronuclei cells frequency between gym users of Brazilian and Spanish, we observed 3.6 times more micronuclei frequency and 2.6 times more nuclear buds frequency (both cells indicative of DNA damage) in Brazilians when compared to the Spanish. These results may be linked to these two countries populations by different eating habits, directly influencing the frequency of cellular changes assessed (FENECH; BONASSI, 2011).

In Brazil, it is known that there is a prevalence of adherence to the "Western diet", which is related to increased diseases since it is rich in saturated fats, simple sugars, and poor in fiber. In addition, the components of this diet can be a triggering factor for obesity (FRANCISCO; ASSUMPÇÃO; MALTA, 2019; HARIHARAN; VELLANKI; KRAMER, 2015; ZINÖCKER; LINDSETH, 2018). We can observe in our study that Brazilians have higher BMI and body fat averages when compared to Spaniards. There are reports in the literature showing that accumulation of body fat from overweight causes metabolic disorders, such as activating inflammatory processes, which can lead to DNA damage and other nuclear anomalies and consequently damage to genome integrity (HOTAMISLIGIL, 2006; KARALIS et al. 2009; LOWELL, SHULMAN, 2005). The increase of micronuclei and nuclear buds frequencies have been observed in numerous diseases such as diabetes, obesity, cardiovascular diseases, neurodegenerative diseases, and some types of cancers (FENECH et al., 2020).

On the other hand, the population of Spain is characterized by the adherence to the "Mediterranean diet", consuming many fruits, whole grains, nuts, fish, white meats, olive oil, and low consumption of red meat, a diet rich in antioxidants, fiber, and monounsaturated fat (GIACOSA et al., 2013; PAUWELS, 2011). This dietary pattern is associated with normal body composition indices, decreased blood pressure, improved lipid profile.It also presents an anti-inflammatory effect that consequently may be associated with DNA protection (GALLEANO et al., 2010; VALLVERDÚ-QUERAL et al., 2013; BONACCIO et al., 2017; KOOLAJI et al., 2020), which in part may be related to the lower frequency of DNA-indicating cells in this population.

Other cellular alterations, such as condensed chromatin and karyorrhectic cells, were observed in Brazilian gym users of the present study. According to Thomas et al. (2009), these mouth cells present a fragmented nucleus, suggesting that these cells may be undergoing a late phase of apoptosis, but this is yet to be proven. Rohr et al. (2020) conducted a study in different Brazilian states and showed that participants in the state of Rio Grande do Sul had higher condensed chromatin and karyorrhectic cell frequencies when compared to other states of Brazil. However, the study was unable to demonstrate whether regional variations could influence the nuclear anomalies of the states analyzed; furthermore, the study suggests that cigarette consumption among study participants from different Brazilian regions was positively associated with higher DNA damage and cell death frequencies.

Regarding other cellular alterations, in the Spanish population, we only observed higher karyolytic cell frequencies compared to the Brazilians. This cellular alteration presents a nucleus completely depleted of DNA and is a general morphological alteration in necrotic cells. However, the mechanism of the generation of karyolytic cells has not yet been clarified (THOMAS et al., 2009; TAKADA, WATANABE, MIZUTA, 2020).

Our study has some limitations, which need to be acknowledged. The study's cross-sectional design cannot establish causality, according to the associations observed between gym users of both countries. Furthermore, we

did not observe cultural, socioeconomic, genetic, and physiological factors. Nonetheless, eating habits, and the degree of exposure to possible agents that cause cellular alterations in both populations are variables that could impact the results of this investigation.

5 CONCLUSIONS

Brazilian gym users showed higher rates of DNA damage (micronuclei and nuclear buds) and cell death (cells with condensed chromatin and karyorrhexis cells), while Spanish gym users showed a higher late-stage cell death frequency (karyolytic cells). Long-term clinical studies are needed to assess cellular alterations and DNA damage in different populations to improve understanding of the factors) that may cause genomic instability in populations from different countries. In addition, strategies must be proposed and interventions aimed at improving genome health should be made.

ACKNOWLEDGMENTS

The authors would like to thank all the physical exercise practitioners from gyms in Santa Cruz do Sul/Brazil and Fitness Centers in Madrid/Spain, who agreed to participate in the study. We also thank FAPERGS, CAPES, CNPq, and UNISC for supporting this research.

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