

PERCEIVED LEVEL OF STRESS AND INSTABILITY GENOMIC AMONG GYM USERS

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KEYWORDS: DNA Damage, Fitness Centers, Exercise, Resistance Training

ABSTRACT

Stress and the practice of physical exercises imply in several outcomes to the health of individuals, including cellular changes. The buccal micronucleus cytome assay (BMCyt) is a biomarker widely used to assess the impacts of lifestyle characteristics such as stress levels on nuclear changes. The aim of this study was to evaluate and relate the perceived level of stress on the occurrence of DNA damage, and other nuclear/cellular abnormalities in gym users. Descriptive cross-sectional study, carried out with 116 gym users from Madrid/Spain. A questionnaire was used to assess the perceived level of stress and BMCyt was used to assess DNA damage and other nuclear/cellular abnormalities. Of the 116 individuals evaluated, 54.3% reported being stressed at sometimes. There was no significant difference in relation to the reported stress level and the biomarkers of DNA damage (cells with micronuclei or nuclear buds), proliferative potential (frequency of basal cells), and cell death (cells with condensed chromatin, karyorrhexis, karyolysis and pyknotic) ($p>0.05$). However, gym users who reported being almost always/excessively stressed had significantly less frequency of binucleated cells (biomarker of cytogenetic defects) compared to individuals who reported being rarely stressed ($p=0.008$). Our results showed that the majority of gym users reported being stressed at sometimes. However, it was found that gym practitioners who reported being almost always/excessively stressed had a lower frequency of binucleated cells.

NÍVEL DE ESTRESSE PERCEBIDO E INSTABILIDADE GENÔMICA ENTRE OS USUÁRIOS DE ACADEMIA

PALAVRAS-CHAVE: Dano ao DNA, Academia de ginástica, Exercícios Físicos, Treinamento de Força

RESUMO

O estresse e a prática de exercícios físicos, implicam em diversos desfechos à saúde dos indivíduos, dentre eles as alterações celulares. O ensaio de citoma de micronúcleo bucal (BMCyt) é um biomarcador amplamente utilizado para avaliar os impactos das características de estilo de vida como níveis de estresse sobre alterações nucleares. O objetivo deste estudo foi avaliar e relacionar o nível de estresse percebido com a ocorrência de dano no DNA e outras anormalidades nucleares/celulares em praticantes de academia. Estudo transversal descritivo, realizado com 116 praticantes de academia de Madrid, Espanha. Um questionário foi utilizado para avaliar o nível de estresse percebido e o BMCyt foi utilizado para avaliar a de dano no DNA e outras anormalidades nucleares/celulares. Dos 116 indivíduos avaliados, 54,3% relataram estar às vezes estressados. Não houve diferença significativa em relação ao nível de estresse relatado e os biomarcadores de dano no DNA (células com micronúcleos ou brotos nucleares), potencial proliferativo (frequência de células basais) e de morte celular (células com cromatina condensada, cariorréxicas, cariolíticas e picnóticas) ($p>0,05$). Contudo, os praticantes de academia que relataram estar quase sempre/excessivamente estressados apresentaram significativamente menor frequência de células binucleadas (biomarcador de defeitos citogenéticos) em relação aos indivíduos que relataram estar raramente estressados ($p=0,008$). Nossos resultados mostraram que a maioria dos praticantes de academia relataram estar às vezes estressados. Entretanto, verificou-se que os praticantes de academia que relataram estar quase sempre/excessivamente estressados apresentaram menor frequência de células binucleadas.

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1 INTRODUCTION

Modern societies have leaved people more subject to high levels of stress. Economic difficulties, family problems, worries about success, uncertainty about their future, and lifestyle (e.g. smooking, alcohol abuse, unhealthy diet, sleep disturbances, and obesity) are the main causes of stress in adulthood (STULTS-KOLEHMAINEN; SINHA, 2014; XENAKI et al., 2018).

The high level of perceived stress decrease well-being, producing negative impacts on general health (XENAKI et al., 2018). In addition, stress may lead to long-term psycho-physiological changes, deteriorating physical and mental health (LEE; KIM; WACHHOLTZ, 2016). It is also well established that genomic damage is produced by environmental exposure to lifestyle factors, as stress (HOLLAND et al., 2008; FENECH; BONASSI, 2011). One of the most widely used biomarkers to study DNA damage, and other cellular changes in the human population is the buccal micronucleus cytome assay (BMCyt). The application of this method, minimally invasive sampling, has been successfully applied to evaluate the impact of lifestyle factors, for studying DNA damage, chromosome malsegregation and cell death (THOMAS et al., 2009).

Evidence has shown that physical exercise increases overall health and sense of well-being, as well as also has some direct stress-decreased benefits (GERBER et al., 2014; SATO, 2017). Among the several benefits of physical exercise are the social (e.g., being with others in gym), and medical benefits (e.g., reduces peripheral risk factors, such as metabolic diseases, and provide a protection against the development of anxiety and depression symptoms) (ZAMAN; HANKIR; JEMNI, 2019). In addition, evidence suggests that lack of regular exercise may significantly contribute to higher micronucleus frequency (HUANG et al., 2009). Therefore, the aim of this study was to evaluate and relate the perceived level of stress on the occurrence of DNA damage and other nuclear/cellular abnormalities in gym users.

2 THEORETICAL FUNDAMENTALS

Stress, according to American Institute of Stress (2021) may be defined as any circumstance or feeling a condition or feeling experienced when a person perceives that demands exceed the personal and social resources the individual is able to mobilize. Although there is good stress, mostly stress is perceived to threaten one's well-being (BUBLITZ et al., 2016). In addition, stress may be a predisposing, triggering or adjunct factor of several diseases (MUSSI et al., 2019).

Several studies have been showed that the perceived level of stress may lead on psychosocial consequences such as increased levels of depression and anxiety as well as reduced quality of life (GERBER et al., 2014; KIM; MCKENZIE, 2014; ZAMAN; HANKIR; JEMNI, 2019). Modern societies-related stressors include family problems, economic difficulties, worries about success, uncertainty about their future, and lifestyle (STULTS-KOLEHMAINEN; SINHA, 2014; XENAKI et al., 2018). Therefore, perceived stress refers to the interaction between the individual and the environment in the presence of a stressor agent (AVILA-PALENCIA et al., 2017).

Failure to maintain health behaviors such as regular physical exercise has also been showed relation with high perceived level of stress (BURG et al., 2017). The benefits of regular physical exercise on health are widely recognized, demonstrating benefits for emotional well-being, health status, and positive effects on stress

sensitivity (REWS; LANDERS, 1987.). One reason for high perceived level of stress is more sedentary behavior as well as everyday life activities (STULTS-KOLEHMAINEN; SINHA, 2014).

The stress may result in a range of physiological, behavioral, psychological changes, and can lead to lowered self-esteem and lowered feelings of control (AVILA-PALENCIA et al., 2017). And, known that regular physical exercise may lessen the physiological impact of stress, improving aspects of stress resilience, this relationship may be notably complex (BURG et al., 2017). In addition, understand the association(s) of perceived level of stress and exercise may reveal interesting and differing patterns for different persons. It is well established that genomic damage is produced by environmental exposure to lifestyle factors, such as stress and physical inactivity (GAJSKI et al., 2018), and BMCyt assay may be a useful and minimally invasive method for monitoring genetic damage in humans (HOLLAND et al., 2008). The BMCyt assay has been used to measure DNA damage biomarkers (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) (THOMAS et al., 2009). Evaluating the occurrence of DNA damage, and other nuclear/cellular abnormalities in blood, Gajski and colleagues verified that subjects who reported regular exercise had a slight but non-significant increase in all parameters evaluated. Despite of results observed, the authors also reported that several environmentally and/or occupationally factors (e.g. stress) should also be taken into account in order to minimize the effect of interfering factors in results.

3 MATERIALS AND METHODS

3.1 Ethical consideration and sample

The present study is a cross-sectional descriptive study 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 National Health Council of Brazil. The study was carried out between May and July 2019, with gym users from Madrid/Spain.

From the estimation of the population of academics in Madrid/Spain (n=3419), the sample calculation of the present study was performed. Using the equation $n = \frac{N \cdot Z^2 \cdot p \cdot (1-p)}{Z^2 \cdot p \cdot (1-p) + e^2 \cdot N - 1}$ and considering the confidence level of 95% and margin of error of 5%, the sample size calculated was of gym users. The sample size of 230 gym users. However, only 116 individuals volunteered to participate, making the present study sample a convenience. All participants consented to participate in the online research (agreement to the informed consent form).

3.2 Perceived level of stress evaluation

Perceived stress level was assessed using an online questionnaire adapted from Rojas (2003), containing the following question: "How do you classify your stress level?" and response options: i) rarely stressed; ii) sometimes stressed; and iii) always or excessively stressed. This questionnaire also included questions about sociodemographic data (e.g. gender, age, schooling and marital status) to characterize the sample.

3.3 Sample collect and BMCyt assay

The BMCyt assay was performed according to the protocol adapted from Thomas et al. (2009). Details of the procedures used in this trial by our research group were recently described in Borba et al. (2019). After making the slides, they were analyzed using a conventional optical microscope with a 400x magnification (Leica DMLB®, Wetzlar, Germany), using the scoring criteria of Thomas et al (2009). To assess different cell types and nuclear/cellular abnormalities. The slides were analyzed by a single examiner (2 slides per subject), evaluating the following cells: basal cells, cells with micronuclei or nuclear buds and cells with condensed chromatin, karyorrhexis, karyolysis and pyknotic. A total of 2,000 differentiated cells were assessed for the presence of DNA damage and a score of 1,000 cells was assessed to determine the frequency of other nuclear abnormalities. Both results were expressed as counts per 1,000 cells and the slides were coded in order to allow blindfolded analysis, not allowing the evaluator to have knowledge of the subject or the study group to which he belonged.

3.4 Statistical analysis

The data were analyzed using the Graphpad PRISM 6.01 software (Graphpad inc., San Diego, CA). The characteristics of the participants were presented with frequency and percentage distributions for categorical variables using Pearson's Chi-square or Fischer's Exact test. Cramer's phi (ϕ) was used to evaluate the effect size of the differences and associations. The data were checked for normality and homoscedasticity. The Kruskal-Wallis test followed by the Dunn test was used to determine if the differences between of DNA damage in relationship the level of stress. Statistical significance was considered when $p < 0.05$.

4 RESULTS AND DISCUSSION

The mental and physical health benefits of exercise are well known. Studies have shown that physical exercise may contribute to improve mental health and decrease the level of stress, enhancing positive emotion (e.g. vigor, pleasure, and energy) and decreasing anxiety, tension, tiredness and anger (GERBER et al., 2014; KIM; MCKENZIE, 2014). Of the 116 gym users evaluated (37.70 ± 14.88 years and female prevalence, 60.3%), 54.3%, 27.6% and 18.1% reported being sometimes, rarely, and almost always/excessively stressed, respectively (table 1). In addition, the majority of gym users who had a complete degree reported that they were sometimes stressed (79.4%), while individuals with a high school education reported that they were rarely stressed (46.8%, $p = 0.042$; $\phi = 0.207$, table 1). These results showed that work and workplace-related issues may be common sources of stress (DÍAZ-SILVEIRA et al., 2020). For example, high education level is related with important positions in a company and greater responsibilities, consequently increase work-related stress level; and therefore use exercise as a daily stress management strategy (STULTS-KOLEHMAINEN, SINHA, 2014).

Table 1. Perceived level of stress with sociodemographic variables.

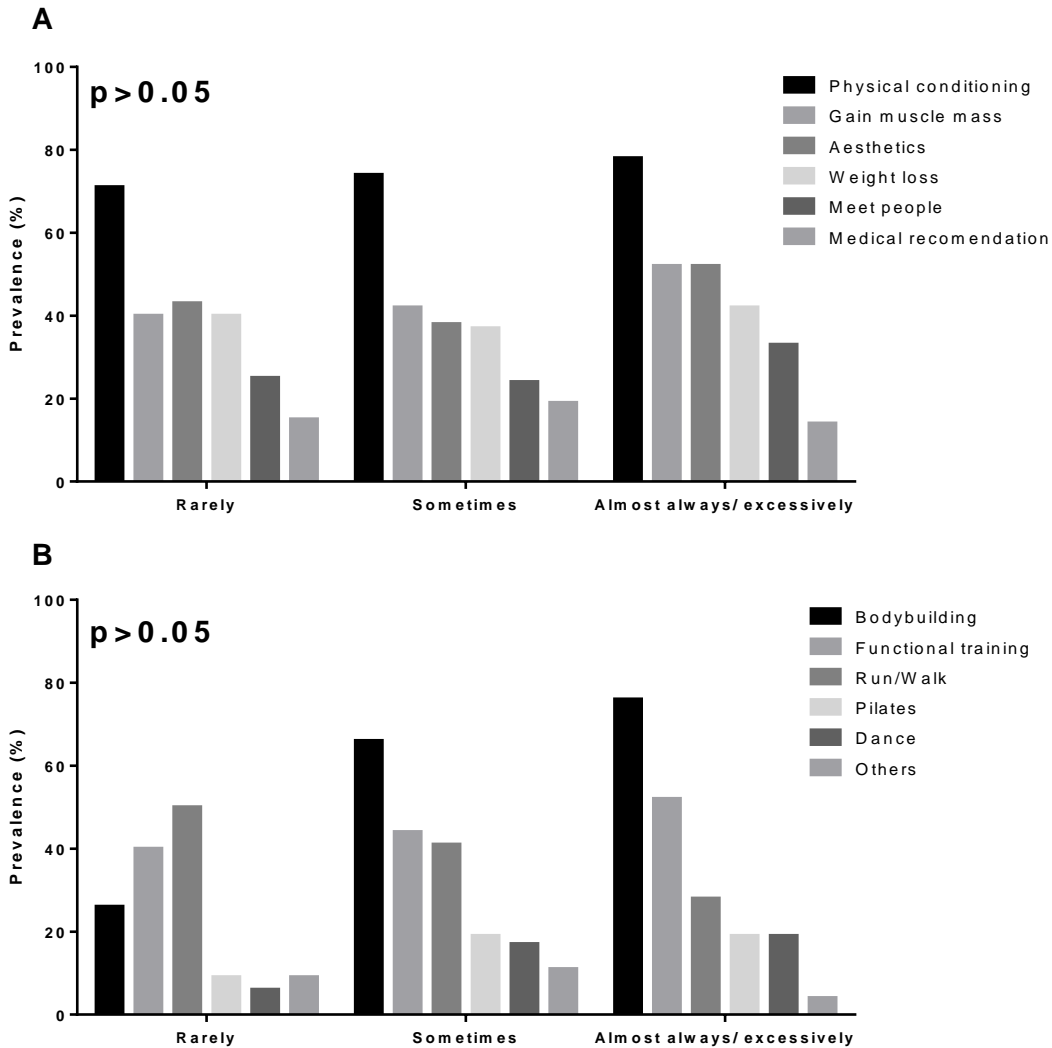
Variables	Stress level			p	φ
	Rarely n (%)	Sometimes n (%)	Almost always/ excessively/ n (%)		
Sex	32 (27.6)	63 (54.3)	21 (18,1)	0.227	0.160
Female	17 (53.1)	37 (58.7)	16 (76.2)		
Male	15 (46.9)	26 (41.3)	5 (23.8)		
Age group				0.253	0.152
18-30 years	10 (31.2)	30 (47.6)	8 (38.1)		
31-50 years	11 (34.4)	21 (33.3)	10 (47.6)		
More than 50 years	11 (34.4)	12 (19.0)	3 (14.3)		
Educational level				0.042	0.207
Elementary school/High school	15 (46.8)	13 (20.6)	6 (28.5)		
Higher education	17 (53.1)	50 (79.4)	15 (71.4)		
Marital status				0.165	0.204
Single	15 (50.0)	42 (68.9)	14 (73.7)		
Married	11 (36.7)	15 (24.6)	3 (15.8)		
Divorced/Widower	4 (13.4)	4 (6.6)	2 (10.5)		
Physical activity level				0.609	0.139
Sedentary	12 (37.5)	29 (46.0)	6 (28.6)		
Moderate	9 (28.1)	18 (28.6)	6 (28.6)		
Vigorous	11 (34.4)	16 (25.4)	9 (42.8)		
Total time of exercise in gym				0.048	0.208
Up to 1 year	7 (21.9)	18 (28.6)	5 (23.8)		
1-5 years	7 (21.9)	27 (42.9)	11 (52.4)		
More than 5 years	18 (56.2)	18 (28.6)	5 (23.8)		
Frequency of exercise				0.019	0.226
Up to 3 times/week	10 (31.2)	26 (41.3)	12 (57.1)		
4 times/week	5 (15.6)	22 (34.9)	4 (19.0)		
5 or more times/week	17 (53.1)	15 (23.8)	5 (23.8)		
Total time of daily exercise				0.082	0.058
Up to 60 minutes	14 (43.8)	31 (49.2)	9 (42.9)		
More than 60 minutes	18 (56.2)	32 (50.8)	12 (57.1)		

Evidence has been showing that psychological stress tends to be associated with reduced exercise. People reporting high psychological stress tend to exercise less than people not experiencing high stress (VAN RAALTE et al., 2019). In the present study, that gym users who reported being rarely stressed frequented the gym longer and more often than individuals who reported being sometimes or almost always/or excessively stressed ($p=0.048$; ϕ 0.208 and $p=0.019$; ϕ 0.226, respectively). These results indicate that the time dedicated to physical exercise seems to be related to the reduction of stress perceived by individuals, which may lead to benefits for mental health. Thus, increase positive emotion may lead to positives health outcomes through strengthened immune, induced by enhancing social ties and health practices including regular physical exercise (KIM; MCKENZIE, 2014). These findings are supported by studies that suggest that people who exercise for a long time, are motivated by the feeling of physical and psychological well-being and positive emotions, as physical exercise may lead to health-promoting behaviors (ISO-AHOLA, 2013, KIM; MCKENZIE, 2014, KANDOLA et al., 2018).

A variety of physical exercises, such as aerobic and anaerobic may have varying degree of influences on mental health and stress managing (KIM; MCKENZIE, 2014). In this study, the most of gym users reported practice physical exercise in fitness center to improve physical conditioning; and the modality more practiced related was

strength training. Kim and Mckenzie (2014) reported that any types of physical activity performed in freely chosen activities would be conducive to enhancing positive emotion, buffering against stress effectively. The authors also indicated that many individuals interviewed reported that at the gym, they not only take their mind off the work, as also move the body, helping in reduce their stress level.

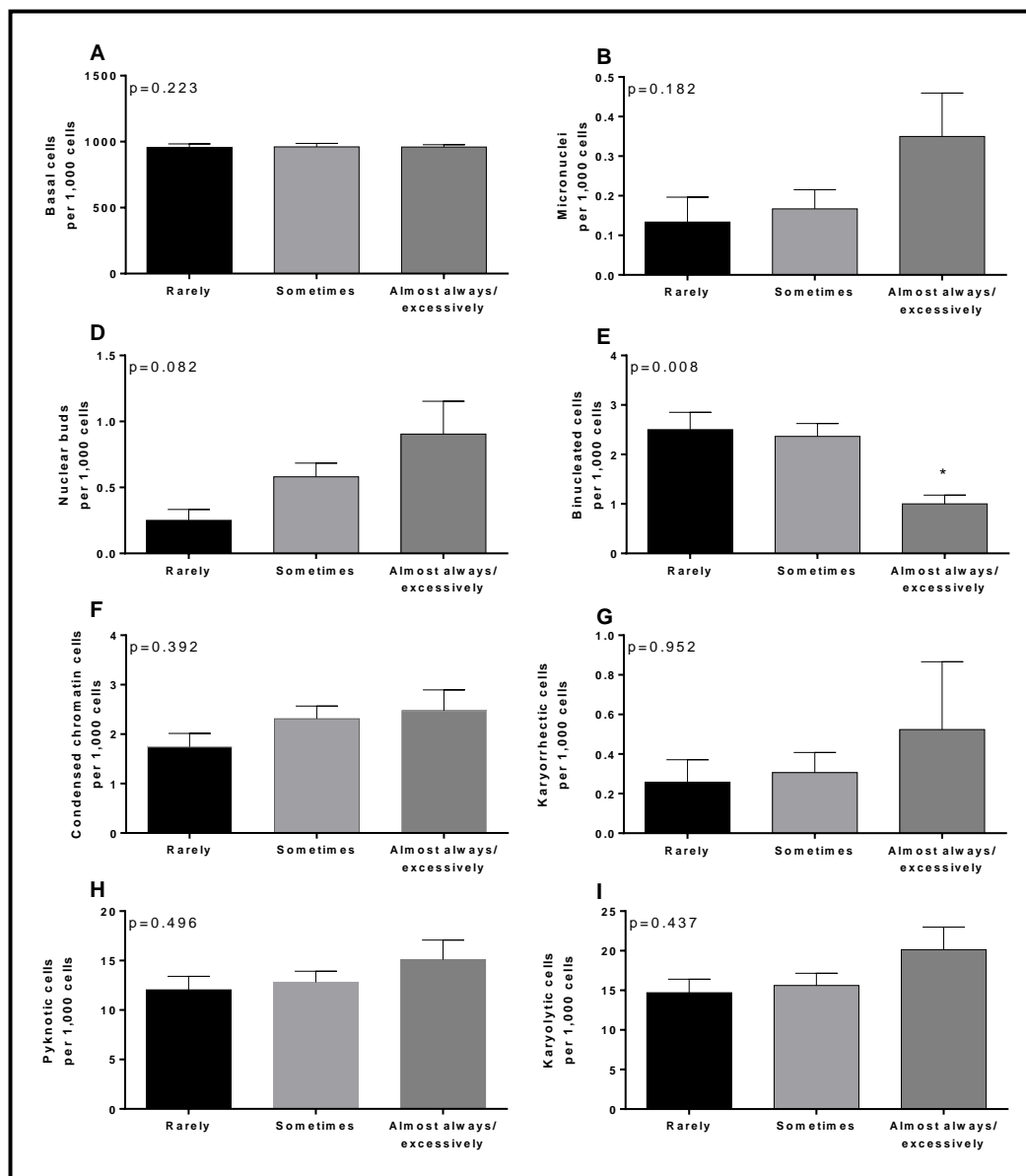
Figure 1. Reason (a) and types of exercises performed (b) according to the perceived stress level. Values presented as percentage distributions. Pearson's Chi-square test was used in the analysis of the variables



Physical exercise and psychological stress seem to have an impact on DNA damage (FRANZKE et al., 2015). However, studies evaluating exercise practice, either aerobic training or resistance exercise, has showed inconsistent results regarding DNA damage (REICHHOLD et al., 2009). In addition, there is a lack of clarity regarding the frequency, intensity, duration, and type of exercises performed that may be involved in improving mental health (GERBER et al., 2014). In our study, we did not observe the perceived stress level differences in frequency of basal cells, cells with micronuclei or nuclear buds and cells with condensed chromatin, karyorrhexis, karyolysis and pyknotic cells scored by the BMCyt assay ($p > 0.005$). However, a frequency of binucleated cells

significantly differ between the levels of stress ($p=0.008$). The results showed that gym users who reported being almost always or excessively stressed presented binucleated cells frequencies 2.4- and 2.5-fold lesser, respectively, compared with who related being sometimes and rarely stressed, indicating that individuals with high stress level had a greater risk of cytokinetic defects, and at least partially induced cytogenetic damage.

Figure 2. Frequency of cells scored by the BMCyt assay according to the level of stress in gym users in Madrid/Spain. Values presented as mean \pm standard error. p : level of significance according to the Kruskal-Wallis test followed by the Dunn post-test. $*p<0.01$ indicates a difference between almost always or excessively stressed with sometimes and rarely stressed.



It is known that physical exercise may reduce the risk of developing many lifestyle-related diseases. However, evidence also concerning the influence of exercise on DNA stability, mainly related to different durations,

intensities, and types of exercise (Reichhold et al., 2009). The CBMN Cyt assay is a test that detects genome instability, including DNA damage (cells with micronuclei or nuclear buds), cytokinetic defects (binucleated cells), proliferative potential (frequency of basal cells), and cell death (cells with condensed chromatin, karyorrhexis, karyolysis and pyknotic). In addition, it is also well established that DNA modulation/stability may be influenced by environmental exposure to lifestyle factors, such as stress (Holland et al., 2008). Although we verified a lower frequency of binucleated cells in gym users who reported being almost always or excessively stressed, it seems that the level of stress tend to associate with a higher rate of DNA damage, proliferative potential, and cell death. However, the influence of stress levels on the occurrence of DNA damage and other nuclear/cellular abnormalities remains unclear, corroborating with the study of Gajski and colleagues (2018).

There are some limitations in the research. In this study the stress level was investigated by self-perception, not by any psychological stress biomarkers, such as salivary cortisol. Therefore, the data may not accurately reflect the true stress level.

5 CONCLUSIONS

Our findings showed that the majority of gym users reported being stressed at sometimes. Regarding the occurrence of DNA damage, and other nuclear/cellular abnormalities in gym users, our results showed that individuals who reported being almost always/excessively stressed had a lower occurrence of binucleated cells frequency, which is an indicative of genotoxic effects of stress. The occurrence of DNA damage and other nuclear/cellular abnormalities (chromosomal instability and cell death) and their relationship with lifestyle factors (e.g., stress) is an important research field. In addition, DNA damage is a critical event in the initiation phase, as well as in the promotion and progression phases, which could be related to carcinogenesis events.

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