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Assessment of Salivary Cortisol and Perceived Stress in Teachers

Regisnei Aparecido de Oliveira Silva[1,*], Lídia Andreu Guillo[2], Nestor Persio Alvim Agrícola[3]

[1]Department of Biological Sciences, Federal University of Goiás (UFG), Jataí, Goiás, Brazil
[2]Department of Biological Sciences, Federal University of Goiás (UFG), Goiânia, Goiás, Brazil
[3]Department of Physical Education, Federal University of Goiás (UFG), Jataí, Goiás, Brazil
*Corresponding author: regisneioliveira@gmail.com

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Abstract **Background:** This study aimed at correlating the levels of salivary cortisol and perceived stress in male teachers, in three periods of the academic year (March, July, December). **Methods:** The collection of saliva for cortisol measurement was performed at 6:30 a.m., 11:30 a.m., and 5:30 p.m. on the same day that the BPSS-10 (Perceived Stress Scale, Brazilian version) was applied. The total concentration of cortisol was calculated using the area under the curve with respect to ground (AUC_g). **Results:** When comparing the periods of the academic year, the mean stress score obtained in the month of March (16.46 ± 5.36) and December (17.54 ± 6.56) showed a significant difference compared with the mean results obtained in July (10.77 ± 5.64; $p < 0.01$ and $p < 0.001$. The AUC_g value showed no significant difference between the periods analyzed (1.145 ± 0.450 µg/dL, 0.916 ± 0.341 µg/dL, and 1.031 ± 0.374 µg/dL, respectively). When analyzing the correlation between BPSS-10 values and total salivary cortisol throughout the school year, no statistical significance was found according to Pearson correlation coefficient ($p = 0.554$). **Conclusions:** The results suggest that the teachers, during the vacation period, might not perceive the physiological reactions of stress, so these reactions were not identified by the BPSS-10 used.

Keywords: *salivary cortisol, stress, teachers, public school*

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1. Introduction

Teachers are among the groups of workers subjected to occupational stress. They face daily stressful situations caused by the intensification and precariousness of the work, mainly by the changes in educational policies experienced in recent decades [1].

Stress may not be observed evenly or present equal intensity throughout the academic year and may, therefore, be subject to variations in periods or according to variables, such as gender, age, length of experience, among others [2]. Research indicates cycles of stress in teachers, with greater intensity at the beginning and end of the academic year [3].

On the issue of gender, it is important to note that, in Brazil, even though the teaching profession involves essentially female workers, the input of male teaching staff in Basic Education is a growing phenomenon in recent years, reaching near gender equality in Secondary Education [4]. This aspect highlights the need for research including male teachers in work situations.

In the past, studies assessing stress at work were carried out with traditional methods, characterized only by questionnaires and/or forms. However, the use of salivary biomarkers has gained popularity in recent decades, mainly in biomedical and psychological research. Among the possible salivary biomarkers, one can highlight enzymes, proteins, immunoglobulins (IgA, IgG, IgM) and cortisol [5,6].

The secretion of cortisol follows a circadian rhythm, reaching its peak half an hour after the individual wakes up and its lowest point near midnight during sleep [7]. Due to its release in bursts, a measurement during a period of 24 hours may reveal 15 or more bursts of cortisol, with the main ones occurring early in the morning, causing variations in its concentration [8]. The variation in cortisol levels during the day was observed in research with academics during assessments, unpleasant video sequences, and public speaking, according to the different stress situations experienced [9]. Increased levels of cortisol were also recorded in response to stress in athletes after Olympic wrestling competitions [10], in children in the period leading up to a dental appointment [11], and in girls with precocious puberty [12]. Therefore, stressor influences may alter the production of cortisol and the individual's circadian rhythm [13].

Research with salivary cortisol has many advantages, such as the possibility of obtaining saliva samples with noninvasive procedures, lack of need of an expert to collect it, and a more accurate assessment as regards collection

via urine. Thereby, researchers can assess cortisol levels without potential reactivity problems and constraints, as is the case of the collection via blood and urine. Moreover, the measurement becomes inexpensive, valid, and reliable from a methodological point of view [13].

Even though cortisol has found a prominent place in the research on stress, both in terms of ease of material collection and result reliability, the complexity of the topic is still a great challenge for researchers [6].

This study aimed to analyze the association between salivary cortisol level and perceived stress in male teachers at different working times during an academic year.

2. Methodology

The research was characterized as a descriptive longitudinal observational study of 13 male teachers distributed over five public state schools of Basic Education in the city of Jataí, Goiás, Brazil. The number of schools corresponds to all public schools in the city offering the level of education assessed in the study. The total number of male teachers in the state school system in the city is 39, according to data from the State Undersecretary of Education.

The research activities were initiated only after approval by the Research Ethics Committee at the Federal University of Goiás, under the number 609.308/2014.

The choice of the volunteers was characterized by a non-probabilistic convenience sample [14], according to inclusion and exclusion criteria. Inclusion: teachers working for at least 1 year in Elementary and Secondary Education. Exclusion: teachers who continuously use alcohol, cigarettes, and drugs influencing the central nervous system, as well as the continued use of medications; teachers who had been diagnosed with a chronic disease; teachers who had been exposed to any stress factors in the month before the collection period (*e.g.*, the death of a family member, family member with a disease).

2.1. Study outline

The academic year in the state of Goiás usually starts in the first week of February and continues until the end of June. The month of July is dedicated to a school recess of 30 days. School activities resume in the first week of August and end in the second week of December.

Data were collected in three periods during the 2014 academic year: 1) the third week of March (MAR); 2) last week of the recess in July (JUL); 3) the last week of the academic year (DEC). The volunteers filled out a questionnaire including a profile analysis, and health and working conditions. They were selected according to the exclusion criteria mentioned above.

The study was conducted with the following: Perceived Stress Scale 10, Brazilian version (BPSS-10), and the collection of saliva for cortisol measurement.

2.2. Perceived Stress Scale

Moments after waking up and before collecting the first saliva sample, the participants filled out the BPSS-10 questionnaire. This instrument was originally developed by Cohen and Williamson [15] and was validated in Brazil by Reis, Hino and Ricardo-Añes [16] on a cohort of 793 teachers from Brazilian universities, named it BPSS-10, with alpha cronbach of 0.83.

The BPSS-10 questions refer to events from the last 30 days with multiple choice answers presented in a Likert-type scale: (0) never, (1) almost never, (2) sometimes, (3) infrequently, and (4) very frequently. Six items are negative (1, 2, 3, 6, 9, 10) and four are positive (4, 5, 7, 8). The sum of the points from the 10 questions yields a score ranging from 0 to 40 points. The score obtained is used as a measurement of perceived stress. A higher score indicates more stress. In this study, we used the categorization according to quartiles into high perception of stress (≥ Quartile 75) and moderate perception of stress (< Quartile 75) [17].

2.3. Saliva of collection

The collect of saliva was carried out by the participants in three specific periods of the academic year (MAR, JUL, DEC). In each specified period, there were three collections during a normal working day, from waking up to the end of the day: 1) approximately 30-40 minutes after waking up (AW), *i.e.*, between 6:00 and 7:00 a.m.; 2) before lunch (BL) between 11:00 a.m. and 12:00 p.m.; 3) in the afternoon (AN) between 5:00 and 6:00 p.m. Before lunch and in the afternoon, samples were collected at the school, with the exception of July, when all samples were taken by the participants themselves in their homes. To prevent forgetfulness or incorrect handling, the volunteers always received, on the day before the collection, three individually packaged kits, wherein the principal investigator detailed instructions and clarified any possible doubts.

The kit was composed of a polypropylene collection tube (2 mL) and a device to fit securely inside the collection tube (Saliva Collection Aid, Salimetrics, USA), for the total collection of the saliva by a passive drainage method.

For the collection, we asked the participants not to make use of drinks, food or any oral hygiene products with the use of cream for 1 hour prior to sample collection. The participants were asked to rinse the oral cavity with non-sparkling mineral water, and salivation was not stimulated.

At the end, the tube should be kept refrigerated until delivery to the principal investigator, who provided the storage at -20℃ until analysis biochemical.

2.4. Measurement of salivary cortisol levels

Salivary cortisol levels were measured by enzyme immunoassay (EIA) with the High Sensitivity Salivary Cortisol EIA kit (Salimetrics, State College, USA), following the manufacturer's protocol. The coefficients of intra- and inter-assay variation were lower than 7% and 11%, respectively, with the minimum detected concentration as 0.007 μg/dL. The standard solutions, internal controls, and saliva samples were analyzed in duplicate.

Since the production of cortisol throughout the day provides more stable values [18], the salivary cortisol levels were calculated using the area under the curve with respect to ground (AUC_g), which is considered to be a measurement related to the total production of the hormone or, in other words, an overall cortisol response measurement [19]. The time interval (in hours) in which the collections were made (7:00 a.m. to 6:00 p.m.) was used to calculate the AUC_g, using the trapezoidal rule [20].

2.5. Statistical Analysis

Data were analyzed using descriptive and inferential statistics, considering statistical significance at $p<0.05$. The values of discrete and continuous variables are presented as mean ± standard deviation (mean ± SD), median and quartiles. In the initial analysis of the distribution of the data, the Shapiro-Wilk test was used. As perceived stress data presented normal distribution, the mean values of perceived stress in different periods of the academic year were compared using analysis of variance (ANOVA) for repeated measures with subsequent application of the Bonferroni test.

Cortisol levels followed a non-normal distribution. After removing outliers, a Gaussian distribution was observed using the Shapiro-Wilk test. Therefore, 110 samples were analyzed for a total of 117 samples collected. For comparison of the measurements performed between 6:30 a.m. and 11:30 a.m., the unpaired *t* test with Welch's correction was used for unequal variances, wherever necessary.

In the AUC_g calculations, the values for the times in the morning and before lunch were added, multiplied by 5, and divided by 2 (trapezoidal rule). Thus, the AUC_g values presented represent the total production of cortisol over 5 hours. After calculation of the total daily production and the removal of an outlier value, the AUC_g values presented normal distribution and equal variances in the Bartlett test. Thus, for a comparison of the total salivary cortisol levels (AUC_g) in different periods of the academic year, one-way ANOVA was carried out.

The correlation of the total salivary cortisol levels (AUC_g) with individual BPSS-10 scores was measured using the Pearson correlation coefficient (r) as suggested by Barros and Reis [21].

3. Results

3.1. Characterization and work situation of the participants

The subjects participating in the survey represent 33% (13/39) of the male teachers working in state public Elementary and Secondary Schools in the city of Jataí-GO. These professionals are trained in different fields of knowledge, working in teaching and coordination. From the total number of teachers, 54% work on a temporary contract basis and only 46% are full-time staff. The characteristics of age, length of experience, number of schools in which they work, and weekly working hours are presented in Table 1.

3.2. Perceived Stress

The data related to the PSS are presented in Table 2. The mean and quartile values of the results obtained at the beginning and end of the academic year were higher than those obtained over the vacation period (July).

The repeated measures ANOVA analysis showed significant differences between the mean values obtained in different periods of the academic year ($p=0.0002$). The subsequent application of the Bonferroni test revealed statistically significant differences between the mean scores from March and July ($p<0.01$) and between July and December ($p<0.001$).

3.3. Salivary Cortisol

The salivary cortisol values at different times of the day, in each period of the academic year, are shown in Figure 1. As expected, the characteristic profile of salivary cortisol secretion was observed in the three periods of the academic year analyzed: higher concentrations upon waking, followed by a gradual decline throughout the day. The salivary cortisol levels were significantly higher at 6:30 a.m. (AW), when compared with the values obtained at 11:30 a.m. (BL): 0.353 ± 0.189 *versus* 0.129 ± 0.037 µg/dL; 0.196 ± 0.077 *versus* 0.132 ± 0.065 µg/dL, and 0.274 ± 0.118 *versus* 0.113 ± 0.052 µg/dL, in March, July, and December, respectively.

Table 1. Characteristics of the participating teachers

	Mean	Standard Deviation	Minimum	Maximum
Age (years)	32	8.2	23	50
Number of schools	1.9	0.9	1	4
Weekly working (hours)	47.2	12.6	20	60
Length of experience (years)	8.5	7.9	1	30

Table 2. Descriptive measures of perceived stress scale (BPSS-10) throughout the academic year

Period of the academic year	Mean	Standard deviation	CI (95%)	Minimum	Maximum	Quartiles		
						Ql	Md	Qu
March	16.46 [ab]	5.36	13.2 to 19.7	7	25	13	18	20
July	10.77 [ac]	5.64	7.3 to 14.1	0	20	6.5	12	15
December	17.54 [b,c]	6.56	13.5 to 21.5	5	27	14	18	23

Analysis of variance (ANOVA) for repeated measures, followed by the Bonferroni test: [a] $p< 0.01$, [b] $p>0.05$; [c] $p<0.001$
Ql : lower quartile; Qu: upper quartile. Md: median.

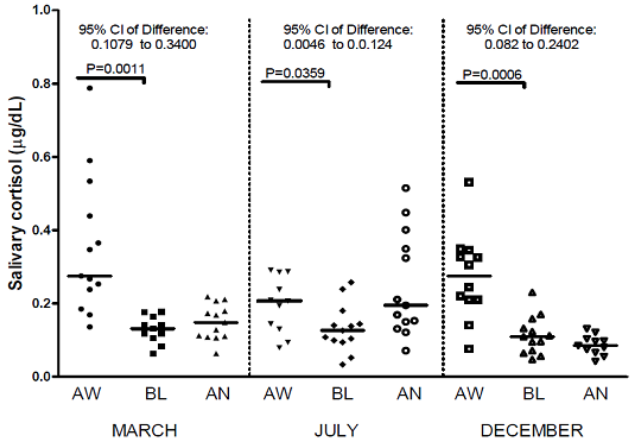

Figure 1. Daily levels of salivary cortisol in the three periods of the academic year. The points represent individual participants; the horizontal bars show the median values. (AW = collection after -waking up BL = before lunch; AN = afternoon). P values obtained by the parametric *t* test, with Welch's correction for unequal variances

The total salivary cortisol data (AUC_g) observed in the three periods of the academic year are presented in Table 3. In the comparison between the periods, there was no significant difference (p=0.3715, ANOVA), although the average values were lower in the month of vacation in relation to the other two periods studied.

Table 3. Values of the area under the curve with respect to ground (AUC_g) for salivary cortisol in different periods of the academic year

	AUC_g[a] (µg/dL)		
	MAR	JUL	DEC
Mean (SD)	1.145 (0.450)	0.916 (0.341)	1.031 (0.374)
Median	0.997	0.910	1.058
Maximum	2.030	1.678	1.710
Minimum	0.547	0.467	0.427
95% CI	0.858, 1.431	0.699, 1.134	0.804, 1.258

[a]Parametric test (one-way analysis of variance), p=0.2480

When analyzing the correlation between perceived stress score and the total salivary cortisol values (AUC_g) for all periods of the academic year, no association was found between these variables, as shown in Figure 2 (Pearson correlation coefficient, p=0.554).

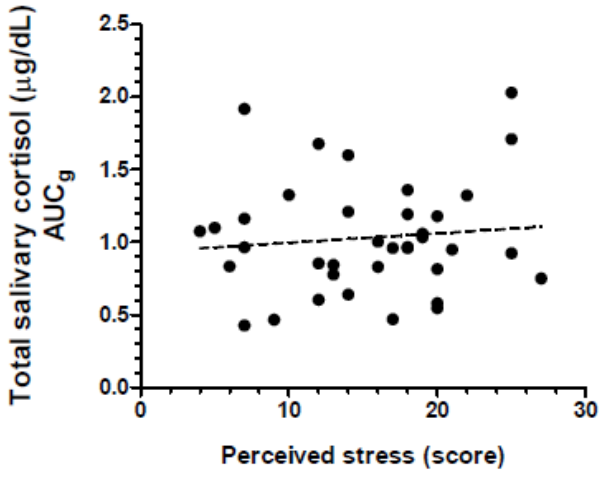

Figure 2. Relationship between perceived stress and total salivary cortisol (AUC_g). The correlation was determined using the Pearson correlation coefficient

4. Discussion

This study included male teachers with a mean of 8.5 ± 7.9 years of professional experience and mean age of 32 ± 8.2 years, who may be considered young professionals. However, one should take into account that the high workload (47.2 ± 12.6 hours), the various work locations (1.9 ± 0.9), added to the functional situation (54% under temporary contract), may generate a psychological and physical demand that compromises the work activities. In this regard, the literature reinforces that the wear of the teacher is determined largely by the type and form of organization of their work [22].

The mean value of the stress score observed considering the entire academic year was 14.92 ± 3.63. This value was lower than the average determined by Reis [17] in 793 Brazilian university professors (17.07 ± 7.37), but higher than the value described by Cohen and Williamson [15] in an American population (13.03 ± 6.35). Research also indicates differences in stress levels between some variables such as profession, age, gender. Male university professors had a lower mean value when compared with females (16.18 ± 7.37 and 18.26 ± 7.20, respectively) [17]. Compared with other professions, it was observed that male teachers tend to be more affected by stress than male firefighters [23]. The corresponding mean score among elderly Brazilians was 15.13 ± 6.45 [24].

The mean stress values in each period of the academic year show that the teachers surveyed present a moderate level of stress, according to categorization in quartiles. Higher stress values were recorded at the end of the academic year with a mean and standard deviation of 17.54 ± 6.56 and a maximum value of 27 points. In the vacation period and beginning of the academic year, the values recorded were 10.77 ± 5.64 (maximum 20) and 16.46 ± 5.36 (maximum 25), respectively. These data are corroborated by research conducted with Basic Education teachers in the United States, which highlight the existence of stress cycles, with greater intensity at the beginning and end of the academic year and the end of assessment periods [2]. Other authors have pointed out the seasonality of stress [21], as well as the end of the year as the moment of increased teacher attrition [25]. Considerable stress levels have been reported by researchers from different countries, like Antoniou et al. [26] in Greece; Chan; Chen; Chong, [27], in China; Fisher [28] in the United States; and Hasan [29] in India. Little is known about the occurrence of stress in the Brazilian population, as most studies are limited to investigating specific professions, restricted contexts, as well as using small samples [17].

Although the mean levels of stress recorded in this study have been moderate throughout the year, they should be taken as a warning sign, because high levels of stress at work reflect psychobiological responses that, in turn, exert a negative influence on the work [30]. High levels of stress can also cause some health problems: pain, anguish, anxiety, depression, tachycardia, hypertension, among others. These problems, when affecting teachers, can jeopardize the development of their professional activities [31].

Regarding the salivary cortisol levels from the three periods of the day (after waking up, before lunch, and afternoon) at different times of year (March, July, December), the teachers included in the study were observed to follow a circadian rhythm. Higher values were observed in the morning periods (after waking up), with a sharp fall 5 hours later (before lunch): 0.353 µg/dL *versus* 0.129 µg/dL; 0.196 µg/dL *versus* 0.132 µg/dL; and 0.274 µg/dL *versus* 0.113 µg/dL, respectively. Higher cortisol values upon waking were found in a study conducted by Campos and David [32], in which they obtained the following results: 0.601 µg/dL (30 minutes after waking up) and 0.271 µg/dL (after lunch). Similar behavior was obtained in studies developed by Steptoe et al. [29] and Pruessner et al. [33], in which high cortisol levels were recorded 30 minutes after waking up, with a sharp fall during the day.

The values obtained in this study among surveyed teachers are in agreement with the reference values for both men and age. The salivary cortisol reference ranges for men follow the following values according to age: between 21-30 years: morning = 0.112-0.743 µg/dL, night = 0.018-0.308 µg/dL; between 31 and 40 years: morning = 0.119-1.551 µg/dL, night = 0.018-0.358 µg/dL; and between 41 and 50 years: morning = 0.177-1.087 µg/dL, night = 0.018-0.329 µg/dL [34].

When considering the salivary cortisol values obtained by AUC_g, the recorded means were 1.145 ± 0.450 µg/dL (March), 0.916 ± 0.341 µg/dL (July), and 1.031 ± 0.374 µg/dL (December). The ANOVA analyzed between the data for cortisol salivary collected at different times of the academic year showed no statistically significant difference (p=0.2480).

There was no statistical significance in the relationship between salivary cortisol concentration and perceived stress levels. A similar result was described by Rocha et al. [35] who reported nonsignificant higher levels of stress and cortisol on working days (355.6 ng/mL) and lower levels on days off (348.6 ng/mL).

Significant correlations were found in studies reporting cortisol and stress levels. Chronically stressed individuals had a significantly greater increase in cortisol (0.561 µg/dL) compared with unstressed subjects (0.329 µg/dL) [36]. Also, male students had a significant correlation between salivary cortisol levels upon waking up and acute stress [33].

The absence of a significant correlation between job stress and salivary cortisol levels observed in the present study suggests that teachers, during the vacation period, fail to perceive or feel the physiological reactions of stress; therefore, these reactions cannot be identified by the BPSS-10. It is possible that some professionals, in certain periods, manage stress with more self-control, so it is not detected by the instruments used [10]. It is believed that subjects when answering the BPSS-10, associate their responses to the perception of receding stress, which differs from the amounts obtained in the cortisol samples. One should take into account that the stress perception report has more significant results when assessed using detailed instruments including daily questions before and after the working day [36] or even before and after stressful situations, such as assessments [37] or dental appointments [11]. Furthermore, authors report negative correlations between salivary cortisol and questionnaires assessing subjective stress in off-duty periods [38].

The association of salivary cortisol levels in research related to stress has become commonplace nowadays [7]. Authors like Gröschl et al. [39] and Kalman and Grahn [40] show that there is scientific evidence that stress is related to increased levels of cortisol; therefore, cortisol is a key regulator of stress mechanisms and consequently a biological indicator of stress.

At the time that this study was completed, the literature lacked studies using the methodological dynamic used in the present research, which assessed the relationship between cortisol and stress in male teachers during 1 year of academic work. Innumerable studies present results of the relationship between cortisol/stress for short periods or between different groups [11,12,23,30,33]. Therefore, the use of salivary cortisol as a stress biomarker in teachers should be considered. However, it should be taken into account the collection of a greater number of saliva samples during the academic year or during short periods and stressful situations, such as during assessment moments.

Despite the nuances, studies such as this can provide valuable information about the pathways through which cortisol contributes to research on health and the consequent impairment of the daily function of the teachers' work [41]. It is important to emphasize that the results herein presented become a warning to the professionals studied and open methodological possibilities for future research on the topic.

5. Final Considerations

The results of this study show that the relationship between salivary cortisol concentration and perceived stress levels were not statistically significant.

The results presented provide a methodological contribution to study the relationship between salivary cortisol and perceived stress at work in Basic Education teachers and in other professions in Brazil and in different countries. Therefore, further research is suggested on the topic to validate the correlation between the perceived stress scale (BPSS-10) and cortisol, using aspects not discussed herein, such as variables related to each individual and the diversification of methodological instruments for data collection. This caution is necessary, because of factors such as the long period researched, the small number of participating subjects and the strict selection criteria of the group studied in this research (only males and essentially healthy individuals).

Compliance with Ethical Standards

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Conflict of Interest

The authors declare that they have no competing interests.

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