Academic stress and practical performance of freshmen in human anatomy

Estresse acadêmico e desempenho prático de calouros na anatomia humana

Lucas Pereira da Silva
Graduating in Physiotherapy
Institution: Universidade Federal do Triângulo Mineiro (UFTM)
Address: Rua dos Andradas, 330, Nossa Sra. da Abadia, Uberaba - MG,
CEP: 38025-200
E-mail: lucasp178kz@gmail.com

Aline Araújo Martins Ferreira
Graduating in Physiotherapy
Institution: Universidade Federal do Triângulo Mineiro (UFTM)
Address: Rua dos Andradas, 330, Nossa Sra. da Abadia, Uberaba - MG,
CEP: 38025-200
E-mail: martinsferreira.aline@outlook.com

Ana Paula Espindula
Post-Doctorate in Health Sciences
Institution: Universidade Federal do Triângulo Mineiro (UFTM)
Address: Rua dos Andradas, 330, Nossa Sra. da Abadia, Uberaba - MG,
CEP: 38025-200
E-mail: ana.espindula@uftm.edu.br

Odival Seabra
PhD in Physiological Sciences
Institution: Universidade Federal do Triângulo Mineiro (UFTM)
Address: Rua dos Andradas, 330, Nossa Sra. da Abadia, Uberaba - MG,
CEP: 38025-200
E-mail: odival.seabra@uftm.edu.br

Paulo César Pio
PhD in Physiological Sciences
Institution: Universidade Federal do Triângulo Mineiro (UFTM)
Address: Rua dos Andradas, 330, Nossa Sra. da Abadia, Uberaba - MG,
CEP: 38025-200
E-mail: paulocesarpio@yahoo.com.br
ABSTRACT

Objective: to evaluate salivary Cortisol and Alpha-amylase levels, relating them to the level of stress and academic performance of calories in the Human Anatomy discipline. Methods: Saliva collection procedures were performed before and after the application of the practical assessment of Human Anatomy. Saliva Cortisol and alpha-amylase concentrations were evaluated by ELISA method. The short version of the Stress Assessment Questionnaire for Nursing Students (ASNS) was used. Results: There was an increase in the students' stress level, with a significant increase of 46.8% in the levels of Cortisol and 46.1% of Alpha-amylase, (p <0.0009 and p <0.0001, respectively) after performing the proof. Initial averages were evaluated, before the start of the evaluation, of 0.440 ± 0.047 µg/dL for Cortisol and 111.19 ± 11.86 U/mL for salivary Alpha-amylase, and final average, after the evaluation was carried out, of Cortisol and final Alpha-amylase of 0.646 ± 0.178 µg/dL and 162.45 ± 44.69, respectively. The level of stress detected by the questionnaire presented significantly and positively (Cortisol: r²= 0.4353; p=0.0272/Alpha-amylase: r²= 0.4413; p=0.0258), with peak Cortisol (0, 21±0.15 μg/dL) and alpha-amylase (162.5 ± 44.7 U/ml). There was a significant and negative glow of academic performance with the peak of Cortisol (r²= 0.4169; p=0.0319) and Alpha-amylase (r²=0.4178; p=0.0316). Conclusion: The increase in declared stress is multifactorial, in which the university routine and emotional instability intensify the increase in anxiety and self-demand, which has repercussions on the performance of academics.

Keywords: academic performance, cortisol, salivary alpha-amylase, stress.
RESUMO
Objetivo: avaliar os níveis salivares de Cortisol e Alfa-amilase, relacionando-os com o nível de estresse e desempenho acadêmico de calorias na disciplina da Anatomia Humana. Métodos: Procedimentos de coleta de saliva foram realizados antes e depois da aplicação da avaliação prática da Anatomia Humana. As concentrações de saliva cortisol e alfa-amilase foram avaliadas pelo método ELISA. Foi utilizada a versão resumida do Questionário de Avaliação do Esforço para Estudantes de Enfermagem (ASNS). Resultados: Houve aumento no nível de estresse dos alunos, com aumento significativo de 46,8% nos níveis de Cortisol e 46,1% de Alfa-amilase, (p <0,0009 e p <0,0001, respectivamente) após a realização da prova. Foram avaliadas médias iniciais, antes do início da avaliação, de 0,440 ± 0,047 μg/dL para Cortisol e 111,19 ± 11,86 U/mL para alfa-amilase salivar, e média final, após a avaliação, de Cortisol e alfa-amilase final de 0,646 ± 0,178 μg/dL e 162,45 ± 44,69, respectivamente. O nível de estresse detectado pelo questionário apresentou significante e positivamente (Cortisol: r²= 0,4353; p=0,0272/Alfa-amilase: r²= 0,4413; p=0,0258), com pico de Cortisol (0,21±0,15 μg/dL) e alfa-amilase (162,5 ± 44,7 U/ml). Houve um brilho significativo e negativo de desempenho acadêmico com o pico de Cortisol (r²=0,4169; p=0,0319) e Alfa-amilase (r²=0,4178; p=0,0316). Conclusão: O aumento do estresse declarado é multifatorial, em que a rotina universitária e a instabilidade emocional intensificam o aumento da ansiedade e da autodemanda, repercutindo-se no desempenho dos acadêmicos.


1 INTRODUCTION
During student life, over half of enrolled students experience emotional problems which may result from academic overload, pressure to succeed, peer competition, and less time to spend on leisure or family. When students experience psychological distress, academic productivity drops. Students who are experiencing depression or other psychological problems are generally found to have trouble maintaining progress, and encounter difficulty adjusting to higher education.¹

Scientific evidence points to a relationship between changes of chronic origin and the stress of academic activities, with high levels of anxiety being observed mainly in undergraduate students and health professionals.²

In response, there is a stimulation of hormone production through the Hypothalamus-Pituitaria-Adrenal (HPA) axis, which provides numerous...
physiological changes and adaptations in the human body. These adaptations are considered a normal reaction to everyday situations, but in recurrent cases and for a long time, they can negatively impact the quality of life.\textsuperscript{(4)} Substances such as Alpha-amylase (AA) and Cortisol are some stress and immunity biomarkers that can be found in saliva. Thus, the findings point to a relationship between the concentration of these salivary biomarkers and the level of stress.\textsuperscript{5}

Cortisol is a predominant glucocorticoid in humans, which performs several physiological functions essential to homeostasis. Among these functions, it has an important role in gluconeogenesis, from amino acids, which directly affects hepatic glycogen deposition. This regulation is essential for the maintenance and release of energy sources, as well as for coping with stressful life events.\textsuperscript{6}

Cortisol levels effectively influence the process of learning, memory and emotions, acting on immune system functions, such as lymphocyte maturation.\textsuperscript{7} In addition, it is related to the secretion of catecholamines, such as adrenaline and noradrenaline, which act on the $\beta$-1 receptor, increasing heart rate and muscle contraction strength; and on the $\alpha$-1 receptor, generating vasoconstriction. Therefore, these factors lead to an increase in blood pressure arterial.\textsuperscript{8}

The secretion of corticotropin-releasing hormone (CRH) by parvocellular neurons in the paraventricular nucleus of the hypothalamus stimulates the HPA axis. After the arrival of CRH in the anterior pituitary, the secretion of adrenocorticotropic hormone (ACTH) occurs. This molecule acts on the adrenal glands, stimulating the production of cortisol, being the main product of the hormonal cascade of the HPA axis.\textsuperscript{5}

It is important to note that cortisol concentrations vary throughout the day as a result of the circadian cycle. This cycle is represented by high levels of this hormone upon waking, maintaining concentrations until the first hour of activity; with a gradual decline, to its lowest level, at night, at bedtime.\textsuperscript{9}

Cortisol can be measured by different biochemical methods and can be quantified in different samples, due to the fact that it is peripherally bound to
transport proteins in blood plasma, urine and saliva, or present in the active form in plasma, free cortisol.\textsuperscript{10} 

There is a consensus in the literature on the variables that are considered as exclusion factors in salivary and blood cortisol surveys, with emphasis on the use of steroids, being in the last trimester of pregnancy, being sick on the day of collection and having evidence of endocrine disorder.\textsuperscript{11} 

Alpha-amylase is an enzyme found in saliva, which acts in digestion and maintenance of oral health. According to the literature, it has been shown that the physical stress there is a peak of enzyme production soon after the onset of the stressor stimulus, followed by regression to baseline levels after 30 to 60 minutes. Although this change has been evidenced in cases of physical stress, this spike in alpha-amylase production has also been associated with psychological stressors.\textsuperscript{(12)} Since the autonomic nervous system stimulates the parotid glands to secrete Alpha-amylase in response to acute stressful stimuli,\textsuperscript{3,12} 

Over the last few years, salivary alpha-amylase has been shown to be an important biomarker of stress level, in which the number of studies aimed at evaluating the relationship between its levels and behavioral disorders resulting from changes in stress has been growing.\textsuperscript{12} Thus, the hypothesis of the study is that the level of stress negatively interferes with academic performance. In this perspective, the objective of the study was to evaluate the levels of cortisol and salivary alpha-amylase, relating them with the level of stress and academic performance of freshmen of the Physiotherapy course, during the first practical evaluation of the discipline of Human Anatomy.

2 METHODS

The study was approved by the Research Ethics Committee, process number 4.492.091. The study consists of observational, cross-sectional, quantitative and longitudinal research. 

We recruited 64 volunteers from the Physiotherapy undergraduate, of both sexes, aged between 18 and 29 years, 45 female and 19 male. As inclusion criteria, the volunteers should be freshmen and be studying the discipline of
Human Anatomy, accepting to participate in the research, by signing the Free and Informed Consent Form. Thus, the volunteer students assumed the commitment to comply with all guidelines and recommendations, mainly related to food restriction conditions in the 12 hours prior to saliva collection.

On that occasion, an interview and physical evaluation were scheduled. The evaluation form addressed personal data, daily life habits, family history, past medical history, psychological and psychiatric condition, sleep quality and medication use. At the end of the interviews, the following exclusion criteria were adopted: male gender, presence of neurological, rheumatological, metabolic, chronic, psychiatric, behavioral diseases and sleep disorders. In addition, participants could not take prednisone, dexamethasone, and other steroids administered orally or intravenously.

Participants were instructed not to use black tea; green tea; energetic drinks; cola-based soft drinks; chocolates and chocolate milk, twelve hours before the collections. In addition, cleaning of the oral cavity with light mouthwash with water was requested. In the presence of possible oral lesions, active or potential bleeding, saliva collections were not performed, with the immediate exclusion of the study participant.

In case of non-compliance with the guidelines and/or recommendations, as well as in case of abandonment, the participants’ data were disregarded in the study. After adopting the inclusion and exclusion criteria, 11 female academics were selected. (Figure. 1).
The practical assessment of human anatomy was applied individually in an intermittent flow, alternating between one minute to answer a practical question and one minute sitting in a chair waiting for the next question, this strategy aimed to relieve the academic from stress.

The participants themselves collected saliva samples, with the help of a previously trained professional, using Salivette tubes. Saliva collection procedures were performed before and after the application of the practical assessment. The samples were identified by letters and numbers, in order to guarantee the anonymity of the academics.

The volunteers were instructed to previously wash their hands, remove the swab (sterile cotton) from the Salivette tube, introduce it into the oral cavity and chew for 60 seconds to stimulate salivation. The professionals assistant extracted the cotton ball, using a sterile clamp, and placed them properly inside the tubes.

Figure 1. Flow diagram

Source: Data from authors.
The samples were centrifuged, with collection of aliquots of the supernatant, being stored in a freezer at -20°C. Aliquots were thawed immediately prior to analysis.

The salivary cortisol and alpha-amylase concentrations were evaluated by the ELISA method, utilizing ELISA kit – 1x96 wells from Salimetrics, LLC. The cortisol assay method has analytical sensitivity <0.007 µg/dl and Alpha-Amylase Elisa Kit Salivar 0.4 U/ml. Both have intra- and inter-assay coefficients of variation <7% and <11%, respectively. Cortisol assays do not show cross-reactivity as, for example, prednisone and 17-hydroxyprogesterone (17OHP). However, it has 0.56% reactivity with prednisolone and 19.2% with dexamethasone.

The reference value of the method at 11 pm for adults, according to the supplier, is up to 0.12 µg/dL for cortisol and 18 U/mL for alpha-amylase. These reference values were verified by determining the cut-off, calculated as the mean of the negative control, plus three standard deviations. Thus, the cut-off calculation for cortisol = 0.0468 + (3 x 0.025), being approximately 0.12 µg/dL. Whereas, the Cut-off for alpha-amylase = 9.23 + (3 x 2.93), ie approximately 18 U/mL.

Sample preparation was performed by coating the plates with anti-cortisol and anti-alpha-amylase (solid phase antibody) monoclonal antibodies. Then, 25 µL of standard and control samples, 25 µL of diluent in the zero well and in the well with NSB (non-specific ligand) were added. Then, 200 µl of conjugate diluted 1:1600 (15 µl of conjugate to 24 ml of diluent) was added. The plates were then homogenized for 5 minutes at 500 rpm and incubated for an additional 55 minutes at room temperature. Subsequently, four washes were performed with 1X buffer. Thus, 200 µL of TMB solution (tetramethylbenzidine) homogenized for five minutes and incubated in the dark for another 25 minutes were added. At the end, 50 µL were added to the stop solution. Absorbance readings were taken 10 minutes after addition of the stop solution in an ELISA reader at 450 nm. The entire reaction was carried out at room temperature.

The short version of the Instrument for Assessment of Stress in Nursing Students (ASNS) questionnaire was used, adapted to analyze the stress level of
students in the health area. The adapted short version has 30 items organized into three explanatory factors: Behavioral - interpersonal relationships and academic-professional experience (items 2-5), Stressors - excess of activities, responsibilities, performance and routine (items 1, 10-16 and 18), Organizational - academic-professional activities and conflict management (items 1, 10-16 and 18) (Figure 2).

Figure 2. Carefully read each item below and mark with an “X” the number corresponding to the intensity of stress that the situation causes you, according to the caption below:

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I don't experience the situation</td>
<td>I don't feel stressed situation</td>
<td>I feel a little stressed the situation</td>
<td>I feel very stressed about the situation</td>
</tr>
<tr>
<td>2</td>
<td>The obligation to carry out extracurricular work</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Carry out care procedures in general</td>
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<td></td>
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<tr>
<td>4</td>
<td>Communication with other professionals in the academic unit</td>
<td></td>
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<tr>
<td>5</td>
<td>The environment of the university clinical unit</td>
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<td></td>
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<tr>
<td>6</td>
<td>Communication with professionals from other sectors of the University</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>Being afraid of making mistakes during practice assessments</td>
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<tr>
<td>8</td>
<td>The form adopted to evaluate the theoretical content</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>Distance between university and place of residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Feeling insecure or afraid when taking theoretical tests</td>
<td></td>
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<tr>
<td>11</td>
<td>Realize the difficulties that involve the relationship with other professionals in the area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Realize professional responsibility when in theoretical and practical classes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>13</td>
<td>Observing conflicting attitudes in other professionals</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>14</td>
<td>Public transport used to get to the university</td>
<td></td>
<td></td>
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<tr>
<td>15</td>
<td>Time required by the teacher to deliver extracurricular activities</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>16</td>
<td>Distance between most campuses and quarters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Lack of leisure time</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>18</td>
<td>Public transport used to get to the university</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Missing time for rest</td>
<td></td>
<td></td>
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</tbody>
</table>

Source: Instrument for Assessment of Stress in Nursing Students (ASNS). Authors' data

Statistical analyzes were performed using the GraphPad Prism® program, version 5.0. The distribution of quantitative variables were performed using the Shapiro-Wilk test and homogeneity using the Bartlett test. The data showed parametric distributions. Thus, the paired t test (t) was used to compare salivary cortisol and alpha-amylase data before and after the application of the practical human anatomy assessment.
In this type of distribution, values were expressed as mean and standard deviation (±). For correlation analysis, Pearson's correlation coefficient (r) was used because the distribution was normal. Statistical differences were considered significant when the probability (p) was less than 5% (p<0.05).

3 RESULTS

Participants had the following means ± standard deviations: age 19.2 ± 1.3 years; height of 1.64 ± 0.05 meters; body mass of 64.3 ± 14.3 meters and BMI of 23.9 ± 4.0 kg/m². The following BMI reference values were adopted: a) normal: BMI=24.9; b) overweight: BMI > 25; c) obesity: BMI > 29.9. Thus, among the participants, 63.6% (n=7) had a normal BMI; 9.1% (n=1) were obese and 27.3% (n=3) were overweight. Of the participants, 36.4% declared themselves white, 36.4% black and 27.3% brown. None of the participants were smokers. Approximately 63.6% (n=7) reported performing regular physical activity (3 times a week) and 36.4% (n=4) reported being sedentary. As for alcohol consumption, 45.5% (n=5) of the students reported using it regularly, with consumption of more than 5 drinks per week. With regard to the history of past family diseases, prostate and breast cancer, high blood pressure, diabetes mellitus, heart attack and stroke stand out. In the clinical history, there were reports of hospitalization cases in 72% (n=8) of the participants; due to viral infections, anxiety, kidney stones, appendicitis, respiratory diseases and minor surgical procedures. Of these, 54.5% (n=6) underwent surgical procedures: septoplasty, appendectomy and tonsillectomy. None of the participants had autoimmune and infectious diseases. One participant reported having chronic migraine of psychosomatic origin. Still, 90.1% (n=10) reported having psychological problems, with 1 (one) undergoing psychological follow-up and three undergoing psychiatric treatment. The drugs most used by the participants were: contraceptives, corticoids and anti-inflammatories. The average weekly sleep of the participants was 32 hours and 17 hours on weekends. Of the 11 participants, 45.5% (n=5) reported having a religion and being a practitioner.
The measurement of salivary biomarkers indicated an increase in the students' stress level immediately after taking the test (Figure 3), in which a significant increase of 46.8% in cortisol levels and 46.1% in alpha-amylase was observed, (p<0.0009 and p<0.0001, respectively). Initial averages were observed, before the start of the evaluation, of 0.440 ± 0.047 µg/dL for cortisol and 111.19 ± 11.86 U/mL for salivary alpha-amylase, and final average, after the evaluation was carried out, of cortisol and final alpha-amylase of 0.646 ± 0.178 µg/dL and 162.45±44.69, respectively.

The data from the evaluation questionnaire was able to determine specific levels of stress for each of the three explanatory factors evaluated: Behavioral; Stressors; Organizational. Thus, regarding behavioral factors, determined based on the scores for questions 2-5, a mild level of stress was detected in 63% of the participants. Still, a moderate level of stress was observed in 57% of the participants, by analyzing questions 1, 10-16 and 18, related to stressors. With regard to organizational factors, a high level of stress was detected in 82% of the participants, according to the scores for questions 6, 7, 9, 17 and 19 (Figure 3). The stress levels detected by the evaluation questionnaire was 35.55±3.59, considering that the score can vary between 0 and 57. There was a positive and significant correlation between stress levels and cortisol and alpha-amylase (cortisol: r²=0.4353; p=0.0272/alpha-amylase: r²= 0.4413; p=0.0258), with peak cortisol (0.21±0.15 µg/dL) and alpha-amylase (162.5±44.7 U/ml). Furthermore, a negative and significant correlation was observed between academic performance and peak cortisol (r²= 0.4169; p=0.0319) and alpha-amylase (r²= 0.4178; p=0.0316).
4 DISCUSSION

With the results of the present study, our hypothesis was validated, as the level of stress negatively interfered with the academic performance of the evaluated university students. Until now, no studies had been carried out that demonstrated the role of salivary alpha-amylase as a marker of academic stress.

In a study carried out with undergraduate students aged between 18 and 24 years old, students answered a questionnaire to assess the level of concern associated with challenges relevant to daily life through the Depression Anxiety and Stress Scale. Results indicated that the top three concerns were academic performance, pressure to succeed, and graduate plans. The authors concluded that mental health problems negatively influenced the success of university students. Another study associated stress, academic performance, physical and mental health and impulse control disorders in young adult students. Study participants were grouped by their overall score on the Perceived Stress Scale as mild, moderate or severe. Severe perceived stress was associated with worse academic performance and worse physical health, as well as higher rates of psychiatric and impulsive disorders. These findings suggested associations between stress and various aspects of mental or physical health in young adults,
which may be an important consideration for individuals working with college students.\textsuperscript{14} The findings of this study corroborate our findings, which demonstrated a negative and significant correlation between the level of academic performance and the increase in the level of stress. However, another study found that mindfulness is inversely associated with depression and stress, but not with academic performance.\textsuperscript{15}

Another study evaluated depression, anxiety and stress in undergraduate medical students using the Anxiety, Depression and Stress Scale (DASS). The results of this study showed that more than half of the respondents were affected by depression (51.3\%), anxiety (66.9\%) and stress (53\%). The morbidity found was higher in 5th semester students than in 2nd semester students, with women reporting higher scores compared to men.\textsuperscript{16} In the present study, we found similar findings, since an increase in the students’ stress was detected using the short version of the Instrument for Assessment of Stress in Nursing Students (ASNS).

However, our study did not evaluate males, as these subjects were excluded throughout the study according to the established exclusion criteria. In another study, the results showed an increase in perceived stress in university students, young adults, during the period of academic evaluations, compared to periods of less demand. More than 40\% of participants stated that there were changes in gastrointestinal habits during periods of stress. Significant correlations were observed between perceived levels of stress and changes in gastrointestinal habits.\textsuperscript{17}

Other authors have compared the responses of three commonly investigated salivary stress biomarkers representing hypothalamic-pituitary-adrenal activity (Cortisol) and sympathetic activity (Alpha-amylase and Chromogranin A), using academic oral presentation as a model of stress. Saliva was collected from 20 graduate students before and after the presentation, and heart rates were also recorded. Results showed that levels of all three biomarkers, as well as heart rate, were significantly higher in the presenter group compared to the audience group. These results suggested a more sensitive
reactivity of cortisol and alpha-amylase levels as academic stress markers.\(^{(18)}\)

Results that are in line with our findings.

In a systematic review, the authors demonstrated that nursing students experience stress, including Burnout. Stress has been associated with several factors, including work-life balance, resources and support, and adapting to change.\(^{(19)}\) This study also corroborated our findings in undergraduates of the physiotherapy course, as there was an increase in cortisol and alpha amylase levels immediately after the application of the practical test of the discipline of Human Anatomy in relation to these levels before the beginning of the evaluation.

In the researched literature, we found a study that evaluated the effect of an active methodology combined with a lecture on undergraduate nursing student learning and stress and anxiety levels. The level of stress was assessed using the concentration of the stress biomarker Cortisol in saliva. Anxiety was assessed using the State-Trait Anxiety Inventory (STAI) questionnaire. The students achieved significantly higher average scores on exams when the active learning strategy was applied compared to traditional lectures. The active methodology resulted in significantly lower levels of stress and anxiety, as well as better student performance, compared to traditional classes.\(^{(20)}\)

Thus, it is believed that there is a need to better understand the stress faced by health academics and to opt for new methodological strategies to deal with the negative consequences of stress. Among these strategies can be included active methodologies for theoretical and practical classes; quality mentorships for health academics; training in building resilience for new entrants; support in collegial relationships in order to reduce bullying; assistance for professional development and research and better support and resources to overcome increased workloads.

4.1 CLINICAL AND EDUCATIONAL RELEVANCE

A strong and resilient academic workforce is essential for the sustainability of future trained healthcare professionals. Organizations should
review their work practices and provide greater work-related empowerment to reduce occupational stressors among healthcare academics.

5 CONCLUSION

The practical evaluative activity of Human Anatomy in the undergraduate course in Physiotherapy triggered a high level of stress demonstrated by the increase in the concentration of Cortisol and salivary Alpha-amylase, which negatively influenced academic performance. The relationship between stress levels and the concentration of salivary cortisol and alpha-amylase found in the present study demonstrated that salivary cortisol and alpha-amylase are important biomarkers as a predictor of stress. Therefore, the increase in stress proved to be multifactorial, in which the university routine and emotional instability intensify the increase in anxiety and self-demand, which negatively affects the performance of academics.

ACKNOWLEDGEMENTS

This research was supported by the National Council for Scientific and Technological Development (CNPq) PQ-2018 (Process number: 302867/2018-0), Coordination for the Improvement of Higher Education Personnel (CAPES), Minas Gerais State Research Support Foundation (FAPEMIG), Uberaba Teaching and Research Foundation (FUNEPU), and São Paulo State Research Support Foundation (FAPESP).
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