Adv Physiol Educ 44: 744–751, 2020;
doi:10.1152/advan.00075.2020.

TEACHING INNOVATIONS

Effect of an active learning methodology combined with formative assessments on performance, test anxiety, and stress of university students

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Submitted 29 April 2020; accepted in final form 1 October 2020

Cardozo LT, Ramos de Azevedo MA, Morais Carvalho MS, Costa R, de Lima PO, Marcondes FK. Effect of an active learning methodology combined with formative assessments on performance, test anxiety, and stress of university students. *Adv Physiol Educ* 44: 744–751, 2020; doi:10.1152/advan.00075.2020.—The aim of this study was to evaluate the effect of an active methodology combined with a lecture on undergraduate student learning and levels of stress and anxiety. The active learning methodology consisted of a lecture of 50-min duration, study at home with a textbook, an educational game activity, and three formative assessments on the topic of the cardiac cycle. In a following class, the students provided saliva samples to evaluate their levels of stress, received an anxiety test, and then undertook an exam to assess their understanding of the cardiac cycle. The traditional teaching methodology consisted of two lectures (~2-h duration) on blood pressure control systems, delivered orally. In the third class, the students provided saliva samples, received an anxiety test, and then undertook an exam to assess their understanding of blood pressure control systems. The level of stress was assessed with the concentrations of the stress biomarkers cortisol and alpha-amylase in saliva. Anxiety was assessed with the State-Trait Anxiety Inventory (STAI) questionnaire. The students achieved significantly higher average scores in exams when the active learning strategy was applied compared with the use of traditional theoretical classes. The active methodology resulted in significantly lower levels of stress and anxiety, as well as improved student performance, compared with the use of traditional lectures.

academic stress; active learning; active methodologies; educational game; formative evaluation

INTRODUCTION

During their time at university, students encounter a variety of stressful situations. For new arrivals, the challenges include the process of adaptation to a different reality, such as living away from home, interacting with new colleagues, balancing their academic responsibilities and their social life, the heavy workloads of the disciplines, work, studying, and exams (47, 66). Furthermore, many students may not enter university with sufficient maturity to cope with the new circumstances and responsibilities (56).

Many of the skills that are required from new students, such as being capable of autonomy and critical thinking, being motivated to learn, and knowing how to organize their study, are not developed during their time at elementary and secondary schools (52). In courses in the area of health, one of the factors causing stress, according to the students, is the need to memorize, in a short time, substantial amounts of information concerning anatomy, physiology, and biochemistry (60). This is because many universities still use traditional teaching methods where the student receives information passively during a series of theoretical classes in which there is little student participation, consequently hindering meaningful learning and the development of critical and reflective thinking (23, 46).

Maintaining attention during lengthy lectures is a challenge for the new generation of students because from an early age they have had access to information and communication technologies, so they feel the need to be constantly connected to a network of contacts, desire immediate gratification, and have difficulty in concentrating (1, 54). An additional challenge related to the traditional teaching method is the use of tests at the end of a series of lectures, since during the teaching-learning process the students are unable to identify their doubts and correct concepts that have been misunderstood, often only perceiving them at the time of the test (29).

Taking tests is one of the main factors that cause stress in students. The inability of the student to master certain topics, the fear of failure, or the frustration of not achieving the expected goals (28, 47, 49) can generate fear and anguish (40), leading to academic stress (28, 47).

The term "academic stress" is used to describe stress related to study, with the associated negative impacts on the academic performance of students (2, 28). Stress factors are perceived differently by each individual, resulting in different individual responses to the same stressor, with or without associated symptomatic manifestations (7). Academic stress can be aggravated when the student feels incompetent in the face of challenges (14), resulting in anxiety disorders (11, 39), and can continue throughout the undergraduate program and during later professional life (50).

During the stress reaction, there is increased secretion of catecholamines by the sympathetic nervous system (SNS) and the medulla of the adrenal gland, together with the release of cortisol from the adrenal cortex. These stress reaction mediators activate physiological responses whose function is to enable the individual to cope with, or adapt to, the stressful situation (26, 59, 68). However, when the stress is very intense or is maintained for a long period, the levels of stress hormones remain high and adaptation may not occur, favoring the appearance of pathologies (44, 59, 64).

Positive correlations are observed between blood and saliva cortisol concentrations (70), as well as between the level of

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stress and the concentration of cortisol in saliva (27, 43). Consequently, cortisol in saliva can be used as a biological marker for stress (27, 30, 58). In addition, the secretion of alpha-amylase is increased by sympathetic stimulation of the salivary glands (48) during the stress reaction, so the concentration of alpha-amylase in saliva can be used as a marker for the activity of the sympathetic nervous system (27, 30, 31, 58).

Studies have found that the stress caused by academic tests leads to increases of the concentrations of alpha-amylase (58) and cortisol (49, 56, 58) in the saliva of university students. It has also been observed that higher levels of stress and anxiety are associated with poorer performance of students in assessments (49, 60). Therefore, it is important to determine whether changes in teaching and evaluation methodologies might be able to reduce the stress and anxiety of students faced with tests.

The achievement of educational objectives requires institutional support of the student, which may include consideration of the teaching and evaluation methods used during undergraduate courses (47). From this perspective, active teaching strategies are considered useful for learning because they favor autonomy, stimulate interest and curiosity, encourage decision-making, and make the student responsible for his/her acquisition of knowledge (5, 8).

Activities involving the use of educational games to improve learning can be used in teaching strategies in combination with theoretical lectures or used after lectures (4, 12, 65). Such games assist in the development of problem-solving skills, increase the interest and motivation of the student in learning a particular topic, enable the application of acquired knowledge, develop the ability to work as a team, and improve cognitive and psychomotor development (4, 25, 38, 65). As an example, a cardiac cycle puzzle was developed for teaching of the cardiac cycle (36, 38). It was found that use of the puzzle improved the learning of university students in the first year of a dentistry course compared with a group who only received theoretical lectures (10). Similar results have been observed with other educational games (9, 34, 55, 62, 63).

However, the lecturer must be aware that the teaching strategy on its own, without evaluation of the process, is not sufficient for learning to actually occur. For the student to be responsible for his/her acquisition of knowledge, it is essential that he/she perceives what has been learned, identifying the progress that has been made as well as any remaining doubts. It is then possible to reevaluate the study plan, adopting the strategies necessary for further clarification.

Formative assessment places value on the experiences of the students and lecturers during the teaching-learning process, enabling the students to identify what they already know and what they have not yet understood as well as to plan future actions for learning of topics and concepts (24, 53). The use of grading is optional in formative assessment, since the objective is not to classify the student as approved or not but rather to assist the teaching-learning process (37). In this way, it is possible to monitor the progress of the students, identifying their difficulties and avoiding the teaching-learning process ending without achievement of the learning objectives (29, 71).

In her teaching practice, the coordinator of the present study found that the use of active methodologies alone did not enable the students to assume coresponsibility for their acquisition of knowledge. Therefore, the active teaching strategies were associated with evaluation procedures, resulting in pragmatic active teaching-learning methodologies (37). The present study was designed along these lines.

It is evident that educational games can improve learning, with formative assessments contributing to the success of the teaching-learning process, and that academic stress can compromise the performance of students in assessments of their understanding. Therefore, the objective of this study was to evaluate the effect of an educational game, combined with theoretical lectures and formative assessments, on the learning and levels of stress and anxiety of undergraduate students in the area of health.

MATERIALS AND METHODS

Experimental design. This study was approved by the Research Ethics Committee of the Faculty of Dentistry of Piracicaba (FOP-UNICAMP, protocol CAAE 10859119.0.00005418). The participants in the study were 56 students enrolled in the second semester of the Dentistry course, studying the Biosciences II discipline. All the students agreed to participate in the research and signed free and informed consent declarations.

All the procedures performed were part of the discipline, so all the students participated. To ensure autonomy and reduce any vulnerability of the students in deciding to participate in the study (67), the students were requested to authorize the use of the research data after divulgation of the grades for the cardiovascular system topic. The invitation to participate in the research was made outside of class hours, at a specific time, by a postgraduate student.

This study included lectures and assessments performed during the integrated Biosciences II discipline related to the cardiovascular system topic. This topic included lectures on anatomy, histology, and physiology delivered by different lecturers. The present study only considered the physiology activities, which included two related topics with similar degrees of difficulty: the cardiac cycle and blood pressure control systems. This strategy enabled the analyses to be performed with the same class of students.

The learning objectives of the cardiac cycle classes were that the students should be able to understand how the morphological and physiological characteristics of the cardiac structures contribute to the continuous pumping of blood and how cardiac changes affect this pumping.

The learning objectives of the classes on blood pressure control systems were that the students should be able to identify changes in blood pressure caused by everyday or clinical situations and to explain the physiological responses activated to return to resting levels of blood pressure.

The cardiac cycle topic was taught with the active methodology (a lecture of ~50 min + educational game activity + home study + formative assessments). The blood pressure control systems topic was taught with the traditional method (lectures of ~2 h each + home study). Comparison was then made of the effects of the active methodology and the traditional method on learning (summative assessment) and the levels of stress and anxiety of the students (Fig. 1).

Active methodology. In this study, the active methodology consisted of the combination of an educational game activity with evaluation procedures, which had previously been used by the coordinator in the teaching of the cardiac cycle. The method consisted of the combination of a lecture of ~50 min, textbook study at home, an educational game activity (2 h), and three tests (formative assessments), as detailed below. The grades obtained in the formative assessments were not considered for the students' approval in the discipline since the objective was to monitor the progress of the students, identifying their doubts and topics that had not been understood.

In the first class, the students completed individual prior knowledge assessment, using a free smartphone application (Socrative Student

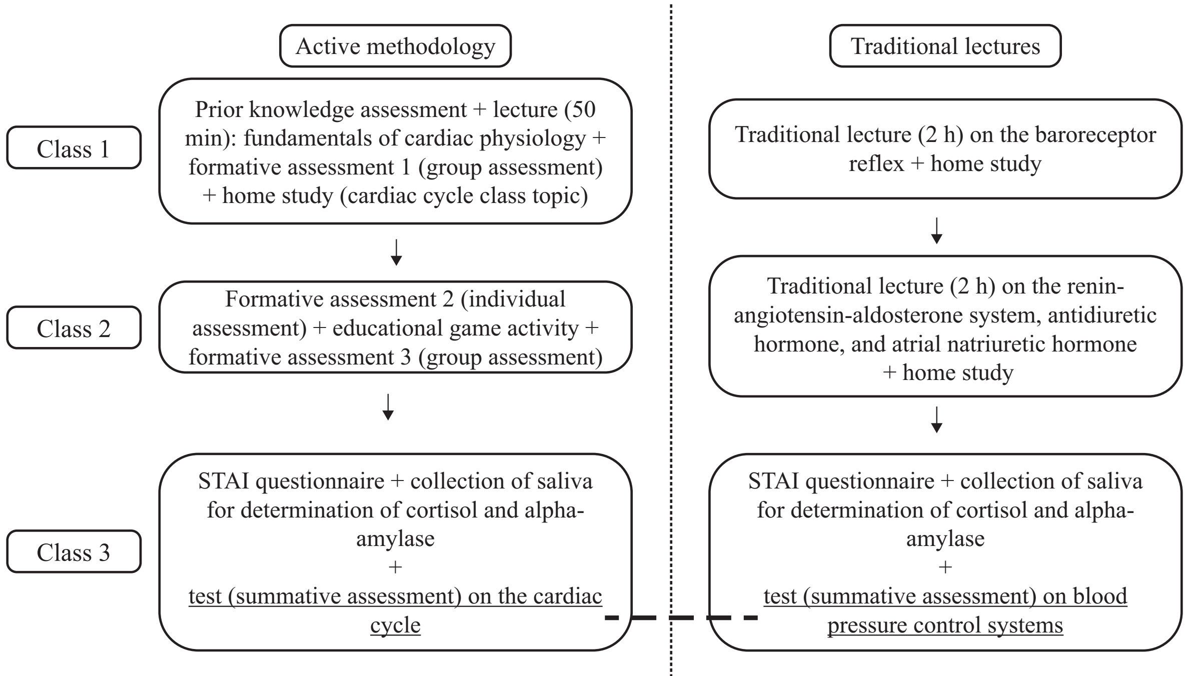

Fig. 1. Description of the activities and assessments performed with the active methodology and traditional lectures.

app), available on Android and iOS app stores. Also, the grades of students in these test were not summative and were not considered for their approval in the discipline. The tests consisted of four multiple-choice questions on physiology topics that had been taught in the previous semester (Supplemental Table S1, *questions 1–3*; see https://doi.org/10.6084/m9.figshare.12654059) or had been studied previously in anatomy and histology classes of the discipline (Supplemental Table S1, *question 4*; see https://doi.org/10.6084/m9.figshare.12654059). For this test, all the students had access to the smartphone application that allowed the lecturer to access the responses of the students in real time (69).

The objective of this test was to determine whether the students had understood previous contents needed for the topic to be taught, enabling the lecturer to discuss and correct any errors identified. As the students responded, the lecturer checked the answers on her smartphone. Most of the students understood the trajectory of blood in the heart, action potential, and coupled excitation-contraction in skeletal muscle cells (Supplemental Table S1; see https://doi.org/10.6084/m9.figshare.12654059). These concepts are necessary for understanding the physiology of cardiac muscle contraction and the cardiac cycle. All questions were translated from Portuguese to English for this article.

After the prior knowledge assessment, a lecture of ∼50 min was then provided, using PowerPoint slides and oral description of the fundamentals of cardiac physiology, addressing the special characteristics of cardiac cells, the physiology of pacemaker cells, the control of these cells by the autonomic nervous system, the difference between the skeletal muscle action potential and the cardiac muscle plateau potential, and the conduction and transmission of the electrical stimulus through the fibers of the heart (10). Since some incorrect answers were given in the prior knowledge assessment, in the oral presentation the lecturer drew attention to the items corresponding to the errors that had been identified, asking if there were any doubts and providing clarification where necessary.

In this class, held in a lecture theater, there was no explanation about the cardiac cycle and no discussion of the relationship between the morphological and functional characteristics of the heart. At the end of the class, the students took, as a group, a test performed with the smartphone application. The purpose of this test (Supplemental Table S2, *formative assessment 1*; see https://doi.org/10.6084/m9.figshare.12654077) was to determine what the students had understood immediately after the theoretical class.

The students were then instructed to use a textbook to study the topic addressed in the classroom as well as the topic of the next class (the cardiac cycle). The lecturer explained that the content taught in the theoretical class, together with reading of the book, would enable the students to understand the topic addressed in the next class. The lecturer also informed students that at the beginning of the next class there would be a new individual test (*formative assessment 2*), followed by an educational game activity. The students were told that grades would be given for this test, with questions related to the content explained in the classroom having greater value than questions related to the content (cardiac cycle) that the students had studied alone at home.

At the start of the second class, the students individually performed a printed test (Supplemental Table S2, *formative assessment 2*; see https://doi.org/10.6084/m9.figshare.12654077), followed by the educational game activity involving a cardiac cycle puzzle (available at https://www.lifescitrc.org/resource.cfm?submissionID=11445#.X6nFX_D4B2g.gmail). A detailed description of this puzzle was provided previously by Marcondes et al. (38). For this activity, the students were divided into groups of five or six participants, defined by the lecturer, based on the averages obtained in the Biosciences I discipline in the previous semester, such that each group included students with performance above and below the average. The students were informed that the purpose of the activity in groups was for them to assist each other in understanding the topics and that during the activity each group should act and respond according to a consensus reached by all the group members, to promote collaborative learning.

Each group was provided with a figure containing illustrations of the phases of the cardiac cycle and was instructed to position the images in the correct sequence. When the group had completed this task, they should ask the lecturer or the monitors (postgraduate students) to check whether the sequence was correct. If it was correct, the students could proceed to the next stage of the activity. If it was incorrect, they should again discuss it within the group, to identify errors (10, 38).

In the next stage, the students were provided with a table consisting of six columns with the following headings: phase of the cardiac cycle,

figure, atrial state, ventricular state, atrioventricular valves, and pulmonary and aortic valves. The columns should be completed with the tokens provided. As in the previous stage, the students should ask the lecturer or monitors to check the table once consensus had been reached on its completion. If it was correct, the group could proceed to the next stage. If any of the pieces were incorrectly positioned, the students should discuss within the group, to identify the pieces that should be repositioned, until reaching the correct solution. Throughout all stages of the activity, the role of the lecturer and monitors was not to indicate where errors lay but to encourage the students to rethink, discuss among themselves, and solve the problem. If, after discussion, the group was unable to find the errors, the lecturer or the monitors asked questions intended to guide the students toward achieving correct positioning of all the pieces.

At the end of this stage, the students received questions, one at a time, to discuss among the members of the group (10). The group discussions constituted a type of formative assessment process, since they enabled the students to identify what they knew as well as to collectively resolve their doubts.

At the end of this activity, the students completed, as a group, a test (with grading) on the topics addressed in the activity with the educational game, using the smartphone application Socrative Student (Supplemental Table S2, *formative assessment 3*; see https://doi.org/10.6084/m9.figshare.12654077).

The three formative assessments enabled evaluation of the learning resulting from the lecture of ~50 min (*formative assessment 1*), the lecture (50 min) and home study (*formative assessment 2*), and the combined use of the lecture (50 min), home study, and the collaborative group activity with the educational game (*formative assessment 3*).

For the next class, the students were instructed to study the content taught in the theoretical class and used during the activity with the educational game and were informed that they would take a test to assess their understanding of the cardiac cycle.

At the start of the third class, the students provided saliva samples for determination of the stress biomarkers cortisol and alpha-amylase, and they were requested to complete a questionnaire to assess their level of anxiety. They then individually performed a printed test (Supplemental Table S3; see https://doi.org/10.6084/m9.figshare.12654086). This test was used as a summative assessment of the students' understanding of the cardiac cycle, to determine whether the learning objectives had been achieved (Fig. 1). It should be noted, however, that all the formative assessments were considered in the analysis of the learning achieved by the students during the period of the study.

Traditional class. Traditional classes are based on the notion that knowledge should be transmitted from the lecturer to the student, with the lecturer being the active agent who presents and "teaches" the contents. The student is a passive agent who should receive the contents and faithfully reproduce them during tests.

In this study, two traditional theoretical classes were held, each lasting 2 h, on the subject of blood pressure control systems, together with home study by the students as described below. The classes were held in a lecture theater, with PowerPoint slides and oral presentation of the topics by the lecturer, addressing the importance of blood pressure regulation, perfusion through the tissues, venous return, and the following blood pressure control systems: local control, baroreceptor reflex, renin-angiotensin-aldosterone system, antidiuretic hormone, and atrial natriuretic hormone. At any time during the classes, the students could ask questions to clarify any doubts. At the end of the second class, the students were asked to study the topics with a textbook before performing a test in the subsequent class (Supplemental Table S4; see https://doi.org/10.6084/m9.figshare.12654107).

At the beginning of the third class, samples of saliva were collected from the students for determination of the stress biomarkers cortisol and alpha-amylase. The students then completed a questionnaire to assess their level of anxiety and individually completed a printed test on the topic of blood pressure control (Supplemental Table S4; see https://doi.org/10.6084/m9.figshare.12654107). This test was used as a summative assessment to evaluate the students' understanding of blood pressure control systems and to determine whether the learning objectives had been achieved (Fig. 1).

Saliva collection and biochemical measurements. For determination of the concentrations in saliva of the stress markers cortisol and alpha-amylase, saliva samples were collected with Salivettes, according to the manufacturer's instructions, at three different times. The first (baseline) was during the first physiology class of the semester, when the students returned from vacation, without performing any other tests. The second collection was made immediately before the test on the topic taught with the lecture (50 min) combined with the cardiac cycle puzzle educational game. The third collection was made immediately before the test on the topic taught with the theoretical classes. The saliva samples were centrifuged, and the supernatants were stored in tubes at −80°C in a freezer before determination of cortisol and alpha-amylase.

Cortisol was determined with a colorimetric enzymatic assay employing a commercial Cortisol Enzyme Immunoassay Kit (Salimetrics, Pennsylvania) with sensitivity of 0.007 µg/dL and coefficient of variation in the range from 7% to 11%.

Alpha-amylase was also determined by a colorimetric enzymatic assay using a commercial α-Amylase Kinetic Enzyme Assay Kit (Salimetrics) with sensitivity of 2.0 U/mL and coefficient of variation below 7.2%.

Assessment of anxiety. The State-Trait Anxiety Inventory (STAI) questionnaire was used to assess the anxiety level of the students at the same times that the saliva samples were collected. The STAI evaluates the occurrence of feelings of tension, nervousness, worry, and apprehension. It consists of 20 questions, with positive and negative items. The student assigns a score, on a Likert-type scale, with the options 1 (none), 2 (low), 3 (moderate), and 4 (high). For quantification and interpretation of the responses, the scores for positive items have inverted weights, where the response options 1, 2, 3, and 4 are assigned the values 4, 3, 2, and 1, respectively (6). In analysis of the responses, a score lower than 33 was considered to indicate mild anxiety, a score between 33 and 49 indicated moderate anxiety, and a score higher than 49 indicated high anxiety (3).

Evaluation of learning. The level of learning was evaluated by comparing the scores obtained in the summative assessments (tests) performed in the third class for the different teaching methods: the active methodology (cardiac cycle) and traditional lectures (blood pressure control systems).

The topics taught were not exactly the same (cardiac cycle and blood pressure control systems), representing a limitation of this study. Therefore, to identify whether any differences in results could be influenced by one topic being more challenging than the other, the students were asked to give their perceptions of the level of difficulty they had in understanding both topics, according to the questions presented in Table 2. Summative assessment scores, saliva concentrations of cortisol and alpha-amylase, and anxiety indexes were evaluated both from all students and, separately, from students who considered the two topics to have the same level of difficulty.

Statistical analysis. The scores obtained in the tests on the topics taught with either traditional lectures or the active methodology were compared with the Student's t test for paired samples ($P < 0.05$).

One-way analysis of variance (ANOVA) for repeated measures, followed by Tukey's test for multiple comparisons of means ($P < 0.05$), was applied to the means for the cortisol and alpha-amylase concentrations in saliva and the scores obtained in the STAI questionnaire at the different times [baseline, before the test on the topic taught with the lecture (50 min) combined with the cardiac cycle puzzle educational game, and before the test on the topic taught with the theoretical classes (2-h duration for each class)]. The data are presented as means and standard deviations (SD). The statistical procedures were performed with GraphPad Prism v. 6.01 software.

Table 1. *Summative assessment scores, saliva concentrations of cortisol and alpha-amylase, and anxiety indexes for students before tests on contents taught with either active methodology or theoretical lectures*

	Baseline	Active Methodology	Theoretical Lectures
Summative assessment score (total score: 10.0)		8.77 (1.24)*	7.22 (2.56)
Cortisol, µg/dL	0.45 (0.22)[a]	0.44 (0.25)[a]	0.66 (0.42)[b]
Alpha-amylase, U/mL	32.30 (20.23)[a]	33.01 (22.09)[a]	49.27 (33.87)[b]
Level of anxiety	38.59 (8.92)[a]	43.57 (9.07)[b]	51.25 (11.76)[c]

Values are shown as means (SD); n = 56 students. Mean scores were obtained in the summative assessment on the contents taught with either the active methodology (lecture + educational game activity + formative assessments) or theoretical lectures. Saliva concentrations of cortisol and alpha-amylase and the anxiety scores determined with the State-Trait Anxiety Inventory (STAI) questionnaire were obtained at 3 different times: baseline (start of the semester, in a week without tests), before a test on the content taught with the active methodology, and before a test on content taught with theoretical lectures. *Significant difference relative to theoretical lectures (Student's t test for paired samples, $P < 0.05$). Different superscript letters indicate significantly different groups (1-way ANOVA for repeated measures; $P < 0.05$). Saliva cortisol and alpha-amylase concentration: theoretical lectures showed higher values compared with active methodology and compared with baseline values ($P < 0.05$). Level of anxiety: active methodology and theoretical lecture showed higher values compared with baseline values ($P < 0.05$); theoretical lecture showed higher values compared with active methodology ($P < 0.05$).

RESULTS

The students achieved a significantly higher average score in the test concerning the content taught with the active methodology compared with the average score for the test about the content taught with traditional lectures (Table 1; $P < 0.05$).

The students presented higher saliva concentrations of cortisol and alpha-amylase before the test on the topic taught with traditional lectures compared with the concentrations of these stress biomarkers observed at baseline and before the test on the topic taught with the active methodology. The values of these variables showed no difference between the baseline and before the test on the topic taught with the active methodology (Table 1).

The mean scores obtained with the STAI questionnaire to determine the levels of anxiety of the students were significantly higher before the tests on the topics taught with both the active methodology and the traditional lectures compared with the baseline (Table 1; $P < 0.05$). The mean score obtained before the test on the content taught with traditional lectures was significantly higher than the mean score obtained before the test on the content taught with the active methodology (Table 1; $P < 0.05$).

The STAI results showed that the students presented a moderate level of anxiety at baseline and before the test on the topic taught with the active methodology, whereas a high level of anxiety was observed before the test on the topic taught with traditional lectures (Table 1).

The evaluation of students' perceptions of the level of difficulty of the heart physiology and blood pressure control systems topics is presented in Table 2. Of 56 students who participated in this study, 26 students considered one topic more difficult than the other one and 30 students considered both topics equally difficult or easy (Table 2). Considering only the data of the 30 students for whom the cardiac cycle and blood pressure control system topics had the same level of difficulty, the grades, salivary concentrations of the stress biomarkers cortisol and alpha-amylase, and anxiety levels, the results (Table 3) remained similar to those from the whole class (Table 1).

Table 2. *Numbers and percentages of the responses of the students concerning their perceptions about the level of difficulty in the physiology of the heart and blood pressure control systems topics*

Options	*N*	%
a) The topic cardiac cycle was more difficult than blood pressure control systems.	9	16.1
b) The topic blood pressure control systems was more difficult than cardiac cycle.	17	30.4
c) Both topics were difficult, with similar levels of difficulty.	27	48.2
d) Both topics were easy.	3	5.4

DISCUSSION

The findings of the present study indicated that the active teaching methodology led to reduced stress and anxiety before the exam, and improved the learning of the university students, in comparison to traditional lectures.

The improvement in learning, as assessed by the exam results, was in agreement with other studies comparing student performance in assessments after the use of educational games or theoretical lessons about cardiac cycle (10), action potential (34), muscle system (33), integration of synapsis, muscle contraction, and autonomous nervous system physiology (9), kinesiology and applied anatomy (42), electrocardiogram (55), and emergency skills training (16). In these studies, the students

Table 3. *Summative assessment scores, saliva concentrations of cortisol and alpha-amylase, and anxiety indexes for students who considered both subjects to have the same level of difficulty before tests on contents taught with either active methodology or theoretical lectures*

	Baseline	Active Methodology	Theoretical Lectures
Summative assessment score (total score: 10.0)		8.80 (1.38)*	6.74 (2.91)
Cortisol, µg/dL	0.44 (0.25)[a]	0.41 (0.24)[a]	0.69 (0.42)[b]
Alpha-amylase, U/mL	32.93 (17.72)[a]	37.73 (26.96)[a]	55.11 (37.84)[b]
Level of anxiety	39.33 (9.02)[a]	43.30 (7.05)[b]	51.80 (11.88)[c]

Values are shown as means (SD); n = 30 students. Mean scores were obtained in the summative assessment on the contents taught with either the active methodology (lecture + educational game activity + formative assessments) or theoretical lectures. *Significant difference relative to theoretical lectures (Student's t test for paired samples, $P < 0.05$). Saliva concentrations of cortisol and alpha-amylase and the anxiety scores determined with the State-Trait Anxiety Inventory (STAI) questionnaire were obtained at 3 different times: baseline (start of the semester, in a week without tests), before a test on the content taught with the active methodology, and before a test on content taught with theoretical lectures. Different superscript letters indicate significantly different groups (1-way ANOVA for repeated measures; $P < 0.05$). Saliva cortisol and alpha-amylase concentration: theoretical lectures showed higher values compared with active methodology and compared with baseline values ($P < 0.05$). Level of anxiety: active methodology and theoretical lecture showed higher values compared with baseline values ($P < 0.05$); theoretical lecture showed higher values compared with active methodology ($P < 0.05$).

reported that the activities with educational games were useful for learning because they promoted interaction and collaboration among the individuals of the group, making the topic clearer and resolving doubts. In collaborative activities, one student explaining to another often seems to be more effective than an explanation provided by the lecturer. This is because when a student who has just learned about the topic explains to another student, emphasis can be given to the key points used to understand the content, in addition to the use of simpler language (41). The use of group activities, such as educational games, allows the students to both learn and teach, developing their ability to listen to colleagues, increasing their interest, and learning to discuss and defend their ideas (22).

Lujan and DiCarlo (35) and Savage et al. (57) suggested that the use of strategies that create a relaxed environment favors interaction among students and with the lecturer, reducing the levels of stress and anxiety while increasing motivation and attention and consequently improving learning. Cooper et al. (13) observed that during group activities there was lower anxiety concerning the tasks faced, because the student realized that his/her difficulty was the same as that of a colleague and they could work together to find the solution. However, in these studies, there was no evaluation of whether, in fact, the use of active teaching strategies led to decreased stress and anxiety, together with improved performance, compared with traditional lectures.

Other authors have observed the relationship between stress biomarkers and assessment activities. Cortisol concentrations increased in exams (56) and oral presentation (45) activities. Alpha-amylase activity showed higher levels in a simulation stimulus (20). In addition, other authors evaluated both biomarkers, cortisol and alpha-amylase, in heralded real-life stressors and observed increased levels mutually (58, 66), as well as anxiety (58).

In a recent study published by our research group (32), academic stress increased alpha-amylase (55.24 U/mL not stressed vs. 97.80 U/mL stressed volunteers) but not cortisol (0.55 μg/dL not stressed vs. 0.44 μg/dL stressed volunteers) levels; similar values were found in this study.

Neuroendocrine stress response might vary depending on personality traits (such as self-esteem and extroversion) and type of stressor (real life or laboratory). The neuroendocrine stress response to an examination is characterized by an anticipatory response of the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis (58), as observed in this study when the test was applied after classes taught by theoretical lectures.

Moreover, the students' academic performances can be impaired by the stress and anxiety caused by exams, compromising the achievement of passing grades. In a dentistry course in Spain, Crego et al. (15) observed that increased levels of anxiety and stress were associated with poor performance of the students in assessments. Ng et al. (49) also observed an association between high levels of stress and poor exam performance in a medicine course.

In the present study, the lower levels of stress and anxiety observed before the test about the topic that had been taught in combination with the active methodology reinforced the hypothesis that active teaching strategies can make the student feel more secure, with less stress and anxiety concerning exams. Consequently, the students showed improved performance compared with the assessment performed after theoretical lectures. Decreased test anxiety and improved performance were also observed in an engineering course that used the active and collaborative "three hundred" methodology developed by Fragelli (21).

The positive effect of the active methodology used in this study could be related to the knowledge assessments performed during the methodology. At most educational institutions in Brazil (both basic and higher education), the strategy adopted involves a sequence of theoretical lessons, individual home study, and an exam. At the end of this process, the student either passes or must take a new exam to obtain a grade. When only this type of summative assessment is used, it is not possible to track the student's progress, because summative assessments only allow determination of the outcome of the learning rather than the evolution of the teaching-learning process (24). Hence, it is not possible to identify doubts and the topics not understood by the students so that such problems can be resolved before the end of a discipline or course (51). Therefore, it is desirable to conduct formative assessments, where the assessment itself provides another opportunity for learning by the student.

In the present study, two formative assessments were applied in the form of tests, while evaluations were also made during the activity with the educational game in the form of questions made to the groups after solving the puzzle. In addition, formative assessments also occurred as informal procedures during the activity with the educational game. For solving the cardiac cycle puzzle, the students had to identify their doubts, ask their colleagues questions, engage in argument, and compare the ideas presented by all the members of the group. In this way, they identified what they had already understood, what was not very clear, and what they had not understood at all until the moment of the activity.

Formative assessment allows students to identify their successes and errors, providing feedback about their progress, doubts, and difficulties with the content, as well as helping them to organize their studies and identify issues that require more attention (53). This assessment also enables the lecturer to accompany the learning of the students, modifying the classroom activities to meet their learning needs (29).

In addition, performing formative assessments at different times enables the students to remember concepts learned previously and to add new information. This process is important for learning, because new memory inputs become stabilized after an initial significant learning, in a process called memory reconsolidation (61). For example, in cardiovascular physiology classes, the students need to remember the anatomical or histological structures of an organ to properly understand its function. The students must retrieve previous knowledge of anatomy and histology, adding new information about the function of specific organs to the existing memory.

It is important to emphasize that active strategies for teaching and the assessment of learning are inseparable. Active teaching strategies alone will not help students unless they are accompanied by assessments that allow the lecturer to accompany the process rather than only considering the final learning result (37). Formative assessments enable the lecturer to monitor the progress of students, modifying the classes so as to best meet their learning needs. For the student, formative assessments enable the identification of doubts, success, errors, and issues requiring more attention, thus assisting the organization of study during the learning process (17, 19, 29). Therefore, the present study indicated that the combination of active learning strategy

combined with formative assessments could make the student more confident of performing well on the summative assessment and achieving the passing grades, decreasing the student's anxiety, as proposed by Downing et al. (18).

The limitation of this study was comparing the scores obtained in assessments of related but not the same topics (cardiac cycle and blood pressure control systems). To evaluate whether this difference could influence the results, we also evaluated only the students to whom both topics were equally difficult or easy. This evaluation also showed that there was a decrease in the scores and increase in salivary stress markers and anxiety levels before the assessment on the topic taught with lectures in comparison to the same parameters obtained before assessment on the topic taught with active methodology. Therefore, the difference in the topics seems not to have influenced the results of the present study. Despite this limitation, at least according to our best knowledge, this is the first study evaluating the effect of active methodology on stress and anxiety induced by assessment.

In a future study, we aim to use another possible experimental design by comparing the two methods to teach the same topic to different students' classes. Since the discipline in question is taught in the second semester of the undergraduate Dentistry course, the study would have to be performed with students who started the course in different years. However, the comparison of different groups of students presents another bias, since different teaching experiences between the groups could also influence the parameters analyzed in the present study.

The findings of the present work showed that active strategies combined with formative assessment resulted in improved student performance due to increased learning and reductions of stress and anxiety. These results suggested that lecturers should be widely encouraged to reappraise their approaches to teaching and assessment.

ACKNOWLEDGMENTS

This study is part of L. T. Cardozo's PhD thesis.

GRANTS

Financial support for this work was provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Grant 2019/05987-4), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.T.C., M.R.d., and F.K.M. conceived and designed research; L.T.C., M.S.C., R.C., P.O.d., and F.K.M. performed experiments; L.T.C., M.S.C., R.C., P.O.d., and F.K.M. analyzed data; L.T.C., M.R.d., M.S.C., R.C., P.O.d., and F.K.M. interpreted results of experiments; L.T.C. and F.K.M. prepared figures; L.T.C., M.R.d., M.S.C., R.C., P.O.d., and F.K.M. drafted manuscript; L.T.C., M.R.d., M.S.C., R.C., P.O.d., and F.K.M. edited and revised manuscript; L.T.C., M.R.d., M.S.C., R.C., P.O.d., and F.K. M. approved final version of manuscript.

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