Teaching baroreflex physiology to medical students: a comparison of quiz-based and conventional teaching strategies in a laboratory exercise

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Berg RM, Plovsing RR, Damgaard M. Teaching baroreflex physiology to medical students: a comparison of quiz-based and conventional teaching strategies in a laboratory exercise. Adv Physiol Educ 36: 147–153, 2012; doi:10.1152/advan.00011.2012.—Quiz-based and collaborative teaching strategies have previously been found to be efficient for the improving meaningful learning of physiology during lectures. These approaches have, however, not been investigated during laboratory exercises. In the present study, we compared the impact of solving quizzes individually and in groups with conventional teaching on the immediate learning during a laboratory exercise. We implemented two quizzes in a mandatory 4-h laboratory exercise on baroreflex physiology. A total of 155 second-year medical students were randomized to solve quizzes individually (intervention group I, n = 57), in groups of three to four students (intervention group II, n = 56), or not to perform any quizzes (control; intervention group III, n = 42). After the laboratory exercise, all students completed an individual test, which encompassed two recall questions, two intermediate questions, and two integrated questions. The integrated questions were of moderate and advanced difficulty, respectively. Finally, students completed an evaluation form. Intervention group I reached the highest total test scores and proved best at answering the integrated question of advanced difficulty. Moreover, there was an overall difference between groups for student evaluations of the quality of the teaching, which was highest for intervention group II. In conclusion, solving quizzes individually during a laboratory exercise may enhance learning, whereas solving quizzes in groups is associated with higher student satisfaction.

active learning; collaborative learning; meaningful learning; mental model

PHYSIOLOGY IS A CONSTRUCTIVIST SCIENCE, in which the ability to apply basic physiological principles for solving problems related to the integrated living organism is regarded as an essential part of mastering the discipline. In the constructivist paradigm, learning involves the active construction of meaning by the learner, by assembling information and meaning into mental models or representations that allow these facts to be applied to solve problems in the real world (12–14). This has been depicted as meaningful learning, a learning experience that occurs when the learner refines existing mental models by interpreting and incorporating new information with existing knowledge (12). Accordingly, a defining characteristic of meaningful learning is that it enables the learner to transfer knowledge, that is, to apply the new information to solve novel problems (12).

In contrast to the classical lecture format, student laboratory exercises potentially provide an ideal learning environment for students to build, test, and modify their mental models of physiological mechanisms (13, 14). Thus, laboratory exercises facilitate student-teacher and student-student interactions, which allows students to repair misconceptions in their mental models and develop more appropriate concepts (13, 14). However, even during laboratory exercises, student involvement varies, and, for some students, the goal of the laboratory exercise is to obtain the prescribed data as quickly as possible and leave, rather than to reflect on the methods and data at hand. Enhancing student involvement in laboratory exercises, and thereby in their own meaningful learning, thus poses an important challenge to the physiology teacher.

Quizzes have proven to be an effective formative assessment tool that enhances meaningful learning during lectures and conventional classroom teaching (15). In the context of physiology education, various collaborative learning strategies have been found to increase student performance on quizzes (3, 5, 9, 18, 19) and to enhance the transfer of knowledge (3, 6) as well as the retention of learned information (4, 8). This is likely because collaborative learning encourages the students to form self-explanations by explicitly articulating their understanding of a topic and enhances student-student interaction, both of which prompt the build-test-refine mental model learning strategy (1, 2). As of now, it is largely unknown whether quizzes and/or collaborative learning strategies improve meaningful learning in the context of laboratory exercises.

In the present study, we implemented two quizzes during a laboratory exercise on baroreflex physiology for second-year medical students. We compared the impact of solving the quizzes individually and in groups with conventional teaching without quizzes (control) on the immediate learning during the laboratory exercise. Immediate learning was assessed in a postexercise test that focused on declarative and procedural learning (recall, intermediate, and integrated questions) related to baroreflex physiology. We hypothesized that 1) quizzes would improve the immediate learning during the laboratory exercise and 2) this would be further enhanced by solving the quizzes in groups.

MATERIALS AND METHODS

Laboratory exercise. The present study was set during a 4-h laboratory exercise on baroreflex physiology, which is mandatory for all second-year medical students as part of a 5-wk cardiovascular physiology course at the University of Copenhagen. It consists of four sections, each lasting ~45 min (Fig. 1). All sections center on a basic model of the baroreflex, in which the aortic and carotid baroreceptors
sense changes in pulse pressure and mean arterial pressure upon various maneuvers (posture changes and intrathoracic pressure manipulations) that affect venous return to the heart; the baroreflex subsequently adjusts mean arterial pressure by modulating heart rate and total peripheral resistance through the parasympathetic and sympathetic sections of the autonomous nervous system. In section 1, the laboratory instructor reviews the fundamental aspects of the baroreflex ex cathedra, based on experimental data from previous studies (16, 17).

In section 2, a volunteering student is instrumentalized (Task Force Monitor System 3040i; CNSsystem) for the online noninvasive monitoring of heart rate, systolic, diastolic, and mean arterial blood pressures, stroke volume, cardiac output, and total peripheral resistance. The student is then instructed to perform five different maneuvers: a Valsalva maneuver for 15–20 s, repeated Valsalva maneuvers interrupted by deep inspirations (modified anti-G straining maneuvers), a 30-s isometric muscle contraction of the palmar flexors of the forearm, an orthostatic maneuver (in which the student squats for ~2 min and then quickly stands up), and finally while lifting the hand with the finger cuffs to measure continuous blood pressure 10–20 cm above the head. While the students observe the hemodynamic effects of these maneuvers in real time on a projector, heart-lung interactions, central command, the exercise pressor reflex, local autoregulation of blood flow, the hemodynamic impact of gravity, as well as potential sources of error and uncertainties in the hemodynamic measurements are discussed.

In sections 3 and 4, the class is divided into two groups. After one section has been completed, the two halves of the class switch and perform the remaining section (Fig. 1). In section 3, students explore baroreflex-mediated cardiovascular adaptations to a posture change from the upright seated position to the supine position. Cardiac output is measured by an inert gas rebreathing method (Innocrin, Innovision, Odense, Denmark), and blood pressure and heart rate are measured with conventional automatic oscillometric equipment in two volunteers in the upright seated and supine positions, respectively.

In section 4, students perform a number of pressure measurements on open and closed water-filled cylinders with an ordinary ruler and a manometer to answer a number of questions that relate to measurement principles and hydrostatics. While the students in section 3 rest between their measurements, the laboratory instructor provides constructive feedback to the students’ thoughts on these questions while highlighting the main learning points.

Students write a final laboratory report, in which they discuss the findings and their implications. This report is to be written in groups of two to six students and handed in within 2 wk after the laboratory exercise.

Study design. We included a total of 155 students, divided between 14 classes with 11 (mean, SD 2) students in each class. Students had a mean age of 22 (SD 2) yr; 52 students (34%) were male and 103 students (66%) were female. One laboratory instructor (either R. M. G. Berg or M. Damgaard) was available per class. R. M. G. Berg taught eight classes, and M. Damgaard taught the remaining six. Before the study, each class had been randomized to one of the following three interventions:

- Intervention I: individual quiz
- Intervention II: group quiz
- Intervention III: conventional teaching (control).

A short questionnaire regarding age, sex, and preparation was completed before the laboratory exercise in all three intervention groups. In intervention groups I and II, quizzes were performed after sections 1 and 2 of the laboratory exercise, respectively. After sections 3 and 4, students in all three intervention groups completed a test individually, followed by an evaluation form.

Quizzes. In intervention group I, students completed the quizzes individually and discussion was not allowed until after the completion of the quiz. Students were to complete the quiz in 10 min. Identical quizzes were used in intervention group II, but here the students were to complete the quizzes in groups of three to four persons, again over a period of 10 min. In both intervention groups I and II, each quiz was followed by a 5-min interactive session, in which the laboratory instructor highlighted the correct responses to each question.

Each quiz consisted of four questions, and they were all of moderate difficulty. Questions were all designed as true/false questions with one or more true answers per question (Table 1). No quizzes were performed in intervention group III.
Table 1. Quizzes A and B

<table>
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<tr>
<th>Quiz A</th>
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<tr>
<td>1. An acute decrease in mean arterial pressure will cause (true or false):&lt;br&gt; A. a decrease in parasympathetic output*&lt;br&gt; B. an increase in sympathetic output*&lt;br&gt; C. reduce the heart rate*&lt;br&gt; D. increase the total peripheral resistance*&lt;br&gt; 2. An acute increase in mean arterial pressure will cause (true or false):&lt;br&gt; A. an increase in parasympathetic output*&lt;br&gt; B. a decrease in sympathetic output*&lt;br&gt; C. reduce the heart rate*&lt;br&gt; D. reduce the total peripheral resistance*&lt;br&gt; 3. Pulse pressure (true or false):&lt;br&gt; A. equals systolic blood pressure − diastolic blood pressure*&lt;br&gt; B. is approximately stroke volume/arterial compliance*&lt;br&gt; C. equals heart rate × total peripheral resistance&lt;br&gt; 4. Mean arterial blood pressure (true or false):&lt;br&gt; A. equals 2/3 diastolic blood pressure + 1/3 systolic blood pressure*&lt;br&gt; B. equals cardiac output × total peripheral resistance*&lt;br&gt; C. equals heart rate × stroke volume × total peripheral resistance*</td>
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<th>Quiz B</th>
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<tr>
<td>1. Transmural pressure (true or false):&lt;br&gt; A. is synonymous to perfusion pressure&lt;br&gt; B. is the pressure difference across a vessel or cardiac wall*&lt;br&gt; C. can be directly measured in the brachial artery by means of an ordinary mercury manometer&lt;br&gt; 2. The heart’s preload (true or false):&lt;br&gt; A. represents the end-diastolic tension in the muscle of the ventricular wall*&lt;br&gt; B. depends critically on the transmural pressure across the ventricular wall*&lt;br&gt; C. does not depend on the venous return to the heart&lt;br&gt; 3. Deep inspiration (true or false):&lt;br&gt; A. increases the intrathoracic pressure&lt;br&gt; B. increases venous return to the heart*&lt;br&gt; C. reduces the transmural pressure across the ventricular wall&lt;br&gt; 4. Forceful expiration (true or false):&lt;br&gt; A. increases the intrathoracic pressure*&lt;br&gt; B. reduces venous return to the heart*&lt;br&gt; C. reduces the transmural pressure across the ventricular wall*</td>
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Quiz A was implemented in intervention groups I and II after session 1 of the laboratory exercise. Quiz B was implemented in intervention groups I and II after session 2 of the laboratory exercise. *True answers.

Test and evaluation. After sessions 3 and 4, all intervention groups completed an individual test on baroreflex physiology. No aids were permitted at this stage. The test consisted of two recall questions, two integrated questions, and two questions that were intermediate between recall and integrated questions. None of the questions related directly to the quizzes that intervention groups I and II had performed, but they all related to the contents of the laboratory exercise. The two recall and intermediate questions were constructed as multiple-choice questions with one or more correct answers (Table 2). The integrated questions were subdivided into multiple-choice subquestions with a single best answer (Table 2). One of the integrated questions was of moderate difficulty and the other of advanced difficulty, and both related to problems that had not directly been addressed during the laboratory exercise. The distinction between moderate and advanced difficulty was based on the supposition that the moderate integrated question merely addressed the impact of gravity on venous return to the heart in a new context, whereas the advanced question required the student to understand and integrate input and output in the baroreflex on multiple levels to be answered appropriately.

After the test, the students completed an evaluation form, in which the students had to 1) rate the academic gain of the laboratory exercise, 2) rate the quality of the teaching, 3) rate their own efforts during the laboratory exercise, and 4) provide an overall assessment of the laboratory exercise. In each instance, students had to choose between poor, below average, average, above average, and excellent.

To maximize overall learning for all three intervention groups after the study, the correct answers to the test were highlighted by the laboratory instructor immediately after the test and evaluation forms had been handed in.

Ethics. Ethical approval was not required for the laboratory exercise per se or for the present study as such. Both were conducted in accordance with Danish and European legislation and were approved by the Faculty of Health Sciences of the University of Copenhagen. The volunteering students were healthy with no previous medical history, and the methods used in this exercise are regarded as safe in healthy subjects. Precautions were made to minimize the potential risk of anaphylaxis.

Table 2. Test

| 1. Where is the venous indifference point in humans? (choose the correct answer/answers)<br> A. At the level of the heart<br> B. At the level of the aortic arch<br> C. 5−10 cm below the heart*<br> D. It varies with posture changes, but is always to be found at the level of the diaphragm<br> 2. Where are the arterial baroreceptors located in humans? (choose the correct answer/answers)<br> A. At the level of the venous indifference point<br> B. In the aortic arch and carotid sinus*<br> C. In the bifurcation between the subclavian and common carotid arteries and in the descending aorta<br> D. In the right atrium and pulmonary veins<br> 3. What is/are the effect/effects of increased sympathetic output in the baroreflex? (choose the correct answer/answers)<br> A. Reduced heart rate*<br> B. Increased heart rate*<br> C. Reduced total peripheral resistance<br> D. Increased total peripheral resistance<br> 4. What is/are the effect/effects of increased sympathetic output in the baroreflex? (choose the correct answer/answers)<br> A. Reduced heart rate*<br> B. Increased heart rate*<br> C. Reduced total peripheral resistance<br> D. Increased total peripheral resistance*<br> 5. When a healthy volunteer in the upright position (choose the correct answer at each point):<br> A. is subjected to high acceleration upward (high G forces), venous return to the heart will decrease*<br> B. is placed in a zero-gravity environment, venous return to the heart will decrease*<br> 6. A healthy volunteer is placed in the supine position with his lower body in a lower body negative pressure chamber. It is observed that his mean arterial pressure remains unchanged, despite that his lower body is subjected to negative pressure. Which physiological adaptations have occurred? (choose the correct answer at each point):<br> A. The venous return to the heart has decreased*<br> B. The transmural pressure in the heart has decreased*<br> C. The stroke volume has decreased*<br> D. The pulse pressure has decreased*<br> E. The parasympathetic output has decreased*<br> F. The sympathetic output has decreased*<br> G. The heart rate has decreased*<br> H. The total peripheral resistance has decreased* |

For each question, every subquestion had to be answered appropriately to be considered correct. One point was assigned per correct answer, yielding a maximal achievable test score of 6 points. Questions 1 and 2 are recall questions and refer directly to facts that had been introduced during the laboratory exercise. Questions 3 and 4 are intermediate questions. Questions 5 and 6 are integrated questions, of which question 5 is of moderate difficulty and question 6 is of advanced difficulty. *Correct answers.
risk, particularly with regard to fainting during the orthostatic maneuver, as subjects were carefully monitored by the laboratory instructors, both of whom were qualified physicians.

Statistical analyses. Apart from age, all data were considered categorical, and nonparametric methods were applied. When student test results were assessed, 1 point was assigned to each correct question only if all subquestions were answered appropriately. Thus, a total test score ranging from a minimum of 0 points to a maximum of 6 points (2 for recall, 2 for intermediate, and 2 for integrated questions) could be achieved. For the statistical analysis of student evaluations, the student ratings were ranked, so that a rating of “poor” was assigned 0 points, below average 1 point, average 2 points, above average 3 points, and excellent 4 points. A Kruskal-Wallis test was applied to assess an overall difference between groups, and, if significant, groups were compared by means of 2 × 2 tables and Fisher’s exact test for binary data and the Mann-Whitney U-test for ordinal data. P values were subsequently adjusted by Holm’s sequential Bonferroni correction for multiple comparisons. All analyses were performed using SAS version 9.2. P values of < 0.05 were considered statistically significant.

RESULTS

Student characteristics. A total of 57 students were randomized to individual quizzes (5 classes, intervention group I), 56 students were randomized to group quizzes (5 classes, intervention group II), and 42 students served as controls by receiving conventional teaching (4 classes, intervention group III). The three groups were similar with regard to age, sex, and preparation (Table 3).

Test results. There tended to be an overall difference in total test scores between groups (P = 0.05), with students performing individual quizzes receiving the highest scores (median: 5, range: 2–6) followed by students performing group quizzes (median: 4, range: 2–6) and the control group receiving the lowest scores (median: 4, range: 1–5).

There were no differences between groups on either recall, intermediate, or integrated questions (Fig. 2). However, when scores in the two different integrated questions were specifically assessed, there was an overall difference between groups for the integrated question of advanced difficulty, with a significant difference between the individual quiz group and the control group (P < 0.05; Fig. 2). Among students that failed to respond appropriately to the integrated question of advanced difficulty, there was an overall difference between groups as to the number of correctly answered subquestions (P < 0.05); hence, students performing individual and group quizzes responded correctly to more subquestions than the control group (both P < 0.05). There were no other significant differences between groups.

Evaluation. Students were generally positive in their evaluations of the laboratory exercise, regardless of the intervention. Thus, more than half of the students rated the academic gain, the quality of the teaching, and the overall assessment of the laboratory exercise above average or excellent, and their own efforts as average or higher, regardless of the intervention. There were no overall differences between groups for academic gain, own efforts, or the overall assessment (Fig. 3); there was, however, a difference across groups for the quality of the teaching (P < 0.05; Fig. 3), and this was significant between the group quiz and control groups (P < 0.05). There were no other significant differences between groups.

DISCUSSION

In the present study, we found that the immediate learning during a laboratory exercise was similar for recall and intermediate questions, regardless of whether students had been subjected to individual quizzes, group quizzes, or conventional teaching. There was, however, a trend for students that had completed quizzes individually to receive higher scores, and the ability to solve an advanced integrated question was significantly higher in this group than in students subjected to conventional teaching. On the other hand, students performing group quizzes rated the quality of the teaching higher than the other two groups, and this was statistically significant compared with conventional teaching.

The finding that students who had performed individual quizzes reached the highest scores in their responses to the integrated question of advanced difficulty is somewhat surprising and is in contrast to our working hypothesis. Our findings suggest that students who had performed individual quizzes were more successful in constructing mental models appropriate for the transfer of knowledge. On the contrary, previous studies have almost unequivocally found that collaborative learning improve student performance on quizzes (3, 5, 9, 18, 19) and enhances the transfer and retention of knowledge (3, 4, 6). There may be a number of reasons for this discrepancy. First, most of the previous studies evaluated collaborative learning strategies in the context of lec-

Table 3. Student characteristics

<table>
<thead>
<tr>
<th></th>
<th>Individual Quiz Group</th>
<th>Group Quiz Group</th>
<th>Control Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of students</td>
<td>57</td>
<td>56</td>
<td>42</td>
<td>NS</td>
</tr>
<tr>
<td>Mean age (SD), yr</td>
<td>22 (SD 3)</td>
<td>22 (SD 1)</td>
<td>23 (SD 3)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>20/37 (35% male:65% female)</td>
<td>18/38 (32% male:68% female)</td>
<td>14/28 (33% male:67% female)</td>
<td>NS</td>
</tr>
<tr>
<td>Has read the protocol, %</td>
<td>75</td>
<td>75</td>
<td>83</td>
<td>NS</td>
</tr>
<tr>
<td>Has studied the subject in physiology textbooks, %</td>
<td>43</td>
<td>32</td>
<td>26</td>
<td>NS</td>
</tr>
<tr>
<td>Total preparation time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None, %</td>
<td>12</td>
<td>14</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>&gt;30 min, %</td>
<td>25</td>
<td>25</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>30–60 min, %</td>
<td>44</td>
<td>41</td>
<td>57</td>
<td>NS</td>
</tr>
<tr>
<td>60–120 min, %</td>
<td>18</td>
<td>17</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>&gt;120 min, %</td>
<td>2</td>
<td>2</td>
<td>0</td>
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NS, not significant.
tures or conventional class room teaching, that is, “passive” learning environments. In contrast, the present study specifically assessed quiz-based learning strategies during a laboratory exercise, that is, an active, discovery-based learning environment, which facilitates student-teacher and student-student interactions and where student “learn by doing” even without any type of formative assessment (12). Perhaps individual quizzes are more efficient in this context, because it forces students to order their thoughts and consider their understanding of the topic at hand. A major limitation of the present study is, however, that retention was not specifically assessed, for example, by comparing exam scores at the end of the term. It has previously been reported that group quizzes improve retention more so than individual quizzes, as evaluated by exam scores in a combined anatomy and physiology laboratory (8). It was not possible to evaluate retention by means of exam scores in the present study, because an integrated exam on the anatomy, biochemistry, and physiology of the gastrointestinal, cardiovascular, and respiratory systems rather than a separate cardiovascular physiology exam is exerted for second-year medical students at the University of Copenhagen. Furthermore, all tests and evaluation forms were completed anonymously by the students, and test scores thus could not be matched with exam scores. Therefore, we cannot rule out that group quizzes specifically enhanced student retention in the current setup.

Fig. 2. Test scores. A: test scores on recall, intermediate, and integrated questions. The abscissa shows the number of correctly answered questions, and the ordinate shows the percentages of students in the given intervention group. B: specific scores on integrated questions of moderate and advanced difficulty. The ordinate shows the percentage of students in each group that responded correctly. *Overall difference between groups (P < 0.05 by Kruskal-Wallis test); †different from the control group (Holm-Bonferroni-corrected P < 0.05 by Fisher’s exact test).

Fig. 3. Student evaluations. A: academic gain. B: quality of the teaching. C: assessment of students’ own efforts. D: overall assessment. *Overall difference between groups (P < 0.05 by Kruskal-Wallis test); †difference between the group quiz and control groups (Holm-Bonferroni-corrected P < 0.05 by Mann-Whitney U-test).
Test scores were generally high in all three intervention groups, and this may to some extent disguise actual differences between groups. Another factor that may obscure the beneficial effects of collaborative learning in the present study is the relatively short time available to solve the quizzes (10 min for 4 questions for each quiz). Collaborative learning requires the integration of multiple concepts and necessitates that students discuss and explore the topic thoroughly, thus generating connections to other information, which requires time (12). It largely rests on the notion that it is much easier to convince someone who is wrong than it is to convince someone who has selected the correct answers for the right reasons (10), and there is sound evidence that it benefits both high- and low-performing students (5). However, the relatively short time available for group discussion in the present study may have forced less-secure students to “sit on the fence” and simply accept the notions of stronger classmates without actually understanding the underlying physiological rationale. A more time-efficient collaborative approach in this context may be so-called peer instruction, in which student solve the same assignments individually and then in pairs of two (10). Although peer instruction only takes a few minutes per assignment, it has been found to enhance both the transfer and retention of physiological knowledge during conventional ex cathedra teaching (3, 4, 6, 18, 19). Peer instruction has, however, not yet been investigated in the context of a laboratory exercise.

Regardless of the impact of collaborative versus individual problem solving, the present study supports previous studies that have found that formative assessment improves meaningful learning by allowing students to test their knowledge, reflect on their understanding, and correct deficiencies in their mental models (12, 15). It is important to recognize that quiz-based teaching strategies depend on constructive feedback (8, 15), as was done in the present study. The teacher must be prepared to take a highly active role by comprehensively rounding up the quiz session and summarize the key learning objectives to the entire class (11). This allows students to reassess their mental models and repair misconceptions. The effects of the feedback from the laboratory instructor on the individual student’s learning neither can nor should be considered separately from the effects of the quizzes per se. To enhance the learning to all students that participated in the study, the laboratory instructors discussed the test and provided constructive feedback to all students, regardless of intervention, after the test and evaluation form had been handed in.

Students generally evaluated the laboratory exercise with high and similar ratings across groups. However, there was a difference between groups on the quality of the teaching, and students performing group quizzes provided the highest ratings on this matter. This concurs with previous studies (3–5, 7, 8, 18, 19) that have shown that collaborative activities are associated with high levels of student satisfaction. This is possibly because many students tend to lose their apprehensions toward speaking in class during collaborative activities, which apart from student-student interaction may improve student-teacher interaction. Collaborative learning has been found to stimulate more positive relationships among students and enhance their psychological well-being, in effect facilitating a more constructive classroom environment (18). Accordingly, students have been found to prefer collaborative activities to other teaching strategies in previous studies (8, 12, 18, 19). A more positive and relaxed atmosphere among students performing group quizzes may thus have influenced their perception of the quality of the teaching in the present study.

In conclusion, we found that quiz-based teaching strategies improved immediate meaningful learning during a laboratory exercise on baroreflex physiology, an effect that was significant for students performing quizzes individually. Furthermore, student satisfaction with the quality of the teaching was higher in students performing quizzes in groups.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: R.M.G.B., R.R.P., and M.D. analyzed data; R.M.G.B. and R.R.P. interpreted results of experiments; R.M.G.B. drafted manuscript; R.M.G.B., R.R.P., and M.D. edited and revised manuscript; R.M.G.B., R.R.P., and M.D. approved final version of manuscript.

**REFERENCES**