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An innovative laboratory research practice supporting an introduction to research methodology in the medical undergraduate curriculum: the students' perspective

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ABSTRACT

Science teaching and practice is relevant in several curriculums. We aimed to introduce research methodology to first-year medical students. A research project was developed in Biochemistry II curricular unit, applying conventional laboratory techniques and establishing correlations with concepts developed in theoretical and practical classes from Biochemistry I and/or Biochemistry II. An anonymous questionnaire provided the feedback from students participation in this project (190 out of 278 students responded). The research project and scientific poster designing, writing and oral presentation were found interesting, contributed to create motivation for basic laboratory scientific research and allowed development/improvement of scientific thinking, communication skills and technical and scientific language. Experimental data analysis and discussion done by students as well as an increase in team work capacity had a good impact. The investigation project allowed the application of theoretical knowledge into practical activities and was recommended to be maintained in future years (as opposed to conventional laboratory classes). Science teaching and practice should be considered in the first year of the medical curriculum.

INTRODUCTION

In the Faculty of Medicine of the University of Porto (FMUP), teaching of Biochemistry to medical students takes place in two curricular units, occurring sequentially in the two semesters of the first year (Biochemistry I and II, respectively). In the curricular unit of Biochemistry II, in the school year 2010/2011, an innovative laboratory investigation project was developed with the purpose of introducing research methodology, scientific thinking, scientific writing and oral and poster presentation of the results as well as critical appraisal to these first-year students of Medicine, while applying conventional laboratory techniques: an enzymatic activity assay - the alkaline phosphatase (ALP) activity assay [1,2], a lipid oxidative stress marker assay - the thiobarbituric acid reactive substances (TBARS) assay [3, 4], and a protein quantification assay - the Bradford method [5]. The project was also used to establish correlations with biochemistry concepts developed in Biochemistry I and/or Biochemistry II theoretical and practical classes, but without adopting a true problem-based learning approach.

The aim of the project was to investigate/characterize some *in vitro* effects of caffeine, based on some previous knowledge concerning this substance [6-9].

METHODS

The laboratory research practice involved all the students enrolled in Biochemistry II (a total of 278 students, divided into 22 classes) for 11 weeks (2.5h per week). The research project proposed to the students was entitled "*In vitro* effects of caffeine upon ALP activity and lipid peroxidation in rat liver homogenates". The overall aim of this project was to investigate some specific *in vitro* properties of caffeine, a methylxanthine present in several beverages of human consumption, relating to theoretical knowledge learned in Biochemistry I and/or II (fat digestion and absorption, lipogenesis and fatty acid esterification, lipoprotein metabolism, redox status, oxidative stress and antioxidants).

The first specific aim of the project related to the fact that caffeine is known to possess an anti-obesity effect [10] and that, besides other mechanisms (eg. appetite suppression and thermogenesis increase), inhibition of fat intestinal absorption might be involved in this metabolic effect of caffeine [6]. Because intestinal ALP limits the intestinal absorption of fat [11] and intestinal ALP increases in the liver after fat feeding [12], one specific aim of this project was to investigate if caffeine affects ALP activity in the liver, *in vitro*.

The second specific aim of the project related to the fact that caffeine possesses antioxidant properties *in vivo* [7-9]. Oxidative stress, which is caused by an imbalance between the production of free radicals and/or reactive oxygen or nitrogen species versus the ability of biological systems to detoxify the various forms of activated species, causes damage to nucleic acid bases, carbohydrates, lipids and proteins, which can severely compromise cellular functions, ultimately leading to cell death. Accordingly, oxidative stress is associated with numerous human conditions, from atherosclerosis to neural degenerative diseases, inflammation, cancer and ageing [13]. The other specific aim of the project, therefore, was to investigate whether caffeine possesses antioxidant properties, *in vitro*.

In the 1st week, a complete explanation of the research project was provided: the theoretical background was described, the aims of the investigation were discussed, the 3 above mentioned techniques were explained and some bibliographic references were given. Spectrophotometry, in what regards absorvance, transmittance and calibration curve concepts as well as the Beer-Lambert-Bouguer law, was explained [14]. The students were introduced to the online search of scientific information [by the use of search engines for scientific information such as PubMed, ISI Web of Knowledge and the online library of the Faculty of Medicine of the University of Porto (http://biblioteca.med.up.pt/)] and to the significance of scientific articles (by taking into consideration the journal impact factor and the number of times a given article is cited).

In the 2nd week, the calibration curves for *p*-nitrophenol (for the ALP activity assay, since *p*-nitrophenylphosphate was used as the ALP substrate [1,2]), malondialdehyde (for the TBARS assay [3,4]) and albumin (for the protein quantification assay [5]) were determined. In each class, the students were divided into small groups (3-4 students per group) and every group determined each one of the calibration curves. These calibration curves were later used in the calculations.

From the 3rd to the 6th week, the students, still divided in groups, performed, under their teacher supervision, the experimental protocols concerning determination of ALP activity, lipid peroxidation levels and protein content in rat liver homogenates. In the ALP assay, caffeine (0.1 and 1 mM) was tested alone or in combination with levamisol [0.5 mM, a specific inhibitor of one of the two ALP isoenzymes expressed in the liver (tissue-non-specific and intestinal ALPs), namely the tissue non-specific ALP, allowing an estimate of the effect of caffeine upon tissue-non-specific and intestinal ALP activities]. The characterization of the caffeine antioxidant effect was evaluated by testing the effect of caffeine alone (0.1 and 1 mM) or in combination with *tert*-butylhydroperoxide (2 mM, an oxidative stress inducer that was also tested alone), upon lipid peroxidation levels, by using the TBARS assay. Since different liver homogenates were used in these two assays, protein content was evaluated in each homogenate with the Bradford method [5]. During these 4 classes (2 classes were assigned for each of the techniques, ALP and TBARS assays, with protein levels of the homogenates being quantified in each class), the students were able to discuss and/or apply with their group members, as well as with their teacher: a) the spectrophotometry concepts acquired in Biochemistry II (in the first laboratory class); b) the enzyme kinetic concepts acquired in Biochemistry I theoretical and practical classes; and c) the articles found by themselves in the online search, to be used for the discussion of the results. Moreover, the students were able to establish correlation between: a) the theoretical background of the project and some metabolic pathways learned in Biochemistry I and II theoretical classes, and b) the relevance, in a first glimpse, of the results being obtained and the cellular redox status, oxidative stress and antioxidants themes learned in Biochemistry II theoretical classes.

In the 7th and 8th weeks, calculations were made, in order to express the results as nmol *p*-nitrophenol/min/mg protein (ALP activity) and as nmol malondialdehyde/mg protein (lipid peroxidation levels). The students, under their teacher supervision, analyzed the results using the appropriate statistical methods (Student's t-test, one-way ANOVA and/or nonparametric tests) and constructed the corresponding graphs [since these specific statistical competencies were acquired by the students during the 1st year curricular units of Introduction to Medicine I (first semester) and Introduction to Medicine II (second semester)].

In the 9th and 10th weeks, every group of students, which were given the task of preparing a poster concerning this investigation project, worked on it. An analysis and discussion of all the differences found (or not) was made, including, if necessary, further bibliographical research to support/explain the results obtained. Moreover, the poster was prepared exclusively by the students (including poster structure and organization, selection of results and the corresponding discussion and conclusions) – but the teacher could be called to solve any problem or to clarify any doubt. The students' involvement and enthusiasm in elaborating the poster were evident and strong since the contact time between students and their respective teacher, for this task, clearly exceeded the 5h period of the two practical classes.

In the 11th week, every group of students presented their poster orally to their colleagues and teacher. In every class, each poster was discussed and the best poster was selected by the students and the teacher. This selection involved a very lively discussion among students and between students and their teacher.

Thereafter, the 22 best posters were exhibited in the Biochemistry Department and evaluated by an independent jury composed by investigators and PhD students of the Department. This poster exhibition could be seen by all the Faculty personnel for 2 weeks. The best of the 22 posters was selected and the result of this evaluation was published in the FMUP web page (http://bioquimica.med.up.pt/ensino/index.html). The group of winning students was given a book written by one the Department investigators.

Additionally, the overall individual performance of each student was evaluated throughout this process and the classification obtained contributed to 10% of the final classification of the curricular unit.

In the last week of Biochemistry II, a questionnaire, including both specific questions (Table 1) and space for free text comments, was distributed to all the students in order to get feedback from the participation in the research project. This questionnaire was anonymous.

RESULTS

The results of the questionnaire are presented in Table 1, in a scale from 1 to 5, corresponding to an increasing agreement/relevance. The questionnaire was filled in by a representative sample (68.3%) of the overall population of the students enrolled in Biochemistry II. No written comments were obtained in the free space of the questionnaire.

DISCUSSION

Laboratory investigation projects, where the students "act as scientists", have been introduced in the two Biochemistry curricular units (Biochemistry I and II), in the Department of Biochemistry of FMUP, more than ten years ago [2,15-18]. All the research projects developed so far aimed to verify a new hypothesis, regarding a subject that both students and teachers were interested in (what creates a common discovery process and facilitates the improvement of the student-teacher relationship) [15,19].

Here, we show that our first-year medical students found the 2010/2011 research project interesting (3.50±0.07; score 4 was chosen by 45% of the students). Similarly, they found that a) its main outcome in terms of student evaluation and recognition of achievement (the preparation and presentation of a scientific poster) was an interesting exercise (3.73±0.08; scores 3, 4 and 5 were chosen by 25, 38 and 26% of the students, respectively), and b) the research project contributed to create motivation for basic/laboratory scientific research (3.46±0.08; score 4 was chosen by 41% of the students). Students have willing and enthusiastic spirits and are highly motivated to learn, which makes them vital participants in research projects and, at the same time and most importantly, active players in a discovery process that is relevant for them, thus positively modulating their cognitive centers [15,20].

Incorporation of a medical school research project in the fourth year of the medical curriculum increases research skills, is a useful replacement for traditional clinical elective rotations and impacts favorably on future medical careers [19]. Similarly, it has been reported that attendance in a course on research methodology by second year medical students is related to a positive, but short-term, effect on students' interest and attitudes toward science and scientific method and research in Medicine [21,22]. According to the authors of that study, maintenance of the positive effect could be achieved by the vertical integration of scientific research in the medical curriculum, which should have

Table 1. Results of the students' evaluation of their participation in the laboratory research project (curricular unit of Biochemistry II), during the second semester of the school year 2010/2011. The questionnaire consisted of a series of questions classified from 1 to 5, corresponding to an increasing agreement/relevance.

Question	Score					Mean	SEM	*n*
	1	2	3	4	5			
1. *The laboratory investigation project allowed the appliance of theoretical knowledge into practical activities*	1.1%	10.6%	29.1%	44.4%	14.8%	3.614	0.066	189
2. *The laboratory investigation project allowed the analysis and the discussion of the experimental data*	0.5%	1.1%	13.2%	53.2%	32.1%	4.153	0.052	190
3. *The laboratory investigation project allowed the development or the improvement of scientific thinking, technical and scientific language and communication skills*	0.5%	6.9%	22.2%	46.6%	23.8%	3.862	0.064	189
4. *The laboratory investigation project was interesting*	4.8%	8.5%	30.3%	44.7%	11.7%	3.500	0.071	188
5. *The laboratory investigation project contributed to create motivation for basic/laboratory scientific research*	4.7%	12.1%	29.0%	40.5%	13.7%	3.463	0.075	190
6. *The laboratory investigation project contributed to increase team work capacity*	1.6%	7.4%	18.0%	48.7%	24.9%	3.874	0.067	190
7. *The preparation and presentation of a scientific poster was an interesting exercise*	4.8%	6.4%	25.0%	38.3%	25.5%	3.734	0.077	188
8. *I would rather prefer the conventional laboratory classes where a specific technique/assay is presented*	35.8%	33.2%	19.0%	5.8%	6.3%	2.137	0.084	190
9. *The laboratory investigation project should be maintained in future years*	4.2%	6.3%	16.9%	37.6%	34.9%	3.926	0.078	189

SEM=standard error of the mean.

an early start in the medical curriculum [22]. Importantly, increasing and/or improving positive attitudes toward scientific research among medical students may contribute to the development of critical thinking abilities and critical appraisal skills needed for learning and practicing evidence-based medicine [19,21-23].

Journal clubs and scientific writing are excellent tools for teaching critical appraisal skills [24-28] – and this was began with our students in the first semester, in Biochemistry I. Writing and publishing determines successful medical career development, creating reputation by disseminating expertise and accomplishments [28]. On two distinct occasions, two previous research project results have been orally presented in scientific meetings by our students [29,30].

In line with this, our students revealed that the investigation project allowed the development or the improvement of scientific thinking, technical and scientific language and communication skills (3.86±0.06; score 4 was chosen by 47% of the students), where a) the analysis and the discussion of the experimental data done by the students, either alone or in collaboration and/or under the supervision of the teacher (4.15± 0.05; score 4 was chosen by 53% of the students), b) the designing, writing and oral presentation of the poster, and c) the increase of team work capacity (3.87±0.07; score 4 was chosen by 49% of the students) had a strong impact. The bibliographic search, and its reading and discussion, were also important factors in the above results. Communication skills are important to clinicians [31] and scientific writing has been reported inseparable from literature-based learning [32], its practice being important in the medical curriculum [33,34].

Engaging students in scientific discovery and methodology improves learning and knowledge retention [35], and our results showed that students considered that the laboratory investigation project allowed the application of theoretical knowledge into practical activities (3.61±0.07; score 4 was chosen by 44% of the students) and should be maintained in future years (3.93±0.08; scores 4 and 5 were chosen by 38 and 35% of the students, respectively) [in opposition to the conventional laboratorial classes (2.14±0.08; scores 1 and 2 were chosen by 36 and 33% of the students, respectively)]. Of note, the students attended some conventional laboratory classes in the previous semester, in the curricular unit of Biochemistry I (e.g. demonstration of the *in vitro* activity of succinate dehydrogenase) and so a direct comparison between the two teaching methodologies could be made.

In conclusion, it is well known that teaching should be approached with the same rigor as science at its best [35] and here we showed that science teaching should be considered in the first year of the medical curriculum.

Declaration: The authors declare that they have no conflict of interest.

REFERENCES

1. McComb RB, Bowers Jr GN, Posen S. Alkaline Phosphatase. 1979. New York: Plenum Press.
2. Mota A, Silva P, Neves D, Lemos C, Calhau C, Torres D, Martel F, Fraga H, Ribeiro L, Alçada MN, Pinho MJ, Negrão MR, Pedrosa R, Guerreiro S, Guimarães JT, Azevedo I, Martins MJ. Characterization of rat heart alkaline phosphatase isoenzymes and modulation of activity. Braz J Med Biol Res 2008; 41:600-9.
3. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52:302-10.
4. Kozmus CE, Moura E, Serrão MP, Real H, Guimarães JT, Guedes-de-Pinho P, Duarte BP, Marques F, Martins MJ, Vieira-Coelho MA. Influence of dietary supplementation with dextrin or oligofructose on the hepatic redox balance in rats. Mol Nutr Food Res 2011; 55: 1735-9.
5. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.
6. Wang S, Noh SK, Koo SI. Epigallocatechingallate and caffeine differentially inhibit the intestinal absorption of cholesterol and fat in ovariectomized rats. J Nutr 2006; 136: 2791-6.
7. Nobre Jr HV, Cunha GM, de Vasconcelos LM, Magalhães HI, Oliveira Neto RN, Maia FD, de Moraes MO, Leal LK, Viana GS. Caffeine and CSC, adenosine A_2A antagonists, offer neuroprotection against 6-OHDA-induced neurotoxicity in rat mesencephalic cells. Neurochem Int 2010; 56:51-8.
8. Inkielewicz-Stepniak I, Czarnowski W. Oxidative stress parameters in rats exposed to fluoride and caffeine. Food Chem Toxicol 2010; 48:1607-11.
9. Prasanthi JR, Dasari B, Marwarha G, Larson T, Chen X, Geiger JD, Ghribi O. Caffeine protects against oxidative stress and Alzheimer's disease-like pathology in rabbit hippocampus induced by cholesterol-enriched diet. Free Radic Biol Med 2010; 49:1212-20.
10. Zheng G, Sayama K, Okubo T, Juneja LR, Oguni I. Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, in mice. In Vivo 2004; 18:55-62.
11. Narisawa S, Huang L, Iwasaki A, Hasegawa H, Alpers DH, Millan JL. Accelerated fat absorption in intestinal alkaline phosphatase knockout mice. Mol Cell Biol 2003; 23:7525-30.
12. Goseki-Sone M, Oida S, Iimura T, Yamamoto A, Matsumoto HN, Omi N, Takeda K, Maruoka Y, Ezawa I, Sasaki S. Expression of mRNA encoding intestinal type alkaline phosphatase in rat liver and its increase by fat-feeding. Liver 1996; 16:358-64.
13. Halliwell B. Biochemistry of oxidative stress. Biochem Soc T 2007; 35:1147-50.
14. Hipólito-Reis C, Alçada MN, Azevedo, I. (Eds.). Práticas de Bioquímica para as Ciências da Saúde, 1st ed. 2002. Lisboa: Editora Lidel.
15. Martins MJ, Alçada N, Azevedo I. A prática da investigação científica na aprendizagem da bioquímica numa faculdade de Medicina. In: Sentidos da pedagogia no ensino superior, ed Leite C, pp. 129-140. 2010a. Porto: CIIE/Livpsic.
16. Martins MJ, Neves D, Rebelo S, Azevedo I. Abordagem multidisciplinar de um problema científico na pré-graduação em medicina. Implicações na aprendizagem. In: La innovación educativa en el contexto actual de la educación superior, ed Barcía I, pp. 241-245. 2010b. Vigo: Vicerreitoría de Formación e Innovación Educativa, Universidade de Vigo.
17. Ribeiro L, Calhau C, Pinheiro-Silva S, Santos A, Alçada M, Guimarães J, Pina A, Maia B, Pereira F, Pimentel F, Lopes H, Santos L, Gonçalves N, Cunha S, Viana P, Almeida J, Lopes A, Azevedo I. Impact of acute exercise intensity on plasma concentrations of insulin, growth hormone and somatostatin. Acta Med Port 2004; 17:199-204.
18. Guerreiro S, Alçada MN, Azevedo I. Sugary drinks and glycemia. Acta Med Port 2010; 23:567-78.
19. Frishman WH. Student research projects and theses: should they be a requirement for medical school graduation? Heart Dis 2001; 3:140-4.
20. Liddell PW, Heuertz RM. Students as vital participants in research projects. Clin Lab Sci 2011; 24:66-70.
21. Hren D, Lukić IK, Marusić A, Vodopivec I, Vujaklija A, Hrabak M, Marusić M. Teaching research methodology in medical schools: students' attitudes towards and knowledge about science. Med Educ 2004; 38:81-6.
22. Vujaklija A, Hren D, Sambunjak D, Vodopivec I, Ivanis A, Marusić A, Marusić M. Can teaching research methodology influence students' attitude toward science? Cohort study and nonrandomized trial in a single medical school. J Investig Med 2010; 58:282-6.
23. Ghali WA, Saitz R, Eskew AH, Gupta M, Quan H, Hershman WY. Successful teaching in evidence-based medicine. Med Educ 2000; 34:18-22.
24. Sandifer QD, Lo SV, Crompton PG. Evaluation of a journal club as a forum to practise critical appraisal skills. J R Coll Physicians Lond 1996; 30:520-2.

25. Edwards R, White M, Gray J, Fischbacher C. Use of a journal club and letter-writing exercise to teach critical appraisal to medical undergraduates. Med Educ 2001; 35:691-4.

26. Alguire PC. A review of journal clubs in postgraduate medical education. J Gen Intern Med 1998; 13:347-53.

27. Wong VS. Write. Acad Med 2010; 85:1724.

28. Derish PA, Maa J, Ascher NL, Harris HW. Enhancing the mission of academic surgery by promoting scientific writing skills. J Surg Res 2007; 140:177-83.

29. Marques ARM, Martins H, Martins MJ. Modulação da actividade da fosfátase alcalina hepática por ácidos gordos. Paper presented at the IX Jornadas Científicas de Estudantes de Medicina. 2006 (April). Porto, Portugal.

30. Martins H, Gonçalves MC, Azevedo I, Martins MJ. Modulação da fosfátase alcalina hepática e cardíaca por ácidos gordos. Paper presented at the V Encontro FIIP. 2006 (September). Porto, Portugal.

31. Jason H. Communication skills are vital in all we do as educators and clinicians. Educ Health (Abingdon) 2000; 13:157-60.

32. Colabroy KL. A writing-intensive, methods-based laboratory course for undergraduates. Biochem Mol Biol Educ 2011; 39:196-203.

33. Shah J, Shah A, Pietrobon R. Scientific writing of novice researchers: what difficulties and encouragements do they encounter? Acad Med 2009; 84:511-6.

34. Goldhamer ME, Cohen AP, Bates DW, Cook EF, Davis RB, Singer DE and Simon SR. Protecting an endangered species: training physicians to conduct clinical research. Acad Med 2009; 84:439-45.

35. Handelsman J, Ebert-May D, Beichner R, Bruns P, Chang A, DeHaan R, Gentile J, Lauffer S, Stewart J, Tilghman SM, Wood WB. Education. Scientific teaching. Science 2004; 304:521-2.