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Pizza and pasta help students learn metabolism

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**Passos, Renato M., Alexandre B. Sé, Vanessa L. Wolff, Yanna
K. M. Nobrega, and Marcelo Hermes-Lima.** Pizza and pasta help
students learn metabolism. *Adv Physiol Educ* 30: 89–93, 2006;
doi:10.1152/advan.00044.2005.—In this article, we report on an ex-
periment designed to improve the learning of metabolic biochemistry
by nutrition and medical undergraduate students. Twelve students
participated in a monitored lunch and had their blood extracted for
analysis *1*) before lunch, *2*) 30 min after lunch, and *3*) 3 h after lunch.
The subjects were divided in two groups. One group had a hypergli-
cidic meal [pasta plus orange juice: 80% carbohydrate, 10% protein,
and 10% lipid (estimated values)] and the other group had a hyperli-
pidic meal (calabresi pizza plus diet soda: 36% carbohydrate, 18%
protein, and 46% lipid). Individual quantities of food were based on
body mass index, age, and sex. The blood parameters analyzed were
glucose, triglycerides (TG), and urea. Glucose remained constant in
the three measurements in both groups. The TG concentration in the
pasta group was constant before and after lunch but increased signif-
icantly during the evening. In the pizza group, TG increased after
lunch and remained constant in the evening. Levels of urea increased
only in the evening, specially in the pizza group. These results were
used for the final biochemistry exam. With the maximum score set as
10, the average score was 6.0 ± 2.4 ($n = 102$). We considered this
activity a unique way of evaluating important issues on metabolism,
because students had several hours to work on the final exam (with
free access to a bibliography). It was also a good didactic experience
(problem-based learning like) for the subject students, because they
had to work in all phases of the experiment (idealization, realization,
and analysis) and participated actively in the elaboration and correc-
tion of the exam.

physiology; biochemistry; nutrition; medical education; problem-
based learning

A PREVIOUS REPORT from Hermes-Lima and co-authors (6) has
described the importance of student seminars on clinically
relevant topics of metabolic biochemistry (including obesity,
diabetes, cholesterol-related diseases, iron metabolism, and
free radicals) for medical and nutrition students at the Univer-
sity of Brasilia (Brasília, Brazil) enrolled in a basic biochem-
istry course. In this article, we report on an experiment de-
signed to improve the teaching/learning of metabolic biochem-
istry. Our goal is to illustrate the basic concepts of human
metabolism through a simple experiment using undergraduate
students as subjects.

The experiment consisted in verifying whether different
meals, one carbohydrate rich (pasta) and another fat rich
(pizza), would have different effects in certain blood biochem-
ical parameters. Our purpose was to demonstrate the behavior,

throughout the day, of plasma glucose, triglyceride (TG), and
urea levels in response to the composition of the meals taken
by the volunteers. The blood results, along with the description
of the experimental protocol, were then converted in questions
for the final biochemistry exam to assess students' comprehen-
sion of human metabolism. Their scores were then analyzed
and are presented here.

The Pizza-and-Pasta Experiment

Twelve healthy students from our Advanced Biochemistry
course (from the first or second year of medicine or nutrition)
volunteered to participate in the experiment. They were di-
vided in two groups, each composed of three men and three
women, arranged in a way so that the physical parameters were
the most homogeneous possible, as shown in Table 1 (there
were no statistical differences between groups). The 12 volun-
teers gave written consent to participate in this study; 11 of
them were Caucasian-Brazilian and 1 was an African-Brazilian
female. The protocols used in this study were also reviewed
and approved by the University of Brasilia Ethical Committee.

The first group was to have a hyperglicidic meal (HG group)
and the other group was to have a hyperlipidic meal (HL
group). The HG meal consisted of spaghetti, tomato sauce, and
orange juice. The HL meal consisted of calabresi pizza and diet
soda. Both meals were isocaloric (averaging ~950 kcal for
men and ~700 kcal for women), covering ~35% of the daily
caloric requirements (this was defined taking into consideration
the weight, body mass index, and sex of each individual). A
certified nutritionist (Janini Ginani) obtained the estimated
nutritional composition of the meals (see Table 2) based on
well-known data from laboratories of dietetic analysis (14) and
calculated the portion of food to be ingested by each subject.

On the day of the experiment (August 15, 2002), subjects
were instructed to eat a light Brazilian-type breakfast (ideally,
the breakfast meal should also have been standardized and
supervised by a nutritionist to ensure full uniformity) and
arrive at the University Hospital laboratory to collect the
baseline blood sample at noon (premeal sample). After that, the
12 subjects and the coordinating teacher (M. Hermes-Lima)
went to a nearby restaurant for the supervised lunch, which
lasted ~1 h, that is, from 1:00 to 2:00 PM. From 2:30 to 3:00
PM, the second sample (postmeal sample) was collected, and
from 6:30 to 7:00 PM, the third sample (evening sample) was
collected. All subjects were instructed not to eat or drink
beverages between the collection of the samples.

Blood Biochemistry: Methods and Results

Methods. The blood samples were collected according to
pattern H3–A3 of the National Committee for Clinical Stan-
dards. Venipuncture was performed by Y. K. M. Nobrega, a

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Table 1. *Physical parameters of the subjects*

	HG Meal		HL Meal		Average	
	Men	Women	Men	Women	Men	Women
Age, yr	18.3 ± 0.9	20.7 ± 0.3	19.3 ± 0.3	19.3 ± 0.3		
Weight, kg[1]	80.3 ± 2.2	53.3 ± 1.7	66.7 ± 4.4	53.0 ± 2.7	66.8 ± 6.1	59.9 ± 3.8
Height, m[2]	1.84 ± 0.03	1.63 ± 0.04	1.76 ± 0.02	1.67 ± 0.04	1.74 ± 0.05	1.72 ± 0.03
Body mass index, kg/m^2	23.7 ± 1.4	20.2 ± 0.9	21.6 ± 1.9	19.2 ± 0.9	22.0 ± 1.0	20.4 ± 1.1

All values are means ± SE; $n = 3$ men and 3 women in each of the hyperglicidic (HG) and hyperlipidic (HL) groups. The HG meal consisted of pasta and orange juice, and the HL meal was pizza and diet soda. None of the parameters were significantly different (one-tailed *t*-tests) when the HG group was computed with the HL group.

registered pharmacist with long-time practice in the procedure. After the collection of blood, the samples were identified, centrifuged, and kept at 5°C. All biochemical determinations were performed on the same day. Determination of glucose, TG, and urea were performed by well-known clinical methods at the University Hospital laboratory. The description of the methods below is just for didactic purposes.

Glucose was determined by the method of glucose-oxidase and peroxidase. Glucose is converted to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide then reacts with 4-amino antipyrine and formaldehyde, in the presence of peroxidase, forming violet-colored quinoneimine, which is then quantified by spectrophotometry at 545 nm.

Determination of TG was performed by means of the enzymatic-colorimetric method of lipid clearance factor. In this method, TG is hydrolyzed by lipoprotein lipase to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol-3-phosphate by glycerol kinase and subsequently converted into dihydroxyacetone-phosphate and hydrogen peroxide by glycerol oxidase. Finally, hydrogen peroxide is determined by means of quinoneimine formation, as described above.

Urea was quantified using urease, which hydrolyzes urea into ammonia and carbon dioxide. Ammonia then reacts with α-ketoglutarate and NADH (catalyzed by glutamate dehydrogenase), forming glutamate, NAD^+, and water. The decrease in absorbance at 340 nm due to the oxidation of NADH to NAD^+ was monitored and compared with the stardard curve of urea.

Biochemical data from males and females eating the same meal were analyzed together to simplify statistics. Results are shown as means ± SE, with $n = 6$ subjects/group. Statistical analysis among different periods (premeal, postmeal, and evening) was performed by one-way ANOVA; when a significant *F*-ratio was found, a Student-Newman-Keuls post-

ANOVA test was performed. Comparisons between different groups (HG and HL) were done by Student's *t*-test. Significance was accepted at $P < 0.05$.

Glucose in plasma. Glucose remained constant throughout the three periods and did not show any statistically relevant difference between groups (see Table 3).

The pattern observed for plasma glucose exemplifies an important mechanism of homeostasis: the regulation of glycemia by the pancreatic hormones insulin and glucagon. Other metabolic principles could also be discussed using these data, including the relevance (or not) of hepatic glycogen synthesis, hepatic and muscular glycogenolysis, and hepatic gluconeogenesis during the three phases of the experiment as well as the need to maintain glucose supply to the brain (4, 12).

Moreover, the relatively long period for glucose determination after the meal was a main cause for the lack of any observed change in blood glucose. The perception of this experimental problem was required for the students' answers. In any case, the description of the role of insulin for glucose metabolism was required.

TG in plasma. The plasma levels of TG are depicted in Fig. 1. These results were very useful because they allowed multiple analyses. In the HL group, as expected, TG was significantly increased right after lunch (from 75.5 ± 7.4 to 92.7 ± 11.1 mg/dl) because of the high fat content of the pizza. During the afternoon, the apparent increase in TG in the HL group (from 92.7 ± 11.1 to 100.7 ± 15.7 mg/dl) was not significant compared with the postmeal sample.

In the HG group, the TG concentration remained unchanged after lunch (premeal: 77.2 ± 3.9 mg/dl; postmeal: 79.2 ± 9.1 mg/dl). This outcome was expected considering the low fat content of pasta with tomato sauce. Interestingly, levels of TG increased significantly in the evening, reaching 96.8 ± 9.6 mg/dl. Hepatic fatty acid biosynthesis along with TG formation resulted from the use of carbohydrates from pasta (in high content) as primary substrates. This must explain the higher levels of TG in the evening. These results are quite illustrative of the dynamic interconnection between fat and carbohydrate metabolism (4, 17).

Table 2. *Composition of the meals*

Meal	Carbohydrate, %	Protein, %	Lipid, %
HG	80	10	10
HL	36	18	46

Estimated percentages used in the biochemistry exams were obtained from well-known tables of dietetic composition in Brazil (14). On October 2005, the authors contacted the restaurant (Felicità) to perform a real analysis of the meals used in the experiment (9, 13) to validate the numbers and discussions presented in this work. For the pizza, the real values were very similar to the estimates (variation of 1.17-fold), and, for the pasta, only the lipid levels showed a relevant difference (17% instead of 10%), due to a greater amount of oil used for pasta preparation in that particular restaurant. However, this fact did not change the interpretation of the results.

Table 3. *Glucose measurements in both groups throughout the day*

Meal	Premeal	Postmeal	Evening
HG	95.7 ± 1.5	96.3 ± 4.1	91.2 ± 3.6
HL	94.7 ± 3.0	97.8 ± 4.1	96.2 ± 1.8

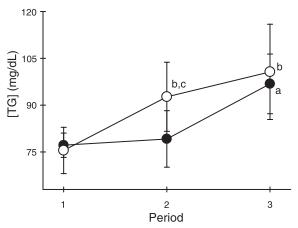Values are means ± SE (in mg/dl). There were no statistically relevant differences among periods or groups.

Fig. 1. Triglyceride (TG) concentration (in mg/dl) during the following periods: premeal (*period 1*), postmeal (*period 2*), and evening (*period 3*). HG, hyperglicidic meal (●); HL, hyperlipidic meal (○). [a]Significantly different from *periods 1* and *2*; [b]significantly different from *period 1*; [c]significantly different from the respective HG group. Values are means ± SE; *n* = 6.

Urea in plasma. The levels of plasma urea are shown in Fig. 2. Urea remained constant in both groups after lunch (pre- and postmeal urea levels in both groups: 24–25 mg/dl) and increased significantly during the afternoon only in the HL group (evening sample: 32.8 ± 2.1 mg/dl). An apparent increase in plasma urea concentration was seen in the HG group (to 27.3 ± 2.0 mg/dl), although it was not significant. These results suggested a greater protein catabolism in the group eating pizza, which was not evident in the HG group because of the lower protein content of that meal (see Table 2).

The Final Biochemistry Exam

As stated previously, the experiment was converted into one exercise (subdivided into three parts) for the final exam of the basic biochemistry course. This 90-h course, named Bioquímica e Biofísica (6), is obligatory for first-semester students of nutrition and medicine at the University of Brasilia. All the basic facts and concepts of the integrated metabolism of carbohydrates, fats, and proteins are covered and discussed during the course [in a problem-based learning (PBL)-like approach, always based on recent scientific papers and related to practical situations, as metabolic diseases and extreme physiological conditions] before the final exam. On the exam, we provided a detailed description of the experimental protocol, depicting all blood biochemistry results in figures and tables (the same as shown in this report). The following three questions were asked:

A. Why did plasma glucose levels remain constant in both groups (HG and HL) throughout the day?

B. Explain the variations in plasma TG levels along the day. Why did plasma TG behave differently (comparing HG and HL groups) after lunch and in the afternoon?

C. Explain the changes in plasma urea levels during the afternoon. Why were these more evident in the HL group?

This final exam is a decade-long tradition in our basic biochemistry course. It is an extensive exam comprising broad questions, always regarding a recent scientific paper, an experiment (as in this present case), or any pertinent theme related to applied biochemistry (18). Students with similar grades from previous exams are allowed to work in pairs and to bring

whatever printed bibliography they feel necessary to be used during the exam. The pizza and pasta questions covered about 30% of the final exam's total grade.

In this model of exam, questions have no exact or perfect answers. Our objective is, instead, to assess students' comprehension of integrated metabolism, working on higher cognitive levels of Bloom's taxonomy: application, analysis, synthesis, and evaluation (2). Thus the answers were assigned a score from 0 to 10 based on the following criteria:

- Correct understanding of fat, carbohydrate, and amino acid metabolism;
- Correct understanding of hormone-regulated and integrated metabolism;
- Correct application of concepts of integrated metabolism in new situations; and
- Correct use of simple statistical concepts applied to blood biochemistry (the teacher previously discussed with students the meaning of simple statistical differences and their clinical application).

Because the students were allowed to consult the bibliography during the exam, it was not required for them to "copy and paste" theoretical details (i.e., metabolic pathways) from the books to answer the questions. On the contrary, we encouraged them to present synthetic and analytic answers. To exemplify that, we include herein one example of each answer that received the maximum score (see the APPENDIX). They describe concisely and thoroughly the metabolic concepts involved in each question, reflecting full comprehension by these students.

The pizza and pasta questions were used in the final exam on 3 occasions, for a total of 102 exams. Mean scores, on a 0–10 basis, were 6.5 ± 2.2 (*n* = 34), 6.0 ± 2.7 (*n* = 35), and 5.6 ± 2.3 (*n* = 33) for the first-semester class of 2002, second-semester class of 2002, and second-semester class of 2003, respectively. The mean score considering the three classes together was 6.0 ± 2.4 (*n* = 102). The relative distribution of total scores is shown in Table 4. About 51% of the students presented scores of 6.1 or above (which we consider a satisfactory score), whereas 24% scored 4.0 or below (very unsatisfactory scores).
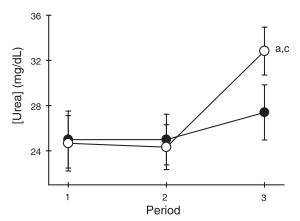

Fig. 2. Urea concentration (in mg/dl) during the following periods: premeal (*period 1*), postmeal (*period 2*), and evening (*periold 3*). ●, HG meal; ○, HL meal. [a]Significantly different from *periods 1* and *2*; [c]significantly different from the respective HG group. Values are means ± SE; *n* = 6.

Table 4. *Scores of the pizza and pasta questions by the classes of the first semester of 2002, second semester of 2002, and second semester of 2003*

	Grades					
Class	0.0–2.0	2.1–4.0	4.1–6.0	6.1–8.0	8.1–10	Total
First semester of 2002	1 (3)	5 (15)	6 (17)	10 (30)	12 (35)	34 (100)
Second semester of 2002	4 (12)	4 (12)	6 (18)	11 (32)	8 (23)	35 (100)
Second semester of 2003	3 (9)	8 (24)	13 (39)	5 (15)	6 (18)	33 (100)
All	8 (8)	17 (16)	25 (25)	26 (25.5)	26 (25.5)	102 (100)

Values are the numbers of exams in each grade interval; numbers in parentheses are gross percentages of the total for each class.

Conclusions and Perspectives

The pizza and pasta experiment described herein made it possible to cover in the final exam various important topics of metabolism and to demonstrate how the body's metabolic machinery reacts to different diets depending on their composition. This was especially valuable for students pursuing a future residence in endocrinology, general clinical medicine, or clinical nutrition (7, 16). Moreover, because students had plenty of time (8–9 h) to work on the final exam, they had the opportunity to apply their knowledge of metabolism (and discuss issues with an exam mate, having books and notes in hand) while answering the questions. Besides, knowing that the exam contemplated a real experiment done with their peers might represent a stimulus for answering the questions, which we call an "emotional bond" between the two groups of students (the ones taking the exam and the ones who participated in the experiment). Notwithstanding, because we did not have a "control group" tested in a conventional method, we cannot conclude that this exam was superior in a pedagogical sense. Still, the analysis of the grades show that the students' performance was satisfactory, and thus the method of evaluation is valid.

We believe the activity was not only helpful for the students taking the exam but, even more so, for those who participated as volunteers. These students, who also worked as peer tutors of Bioquímica e Biofísica (6) and were enrolled in our Advanced Biochemistry course, participated in all levels of the experiment: planning, execution, and elaboration of the exam and the overall analysis of the data, similar to other teaching experiences reported in the literature (3, 8). Moreover, it is relevant to mention that the correction of the final exam was done as a team activity between M. Hermes-Lima and the peer tutors, making this a rich didactic experience for the latter. Each semester, the best students from Bioquímica e Biofísica (about 20%; see Ref. 6) are selected for the Advanced Biochemistry course, where they generally have the opportunity to participate in activities of similar nature to the one currently reported (including a broad discussion of real results from the biomedical literature). We consider this to be another stimulus for the learning of metabolic biochemistry.

This system of teaching/learning and evaluation fits in the greater philosophy of PBL used in many medical schools throughout the world, especially in the United States and Canada (10, 15, 19). The main difference, in our case, is that students are also evaluated in a problem-based context, besides being taught in a classical PBL approach, already used in our course. It is reasonable to say, though, that the students who participated in the experiment benefited from a genuine PBL-like experience, because they had to work in all stages of it, as stated above. Moreover, for the students of the first-semester class of 2004, second-semester class of 2004, and first-semester class of 2005, the pizza and pasta questions were incorporated into class-time discussion, because they provided a broad and synthetic view of integrated metabolism. Indeed, these students demonstrated a great interest about the experiment, discussing and suggesting metabolic interpretations for the results.

For future classes, we intend to expand the scope and usefulness of the pizza and pasta results. Several other topics can be discussed, including what would be expected in the blood biochemistry (or even urinalysis) of diabetic and obese subjects or even elite athletes. Interestingly, it has been reported in the literature a case of exaggerated hyperglycemia after a pizza meal in diabetic patients (1), which is an excellent topic for class discussion. The experiment can also be used as a health education exercise for medical and nutrition students on the potentially harmful effects of high-fat or high-carbohydrate diets.

When the pizza and pasta experiment was presented at the Brazilian Biochemistry and Molecular Biology Annual Meeting of 2003 (11), many biochemistry and physiology professors from numerous universities countrywide became interested in using this type of applied and functional pedagogical tool in their courses. This indicated that this teaching method is of valuable relevance for the learning of metabolic biochemistry for undergraduate students. Indeed, considering the importance of the subject (5), a similar experiment was carried out in 2003, when students were given two types of breakfast (high carbohydrate or high fat, simulating a Brazilian-type and an American-type of breakfast, respectively). Unfortunately, the blood results (glucose, cholesterol, TG, and uric acid) turned out to be highly complex, and the interpretation of the data was beyond the level of basic biochemistry for first-year undergraduate students.

Finally, it is important to emphasize that the main objective of the pizza and pasta experiment was an attempt to enrich the lecture-type format used in most "classic" biochemistry/physiology courses worldwide.

APPENDIX: EXAMPLES OF PERTINENT ANSWERS BY THE STUDENTS

Answer to question A. The blood glucose levels are regulated by the ratio insulin/glucagon during the day. This way, on the post-meal period, glycemia would not be changed because of insulin action. It stimulates glucose absorption by the peripheral tissues (muscles and fat) and activate the synthesis of glycogen and lipids, not allowing an increase in blood glucose levels. During the evening period, glycemia does not fall due to

a decrease on the ratio insulin/glucagon. This glucagon release stimulates hepatic glycogenolysis and gluconeogenesis, maintaining the blood glucose levels constant. (by Aline Catunda and Karen Goulart)

Answer to question B. In the group with the HL diet, there is a faster increase in blood TGs because they are absorbed from the meal under the chylomicron form. These levels are kept constant because, in this case, there is not significant synthesis of TGs from carbohydrates during the afternoon. In the group with HG diet, there is a slower rise on triglycerides levels because they come mostly from carbohydrates absorbed from the diet. The ingestion of a large quantity of carbohydrates will activate the production of Acetyl-CoA, that is used in the synthesis of fatty acids and TGs. (by Aline Catunda and Karen Goulart)

Answer to question C. The increase in plasma urea levels occurs due to the metabolism of the amino acids from proteins and the subsequent removal of their amine groups. Because the amount of protein is higher in the HL than in the HG meal, the urea concentration in the evening period was higher in the first group. This increase in urea only showed in the evening period because some time was necessary for the digestion of the proteins and the metabolism of the amino acids that constitute them. (by Cecilia Kinoshita and Raquel Nunes)

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