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Hands-on experiments on glycemia regulation and type 1 diabetes

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Mingueneau M, Chaix A, Scotti N, Chaix J, Reynders A, Hammond C, Thimonier J. Hands-on experiments on glycemia regulation and type 1 diabetes. *Adv Physiol Educ* 39: 232–239, 2015; doi:10.1152/advan.00047.2015.—In the present article, we describe a 3-day experimental workshop on glycemia regulation and type 1 diabetes that engages students in open-ended investigations and guided experiments leading to results that are not already known to them. After an initial questioning phase during which students observe PowerPoint slides depicting the glycemia (blood glucose levels) of individuals in various situations, students design, execute, and interpret experiments to address one of the following questions: *1*) Which criteria must an animal model of diabetes fulfill? *2*) How do pancreatic cells maintain glycemia constant? and *3*) Is there a way to produce an insulin protein similar to the one released by human pancreatic cells? Students then *1*) measure glycemia and glycosuria in control mice and in a mouse model of type 1 diabetes (Alloxan-treated mice), *2*) measure the release of insulin by pancreatic β-cells (INS-1 cell line) in response to different concentrations of glucose in the extracellular medium, and *3*) transfect Chinese hamster ovary cells with a plasmid coding for green fluorescent protein, observe green fluorescent protein fluorescence of some of the transfected Chinese hamster ovary cells under the microscope, and observe the characteristics of human insulin protein and its three-dimensional conformation using RASMOL software. At the end of the experimental session, students make posters and present their work to researchers. Back at school, they may also present their work to their colleagues.

inquiry-based learning; blood glucose physiology; diabetes; high school students; undergraduate students

THE OBJECTIVE of the present workshop was, via hands-on experiments, to help students understand the regulation of glycemia and the origin of type 1 diabetes. Students also explore the link between fundamental research and therapeutic innovations.

After an initial questioning phase where students observed Microsoft PowerPoint slides and asked questions about glycemia (blood glucose levels) of individuals in various situations, they then design, execute, and interpret experiments to address one of the following questions: *1*) Which criteria must an animal model of diabetes fulfill? *2*) How do pancreatic cells maintain glycemia constant? and *3*) Is there a way to produce an insulin protein similar to the one released by human pancreatic cells? At the end of the experimental session, students make posters and present their work to researchers (Fig. 1).

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Background

The blood sugar concentration is maintained around a stable value in humans (1 g/l) thanks to two pancreatic hormones, insulin and glucagon (1). Insulin is produced and released by β-cells, which account for 90% of endocrine pancreatic cells, and glucagon is produced and released by α-cells, which account for the remaining 10%. These cells sit in tiny groups, called pancreatic islets, scattered throughout the tissue of the pancreas. Like all endocrine cells, they release their products, insulin or glucagon, directly into the bloodstream (10, 13). Because the regulation they facilitate is continuous and dynamic, these two hormones keep tight control of blood glucose levels so that they do not rise or fall outside healthy limits. Although glucose entries are discontinuous and vary widely depending on many factors (what we eat, how much we exercise, etc.), blood glucose levels stay constant thanks to these regulations. To reduce the concentration of glucose, insulin is released into the blood, *1*) promoting glucose uptake in insulin-sensitive tissues, primarily skeletal muscle, adipose tissue, and the liver, *2*) stimulating glucose storage in the liver, and *3*) inhibiting the secretion of the antagonist hormone glucagon (8). Conversely, when blood sugar levels become too low, during a fasting period, glucagon acts to mobilize glucose from the glycogen stocks present in the liver (or in the kidney in conditions of extreme starvation) to the blood.

Diabetes mellitus is a disease in which the body cannot regulate the amount of glucose in the blood, resulting in abnormally high levels of blood sugar (hyperglycemia). The two most common forms of diabetes mellitus result from a dramatically decreased production of insulin due to the progressive death of pancreatic β-cells (type 1 diabetes) (3) and a decreased sensitivity of insulin receptors to insulin and pancreatic β-cell dysfunction (type 2 diabetes) (16). Both deficiencies lead to hyperglycemia, which largely causes the acute signs of diabetes, among which are excessive production of urine (diabetes mellitus comes from the two Greek words "to pass through" and "honey," referring to excessive glucose-laden urination) and changes in energy metabolism. Diabetes has both environmental (e.g., infections, diet, and stress) and genetic origins (3). As of 2010, the prevalence of diabetes was 11.7% in North America (12.3% in the United States) and 8.5% in Europe (9.4% in France) (7).

What phenomena does the activity explore? The present workshop explores some of the steps researchers had to fulfill before designing treatments for type 1 diabetes. Students think about the need for cellular and animal models to understand pancreatic physiology and dysfunction in the course of the

Day	Schedule	Group #1	Group #2	Group #3
Day 1	9am–12pm	**Observations and Discussion**		
	2pm–5pm	Glycemia and glycosuria measurements	-INS1 cell stimulation ELISA : - primary antibody coating	CHO preparation and transfection
Day 2	9am–12pm	- Preparation and observation of pancreas sections - INS1 treatment with alloxan	ELISA: - incubation with insulin and INS1 culture supernatants	CHO observation
	2pm–5pm	INS1 viability test	ELISA : - secondary antibody incubation - enzymatic revelation and absorbance measurements	Bioinformatics : analysis of insulin structure and plasmid design
Day 3	9am–12pm	Results, analysis, discussion and slide preparation for oral and poster presentations		
	2pm–5pm	**Oral presentation** (chimeric groups). **Poster preparation** and **discussion** with researchers.		

Fig. 1. Schedule of the workshop. CHO, Chinese hamster ovary.

disease. They have to identify the criteria that an animal model of type 1 diabetes must fulfill (high glycemia, poor glycemia regulation, typical histology of endocrine pancreas, and lack of insulin in pancreatic β-cells) and test whether the model that we provided meets these criteria. They have to understand the molecular nature of insulin (protein) and identify the mechanism by which insulin secretion by pancreatic cells ensures the maintenance of glucose homeostasis despite the natural variability of glucose entry into the organism. They find out how to produce large quantities of human insulin: since it is a protein, the gene coding for it can be introduced into a microorganism or a cell line to engineer a genetically modified organism, but will this insulin be similar to the insulin produced by pancreatic β-cells? What methods could be derived to check whether this recombinant insulin will work in mammals?

What physiological parameters are measured or observed? To test whether Alloxan-treated mice (9) are a good animal model of type 1 diabetes and how to treat them, students were divided into the following three groups:

- Students from *group 1* measure the concentration of glucose in the blood (glycemia) and in the urine (glycosuria) of control and Alloxan-treated mice in two situations: after a 4-h fast and after glucose injection (intraperitoneally). Students then observe the histology of the pancreas and the presence (or not) of insulin-producing cells.
- Students from *group 2* measure the release of insulin pancreatic β-cells (INS-1 cell line) in response to different concentrations of glucose in the extracellular medium.
- Students from *group 3* transfect cells with a plasmid coding for green fluorescent protein (GFP) and observe the GFP fluorescence of some of transfected Chinese hamster ovary (CHO) cells under the microscope. They observe the characteristics of human insulin protein and its three-dimensional (3-D) conformation using RASMOL software.

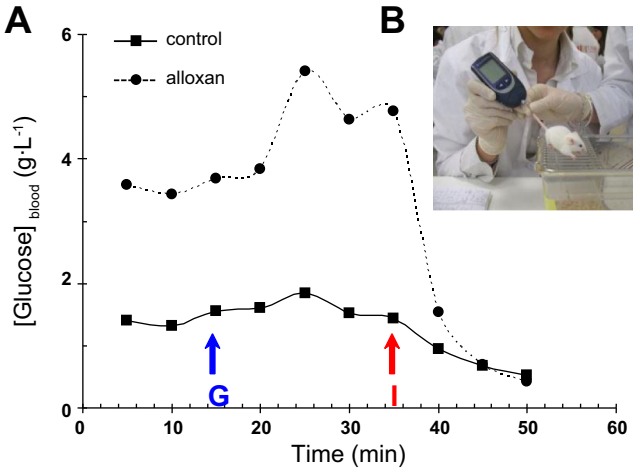
Learning Objectives

After completing this activity, students are able to explain data on glucose regulation and dysregulation and to analyze all their data. They are also able to propose the general outline of a protocol and design control experiments.

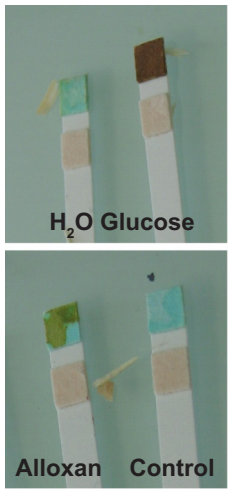
Specific Objectives

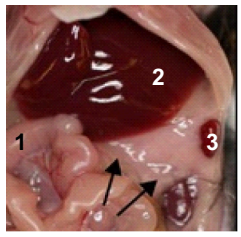
The specific objectives of the workshop were as follows:

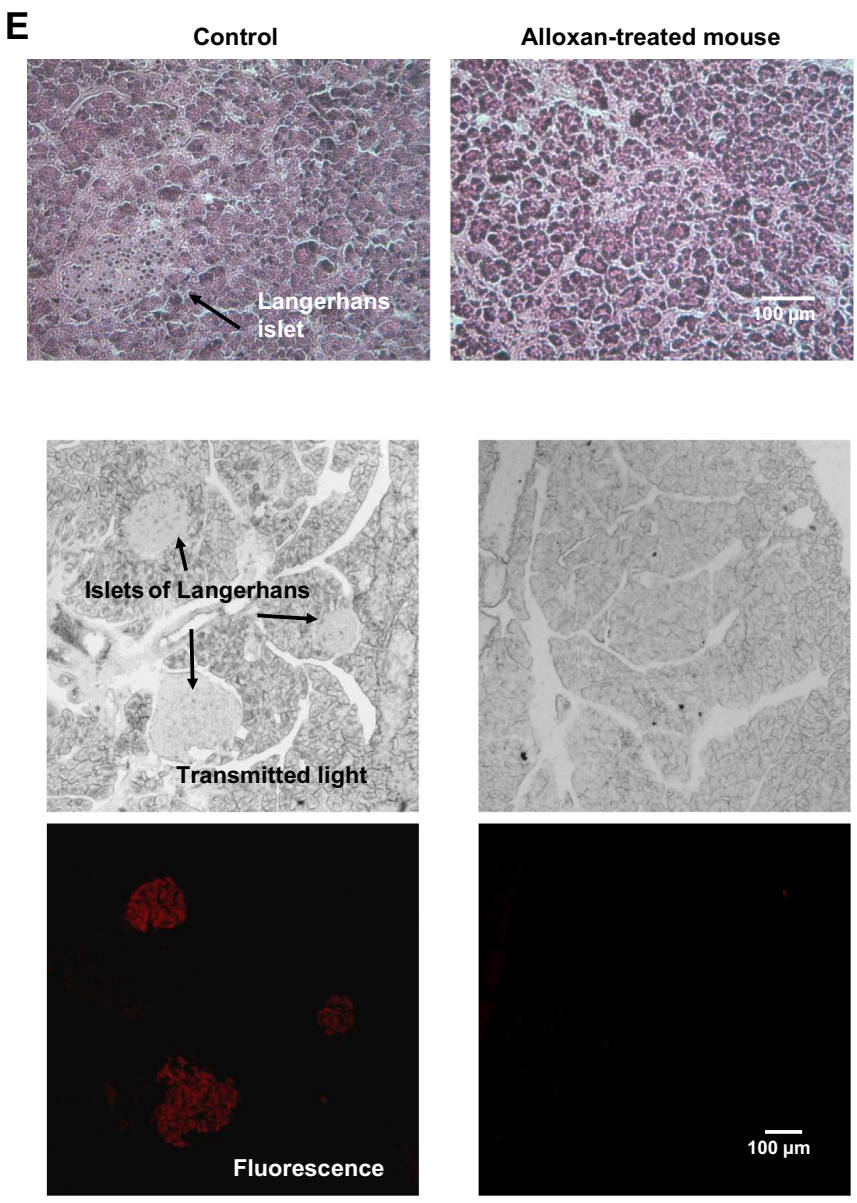
- In *group 1*, students prepare chemical solutions (they learn principle of dilutions and unit conversions) and draw curves of blood glucose concentration as a function of time (they learn mathematics needed to extract meaningful information from noisy biological data: linear regression, notion of population distribution, and calculation of means and SDs). They draw histological characteristics of the pancreatic slices observed under microscopy and describe the presence (or not) of insulin-producing cells.
- In *group 2*, students measure and quantify insulin release by a pancreatic β-cell line in culture using an ELISA technique, which involves multidisciplinary skills. They explain the immunological (antigens and antibodies), chemical (nature of the antigen-antibody interaction and notions of affinity and specificity), and physical (light, photons, absorbance, wavelength, and Beer-Lambert law) concepts underlying the ELISA technique.
- In *group 3*, students explore genetics and genetic engineering (gene structure, gene expression, and gene-protein coding mechanisms), bioinformatics (use of public databases like the Ensemble Protein Data Bank and molecular visualization software like RASMOL), chemistry (concepts of chemical bonds ranging from covalent peptidic and disulfide bonds to noncovalent hydrophobic interactions and hydrogen bonds), physics (fluorescence principles and emission

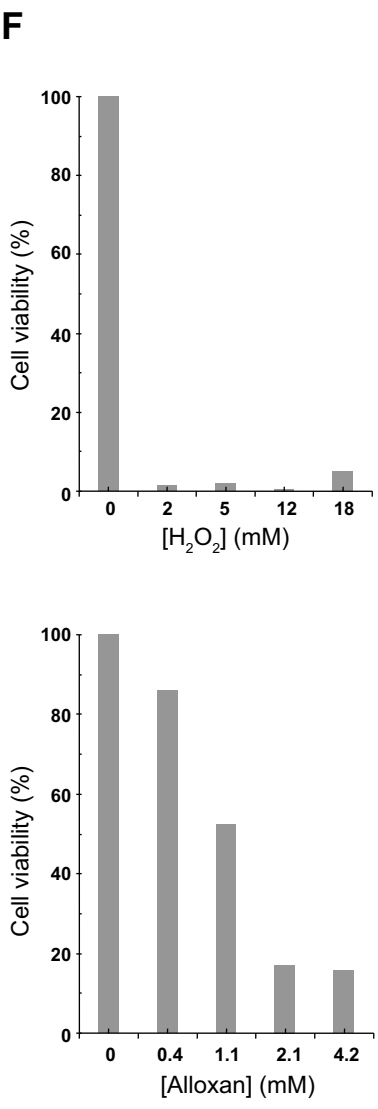

A
control
alloxan
[Glucose]blood (g·L-1)
G
I
Time (min)

B


C
H2O Glucose
Alloxan
Control


D
1
2
3
1 = intestine
2 = liver
3= spleen


E
Control
Alloxan-treated mouse
Langerhans islet
100 µm
Islets of Langerhans
Transmitted light
Fluorescence
100 µm


F
Cell viability (%)
[H2O2] (mM)
Cell viability (%)
[Alloxan] (mM)

and excitation spectra), and biochemistry (primary and tertiary protein structures, atomic and elementary composition, and protein folding) concepts underlying transfection, gene expression, and control of gene expression. With the use of RASMOL software, students analyze the 3-D conformation of a protein.

Activity Level

This activity is suitable for high school students of the 11th and 12th grades as long as they have access to a well-equipped laboratory. It cannot be directly implemented in a classroom setting, given that it requires laboratory equipment not usually available in high schools. However, the educational approach could be adopted using published documents (2, 6, 17, 18). As discussed in more detail below, this activity is also suitable for Bachelor of Science students (first or second year). In the latter case, the open inquiry phase of the workshop can be extended to the experimental design phase so that students propose experimental strategies to answer the questions they initially identified. The "Tous Chercheurs" association also successfully proposed this type of workshop to the general public as well as patients. The current activity could thus be taught to diabetic patients or to "nonscientific adults."

Prerequisite Student Knowledge or Skills

Before doing this activity, students should have a basic understanding of general biology and basic knowledge of cell biology, DNA, genes, DNA transcription, protein synthesis, genotype, phenotype, antigens and antibodies, hormones, blood circulation, and digestion (pancreas included) and excretory systems. It is not compulsory that students have attended a theoretical course on glycemia regulation before coming to the workshop; this course can take place after the workshop. Prior awareness of diabetes is possible but not required. If students have no prior knowledge of diabetes, they are told that diabetes results from the inability to transport, use, and store glucose acquired from food. None of the students who attended the workshop had had a theoretical course on glycemia regulation before the workshop.

Students are not required to have previous experience of laboratory practice since they are tutored by PhD students (1 tutor for 6–8 students). Tutors receive individual training in inquiry-based learning and experimental protocols on diabetes before the sessions from the Tous Chercheurs team. None of the students who attended the workshop had had previous experience of laboratory practice.

Time Required

This workshop requires 3 full days (Fig. 1). Because of the time required to complete each experimental activity, students do not have the possibility to address all three questions, and each group of students focuses on one question. As discussed in more detail below, each group of students explains the question they have investigated to the other groups on the afternoon of the third day (Fig. 1). In addition, students are reshuffled into three or four new groups on the third day to form the "chimera groups" containing students from each of the previous groups. These chimera groups design a poster summarizing the three questions and experiments, thus allowing each student to think about all three questions.

METHODS

Equipment and Supplies

All groups need to use a cell incubator, a laminar flow hood (to manipulate live cells), and a computer. Depending on the group, the following additional equipment is required:

- *Group 1* requires a fume hood (for manipulating toxic products), a spectrometer, fluorescence microscope (Axiovert 25, Zeiss), a digital camera (Axiocam ICc, Zeiss), and imaging software (AxioVision, Zeiss).
- *Group 2* requires a spectrophotometer (ELX 800, BIO-TEK Instruments).
- *Group 3* requires a fluorescence microscope (Axiovert 25, Zeiss), a digital camera (Axiocam ICc, Zeiss), and imaging software (AxioVision, Zeiss).

For small equipment, see *laboratory protocols 1–3* in the Supplementary Material.[1]

Animal Subjects

All experiments were approved by the Ethic Committee of Provence (CEEA14), by the Institut National de la Santé et de la Recherche Médicale animal care and use agreement (D-13-055-19), and the European community council directive (2010/63/UE). Animals had access to food and water ad libitum and were housed in our institutional animal facilities under a 12:12-h light-dark cycle at 22–24°C.

Instructions

Classes are divided into three or four groups of six to eight students per group by the teacher before the workshop. At the end of the first

[1] Supplemental Material for this article is available at the *Advances in Physiology Education* website.

Fig. 2. Experimental results from *group 1*. *A*: glycemia as a function of time before and after an intraperitoneal injection of 15 mg glucose (blue arrow) in control and Alloxan-treated mice. The mean value of three sets of measurements during 120 min showed that the mean basal glycemia of control and Alloxan-treated mice was centered around 1.3 and 3.7 g/l, respectively. Glycemia increased very moderately in the blood of control mice after glucose injection (time = 15 min; this injection mimics a sweet meal), whereas the same glucose injection induced a much stronger increase of the glycemia of Alloxan-treated mice. In control mice, glycemia returned to *time 0* levels in ~20 min, whereas glycemia of Alloxan-treated mice did not return to basal levels during this same timeframe. Injection of insulin (5 UI/ml, time = 35 min, red arrow) strongly decreased glycemia in Alloxan-treated mice to the same value as observed in control mice. Students compare their results with the results shown in Fig. 1. *B*: measurement of glycemia with Accucheck. *C*: measurement of glycosuria. *D*: mouse dissection after euthanasia to locate the pancreas (arrows). *E*: pancreas slices from control (*left*) and Alloxan-treated (*right*) mice showing the disappearance of the pancreatic islets after Alloxan treatment (*top*: hematoxylin/eosin staining; *middle* and *bottom*: transmitted light and insulin immunostaining). Fluorescence emitted by the fluorochromes linked to the secondary antibody was only detected in the control pancreas and was localized in the islets of Langerhans. *F*: percentage of pancreatic β-cells (INS1-E cell line) alive [as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction; see *laboratory protocol 1*] as a function of the concentration of H_2O_2 (cell death control, *top*) or Alloxan (*bottom*) in the culture medium.

morning (after *phases 1* and *2*; see below), each group is assigned an experiment, with their consent. In larger classes, two groups address the same experimental question.

Phases 1 and 2 of the workshop: open-ended questioning and hypothesis generation and experiment planning (day 1). Phases 1 and *2* of the workshop are shown in Fig. 1. At the end, students are asked how they would proceed to understand the physiology of pancreatic cells, how pancreatic cells regulate glycemia thanks to insulin, and how to treat individuals they have previously identified as diabetic.

Phase 3 of the workshop: experimentation, data analysis and interpretation (days 1–3). Phase 3 of the workshop is shown in Fig. 1. Students experiment for 2.5 days, during which they learn to read and follow a protocol, design control experiments, manipulate models, quantify results, discuss, interpret data, and draw conclusions. In parallel, they make slides summarizing their hypotheses and experimental work. The three groups of students explore the following questions:

- *Group 1* explores the criteria that an animal model of type 1 diabetes must fulfill (5, 9, 12, 15). Tutors explained that one model, the Alloxan model (5, 9), is available in the laboratory and researchers want to know whether it is a good model for studying diabetes and designing a therapeutic strategy. After discussions both within the group and with the tutor, the students usually addressed the following questions: *1*) Do Alloxan-treated mice show hyperglycemia and glycosuria? *2*) Are pancreatic β-cells present and/or altered in any way in this model? and *3*) What is the mechanism of action of Alloxan (15)? The protocols of the experiments performed to answer these questions are described in *laboratory protocol 1*.
- *Group 2* explores how pancreatic β-cells maintain glycemia constant while entries and consumption of glucose in the organism are highly variable (4, 11). Students began the session by observing histological slices from the pancreas of control and Alloxan-treated mice. They usually identified a loss of pancreatic cells after comparing sections from both groups. Tutors explained that the cells that were lost in Alloxan-treated mice are called pancreatic β-cells and are responsible for synthesizing and releasing the hormone insulin. To understand the conditions for insulin release and how this release keeps glycemia constant, tutors explained the concept of mammalian cell culture and cell lines. Students knew what a cell is and that organs are constituted by cells that may or may not be homogenous. We then explained that to study the system we needed to simplify it, i.e., to study in isolation the type of cell that releases insulin (β-cells). To this purpose, researchers either cultured β-cells isolated from a pancreas (primary culture) or a pancreatic β-cell line that can proliferate indefinitely. Since primary cultures are difficult to achieve, we proposed using INS-1E cells, a rat pancreatic β-cell line, as a tool to address this question (11). The following are examples of questions generated by students: What are the microscopic characteristics of INS1-E cells? What are the molecular components contained in food that trigger insulin secretion? Do they all trigger insulin secretion with the same efficiency? Do they trigger secretion in a binary (on/off response) or continuous manner (dose-response relationship)? These questions were addressed using ELISA-based methodology (4). Detailed protocols are described in *laboratory protocol 2*.
- Group 3 explores if there is a way to engineer a genetically modified organism that would produce an insulin protein similar to the one released by mammalian pancreatic β-cells (14). Tutors asked students to think about the following question: "How can we design an applied research protocol aimed at producing recombinant insulin?" After discussions within the group and with the tutor, the students generally proposed different experimental plans to produce insulin: for example, by extraction methods from other species, by chemical reactions, or by biologically catalyzed reactions using either enzymes or microorganisms or mammalian cells. This question quickly drove them to ask about the chemical nature of insulin. Once they knew it was a protein, they proposed transferring the gene that encodes this protein. The questions were then "How can a gene be transferred from one cell to another? Directly or using a vector? Should the gene be transferred into prokaryotic or eukaryotic cells?" This genetic engineering component requires a lot of tutoring, because usually students have never studied it before. After students performed experiments aimed at producing human insulin in the CHO cell line (14), tutors discussed with students the advantages of the various strategies they initially proposed and compared bioengineering methods with insulin extraction strategies from the bovine/porcine pancreas. Tutors also discussed with students the differences in the resulting insulin molecule when produced by microorganisms like bacteria or yeast versus mammalian cells. Protocols for testing these questions are shown in *laboratory protocol 3*.

On the afternoon of the third day (Fig. 1), each group of students explains the question they have investigated to the other groups as well as the results obtained and conclusions drawn. Students are then reshuffled into three or four new groups (chimera groups)

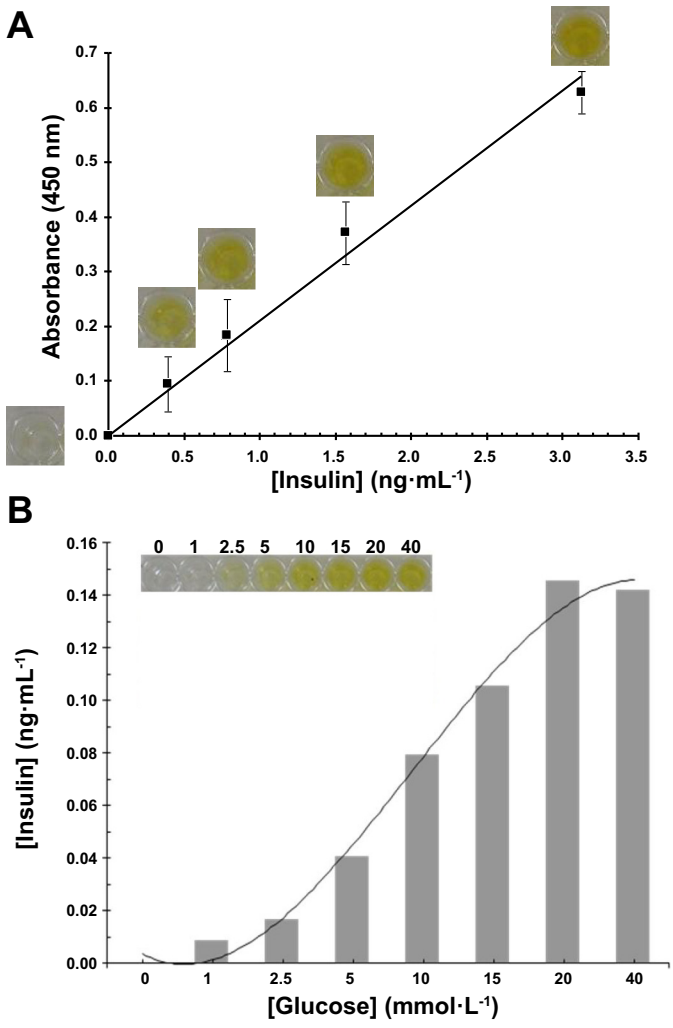


Fig. 3. Experimental results from *group 2*. Quantification of insulin in the culture medium of INS1-E cells was performed using sandwich ELISA based on *1*) the capture of insulin released in the culture medium by specific monoclonal capture-antibodies adsorbed to a plastic surface, *2*) the detection of captured insulin by biotinylated anti-insulin detection antibodies, *3*) the binding of horseradish peroxidase-conjugated streptavidin to the biotinylated detection antibodies, *4*) the detection of horseradish peroxidase enzymatic activity using the tetramethyl benzidine substrate, and *5*) the reading of the absorbance at 450 nm resulting from the conversion of the substrate into a colored product. *A*: standard curve. *B*: quantification of insulin released by INS1-E cells as a function of glucose concentration added in the culture medium.

containing students from each of the previous groups to design a poster summarizing the three questions and experiments.

Troubleshooting

Troubleshooting always comes from errors made by students during manipulation because Alloxan-treated mice, INS-1 or CHO cell cultures, and pancreas slices are prepared in advance by the teaching team and the equipment is regularly checked.

Safety Considerations

The Tous Chercheurs laboratory follows the safety rules of the Institut National de la Santé et de la Recherche Médicale (INSERM) and is under the supervision of the INSERM engineer in charge of hygiene and safety in the institute. Biological and chemical wastes are collected in specific containers. Animals are manipulated by researchers having a diploma for animal experimentation. In the case of injury, a first aid kit is present in the Tous Chercheurs laboratory. All students wear a laboratory coat and are taught basic safety rules associated with the work in research laboratories at the beginning of the experimentation phase of the workshop (*phase 3*).

- In *group 1*, students wear gloves to manipulate INS-1 cells, Histolemon (for histology), and diluted solutions of Alloxan, the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and Dakopen under the hood.
- In *group 2*, students wear gloves to manipulate INS-1 cells and diluted solutions of 3,3′,5,5′-tetramethylbenzidine and HCl under the hood.
- In *group 3*, students wear gloves to manipulate CHO cells under the hood.

RESULTS

Expected Results

Representative results obtained by the students following *laboratory protocols 1–3* are shown in Figs. 2–5, respectively. When discussing the etiology of diabetes, students often suggested that the number of diabetic people in a country varies as a function of what they eat, how much they exercise, and their genetic background. On the question of how to study an illness and find medications to cure it, a high proportion of students

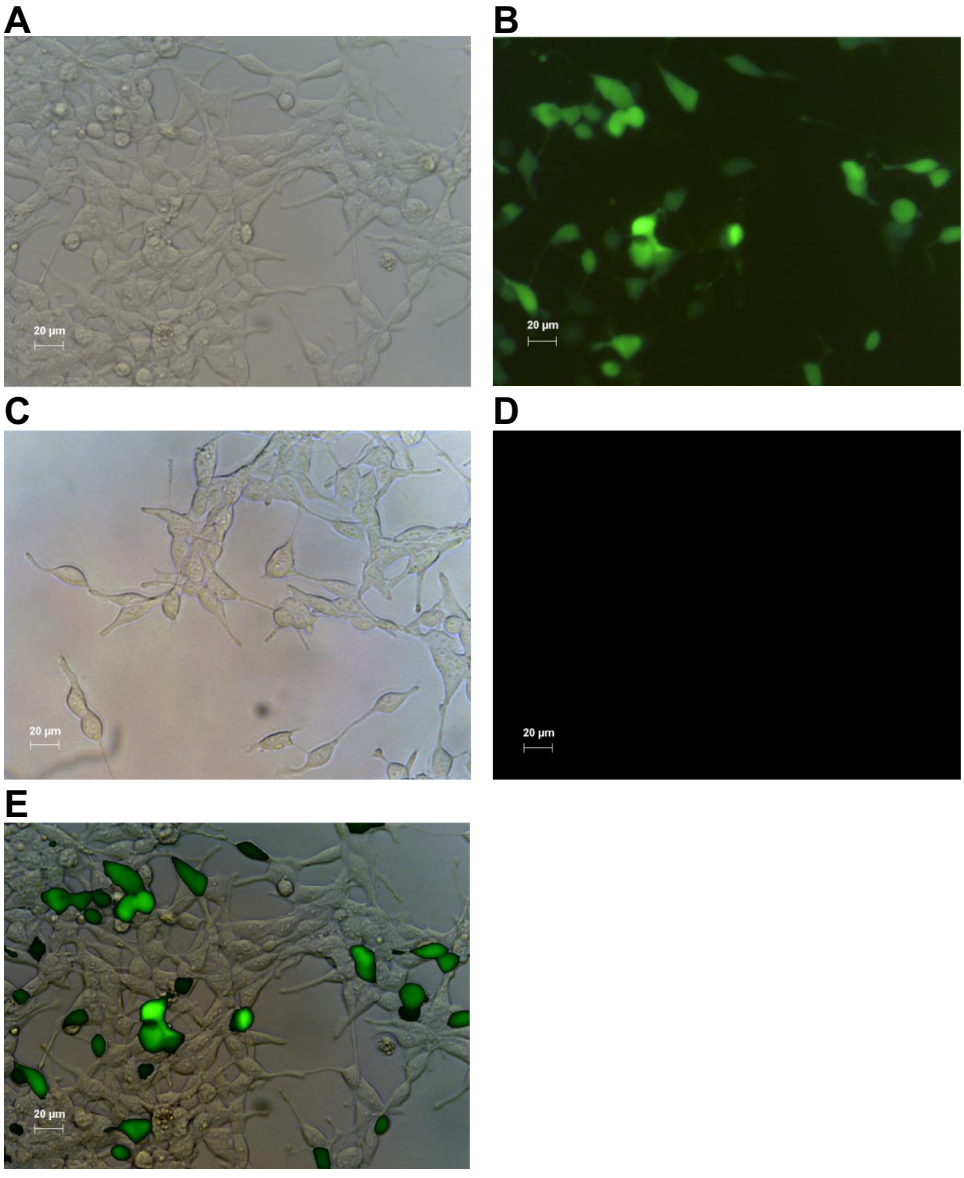


Fig. 4. Experimental results from *group 3*. Cells were transfected with a plasmid encoding insulin and green fluorescent protein (*A* and *B*), and control cells (*C* and *D*) were observed 24 h after transfection. *A–D*: transmitted light (*A* and *C*) and fluorescence microscopy (*B* and *D*). Students proposed to overlay the two types of images (*E*) to evaluate transfection efficiency.

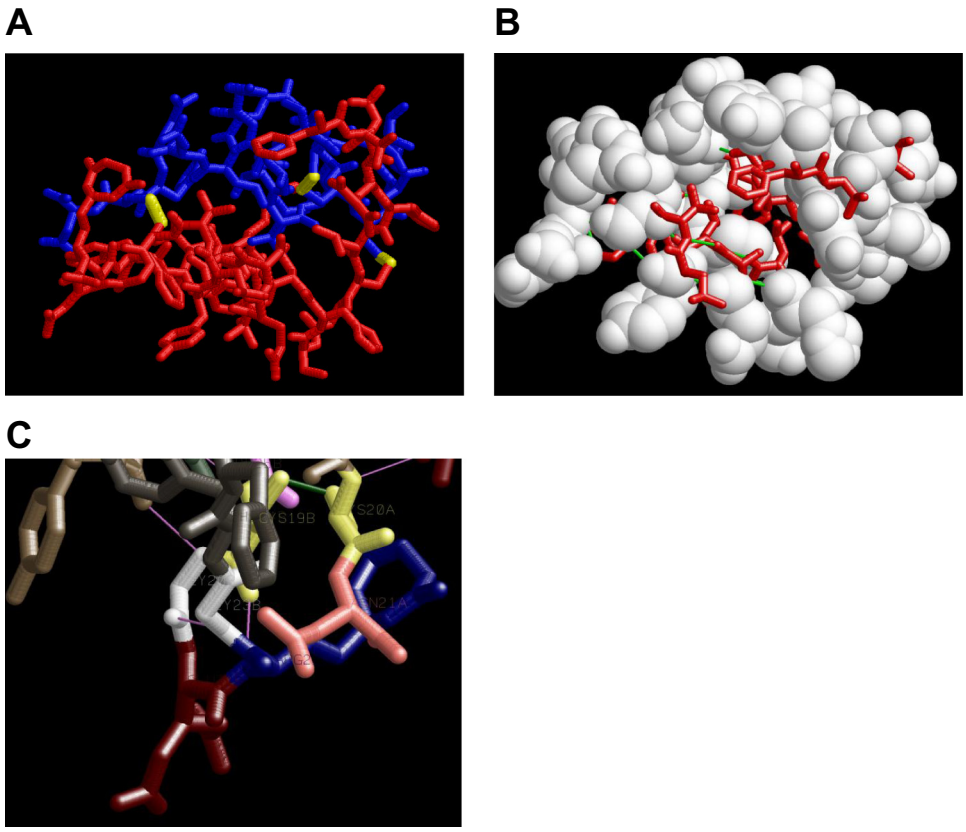


Fig. 5. Bioinformatic analyses performed by *group 3*. The molecular nature and properties of insulin (Protein Data Bank no. 4INS; Baker et al.) were investigated using RASMOL software. The presence of two chains was revealed by attributing a color to each chain and visualizing the nature of the molecular links between these chains (disulfide bonds are shown in yellow) *A*: to better understand the tridimensional shape of the molecule and the mechanisms leading to protein folding, hydrophobic parts of the molecule are shown in red (stick representation) and hydrophilic in white (spacefill representation). Hydrogen bonds are shown in green. Hydrophobic residues mostly reside deep in the protein core, whereas hydrophilic residues are found on the protein surface, strongly suggesting that hydrophobic forces play a major role in insulin folding in aqueous environments (*B*). The elementary elements constitutive of insulin are shown in color for a small part of the molecule at the interface between the two chains. This allows identification of the building blocks of the molecule, i.e., amino acid residues. The names of these residues are shown as well as hydrogen bonds (pink) and disulfide bonds linking cysteine residues 19 and 20 (green) at the interface between the two chains (*C*).

mentioned that they would have to test any medication before using it on humans. They often suggested doing in vitro experiments or using animal models of the disease to understand the illness and test therapeutic strategies. The discussion often focused on the advantages and limitations of various kinds of experimental models (e.g., animal, cellular, and molecular models) depending on the nature of the question (ranging from general glucose physiology questions to the function of the pancreatic cells or the evaluation of potential treatments). These discussions are also the opportunity for the tutors to discuss with the students ethical questions associated with animal experimentation and define the ethical rules for animal use in experimentation. The tutors discuss the principles of replacement, reduction, and refinement (most often referred to as the 3Rs) as the key strategies to provide a systematic framework to achieve the goal of humane experimental techniques and reduce animal experimentation to the situations where in vitro approaches are not modeling accurately the biological or pathological process under investigation. To better understand the function of the pancreas and pancreatic cells, they often proposed taking out the pancreas from animal models, giving them a glucose-rich diet, or injecting animals with a virus. Regarding the design of a therapeutic strategy, most students suggested injecting insulin, grafting a pancreas, or using stem cells to replace damaged pancreatic cells.

Misconceptions

Before the workshop, students often think that diabetes always implies obesity. This misconception is legitimate given what they hear and see in the media about the association between obesity, cardiovascular problems, and diabetes. Importantly, students realize during the workshop that diabetes is not always associated with obesity when they weigh control and diabetic mice and observe that these mice are not obese but still have diabetes. This is a good opportunity for students to discuss with their tutors about various types of diabetes. They often ask about mouse models to study type 2 diabetes, and tutors can introduce the *ob*/*ob* mouse model, which is deficient in the leptin gene and shows hyperglycemia and insulin resistance.

Evaluation of Student Work

The efficacy of the workshop was assessed using two different methods: *1*) a questionnaire filled out by students before and after the workshop and *2*) a poster produced by students at the end of the workshop.

Inquiry Applications

The workshop is designed to promote exploration, stimulate creativity, and generate enthusiasm about a topic that can be relatively abstract and poorly motivating for students when taught in a traditional learning context.

- Based on their observations, students propose to explore several questions. The tutor selects and proposes one of them with the students' agreement.
- The precise protocol is provided by the teaching team. However, before experimenting, students must propose a basic procedure to be followed. During the workshop, students often ask to test some of their ideas. We encourage them to do so.

- Students carry out the experiments and analyze their data.

During the first morning, the inquiry level is open inquiry since students generate the question and propose the general outline of the protocols to test their question. During experimentations (afternoon of *day 1* and morning of *days 2* and *3*), the inquiry level is facilitated inquiry since the teaching team provides guidelines for experimental design and students expand on the methods.

The whole workshop can be easily shifted to the open inquiry level for all stages. It is just a question of time. When given more time and, in particular, at the university, undergraduate students can design experiments. This workshop can be considered as *level 1* for high school students. A followup *level 2*, entirely open inquiry, can be then proposed on another subject at the university.

Wider Applications

This workshop can be applied to undergraduate students and can also be adapted to a several-week-long university project. To this aim, protocols will not be given to the students; instead, they will have to create them.

Additional Resources

For additional information on the topic of diabetes, please see Refs. 1, 3, 5–10, 13, and 16. For additional information on the techniques used, please see Refs. 4, 11, 12, 14, and 15.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: M.M., A.C., N.S., J.C., A.R., C.H., and J.T. conception and design of research; M.M., A.C., N.S., J.C., A.R., C.H., and J.T. performed experiments; M.M., A.C., N.S., J.C., A.R., C.H., and J.T. analyzed data; M.M., A.C., N.S., J.C., A.R., C.H., and J.T. interpreted results of experiments; M.M., C.H., and J.T. prepared figures; M.M., C.H., and J.T. drafted manuscript; M.M., C.H., and J.T. edited and revised manuscript; M.M., A.C., N.S., J.C., A.R., C.H., and J.T. approved final version of manuscript.

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