Laboratory Exercise

Comprehensive Experiment—Clinical Biochemistry: Determination of Blood Glucose and Triglycerides in Normal and Diabetic Rats

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Abstract

For second year medical students, we redesigned an original laboratory experiment and developed a combined research-teaching clinical biochemistry experiment. Using an established diabetic rat model to detect blood glucose and triglycerides, the students participate in the entire experimental process, which is not normally experienced during a standard clinical biochemistry exercise. The students are not only exposed to techniques and equipment but are also inspired to think more about the biochemical mechanisms of diseases. When linked with lecture topics about the metabolism of carbohydrates and lipids, the students obtain a better understanding of the relevance of abnormal metabolism in relation to diseases. Such understanding provides a solid foundation for the medical students’ future research and for other clinical applications. © 2014 The Authors Biochemistry and Molecular Biology Education published by Wiley Periodicals, Inc. on behalf of International Union of Biochemistry and Molecular Biology, 43(1):47–51, 2015.

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Introduction

Diabetes mellitus (DM) is a common clinical disease. The International Diabetes Federation has projected that the number of individuals with diabetes worldwide will increase from 382 million in 2013 to 592 million by 2035 [1], and according to the 2012 data from the world organization, diabetes is the eighth leading cause of death in the world. There are two primary types of DM: Type I and Type II DM. Type I DM results from the absolute deficiency of insulin due to the dysfunction of the β cells in the pancreas, and its cause is unknown [2]. Type II DM begins with insulin resistance, in which the body’s cells cannot properly respond to insulin [3]. The cause of type II DM is related to obesity and an unhealthy lifestyle. Nearly, 90% of all DM patients have Type II DM. Insulin regulates blood glucose levels by facilitating the uptake of glucose into the cells, followed by the catabolism of the glucose into CO2 and H2O or entry into other metabolic pathways. The overall effect of insulin is to decrease blood glucose levels back to a normal range following a meal and to promote the use of glucose by the body’s cells to provide energy. The eventual consequence of both Type I and Type II DM is absolute or relative insulin insufficiency. Insulin is a unique hormone that decreases blood glucose in the human body. The net effect of DM is persistently high levels of blood glucose, which causes a series of metabolic disorders, including enhanced lipid catabolism. High blood triglycerides result from enhanced lipid catabolism, which is used to provide energy to the cells when glucose is unavailable. This enhanced lipid catabolism leads to other disorders as well, such as acidosis. The typical clinical symptoms of DM are weight loss, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased appetite).
The molecular pathogenesis of DM is related to the biochemical “metabolism of carbohydrates and lipids.” Therefore, to help students appreciate DM from a basic medical perspective, we apply basic DM research into their laboratory practices to make this section more understandable to students and to further aid the students’ study of basic medicine by linking it to a clinical application. The use of clinical biochemistry experiments is an example used to obtain this goal.

The determination of blood glucose and triglyceride levels is a major aspect of clinical biochemistry for second year medical students. The laboratory exercise shows students the importance of carbohydrate and lipid metabolism in diseases such as DM, hyperlipoproteinemia, and arteriosclerosis. We previously established an obese rat model to help the students observe blood glucose and lipid disorders, but there were three limitations of the previous laboratory practice: 1) It took at least 4–6 weeks to induce the obese rat model using a high fat diet, and some of the rats were resistant to the high fat diet. 2) The students were not involved in the entire process because the induction of the obese rat model was set up by a technician. Therefore, by linking current diabetes studies with basic research, we redesigned the experiment and developed a relatively complete, brief and reliable clinical biochemistry experiment. The reformed laboratory practice has been conducted in two student sessions.

This laboratory experiment includes two major parts: activity 1 and activity 2. Activity 1 lasted 4 teaching hours, which were distributed over 4 days, and was started at the end of the last experiment of the course. On day 0, a tail bleeding was first performed. Second, the blood glucose concentration was determined using a kit to confirm the starting blood glucose level of the rats. Next, the rats were divided into the control group and the diabetic group. Finally, the rats in the diabetic group were treated with a single intraperitoneal injection of streptozotocin (STZ), and the rats in the control group were treated with a single intraperitoneal injection of citric acid. At noon on the following 3 days (day 1, day 2, and day 3), the blood glucose levels of the two groups of rats were determined, and the rats in the diabetic group with blood glucose levels greater than 13.8 mmol/L were identified as diabetic rats.

Activity 2 began two weeks after day 0 from activity 1 and was performed throughout the regular course. First, the blood glucose and triglyceride levels were determined and compared between the two groups of rats, and insulin was injected to observe its hypoglycemic effect. Finally, all of the data were collected and discussed.

STZ can be used to construct a diabetic rat model for basic medical research. STZ is a toxic cellular compound, and its chemical formula is shown in Fig. 1. It can be targeted to the β cells of the pancreas and induces type I diabetes by causing DNA damage [6]. In this laboratory practice, the students compared the blood glucose and triglyceride levels in control rats and diabetic rats induced by STZ. As STZ is also a carcinogen, the students are warned to be careful when performing the STZ injection.

Course Information [7]
Carbohydrate and lipid metabolism are important topics in the chapter regarding material metabolism in Biochemistry for medical students. The related metabolic disorders are associated with many clinical diseases, especially diabetes and hyperlipidemia. To increase the interest of the students and to help them to develop a better clinical link, we developed this relatively integral clinical biochemistry experiment. Due to the strong damaging effects of STZ in the β cells of the pancreas and by reproducing a diabetic animal model, the students can observe the progress of the disease, which will inspire thoughts regarding the biochemical mechanism of the disease.

This clinical biochemistry experiment is one of nine experiments designed to be part of the course “Life Science Integral Lab Practice I” and is required for all second year medical students. The class meets twice a week for 6 teaching hours during an 18-week semester. The exercise described here takes a total of 8 teaching hours. If organized properly, the first 4 hr can be distributed into times outside of the scheduled laboratory time. The latter 4 hr occur during the scheduled laboratory time. A timeline outlining the

| TABLE I | The clinical biochemistry experiment timeline |
|---------------------------|------------------------------------------------|
| Activity 1: Establishing the diabetic rat model (4 discontinuous teaching hours). |
| Activity 2: Blood glucose determination preinsulin and postinsulin injection; blood triglyceride determination; and data analysis (4 continuous teaching hours in regular time). |
The experiment described here can be found in Table I. The typical class enrollment is 16 students. The students work in pairs, but each individual is required to maintain their own notebook and write a laboratory report. The students are evaluated based on a written examination (30%), laboratory reports (50%), and performance and attendance (20%). All of the steps, including the tail bleeding, STZ and insulin injection, and blood glucose and triglyceride determination, are performed by the students under the guidance of the instructor.

Activity 1: Establishing the Diabetic Rat Model
All of the animals were treated in accordance with the regulations of the Animal Care and Use Committee at Tongji University. Male SD (Sprague-Dawley) rats were purchased from Shanghai Slac Laboratory Animal Company (Shanghai, China). The rats were housed with a 12/12 hr light/dark cycle in the animal center and were fed by professional technicians. When it was time for activity 1 and activity 2, the rats were brought to the Teaching Laboratory Center of Medicine and Life Science, where the students attended all of their laboratory practices. There were total 12 rats for each class, and two students worked with 1 rat. Normally, 8 rats were sufficient for a class of 16 students, but the extra 4 rats were prepared for use following any unforeseen circumstances (i.e., death or STZ resistance).

Activity 1 can be divided into 3 parts:

- Part 1: The students were told by the instructor that the goal of the experiment was to establish a diabetic rat model and to further compare the blood glucose and triglyceride levels between the control and diabetic groups. The instructor illustrated the operational methods of tail bleeding and intraperitoneal injection to the students by playing a video, which was made by an experienced teacher.
- Part 2: Food intake can interfere with the effects of STZ; therefore, the SD rats, weighing approximately 200 g, were fasted overnight prior to activity 1. The students first performed the tail bleeding and then determined the blood glucose concentration.

The bleeding procedure was based on and developed in reference [8]. Briefly, the very tip of the rat’s tail (5 mm from the end) was snipped and gently squeezed to obtain a drop of blood at the incision site (200–300 μL of blood was sufficient). Two students worked with 1 rat, with one student holding the rat and the other performing the tail bleeding.

For the blood glucose assay, the serum was first separated from the whole blood by centrifuging at 3000 rpm for 5 min, and the supernatant was transferred to another tube for further determination. Then, the students continued to determine the blood glucose concentration according to the kit’s protocol (Table II). The results were recorded as day 0 (Table III). All of the reagent kits (for the glucose and triglyceride assays) were purchased from Beyotime Institute of Biotechnology.

The rats were then randomly divided into the control group and the diabetic group, with four rats per group. The diabetic group was treated with STZ (Sigma) dissolved in a chilled citric acid buffer via an intraperitoneal injection at a dose of 60 mg/kg of body weight [6, 9]. The control group was treated with the equivalent volume of 0.05 mol/L citric acid solution.

### Table II: Blood glucose determination (GOD-POD method)*

| Reagent (μL) | Blank | Day 0 | Days 1–3 | Standard |
|-------------|-------|-------|----------|----------|
| Serum       | –     | 10    | 10       |          |
| Glucose standard (5.05 mmol/L) | –     | –     | –        | 10       |
| ddH2O       | 10    |       |          |          |
| Glucose reagent | 1,000 | 1,000 | 1,000    | 1,000    |

*The tubes were prepared and marked with “blank,” “day 0: 1–8,” “day 1–3: 1–8,” or “standard.” Each reagent was added according to Table II. The tubes were gently shaken and incubated at 37 °C for 10 min. The spectrophotometer was blanked at 505 nm against a blank sample (blank), and the absorbance value was recorded for each sample.

### Table III: Blood glucose levels in the control and diabetic groups

| Group    | Day 0  | Day 1  | Day 2  | Day 3  |
|----------|--------|--------|--------|--------|
| Diabetic | 8.24   | 18.49  | 19.7   | 22.03  |
|          | 4.82   | 17.34  | 17.83  | 20.18  |
|          | 4.51   | 16.32  | 18.16  | 18.98  |
|          | 8.01   | 13.9   | 18.92  | 21.77  |
| Control  | 6.66   | 6.69   | 9.33   | 5.75   |
|          | 5.57   | 5.05   | 8.22   | 7.8    |
|          | 6.18   | 5.22   | 4.88   | 4.55   |
|          | 5.53   | 6.44   | 4.35   | 5.35   |

1. Day 0 refers to the blood glucose level prior to the injection of citric acid or STZ.
2. Days 1, 2, and 3 refer to the blood glucose levels ~24, 48, and 72 hr after the injection of citric acid or STZ.
At noon on the 3 days (day 1, day 2 and day 3) following the STZ injection (day 0), the blood glucose was measured (Table II). The rats in the diabetic group were identified as diabetes if their blood glucose concentration was greater than 13.8 mmol/L for three consecutive days (day 1, day 2, and day 3 measurements). In Table III, we can see that the diabetic rat model was successfully established among the four rats treated with STZ.

Principles of the GOD-POD method: the glucose oxidase (GOD) method is used exclusively to determine blood glucose levels in the clinic because of its high specificity, low cost and convenient operation. Glucose is oxidized by GOD to produce gluconate and H$_2$O$_2$. H$_2$O$_2$ is then oxidatively coupled with 4-amino-antipyrene (4-APP) and phenol in the presence of peroxidase (POD) to yield a quinoneimine dye that is measured at 505 nm. The absorbance at 505 nm is proportional to the glucose concentration in the sample.

Activity 2: Blood Glucose Determination Preinsulin and Postinsulin Injection, Blood Triglyceride Determination, and Data Analysis

Prior to activity 2, the rats were fasted overnight. This entire activity can be divided into 4 parts:

- **Part 1:** The first tail bleeding. All of the samples from the eight rats from both groups were marked as “Pre 1–8.”
- **Part 2:** Administration of the insulin injection and the second tail bleeding. The rats were treated with insulin via an intraperitoneal injection at a dose of 0.75 IU/kg (60 U/kg) of body weight. Thirty minutes after the injection, a second tail bleeding was performed, and the samples were marked as “Ins 1–8.”
- **Part 3:** Blood glucose and triglyceride determination. The blood glucose assay was performed using the protocol from Table II in activity 1. The blood triglyceride assay was performed using a kit and according to Table IV.

It is important to note that the blood triglyceride determination was only performed on the samples from day 0 from activity 1 and on the Pre1–8 samples from activity 2. The primary goal was to compare the blood triglyceride levels between prediabetes and postdiabetes.

Principles of the colorimetric method: Triglyceride is oxidized by lipoprotein lipase to produce glycerol and fatty acids. Glycerol is further phosphorylated into 3-phosphoglycerol by glycerol kinase. 3-phosphoglycerol is then oxidized by glycerolphosphate oxidase to produce H$_2$O$_2$. H$_2$O$_2$ is then oxidatively coupled with 4-APP and EHSPT (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine) in the presence of POD to yield a quinoneimine dye that is measured at 546 nm. The absorbance at 546 nm is proportional to the triglyceride concentration in the sample.

- **Part 4:** Data analysis and discussion. All of the data were collected and are shown in Table V. In Table III, we can

### TABLE IV

**Blood triglyceride determination (Colorimetric method)**

| Reagent (µL) | Blank | Day 0: 1–8 | Pre 1–8 | Standard |
|-------------|-------|------------|---------|----------|
| Serum       | –     | 10         | 10      | –        |
| Triglyceride standard (2.26 mmol/L) | – | – | – | 10 |
| ddH$_2$O     | 10    |            |         |          |
| Triglyceride reagent | 1,000 | 1,000 | 1,000 | 1,000 |

*The tubes were prepared and marked with “blank,” “day 0: 1–8,” “pre 1–8,” or “standard.” Each reagent was added according to Table IV. The tubes were gently shaken and incubated at 37 °C for 10 min. The spectrophotometer was blanked at 546 nm using a blank sample (blank), and the absorbance value was recorded for each sample.

### TABLE V

**Blood glucose and triglyceride levels**

| Groups | Blood glucose (mmol/L) | Blood triglycerides (mmol/L) |
|--------|------------------------|-----------------------------|
|        | Pre | Ins | Day 0 | Pre |
| Diabetic | 28.7 | 17.41 | 1.2 | 9.37 |
|         | 22.92 | 14.8 | 3.37 | 10.26 |
|         | 18.91 | 11.32 | 1.51 | 6.09 |
|         | 29.9 | 15.67 | 1.76 | 4.97 |
| Control | 6.20 | 4.27 | 1.04 | 1.44 |
|         | 7.80 | 3.48 | 1.53 | 3.04 |
|         | 5.20 | 3.66 | 2.59 | 1.53 |
|         | 6.10 | 3.88 | 1.18 | 1.30 |

*Day 0 refers to the blood glucose level prior to the injection of citric acid or STZ in activity 1.

"Pre" refers to the blood glucose or triglyceride levels prior to the injection of insulin in activity 2.

"Ins" refers to the blood glucose levels following the injection of insulin in activity 2."
see that the diabetic rat model was successfully established when compared with the control group in activity 1. Accordingly, two weeks later in activity 2, the blood glucose levels in the diabetic group were consistently higher than 13.8 mmol/L (Table V), and the blood triglyceride levels were also increased when compared with the original levels on day 0. The insulin injection led to a decreasing effect on the blood glucose levels in both groups and displayed an especially therapeutic effect in the diabetic group.

**Calculation**

\[
\text{Blood glucose/triglyceride (mmol/L)} = \left[ \frac{\text{Absorbance of sample (A)}}{\text{Absorbance of standard (A)}} \right] \times \text{concentration of standard}
\]

**Discussion**

It is our aim to link current basic research with the laboratory practices performed by medical students. Producing a diabetic rat model using STZ is a common method to study DM. The entire experiment is easy to address and can be performed in a short period of time. In this laboratory practice, from the establishment of the diabetic rat model to the data analysis, it took two weeks, and the students obtained a general idea regarding the study of diseases and the abnormal metabolic changes that occur in diabetes.

In activity 1, the students first injected STZ into adult rats. Under the guidance of the instructor, the students observed some of the habits of rats and learned proper rat-handling methods. During the tail bleeding performed on the following 3 days, the students observed the obvious increase in the blood glucose concentrations in the STZ-treated rats.

In activity 2, which was performed two weeks after activity 1, the students observed the obvious weight loss in the diabetic group. The students had direct knowledge regarding the weight of the normal and diabetic rats and were impressed with the typical symptoms of diabetes. After administering an insulin injection and repeating the tail bleeding, the students were well trained and cooperated closely. Further determinations of the blood glucose and triglyceride levels made the mechanism of diabetes clear to the students and underscored the significance of high blood glucose and triglyceride levels. The students realized that the lack of blood glucose control in diabetes causes the increased blood triglyceride levels. Insulin, as a hypoglycemic hormone and treatment drug in the clinic, was shown to decrease the blood glucose levels in the rats in activity 2. In a final discussion, the students obtained a clear idea regarding the effect of the abnormal metabolism caused by diabetes and also realized the importance of the prevention of the disease in their daily life. The instructor summarized the experiment and linked the biochemical knowledge to other clinical diseases aside from diabetes, such as hyperlipidemia. One of the benefits of this laboratory exercise is that the students combine their lifestyle with the prevention of the disease and obtain a better understanding of the clinical applications of biochemistry.

This laboratory exercise provides our students with an excellent experience to emphasize the importance of research and the relevance of biochemistry in clinical applications. As a result of the exposure to this laboratory exercise, the students have acquired operational training and the ability to cooperate with faculty and are able to put biochemical concepts into practice in regards to clinical diseases. This comprehensive clinical biochemistry exercise can be widely applied to all medical students.

**References**

[1] Shi, Y., and Hu, F. B. (2014) Te global implications of diabetes and cancer. Lancet 383, 1947–1948.
[2] Vehik, K., Ajami, N. J., Hadley, D., Petrosino, J. F., and Burkhardt, B. R. (2013) The changing landscape of type 1 diabetes: recent developments and future frontiers. Curr Diab Rep 13, 642–650.
[3] Saini, V. (2010) Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. World J Diabetes 1, 68–75.
[4] David, W., Cooke, M. D., and Leslie Plotnick, M. D. (2008) Type 1 diabetes mellitus in pediatrics. Pediatr Rev 29, 374–385.
[5] Laakso, M., and Kuusisto, J. (2014) Insulin resistance and hyperglycaemia in cardiovascular disease development. Nat. Rev. Endocrinol. 10, 293–302.
[6] Deeds, M. C., Anderson, J. M., Armstrong, A. S., Gastineau, D. A., Hiddinga, H. J., Jahangir, A., Eberhardt, N. L., and Kudva, Y. C. (2011) Single dose streptozotocin induced diabetes: considerations for study design in islet transplantation models. Lab. Anim. 45, 131–140.
[7] Beers, M., Archer, C., Feske, B. D., and Mateer, S. C. (2012) Using biocatalysis to integrate organic chemistry into a molecular biology laboratory course. Biochem. Mol. Biol. Educ. 40, 130–137.
[8] Shek, P. N., and Howe, S. A. (1982) A novel method for the rapid bleeding of rats from the tail vein. J Immunol Methods 53, 255–260.
[9] Babu, P. S., and Srinivasan K. (1999) Renal lesions in streptozotocin-induced diabetic rats maintained on onion and capsaicin containing diets. J. Nutr. Biochem. 10, 477–483.