Glycemia regulation and measurement

Glucose tolerance test, Glycemia profile curve

Glucose is a very important convertible energy source in human metabolism. Some tissues (e.g. brain and erythrocytes) are largely or absolutely glucose-dependent.

AIM of the lab

- Practical demonstration of some blood glucose regulatory mechanisms
- Understanding the importance of glucose homeostasis
- Protective mechanisms of hypoglycemia
- Experience fasting and self-blood glucose testing by glucometer

REQUIRED KNOWLEDGE

- Blood Glucose (def., norms)
- Blood glucose regulation (insulin, glucagon, epinephrine, cortisol, …)
- Glucose content calculation if concentration in mmol/l, %, g/L or other measures (osmolarity) is given
- Determination of energy load in glucose solutions of various concentrations
- Plasma osmolarity, how glycemia contribute to plasma osmolarity
- Oral glucose tolerance test (OGTT), procedure and purpose
- Diabetes, what is it, basic knowledge

TASKS

1. Observe the response of a healthy volunteer (fasting overnight), to following drink and food:
    a. orange juice
    b. bread
    c. lentil
    d. instant noodle soup

    with carbohydrates dose equivalent to GTT (= glucose tolerance test) 1g/kg body weight (orange juice and bread). Carbohydrates load in the soup and lentil is lower than acquired for GTT. The aim of exposure to meals c. and d. is to compare glycemic profile after two different dishes with the same carbohydrates load.
2. Estimate blood glucose in capillary blood by glucometer in 10 minutes intervals as described below.
3. In parallel, take samples of capillary blood in a person that after overnight fasting experienced 15 minutes of exercise with performance load 1.5 W/kg.
4. Learn about continuous glucose monitoring (CGM) and its importance in diabetic people.

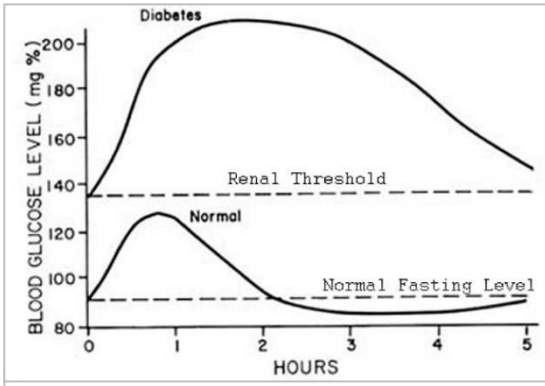
WHAT

1. **Glucose tolerance test**
   Definition according to *PubMed:* A test to determine the ability of an individual to maintain HOMEOSTASIS of BLOOD GLUCOSE. It includes measuring blood glucose levels in a fasting state, and at prescribed intervals after oral glucose intake (1 g/kg) or intravenous infusion (0.5 g/kg).
2. **Blood Glucose (Glycaemia)**
   Definition: Glucose in blood.
   Normal fasting values around 5 mmol/l (90 mg/dL)

3. **Diabetes Mellitus**
   Definition according to *PubMed:* A heterogeneous group of disorders characterized by HYPERGLYCEMIA and GLUCOSE INTOLERANCE.

WHY

1. …… is glucose tolerance test performed clinically?
   a. To help in diagnosing *diabetes* or *impaired glucose tolerance* – IGT ("prediabetes")
2. … do we perform the lab?
   a. To discuss glycemia regulation mechanisms
   b. To perform and to discuss the most common methods of blood glucose estimation
   c. To appreciate benefits of continuous glucose monitoring for the individual with DM
   d. To learn that regular exercise (physical activity) help to keep glycemia within optimal range, prevents development of metabolic syndrome including DM II.
   e. To see that not only carbohydrates and energy load but also food composition and processing have impact on glucose profile curve.



Sample glucose profile curve during oGTT (oral glucose tolerance test):

Task 1: Blood glucose determination after food or exercise challenge

HOW

In Principle:

- Blood glucose is measured in capillary blood of student-volunteers fasting overnight.
- After assessment fasting blood glucose, 3 volunteers choose one of following procedure according to our recommendation:

Time of the lab	Set of the food/drink	Volunteer 1	Volunteer 2	Volunteer 3
7.30 - 10.30	A	Orange juice 10 ml/kg BW	"keiserka" bun 1 piece/25 kg BW	15 min cycling on cycloergometer, performance 1,5W/kg
10.45 – 13.45	B	Instant noodle soup: 50 g carbohydrates/ package/ person	Lentil: 50g carbohydrates/ serving/person	
14.15 – 17.15	A or B			

Tab.1: glucose and exercise load scheme, BW = body weight

- Blood glucose is estimated by personal glucometer according to following scheme: Tab.2. Personal glucometer works in principal as a spectrophotometer. An enzymatic assay (glucose-oxidase reaction of

the sample on the test strip, optical absorption measurement). The glucose profile curve is constructed and discussed.

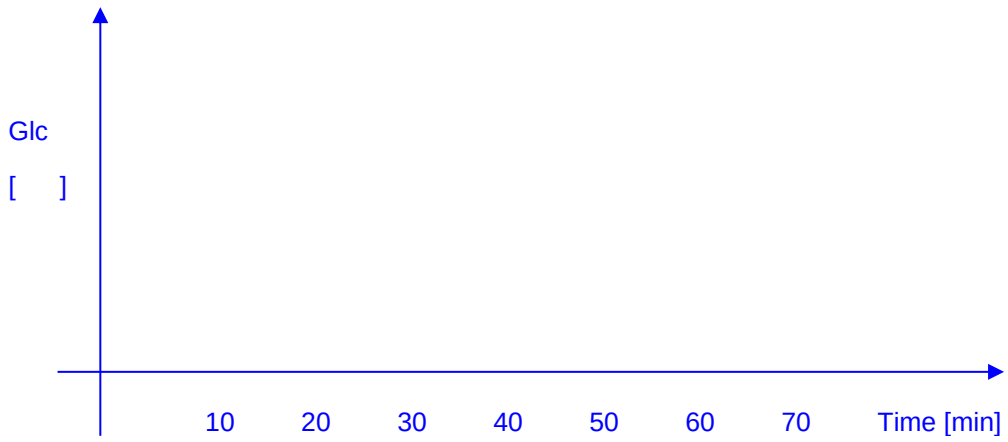
Time of blood sampling	Volunteer 1	Volunteer 2	Volunteer 3
0	x	x	x
procedure	Eating or drinking		15 min exercise
20 min after the end of procedure	x	x	x
30 min	x	x	x
60 min	x	x	x
90 min	x	x	x
120 min	(x)	(x)	(x)

Tab.2: blood sampling scheme

RESULTS

Time [min]	**Fasting Blood glucose**	**10**	**20**	**30**	**40**	**60**	**120**
juice							
bun							
Instant noodle soup							
lentil							
exercise							

Tab.3: blood glucose, mmol/l



Task 2: Glucose profile curve analysis in a healthy person, outcome of continual glucose monitoring (CGM) for diabetic people

Recommended for further reading: Gary Scheiner, CGM Retrospective Data Analysis

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4717507/

Continuous glucose monitoring (CGM) in interstitial fluid of subcutaneous tissue improved daily management of food intake and insulin dosing in people with diabetes enormously. Achieving more stable glycemic curve fluctuating in satisfactory range between 3.5 and not for long much above 10 mmol/l leads to better clinical outcome. Curve analysis provides valuable data that include:

- Postprandial glucose pattern
- Overnight/basal stability (presence/absence of dangerous night hypoglycemia)
- Appropriate insulin timing before meal and duration of bolus insulin action
- Timing of hypoglycemic episodes
- Efficacy of meal (appropriate for the individual) and correction insulin doses
- Impact of variety of lifestyle activities

CGM importance

Permanent availability of actual glycemia data helps people with diabetes to stay within the target range much longer than without. It means shortening periods of hyperglycemia (that lead to development of chronical diabetic complication) and limitation of hypoglycemia (life threatening situations). Moreover, achieving more stable glycemic profile (excluding enormous fluctuation) slows down development of diabetic angiopathy.

In this lab CGM curves of both, healthy and diabetic people, are provided. Students are expected to look at:

1. Time of glycemia of tested individual in target range (3.9-10 mmol/l)
2. Standard deviation that reflects glycemia fluctuation
3. The low end the upper end of achieved range. Which of two edges is controlled more strictly in a healthy individual and in diabetic person. Why?
4. Search for all hypoglycemia episodes in both healthy diabetic reports. In which person have you found it? Why? When did they happen? During the day or during nights?
5. Look at 7 days overlay of CGM. Describe remarkable differences between healthy and diabetic person.
6. Look at composition and carbohydrates load of meals in individual days of healthy person.
   How are particular meals reflected by glycemic curve?
   Is the same meal reflected always by the same glycemic curve shape in one healthy person?
   What else can influence achieved glycemia? How do you understand it?

Healthy person CGM: tasks and comments

Septermber 4th

The same meal was eaten at 2 and 9 p.m. but different glycemic profile was achieved. What could be the reason.

September 5th

Stress is an important factor that worsen glycemia regulation. Also, every illness leads to destabilization of blood glucose profile in diabetic people.

Analysis of CGM data in DM patients aims to fine tuning of food, insulin and life style to achieve the best clinical outcome. Days under stress, sleep deprivation, illnesses as well as unusual events like restaurant visit or celebrations should be marked in the report. Conditions of data collection has to be taken into consideration.

September 5th was the day of mild stress for tested person. How is it reflected by glycemic curve?

Week days versus weekends

Glycemic curves taken during week days and weekends usually differ. Could you explain how and why? How is it in provided data of monitored healthy person?

Mo-Thu

There is usually a remarkable spike during the lunch time. Look at similarities and differences and try to explain them.

Exercise

Find all marked physical activities in the report. Try to summarize what is the effect of exercise on glycemic profile.

Supplement – working sheet for glycemia lab preparation

The topic ……Glycemia regulation…… opens plenty of clinically related questions that can´t be covered in one lab but are very useful to discuss. We offer below some additional tasks and materials for self-study.

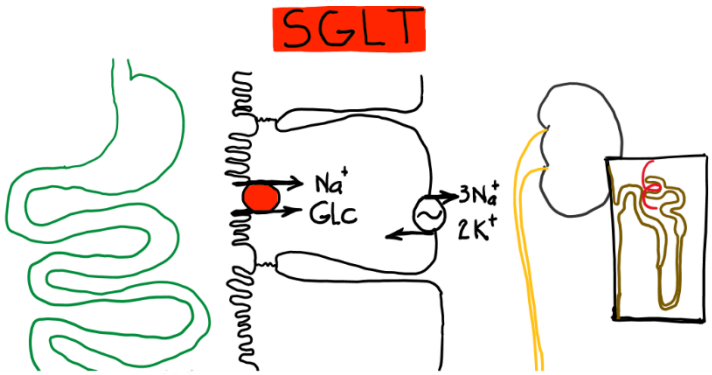
Glycemia in a healthy individual

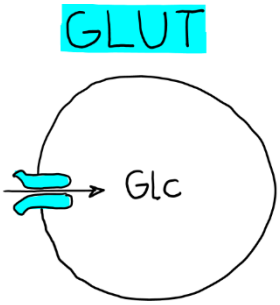
Glycemia fluctuates between …….. and……mmol/l.

- Why should it not drop physiologically under the lower limit?
  …
- Why should it not exceed physiologically the upper limit?
  …
  …
  …
  …
- How does the human respond to hypoglycemia?
  …
  …
  …

Glucose transport mechanisms:

Do you have any idea why the enterocytes and tubular cells in the kidney are equipped with secondary active Glc transporters (SGLT) and the other cells with passive ones (GLUT)?

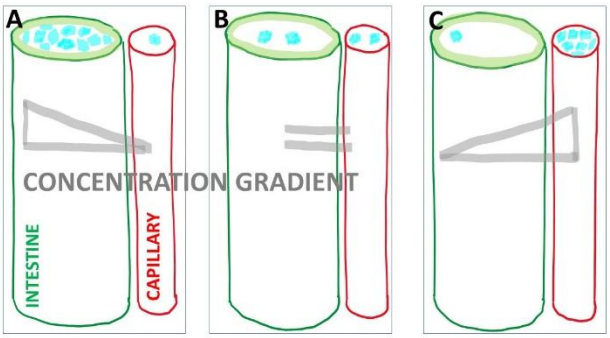




PUSHING FORCE	PUSHING FORCE
Na/K ATPase	Glucose concentration gradient
works also against concentration gradient	works only along concentration gradient

Fig.: Glucose transport in the intestine
Glucose concentration gradient in the intestine does not always favor transport out of the GUT lumen towards cells and capillary. There are three possible situations (A, B, C) in the picture that can occur during food absorption. Active transport prevents wasting of the glucose (or other precious nutrients).

Similar situation is in the kidney tubules.



Fasting

Human beings have a very long history of limited food resources (millions of years compared to the couple centuries of sufficiency and decades of abundance for some populations). That is why mechanisms preventing hypoglycemia had to be evolved very reliably.

- Recall the hormones that cause an increase in glycemia:
  …
  …
  …
- Recall mechanisms of blood glucose elevation:
  …
  …
  …
- Recall substances that can be turned into glucose in the process called gluconeogenesis:
  … … … …
- Recall tissues that are specialized for effective gluconeogenesis:
  …
  …

- Could glycogen in skeletal muscle become the source of blood glucose?
  …
- Compare the effects of epinephrine and cortisol. How do they differ in terms of glycogen synthesis or lysis?
  …
- How do you understand the physiological role of this difference?
  …
- Which one (epinephrine vs. cortisol) serves to boost immediate glucose availability?
  …
- Which one promotes the glucose storage for long lasting fasting?
  …

- Hypoglycemia stimulates adrenal medulla to epinephrine release. What is the effect of epinephrine in larger doses on arteriolar sphincters in skeletal muscle and adipose tissue?
  …
- How does this effect influence blood flow and glucose uptake in these areas?
  …
- What is the effect of epinephrine on insulin release?
  …
- What is the aim of described epinephrine effects in skeletal muscle and adipose tissue on glycemia?
- Why is the glucose level so precisely regulated if there could be also other energy substrates in our body used by cells?

B R A I N

The metabolic rate of the brain is relatively constant and corresponds to 1/5 of BMR. It is a lot of energy that has to be covered largely by glucose. The secondary fuel for the brain is ketone bodies. Ketone body production increases in response to glucagon and cortisol during fasting. Development of adaptation processes that would compensate for the lack of glucose with sufficient quantity of other substrates takes some time (days). Tissues which are utilized preferentially are skeletal muscles and adipose tissue. Both gluconeogenesis and ketone body production occur mainly in the liver and have sufficient capacity to keep healthy fasting individual conscious (with sufficient nutrients delivery to the brain). Obviously, the period of fasting cannot be too long and has to be followed by energy resources replenishment.
What condition leads to un-consciousness in a hypoglycemic individual?

Related to patients on insulin:
Think about the SPEED of hypoglycemia onset if insulin is administered without an adequate food intake. It is the most common cause of hypoglycemia with insufficient brain glucose supply.
(See also the part concerning NON-physiology individual below)

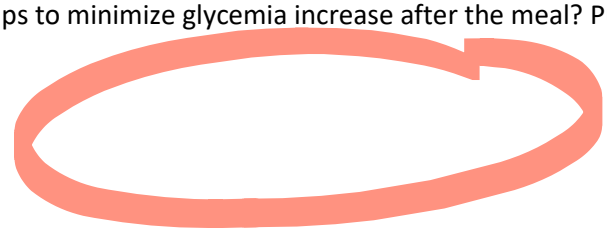
Eating, drinking and internal environment

Consider, how does food ingestion influence various parameters of blood compartment and subsequently extra and intracellular fluid composition.

- How does nutrient absorption contribute to osmolarity fluctuation?
- What is the normal range for plasma osmolarity?
- What is the normal glycemia variations between fasting and a big meal ingestion rich in saccharides?
- How does this fluctuation affect osmolarity?

NOTE: plasma osmolarity estimation: 2x [Na+] + [Glc] + [urea] + others

- Compare the range for normal osmolarity changes and normal glycemia changes.
- Compare physiological range of glycemia and kidney capacity for glucose reabsorption.
- Which hormone helps to minimize glycemia increase after the meal? Put its name into the red circle.



- What tissue is insulin dependent (it can receive glucose only in the presence of insulin)?

The purpose of following questions is to consider how potent insulin is in glycemia maintenance:

If your answer is correct, you have realized, there are only two types of tissue (both serving as major storage of energy substrates) that allow glucose influx only in the presence of insulin.

- How big is their volume compared to body size?
- What type of glucose transporter do they use?
- What are the possible fates/metabolization of glucose entering these cells?

…….. or …….

- How do the utilization of glucose (glycolysis), storing as glycogen or rearrangement into fat affect the trans-membranous concentration gradient?
  Choose the more probable answer:

A: Glucose concentration in extracellular and intracellular space equilibrates quickly and the influx stops.

B: Utilization and storage of glucose favor concentration gradient towards intracellular space and so the influx of glucose can last as long as the GLUT4 transporter is available and there is at least some glucose in plasma.

Now think about injecting of insulin that is not appropriately counterbalanced by food in terms of glucose distribution mechanisms explained above. How fast and how profound can the glycemia drop be? How does the considerable drop of glycemia influence the concentration gradient of glucose between blood and brain? Is the transport mechanism of glucose into brain tissue active or passive?

Besides skeletal muscle and adipose tissue, the **liver** can be profoundly influenced by insulin in terms of glucose uptake (expresses GLUT 4 transporters in response to insulin). In contrast to muscle cells and fat cells, glucose can enter hepatocytes also via other GLUT transporters that are not insulin dependent.

The questions and explanations above were focused on glucose. However, you must consider other relevant components while thinking about the role of insulin in nutrients homeostasis. Examples: AA, FAA, ions.

- What ion in extracellular fluid is most tightly regulated and why?
- How does insulin contribute to this ion distribution in organism after eating?

Glycemia in a person who has impaired glucose tolerance or diabetes

If glycemia regulation is impaired, the organism is not able to keep blood glucose within normal range.
Oral glucose tolerance test is designed to recognize this situation.

- What dose of glucose is given to the patient in this test?.....
- Compare this dose with some ordinary food:

Examples:
3x0,5 l 12°beer (5g saccharides/100 ml)
or

300 g of plain cooked pasta (25g saccharides/100g)
or

100 g of biscuits (75 g saccharides/100g)

Compare with the composition and size of an ordinary big meal:

Pizza Margherita 30 cm diameter

You have realized that oral glucose tolerance test does not represent an extraordinary but quite normal load of saccharides per meal.

Glycemic index (GI), stimulated insulin secretion

- What is GI?
  Find the GI of food examples given above (beer, pasta, biscuits).
- Consider the differences in glycemia profile curve development after intake of each of this food.
  100 g of biscuits contains the same saccharide load as 300 g of pasta.
  Why is diabetic person not recommended to eat the biscuits but can eat pasta?
  Could you find an example of a situation when the diabetic person should eat biscuits rather than pasta?
- Is the glycemic index a sufficient criteria for food selection in diabetics or people at risk of DMII development? Why?
  What other criteria are important?
  Some food does not contain saccharides but still can stimulate insulin release due to AA and lipids content.
- How much insulin do β cells produce per day in a healthy individual?
  Quantify basal secretion……..and stimulated secretion……
- Is the amount of insulin released per nutrient mass/type the same for everybody?
  YES and NO: It will vary in some physiololgical range in healthy individuals.
  NO: if pancreatic β cells are destroyed or if insulin resistance develops.

Review on mechanism of insulin effect and insulin resistance development: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6170977/

Hyperglycemia

- What value of blood glucose is considered hyperglycemia?
  What is the upper limit for fasting glycemia in a healthy individual?
  What is the upper limit for postprandial glycemia in a healthy individual?
- Is hyperglycemia dangerous?
  Why?
- What is more dangerous, hypoglycemia or hyperglycemia?
  Why?

Hypoglycemia

- What is the lower limit for normal glycemia?
- What event can cause hypoglycemia below this limit?
- What are the clinical symptoms of hypoglycemia?
  Separate the symptoms into two groups:
  A: Related to low glucose delivery to Glc dependent tissue:
  B: Related to epinephrine release:
- What consequences do you expect if acute hypoglycemia is not cured immediately?
- Is there any risk of permanent damage?

What would you do in case of hypoglycemia?

Task 1:

It is Monday 11 a.m., you are working in the hospital as a nurse. This time the diabetic patients are administered insulin (15 minutes before regular lunch dispensing) every day. Today patients were given insulin, but accidently the lunch patrol is delayed because some technical issue.
Offer the solution of this situation.
What development of patients´ status do you expect if insulin administration is not counterbalanced with food immediately?

Task 2:

There is an unconscious person admitted to hospital.
Hypoglycemia is one of the possible causes of unconsciousness. Choose the best way of glycemia assessment:

1. From capillary blood sample by personal glucometer bedside test (result in seconds)
2. From venous blood sample sent to the central lab (result in 20 minutes but offers a wider range of analyzed blood parameters)
3. Any measurement introduces useless delay, so it is better to administer glucose i.v. without checking blood glucose

Task 3:

An unconscious person has glycemia of 1.9 mmol/l. Select an appropriate glucose solution: µl

a. 5%, 500 ml i.v.
b. 10%, 500 ml i.v.
c. 20%, 500 ml i.v.
d. 40%, 80 ml i.v.

- Discuss the speed of glucose elevation achieved by administration of particular solution.
- Discuss osmolarity of each solution and consequences for safe administration.
- Determine glucose load in each solution.
- Determine the energy load in each solution. Give an example of food with the same energy content.

Glycemia measurement – lab analysis, monitoring for clinical purposes

Capillary blood analysis detected by glucometer

Principle: glucose oxidase reaction
Advantage: accurate enough, bedside - fast (seconds)
Disadvantage: provides only the glycemia value and nothing else regarding the blood sample

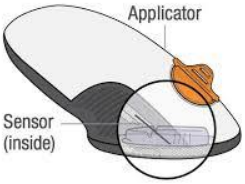
Venous blood analysis in the lab

Principle: glucose oxidase reaction detected by spectro-photometry
Advantage: accurate, can analyze the same sample for more parameters (ions, enzymes…)
Disadvantage: performed in the central lab, lasts at least 20 minutes

Continuous monitoring of glucose concentration in interstitial fluid by electronic chips

Advantage: accurate enough, much broader outcome/medical inference:

- Current glucose measurement each 1 - 5 minutes non-invasively (a fine wire inserted in the skin and subcutaneous tissue)
  sensor exchanged roughly every 10 days



- Shows glycemic trends/profiles
- Shows consequences/insulin -food dependencies
  (relationship between eating and insulin dosing)
- Can be coupled with insulin pump and adjust insulin dosing according to actual glycemia
- Used in everyday routine easily, data collected by mobile phone

Disadvantage: Interstitial fluid glucose fluctuation is delayed behind systemic capillary blood glucose (20 minutes) and even more behind portal blood glucose during nutrients absorption