Carbohydrate Metabolism

Synopsis of Glycolytic Enzyme Deficiencies

The video that accompanies the texts in this segment begins on page 44. However, a quick review of the rest of the materials is highly recommended prior to watching the video.
Glycolytic Pathway

Glycogen → Glucose 1-phosphate
Glucose → Glucose 6-phosphate
Fructose 6-phosphate → Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate ↔ Glyceraldehyde 3-phosphate
1, 3-Diphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Lactate ↔ Pyruvate
Acetyl-CoA

Cytoplasm
Mitochondria

*Stars indicate key controlling enzymes and steps of glycolysis. The light-color star connecting pyruvate to acetyl CoA is not a glycolytic step. It marks the pyruvate dehydrogenase enzyme (PDH) that is required for aerobic respiration of glucose.

**Glycolytic Map**

**Glucose 6-Phosphate**
- Glycogen phosphorylase catalyzes conversion of glycogen to glucose 1-phosphate.
- Hexokinase and/or glucokinase catalyze conversion of glucose to glucose 6-phosphate.
- The two glucose phosphates—glucose 1-phosphate and glucose 6-phosphate, are readily interchangeable—using phosphoglucomutase as their catalyst.

**Hexokinase (HK)**
- Most tissues have hexokinase
- Broad Specificity (acts on most sugars).
- Affinity for glucose is high (low $K_m$); it works maximally during fasting glucose conditions.
- Helps to provide G6P to tissues during low blood glucose levels.
- Low Vmax (cannot phosphorylate high glucose quantities). It has low capacity for working on glucose.
- Inhibited by the product (G-6-P); hence, it works better if products are utilized quickly (e.g. fasting conditions).

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38. Northwestern Medical Review, Bare Minimum Review Series, 2012
Addition of phosphate to hexoses would trap glucose within the cells. It also energizes the molecule in preparation for further enzymatic activities. As we will see later in the absence of alternative pathways to utilize the phosphorylated sugars, this trapping will increase the osmotic pressure and lyses or damages the cells.

**Glucokinase**
- Glucokinase is the unique liver brand of hexokinase.
- Shows affinity for glucose when glucose level is very high (high $K_m$).
- Handles huge incoming venous portal glucose after meals (high $v_{max}$). It has high capacity for working on glucose.
- Synthesis is stimulated by insulin.
- As opposed to hexokinase, it is not inhibited by glucose 6-phosphate.

**Note:** The only other cells in the body with glucokinase are the pancreatic beta-cells.

1. Why do people not get hyperglycemic after carbohydrate meals?

**Hexokinase (HK) Deficiency**
- Autosomal recessive disease
- Hemolytic anemia
- Decreased formation of 1, 3 DPG (1,3 diphosphoglycerate).
- 1,3 DPG is normally converted to 2,3 DPG by a mutase in the red cells.
- Hence, in hexokinase deficiency the 2,3 DPG level is decreased.

**Note:** Normally about 10-20% of red cell glycolytic pathways are shunted toward 2,3 DPG formation. This is an important shunt in the red cells and it is normally stimulated on the tissue sides as a result of accumulation of metabolic byproducts such as CO2 and hydrogen ions that drop the pH.

**2,3 Diphosphoglycerate**
- Most abundant organic phosphate source of RBC
- Regulates binding of oxygen to hemoglobin
- If absent, Hb (hemoglobin) shows a high affinity for oxygen; if present, Hb unloads oxygen.
- $\text{HbO}_2$ (oxyhemoglobin) + 2,3DPG $\rightarrow$ Hb2 (deoxyhemoglobin) + O2 + 3PG
- 2,3DPG level in RBC increases in chronic hypoxia, COPD, asthma, and high altitude.

**Note:** It is postulated that acidity of blood at the tissue level redirects part of red blood cell glycolytic pathways toward 2,3 DPG formation via activating a mutase that converts 1,3 DPG to 2,3 DPG. As a result red cells unload their oxygen to the tissues and become deoxygenated. Deoxyhemoglobin, on the other hand, is a perfect buffer and it buffers the acids. As a result of drop in the acidity a phosphatase enzyme gets activated and converts 2,3 DPG to 3-phosphoglycerate (i.e. back to the main glycolytic pathway). Also note that in the lung vasculature due to lower levels of 2,3 DPG and less acidity of the blood hemoglobin can more aggressively load oxygen. The same mechanism also explains why in COPD and asthma (which are associated with respiratory acidosis and high CO2 levels) there is a higher level of 2,3 DPG in red cells.
Findings in Hexokinase (HK) Deficiency
- Low 2,3-DPG of the red cells causes high oxygen affinity of hemoglobin for oxygen. At the same time it makes unloading of oxygen to tissues very difficult. This leads to left shift of the hemoglobin oxygen dissociation curve and tissue anoxia (or tissue hypoxia).
- Lack of ATP formation due to failure of glycolytic pathways cause hemolytic anemia.

Generalization
All glycolytic enzymes share one aspect in common; their deficiency is associated with hemolytic anemia!

 Autosomal Recessive and Dominant Defects

<table>
<thead>
<tr>
<th>Recessive</th>
<th>Dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Often cause enzyme deficiencies (e.g. hexokinase or glucose 6 phosphate dehydrogenase deficiencies)</td>
<td>Often cause structural protein defects (e.g. collagen in Marfan’s, or spectrin in hereditary spherocytosis)</td>
</tr>
<tr>
<td>100% penetrance (i.e. all patients exhibit all symptoms)</td>
<td>Partial penetrance (i.e. not all patients exhibit all symptoms)</td>
</tr>
<tr>
<td>Early age of onset (e.g. infancy to early childhood)</td>
<td>Variable age of onset (early childhood through middle ages)</td>
</tr>
<tr>
<td>Both parents must be carriers for the defective gene</td>
<td>One of the parents must have the disease</td>
</tr>
<tr>
<td>Often more serious</td>
<td>Often less serious</td>
</tr>
</tbody>
</table>

Mechanism of Hemolytic Anemia
- Red blood cells are 100% dependent on anaerobic glycolysis for making their ATP.
- Failure of glycolytic enzymes impedes ATP production.

Findings in Hemolytic Anemia
- Premature RBC destruction
- Accumulation of hemoglobin catabolic products (e.g. bilirubin).
- Marked erythropoiesis and reticulocytosis.

Lab Characteristics of Hemolytic Anemia
- Hemoglobinemia, hemoglobinuria and Hemosiderinuria (Iron overload)
- Normocytic anemia (acute cases)
- Jaundice (conjugated hyperbilirubinemia)
- Reduced serum haptoglobin. Haptoglobin is made by the liver and binds free hemoglobin and the bound form is removed by the reticuloendothelial system and spleen.

2. A patient is diagnosed with hexokinase deficiency. What is the most likely age of the patient?
   A. 8 months  
   B. 8 years  
   C. 16 years  
   D. 32 years  
   E. 50 years

3. Why does failure of the Na⁺/K⁺-ATPase pump cause hemolysis of the red cells?

4. What is the only source of energy for the red cells and why?

5. In what form is iron absorbed from the GI?

6. How is iron transported in the blood?

7. What is the name of the storage form of iron?

8. What commonly tested disease is associated with high iron deposits in tissues and it is characterized by diabetes, cirrhosis, and dilated (congestive) and/or restricted cardiomyopathy?

9. What is hemosiderin made up of?

10. What is normal reticuloctic index and when does it increase or decrease?

11. What is the term used to describe diabetes as result of hemosiderins deposit in the pancreas?
12. What is the treatment for hemochromatosis?
____________________________________

13. Diagnosis of hemochromatosis quite often begins by measuring the serum levels of transferrin and ferritin. What are the two common tests performed on the liver to diagnose hemochromatosis?
____________________________________

14. What tissue of the body is most adversely affected by glycolytic enzyme deficiencies?
____________________________________

Parthenogenesis of Hemochromatosis

Factors Affecting Integrity of Red Blood Cells
- Increased level of glucose-6-phosphate dehydrogenase (indirect positive effect)
- Adequate availability of ATP
- Proper activity of pentose monophosphate shunt
- Maintenance of glutathione in reduced state

G-6-Phosphate: The Gateway to Other Pathways

Glucose 6-phosphate is the substrate of three enzymatic pathways; glycogen synthesis and degradation (the left side pathway in the above diagram), glycolysis (in the center) and hexose mono-phosphate shunt (on the right).

Biochemical Control of PFK
- The purpose of glycolysis is to produce energy
- High titer of cellular AMP levels (i.e. low energy) will activate the glycolytic enzymes in general and PFK in particular.
- High levels of intracellular ATP will shut-off the PFK
- Citrate is the first compound in the Kreb’s cycle, and it is among the few compounds that can exit the mitochondrial membrane and get into the cytoplasm wherein the glycolytic enzymes are located.
- The titer of citrate increases in the mitochondria whenever there is a high level of ATP production by the Kreb’s cycle. Appearance of the citrate in the cytoplasm will then convey the message of high ATP levels to the PFK.
- The next potent activator of the PFK is fructose 2,6 bisphosphate (see further below)
Fructose 2, 6-Bisphosphate

- In the Liver, F2,6-BP is formed from F6-P by PFKII.
- F2,6-BP activates PFKI.
- It inhibits F1,6-bisphosphatase, that is, the enzyme of the reverse reaction (gluconeogenesis).
- It is an intracellular sensor of glucose level.
- Hepatic levels are decreased with elevated glucagon or during fasting.
- Levels are increased with low glucagon and in the fed state.
- Insulin greatly increases synthesis of PFKII and as a result stimulates the glycolytic pathways.

15. Which hormone stimulates and one inhibits PFK II?

____________________________________

16. Which of the two has a higher effect in raising output of insulin from the pancreas; a high carbohydrate diet or IV injection of glucose?

____________________________________

17. List major causes of rhabdomyolysis:

____________________________________

18. What is the rate-limiting enzyme of glycolysis?

____________________________________

Phosphofructokinase (PFK) Deficiency

- AKA. Type VII Glycogen Storage Disease.
- Inefficient muscle glucose supply causes cramps.
- Glucose is mainly supplied by gluconeogenesis.
- Increased muscle glycogen stores (glucose is shunted toward glycogen production due to improper glycolytic functions).
- Mild Hypoglycemia. Breakdown of glycogen is slowed down and glycogenesis is enhanced.
- Rate of ATP formation (by Kreb’s Cycle) decreases.
- Red cells are deprived of ATP and there is failure of sodium-potassium-ATPase pump. Hence, sodium stays within the red cells and this leads to hemolytic anemia.
- Increased muscle glycogen causes myopathy, rhabdomyolysis, increased serum CPK, myoglobinemia and myoglobinuria.
- Substrates before the step increase in muscle. For example, fructose and glucose 6-phosphate increase, whereas, products after the step such as Fr 1,6-diphosphate and 1,3 DPG decrease.

Phosphofructokinase (PFK) Deficiency

Glucose 1-phosphate

Glucose 6-phosphate

Fructose 6-phosphate

Fructose 1,6-bisphosphate

Phosphofructokinase (PFK) Deficiency

Glycogen

Glucose 1-phosphate

Glucose 6-phosphate

Fructose 6-phosphate

Fructose 1,6-bisphosphate

Fructose 2,6-BP is made in the liver by PFK II and it promotes glycolysis via stimulating Phosphofructokinase I and stops gluconeogenesis.

Last Unique Step of Glycolysis

2 ADP

Pyruvate Kinase

2 ATP

2 Pyruvate

Sugars make pie!

Pyruvate (the pie!) is the end result of carbohydrate metabolism.

Pyruvate is also a pirate because it pirates 3 carbon sugars from cytoplasm into the mitochondria.

(We will see more on this later!)
19. A 25-year-old woman with history of heavy menstrual bleeding complains of fatigue and exercise intolerance. Blood analysis is significant for hypochromic microcytic anemia. Which of the following two iron preps would you prescribe for her, ferric or ferrous sulfate?

**Formation of Pyruvic Acid**
Formation of two pyruvic acid molecules from two PEP molecules in the cytoplasm marks the end of glycolysis. But in order for ATP (energy) to become available pyruvate (the pie!) must be further oxidized (eaten!) either under aerobic or anaerobic conditions (see further below for details).

**The last Step of Glycolysis**

Pyruvate kinase is the last key-controlling enzyme of glycolysis.

**Generalization**
The purpose of glycolysis is to make energy. As a general rule, accumulation of downstream products and abundance of sources of cellular energy such as ATP and NADH inhibit the glycolytic pathway. Conversely, abundance of uphill substrates (that is, the preceding compounds) promotes the pathway.

As we will see later, this principle also holds for the Kreb’s cycle.

**Regulation of Pyruvate Kinase**

Pyruvate Kinase (PK) Deficiency
- Normal RBC lacks mitochondria, and it is fully dependent on anaerobic glycolysis for ATP.
- Deficient individuals have a low Pyruvate Kinase level in their red blood cells
- Hence, the patients have low RBC glycolysis, and low ATP production. Low ATP affects K+/Na+-ATPase pump, and leads to membrane damage, lysis and hemolytic anemia.
- Consequence is anemia, marked increase in reticulocytic number and mild jaundice.
- Failure of the last step of glycolysis
- Abnormal accumulation of glycolytic intermediates before the step
- High 2,3-DPG production and abnormally low oxygen affinity of Hb (right shift of the Hb-oxygen dissociation curve)
- **Treatment Options:** Oxygen therapy, blood transfusion (serious cases), folate or B12 administration and splenectomy.

**Note:** Spleen gets sequestered with and lyses the PK deficient red cells. As such removal of spleen would help to minimize the rate of hemolysis. Furthermore, due to high levels of red cell breakdown these patients are at risk for splenomegaly and rupture of spleen.

20. Where in the cell does the glycolytic pathway take place?
Summary of Glycolysis

[Diagram of glycolysis: 1 Glucose → 2 Pyruvate → 2 NADH, 2 ATP]

Right shift of hemoglobin (Hb) dissociation curve increases P50 (partial pressure of oxygen that saturates 50% of available Hb sites). That is for any PO2 level saturation of Hb will decrease.

Associations of Hemoglobin-Dissociation Curve

<table>
<thead>
<tr>
<th>Left Shift</th>
<th>Right Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Low 2,3 DPG in RBC</td>
<td>- High 2,3 DPG in RBC</td>
</tr>
<tr>
<td>- High hemoglobin oxygen affinity</td>
<td>- Low hemoglobin oxygen affinity</td>
</tr>
<tr>
<td>- Low unloading of O2</td>
<td>- High unloading of O2</td>
</tr>
<tr>
<td>- Carbon Monoxide</td>
<td>- Increased acidity</td>
</tr>
<tr>
<td>- Methemoglobinemia</td>
<td>- Increased PCO2</td>
</tr>
<tr>
<td>- Fetal hemoglobin</td>
<td>- High Temperature</td>
</tr>
<tr>
<td>- Hexokinase and phosphofructokinase Deficiencies</td>
<td>- Chronic hypoxia</td>
</tr>
</tbody>
</table>

21. Both carbon monoxide poisoning and methemoglobinemia cause left shift of hemoglobin-oxygen dissociation curve and make unloading of oxygen more difficult. Why oxygen therapy is still the mainstay therapy in both conditions?

A 4-year girl is being evaluated for anemia and jaundice. Lab results are significant for increased reticulocytes, reduced serum haptoglobin, and increased urinary hemosiderins. No sickle cells or spherocytes are identifiable in the child’s blood. She has mild splenomegaly and lacks hepatoomegaly. There is no history of any recurrent infections.

22. What is the best descriptive term for explaining above finding?

23. What are your first impression top differentials?

24. What are your second impression top differentials?

25. What makes above enzyme deficiencies similar?

26. Which of the three is the most likely cause of the above findings and also the most commonly tested one on the COMLEX and USMLE exams?

27. What is by far the most common cause of hemolytic anemia as a result of an enzyme deficiency?

28. What unique clinical finding will set PFK apart from the other two glycolytic deficiencies?

29. In which of the aforementioned glycolytic deficiencies the level of 2,3DPG increases in the red cells and the patient assume a right shift of the hemoglobin dissociation curve?
30. Why patient with hexokinase deficiency present with the most profound hemolytic anemia among the three glycolytic deficiencies?

31. Which of the patients in the above three glycolytic enzyme deficiencies will be more benefitted from oxygen therapy?

Summary of Glycolytic Enzyme Deficiencies

<table>
<thead>
<tr>
<th>HK</th>
<th>PFK</th>
<th>PK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. All are major glycolytic enzyme deficiencies</td>
<td>2. All are rare autosomal recessive defects</td>
<td>3. Hemolytic anemia</td>
</tr>
<tr>
<td>3. Treatment Options: Blood transfusion, splenectomy, and foliate and vitamin B12 administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Most fatal hemolytic anemia among common glycolytic enzyme deficiencies</td>
<td>• Glycogen storage disease VII</td>
<td>• The most common glycolytic enzyme deficiency</td>
</tr>
<tr>
<td>• Left Shift</td>
<td>• Rhabdomyelysis, myoglobinemia and high serum CK</td>
<td>• Right shift of Hb-oxygen dissociation curve and difficulty with loading oxygen on hemoglobin</td>
</tr>
<tr>
<td>• Treated with blood transfusion</td>
<td>• Left Shift of Hb-oxygen dissociation curve</td>
<td>• Oxygen therapy</td>
</tr>
</tbody>
</table>

32. What is the major source of energy for the red cells during serious starvation?

33. What is the homeostatic value of maintaining blood glucose levels at about 90 mg/dl?

34. You are looking at the blood smear of a 6-year-old child with hemolytic anemia due to pyruvate kinase deficiency. What type of anemia based on the size of red cells do you expect to see?

35. What two clinical conditions are common causes of the same type of anemia based on the size of the red cells?
1. Under the influence of insulin, glucokinase traps (sequesters) glucose in the hepatocytes and convert them to glycogen.

2. Autosomal recessive genetic conditions often are diagnosed at early age. Therefore the best answer is [A].

3. The pump extrudes sodium and returns potassium back into the red cells. In the absence of proper pump function sodium is retained in the red cells and increases osmotic pressure. This leads to swelling and early destruction of the cells.

4. Mature red cells do not have mitochondria. Therefore they are not capable of performing aerobic respiration. For this reason they cannot use fatty acids and ketone bodies as a source of energy. The only energy producing mechanism available to the red cells is anaerobic glycolysis that yields two ATP molecules for every glucose molecule that they consume.

5. Iron is absorbed in ferrous form (Fe++)

6. Transported by transferrin that is a protein made by the liver in the ferric form (Fe+++)

7. The storage form of iron is known as ferritin. It is a protein that combines with iron.

8. Hemochromatosis! The genetic form of the disease is associated with excessive absorption of iron from gut. It is due to mutation in the HFE gene. It is more common among the Caucasians and in particular Irish population.

9. Hemosiderins are made up of iron, protein and polysaccharides. They accumulate in various tissues including heart, pancreas, liver and GI mucosa and often cause death of the tissues.

10. Reticulocytes are immature red cells. Typically about 1% of the red cells are reticulocytes. Reticulocytes develop and mature in the red bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells. Like mature red blood cells, reticulocytes do not have a cell nucleus. They are called reticulocytes because of a reticular (mesh-like) network of ribosomal RNA that becomes visible under a microscope with certain stains such as new methylene blue. In hemolytic conditions the reticulocytic index is more than 1. In contrast in bone marrow diseases it may drop to values less than 1.

11. Bronze diabetes

12. Periodic phlebotomy and oral administration of zinc. Zinc competes with iron for absorption from GI.

13. Liver is one the main organs for accumulation of iron deposits. Ferri-Scan is a MRI-based test to non-invasively and accurately measure liver iron concentrations. It identifies iron deposits in the hepatocytes. Currently it is a preferable choice over the widely used liver biopsies (and staining of the tissue with Prussian blue).

Note that serum transferrin and transferrin saturation are commonly used as screening for hemochromatosis. Fasting transferrin saturation values in excess of 45% is suggestive of hemochromatosis. Transferrin saturation greater than 62% is suggestive of homozygosity for mutations in the HFE gene. Serum ferritin provides another crude estimate of whole body iron stores though many conditions. But it involves a high rate of false positive cases because inflammation, chronic alcoholism, and fatty liver diseases also elevate serum ferritin. Normal serum ferritin values for males are 12–300 ng/ml (and less in females). Serum ferritin in excess of 1000 ng/ml of blood is most likely due to hemochromatosis.

14. Glycolytic enzyme deficiencies most adversely affect the red cells as they can only utilize glucose as the (sole) source of energy.

15. Insulin (fed state) stimulates PFK synthesis whereas glucagon (fasting state) inhibits it.

16. High sugar diet has more profound effect on stimulating the insulin release because in addition to raising sugar levels it activates parasympathetic nervous system. Activation of parasympathetic system greatly activates pancreatic insulin release.

17. Causes of rhabdomyolysis: (1) Muscle Crush (Car accident); (2) Snake Venom; (3) Enzyme Deficiencies: PFK deficiency, McArdle’s disease, Carnitine Acdyltransferase and Carnitine deficiency, and MCAD deficiency; (4) Drugs such as Statins, Fibrac acid derivatives, Tubocurarine plus halothane, haloperidol, and chlorpromazine; (5) Others: Tetanus, UMN Deficits and grand mal seizures.

18. PFK

19. Ferrous sulfate, because iron is absorbed in ferrous form.

20. In the cytoplasm!

21. Patients who breathe hyperbaric oxygen (pressurized 100% oxygen) show improvement. Although many scholars question the utility of the procedure. It is speculated that by pressurized oxygen we are able to bypass the contributions of the hemoglobin and force feed oxygen into the mitochondria.
22. Hemolytic anemia

23. All genetic causes of hemolytic anemia: Hexokinase deficiency, Phosphofructokinase deficiency, Pyruvate kinase deficiency, Glucose 6 phosphate dehydrogenase deficiency, Hereditary Spherocytosis, Sickle Cell disease, Beta-thalassemia (also alpha-thalassemia, and Hereditary elliptocytosis

24. Hexokinase deficiency, Phosphofructokinase deficiency, and Pyruvate kinase deficiency

25. They are key glycolytic enzyme deficiencies. They all autosomal recessive. All three deficiencies cause hemolytic anemia!

26. Hexokinase deficiency

27. G6PD deficiency

28. Rhabdomyelysis as a result of glycogen build-up in the cytoplasm of striated muscle that leads to myoglobinuric, high serum CK and creatine.

29. PK deficiency. The other two are associated with low 2,3DPG and a left shift

30. Hexokinase is also required for HMP shunt function in the red cells. The shunt makes NADPH that safeguards red cell membrane against oxidizing agents via increasing the level of reduced glutathione. So these patients in addition to osmotic damage of the red cells that is shared among all three are at further risk of oxidizing damages to red cell membrane. Note that the red cells due to 100% reliance on glucose can only use hexose monophosphate shunt and anaerobic glycolysis as the source of energy.

31. Patients with PK deficiency as a result of right shift of the oxygen dissociation curve.

32. Glucose. They cannot utilize any other food source.

33. Even though the brain cells under normal conditions and early starvation rely on glucose, the major contribution of maintain glucose is to nourish red cells and promote respiration that is vital to survival.

34. Normocytic anemia. Hemolytic anemias commonly cause normocytic anemia

35. Kidney diseases due to lack of erythropoietin and hemorrhage cause normocytic anemia.