LECTURE 7-METABOLIC REGULATION

Friday, April 25, 2008
10:06 AM

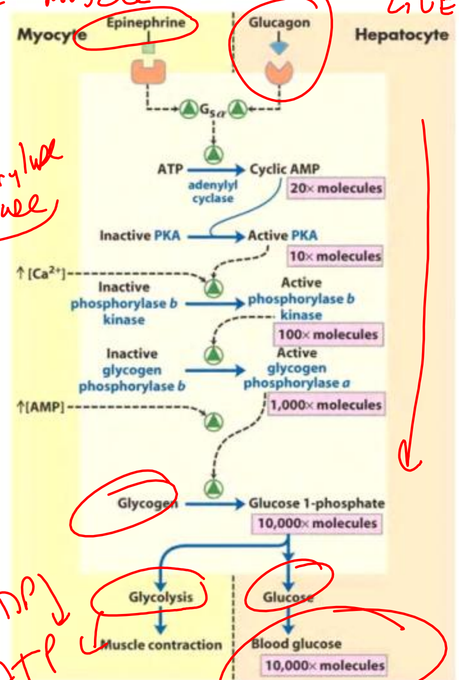
NOTE: The room has changed for the final on Sat. May 17th from Wheel Aud to 100 Haas Pavil.

Bryan Krantz: University of California, Berkeley
MCB 102, Spring 2008, Metabolism Lecture 7
Reading: Ch. 15 of *Principles of Biochemistry*, "Principles of Metabolic Regulation, Illustrated with Glucose and Glycogen Metabolism."

Epinephrine & the cAMP Cascade. Epinephrine is secreted. There are many epinephrine receptors on the surface of muscle cells. Muscle is a characteristic organ where the effect of epinephrine takes place. The **cyclic-AMP (cAMP) cascade** begins with the production of cAMP, which is a pure signaling molecule, i.e., not a metabolism intermediate.

cAMP binds and activates **protein kinase A (PKA)**. PKA converts phosphorylase-*b* into phosphorylase-*a*, which is phosphorylated and active, in two steps. PKA converts glycogen synthase-*a*, which is active to glycogen synthase-*b*, which is phosphorylated and inactive. The end result is that you stimulate glycolysis by increasing the degradation of glycogen and stop synthesis of new glycogen. All the excess glucose goes through glycolysis. This is catabolic metabolism.

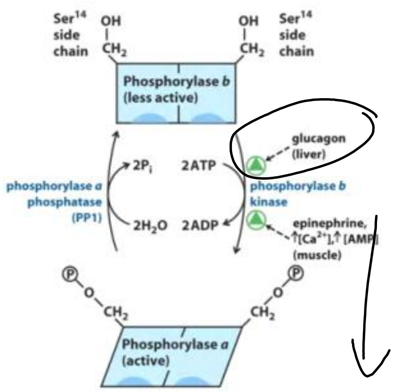
All these phosphorylations occur at the same time. The cAMP cascade results in the phosphorylation of multiple enzymes. The phosphorylation on the protein occurs on a serine or threonine residue in the protein. As a result, these kinases are also **serine/threonine kinases**.



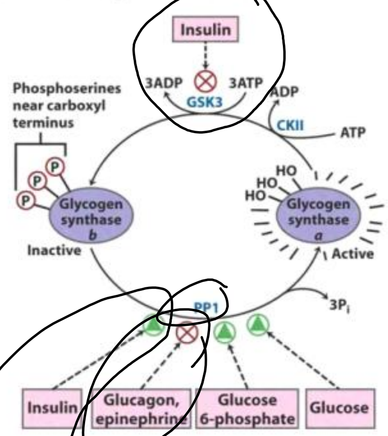
Reciprocal Regulation

Amplification. You do this in many steps to amplify the effect. You come in with one signal molecule of epinephrine, which will activate the receptor. The receptor will activate many G-proteins. Each of these G-proteins will activate adenylylate cyclase. cAMP will activate many PKA enzymes. At every step, there will be many-fold amplification. **One single hormone molecule → activate hundreds of phosphorylations** Unlike allosteric regulation, you produce a "permanent" change in the enzyme molecule, so the effect can be lasting.

Reciprocal Regulation. The reversal of the enzyme phosphorylation modification may require either a kinase or a phosphatase enzyme and these are regulated in a reciprocal fashion.



Glycogen Breakdown



Glycogen Synthase

Regulation of Glucose Homeostasis under Normal Conditions

Regulation vs. Control. These are two terms for specific situations.

Metabolic regulation — maintaining [metabolite]; homeostasis.

Metabolic control — in response to stimulus the output through the metabolic pathway is altered

"Fight or Flight". Brain senses danger; muscles prepare to run.

Hypoglycemia. *What happens when the blood sugar is too low?* Mental impairment, impaired judgement, nonspecific dysphoria, anxiety, moodiness, depression, crying, negativism, irritability, belligerence, combativeness, rage, personality change, emotional lability, fatigue, weakness, apathy, lethargy, daydreaming, sleep, confusion, amnesia, dizziness, delirium, headaches, seizures, and eventually coma. *The brain is very affected; and this is very bad.*

Diabetes. *What happens when the blood glucose level is too high?* Recall C1 of glucose is an aldehyde. It is a reactive and produces an addition compound with proteins; glucose adducts of hemoglobin that can be quantified in a laboratory test. Obviously glucose-modified proteins are not a good thing and diseases, which cannot regulate [glucose] in the blood can be harmful if not managed. To assess whether there is diabetes, a doctor can test for the amount of glucose after fasting. Doctors also want to know what the average level of glucose is in your blood stream while you are carrying on daily chores. The amount of glucose-modified hemoglobin is a very good indicator.

Homeostasis of Blood [Glucose]. Goldy Locks says, "*The glucose concentration is just right!*" Glucose levels should be in the range of ~4 to 8 mM.

Glucagon. Low blood glucose levels can also be catastrophic, because your brain can only really utilize only glucose. (It can utilize ketone bodies in a crisis.) The brain requires a constant supply of glucose, else it will stop functioning and confusion will set in.

The pancreas senses low blood glucose levels and releases **glucagon**, a polypeptide hormone, into the blood. Glucagon mostly affects the liver because liver cells have a large number of glucagon receptors. By changing the number of receptors, you can produce organ-specific regulation.

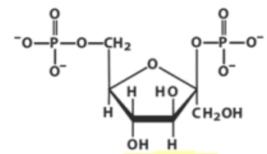
Glucagon:
His–Ser–Gln–Gly–Thr–Phe–Thr–Ser–
Asp–Tyr–Ser–Lys–Tyr–Leu–Asp–Ser–
Arg–Ala–Gln–Asp–Phe–Val–Gln–Trp–
Leu–Met–Asn–Thr–

Glucagon → receptor → cAMP cascade → Phosphorylation reactions → Glycogen breakdown

Glycogen via phosphorylase → Glucose 1-phosphate → Glucose 6- phosphate

For muscle, the glucose-6-phosphate goes into glycolysis and will generate energy.

For liver, the degradation of glucose-6-phosphate through the glycolytic pathway is inhibited. Glucose 6-phosphate accumulates. The liver hydrolyzes glucose-6-phosphate into free glucose, which is then released into the bloodstream for other organs, like the brain.



Fructose 2,6-bisphosphate

Glycolysis Regulation. *How does glycolysis become inhibited?* By the cAMP cascade, many enzymes get phosphorylated, including a liver enzyme that makes yet another signaling molecule, called **Fructose 2,6-bisphosphate**. This is a signaling molecule, unique from fructose 1,6-bisphosphate—a direct metabolite of glycolysis and gluconeogenesis.

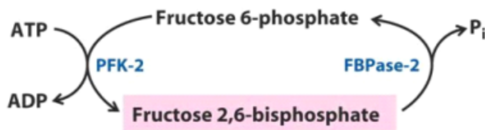
Phosphofructokinase-2. **PFK-2** is like PFK-1 except catalyzes this phosphoryl transfer reaction.

Fructose 6-phosphate + ATP → Fructose 2,6-bisphosphate + ADP

This enzyme occurs in the same large polypeptide chain with an enzyme that catalyzes the backward reaction, **Fructose bisphosphatase-2 (FBPase-2)**.

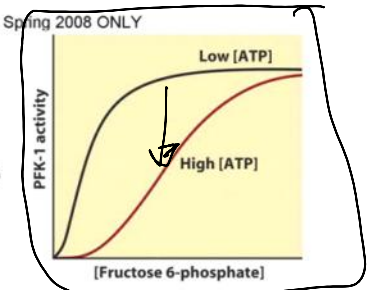
Fructose 2,6-bisphosphate → Fructose 6-phosphate + Pi

This regulatory enzyme contains two distinct enzyme activities in two active sites on one single polypeptide chain.



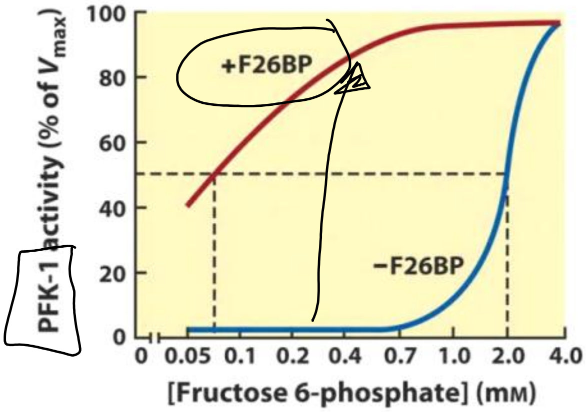
FPK-2 gets phosphorylated in the cAMP cascade in the liver. The effect of phosphorylation generally depends on the enzyme, but usually activity either goes **up** or **down**. When this giant enzyme gets phosphorylated, PFK-2 activity goes down and FBPase-2 activity goes up. The end result is that the concentration of fructose 2,6-bisphosphate goes down.

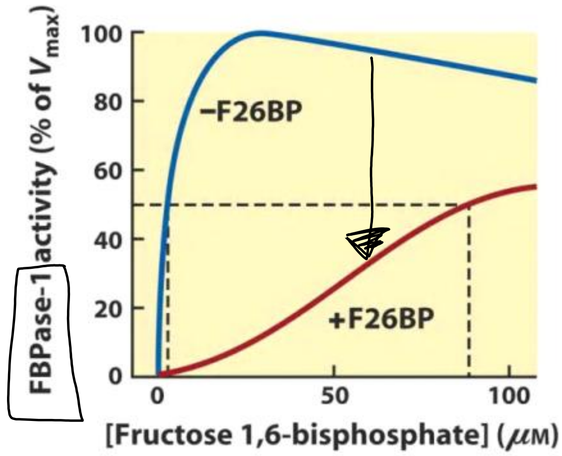
cAMP → phosp → kinase ↓ ↑ phosphatase F-2,6-bP ↓



<u>Fructose 2,6-bisphosphate is the most powerful regulator of the activity of PFK-1</u>. We talked about how PFK-1 activity is regulated by ATP and AMP. Fructose 2,6-bisphosphate is far more powerful than AMP & ATP. When [fructose 2,6-bisphosphate] goes down, glycolysis is inhibited, and glucose enters into the blood.

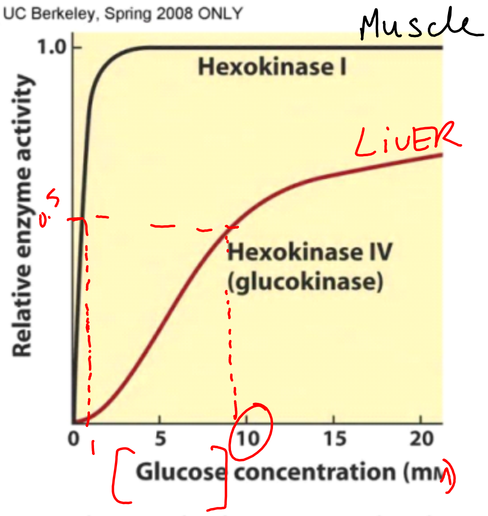
Without fructose 2,6-bisphosphate in the liver, glycolysis cannot occur.**





High [Glucose]

Hexokinase. Michaelis-Menton plots of the velocity of the hexokinase reaction versus glucose concentration show that muscle and liver hexokinase differ in K_ms.



When you look at what happens with the liver hexokinase, you get a curve that is less steep. When you take the substrate concentration at half the maximal rate, then that is the K_m. The K_m value in the liver is about 10mM. The affinity of the liver hexokinase (glucokinase) toward glucose is lower with the liver enzyme. The normal levels of blood glucose are close to 5mM. It is much lower than the K_m.

Liver hexokinase can respond to changes in [glucose]. When the substrate concentration is much lower than the K_m, the reaction rate is almost proportional to the concentration of substrate. When the glucose levels go up, the levels of phosphorylation of glucose by the liver enzyme also go up, which allows the liver to take care of excess glucose. If you have lots of glucose, then the liver enzyme will function much faster and take away glucose.

Glucagon levels goes down in response to high [glucose]. The opposite regulation of the PFK-2/FBPase-2 will occur. The enzyme will become dephosphorylated, which results in the activation of the PFK-2 activity. You will now have much more fructose-2,6-bisphosphate. The glucose-6-phosphate that your liver glucokinase generated by using the extracellular glucose in the bloodstream will go down rapidly through the glycolytic pathway. At the end of glycolysis, most of the product will be converted to fat.

High [Glucose] ↑ F26BP → Glycolysis ↑ →

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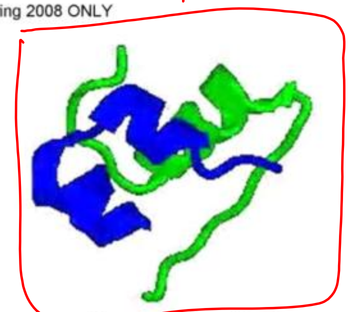
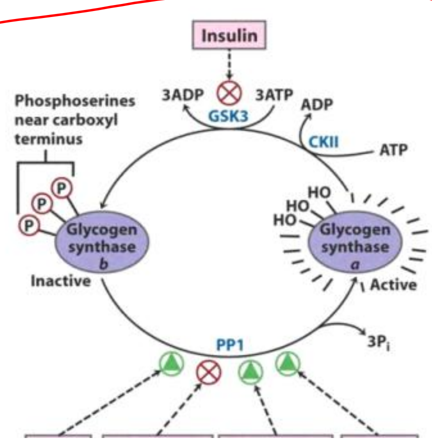
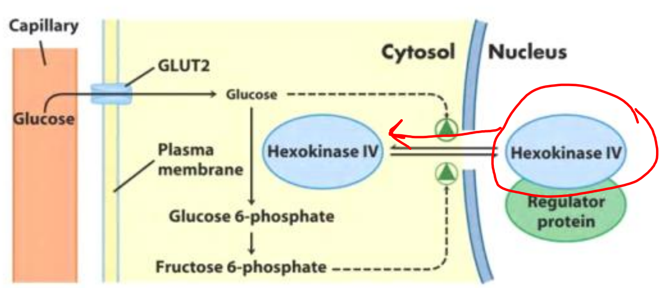
Insulin response to high [glucose]. The secretion of the protein hormone, insulin, from the pancreas occurs. It goes through totally different regulatory pathways in the liver cell. The insulin receptor gets stimulated, and that causes a decreased uptake of glucose by many different cells in the body.



Insulin affects glucose uptake and metabolism. Signal transduction is employed in the regulatory mechanism. Insulin binds to its receptor which in turn starts protein activation cascades. These include: translocation of GLUT-4 transporter to the plasma membrane and influx of glucose, glycogen synthesis, glycolysis, and fatty acid biosynthesis in fat storing cells, adipocytes.





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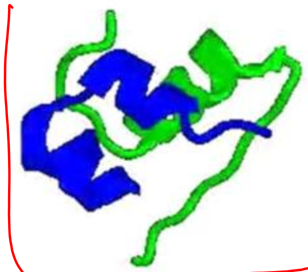
INSULIN



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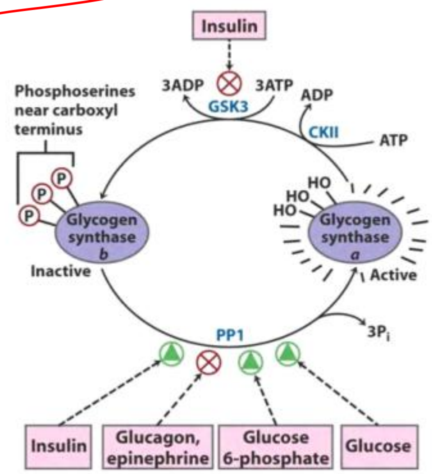
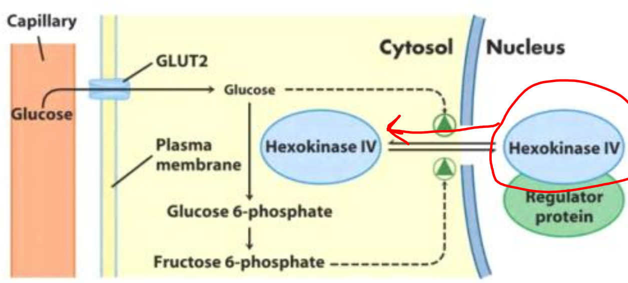
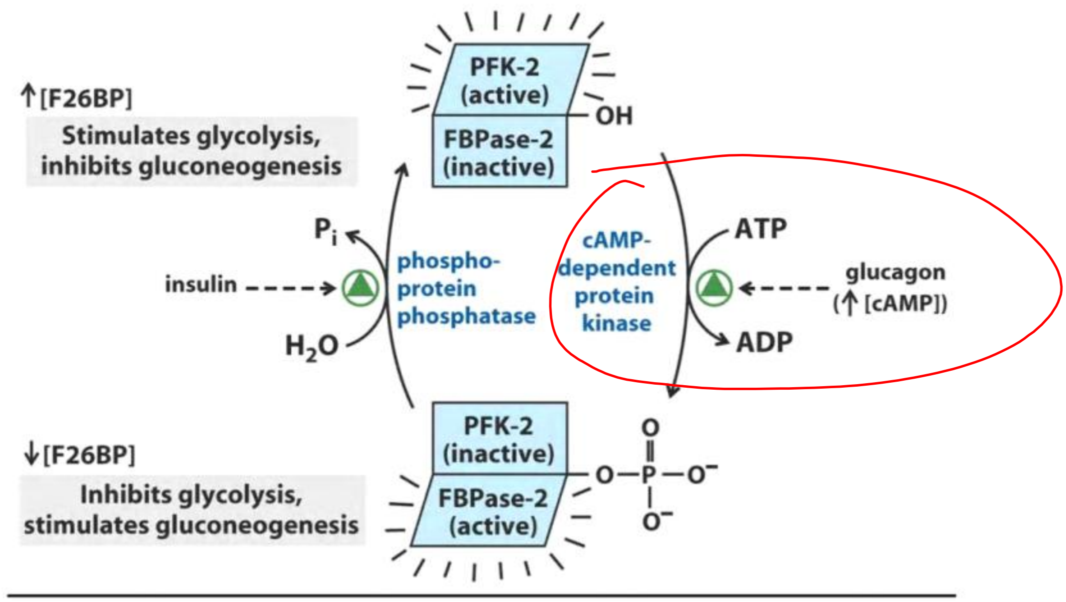


LIVER





↑[F26BP]
Stimulates glycolysis, inhibits gluconeogenesis
PFK-2 (active)
FBPase-2 (inactive)
OH
Pi
insulin
H2O
phospho-protein phosphatase
cAMP-dependent protein kinase
ATP
ADP
glucagon (↑[cAMP])
↓[F26BP]
Inhibits glycolysis, stimulates gluconeogenesis
PFK-2 (inactive)
FBPase-2 (active)

CITRIC ACID CYCLE

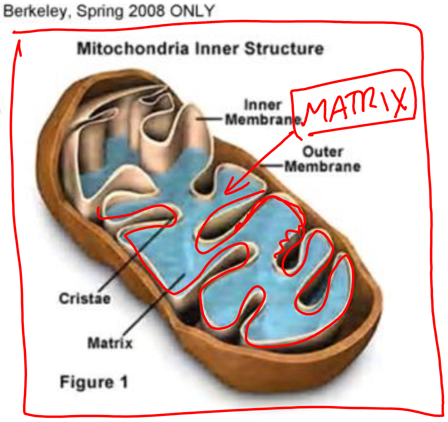


Figure 1

Mitochondria. The **citric acid cycle (CAC or TCA cycle)** occurs in mitochondria, not in the cytosol. **Mitochondria**—the powerhouses of the cell—are likely descendants of ancestral bacteria. A mitochondrion has its own circular DNA, ribosomes, and does cell division! Mitochondria have two membranes, like most Gram negative bacteria, the outer and inner membranes. The inner membrane folds to create **cristae**, increasing surface area for enzymes involved in the **electron transport chain**. The internal space is the **mitochondrial matrix**, where CAC occurs.

Cellular respiration. Aerobic catabolism takes place in mitochondria.

$$\textbf{Glucose} + O_2 \rightarrow CO_2 + H_2O + \textbf{Work} + \textbf{Heat}$$

Oxidation. *Why does all the metabolism of citric acid cycle and oxidative phosphorylation take place in the mitochondria?* We are now getting into oxidative metabolism. Oxidation is a dangerous process that generates a number of highly reactive molecules. If you want to reduce a molecule of oxygen, it will eventually become water.

To reduce oxygen, you have to add electrons one by one. Some of the intermediates are very dangerous, like super-oxide (O_2^-). O_2^- is a reactive species that destroys biomolecules. Thus the confined space in the matrix of the mitochondria is a way to keep the bad guys contained.

History of the TCA Cycle Discovery

Dicarboxylic Acid Cycle. The notion of the citric acid cycle was originated in the observations of a man called Sgent-Gyöngy. He was studying the oxidation carried out by minced pigeon muscle. He was interested in what happens when you added dicarboxylic acid. He added fumarate into this oxidizing pigeon muscle preparation. He could calculate that if fumarate became oxidized completely into CO_2 and H_2O, then the use of one micromole of fumarate results in the consumption of three micromoles of oxygen. When he experimentally added a small amount of fumarate, this resulted in the increase in the oxygen consumption of 23.6 micromoles of oxygen. He came up with the idea that these dicarboxylic acids could be working as catalysts in the oxidation of other molecules.

Tricarboxylic Acid Cycle. Hans Krebs has changed this idea. He decided that this is a **tricarboxylic acid cycle**. The intermediates of the citric acid cycle act as true intermediates and not as catalysts.

Hans Krebs, 1900–1981

Phases of the TCA Cycle.

[1] Acetyl-CoA production: Organic fuels → Acetyl-CoA

[2] Acetyl-CoA oxidation: Acetyl-CoA enters TCA and is enzymatically oxidized, but energy is conserved in electron carriers, NADH $FADH_2$

[3] Electron transfer: energy rich e^- in NADH $FADH_2$ reduce O_2 to H_2O