Gluconeogenesis

Suggested Reading:
Lippincott's Illustrated reviews: Biochemistry

The name of this pathway as it appears in front of you is made from three parts "gluco" stands for glucose, "neo" means new, and "genesis", which means creating or synthesis, so gluconeogenesis means synthesizing glucose again from non-carbohydrate sources, gluconeogenesis is making carbohydrates from non-carbohydrate sources.

Glucose Synthesis is Required for Survival

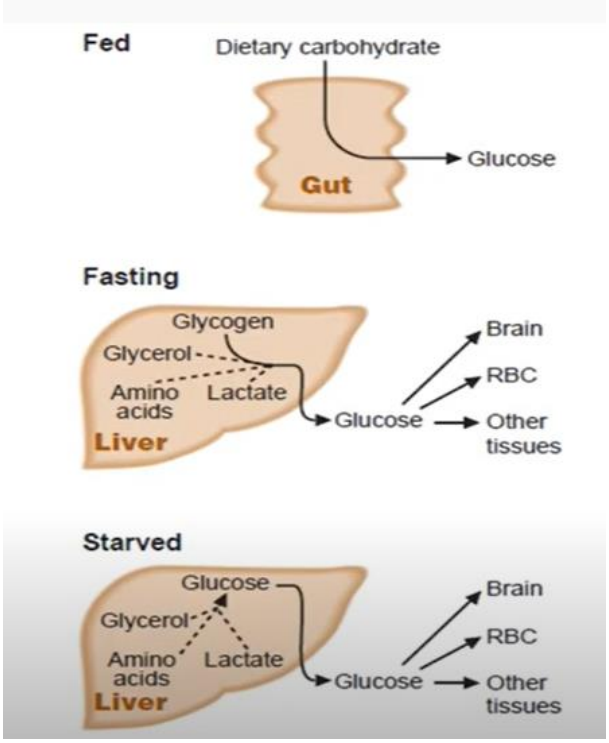
What was mentioned in the slide:

- Brain is dependent on glucose 120g/day
- Body glucose reserve is limited
  = 20 g (extra cellular fluid)
  = 75 g (liver glycogen); enough for 16 hours
  = 400 g (muscle glycogen); for muscle use only
  Main source of energy for resting muscle in postabsorptive state
- 70 Kg man has = 15 Kg fat
  - Fatty acids cannot be converted to glucose
  - Utilization of FA is increased 4–5 X in prolonged fasting
  - In prolonged fasting; FA → ketone bodies at high rate

What was mentioned in the lecture:

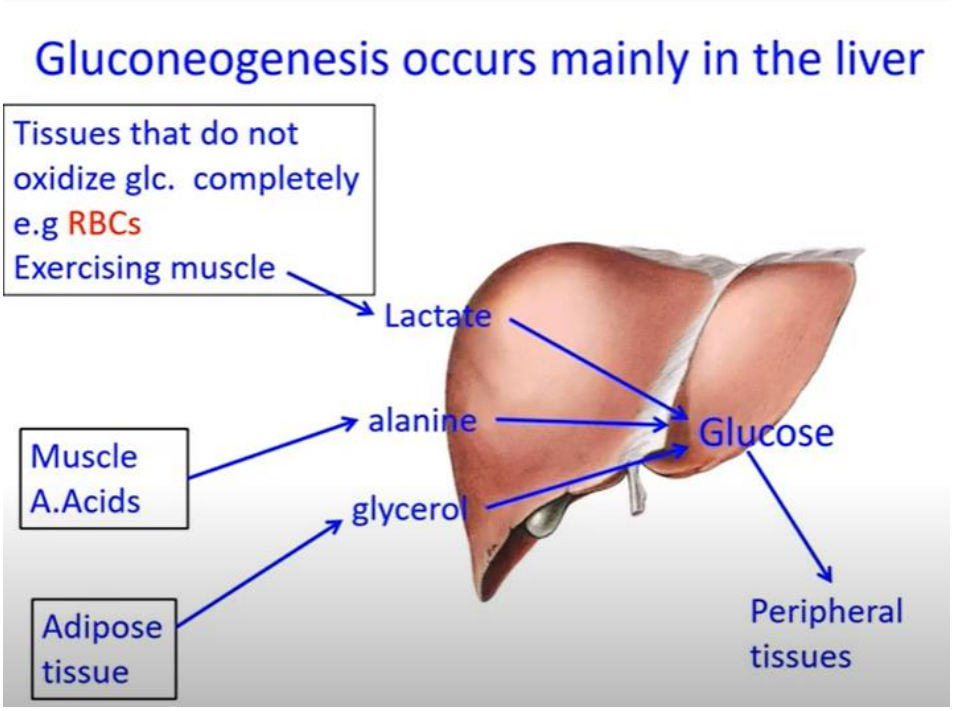
why glucose is required? glucose synthesis is required for survival, our brain depends on glucose, the brain requires about 120 grams per of glucose per day, which represents most of the consumed glucose, and almost it is absolute requirement during a normal life, the body's glucose reserve is limited, the amount of glucose that is found in the blood and the extracellular fluids is about 20 grams, while the amount that is found in the liver as liver glycogen is about 75 grams, in skeletal muscles (the whole skeletal muscular system) is about 400 grams, the glycogen in muscles is only for muscle use (muscles are selfish).
So the brain should get the glucose, but glucose storage is limited, the amount that is found in the liver is about 75 grams, and when we say that the brain requires 120 grams, then the liver glycogen is sufficient only for 16 hours, on the other hand, the glucose is the main source of energy for the resting muscle in the postabsorptive state (postabsorptive state means that the GIT is empty (after eating)).
on the other hand, a man who is 70-kilogram- weight has about 15 kg of fat, but the problem is that the fatty acids cannot be converted to glucose, So how can we get the glucose If the fatty acids cannot be converted to glucose and there's a plenty of fatty acids in the body? Utilization of fatty acid is increased in prolonged fasting, in the case of prolonged fasting, fatty acids can only be converted to ketone bodies, in

that case, ketone bodies can replace some of the glucose requirements in the brain, during prolonged fasting, we also can get glucose from proteins and amino acids.



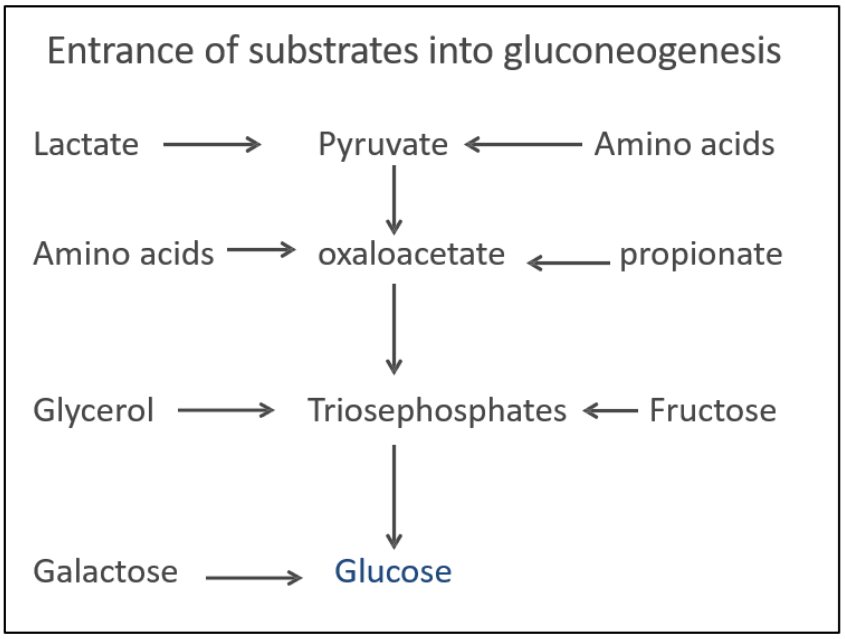
What was mentioned in the lecture:

we can say that we have three biological states, during the fed stage (directly after food intake, during absorption of food) the dietary glucose is the major source of glucose, in this case, small intestine is passing the glucose into the blood and the glucose is the available from the dietary carbohydrate, but during fasting, glucose is no longer coming from this small intestine, So it has to be obtained from glycogen, so glycogen becomes the major source of the glucose in the blood, and this glucose is utilized by a brain and RBCs, remember that RBCs have no other choice except for glucose, and for other tissues, during prolonged fasting or starvation, after all glycogen is consumed, the liver produces glucose from non-carbohydrate sources, these non-carbohydrate sources include glycerol from triacylglycerol, amino acids from muscle protein and lactic acid that is produced in some tissues.



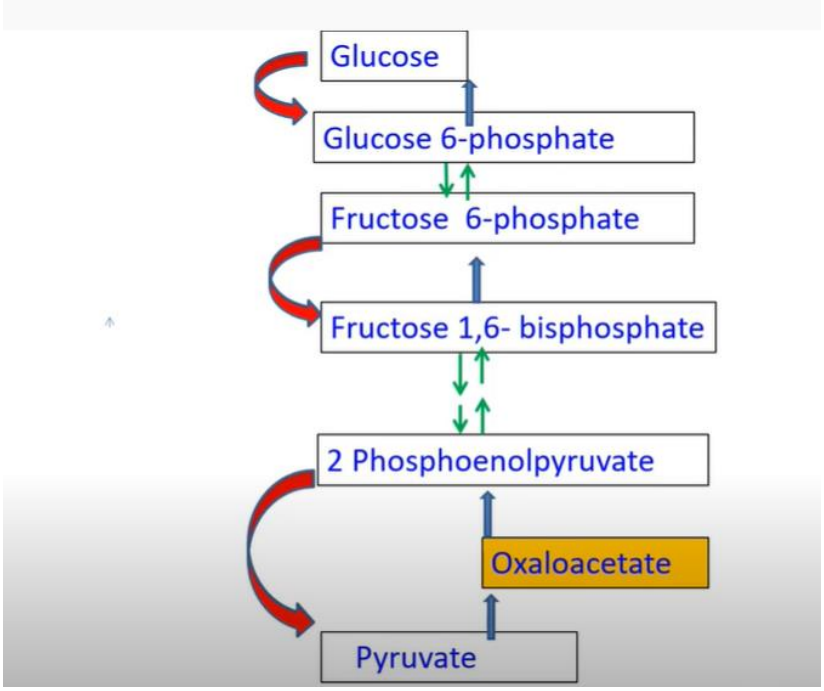
What was mentioned in the lecture:

Gluconeogenesis, which is synthesis glucose from non-carbohydrate sources, occur mainly in the liver, sources for gluconeogenesis include lactate, lactate is produced from tissues that do not oxidize glucose completely (like exercising muscles and RBCs in anaerobic conditions), lactate then is carried in the blood to reach the liver, also degradation of muscle proteins produces amino acids, amino acids are transported in different ways, one of them is to produce alanine, alanine then reaches the liver along with other amino acids to be used as a substrate for glucose synthesis, adipose tissue, adipose tissue contains a plenty of triacylglycerol, which is a glycerol that is esterified to three fatty acids, fatty acids cannot be converted to glucose, but glycerol, which is only a small part of triacylglycerol, (three carbons out of almost 60 carbons), can be converted glucose in the liver by the process of gluconeogenesis to produce glucose, glucose is then carries to the peripheral tissues, mainly the brain and RBCs .



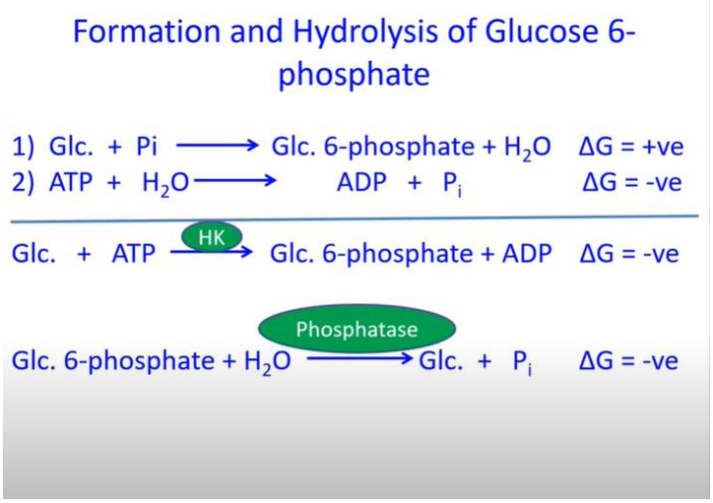
What was mentioned in the lecture:

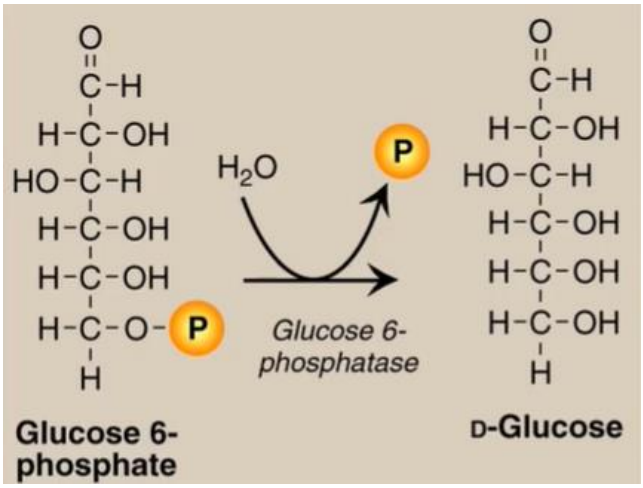
Regarding entrance of substrates into gluconeogenesis, substrates for gluconeogenesis include lactate, pyruvate, certain amino acids, propionate and glycerol, how these can be converted into glucose? Lactate and certain amino acids enter the gluconeogenesis as pyruvate, pyruvate is then converted to oxaloacetate, oxaloacetate then continues in the reverse direction of the glycolysis pathway until we reach triosephosphates (glyceraldehyde 3- phosphate and dihydroxyacetone phosphate), so gluconeogenesis starts with pyruvate and ends with glucose, lactate can be easily converted by oxidation to pyruvate, while some amino acids like alanine can be easily converted to pyruvate by transamination, bit can be converted to proper unit, other amino acids can be directly converted to oxaloacetate, propionate which is produced by certain pathways also enters gluconeogenesis as oxaloacetate, while glycerol enter at the level of triosephosphates, fructose and galactose can also be converted into glucose, but do we consider this a gluconeogenesis? No, because this is not synthesis of glucose from non-carbohydrate sources, we are just converting one sugar to another.



What was mentioned in the lecture:

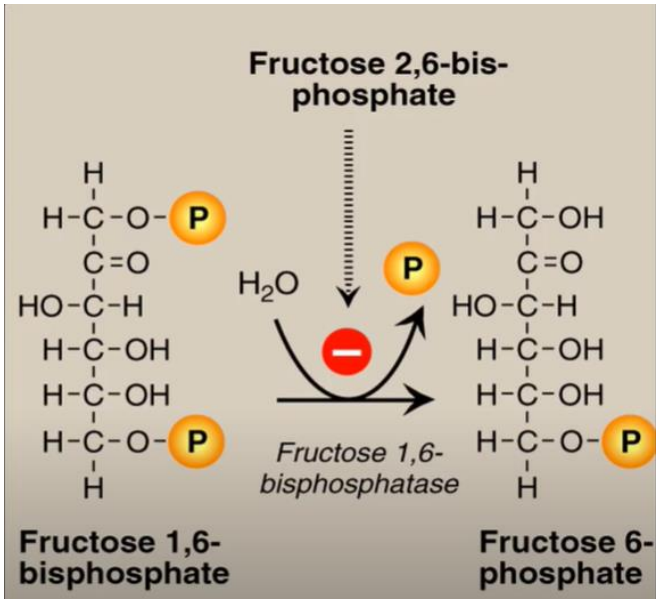
Glycolysis is formed from ten reactions, seven of the reactions are reversible while three of them are irreversible, irreversible reactions are depicted here by the red arrows, they are glucose→ glucose 6-phosphate, fructose 6-phosphate → fructose 1,6-bisphophate and phosphoenolpyruvate → pyruvate, gluconeogenesis is conversion of pirate to glucose, it is like you're passing glycolysis but it the opposite direction, Can we use the 7 reversible steps of glycolysis in gluconeogenesis? Yes, we can, reversible steps can be common between glycolysis and gluconeogenesis, but the irreversible slips cannot be used by gluconeogenesis, it is like you are in a road and some parts of it are bidirectional while the others are one directional, how can you get back? By using an alternative route, the same applies here, so to convert pyruvate back to phosphoenolpyruvate, we must use a different reaction than the one catalyzed by pyruvate kinase, because this is a different step in glycolysis, the same applies for fructose 6-phosphate →fructose 1,6- bisphosphate and for glucose →glucose 6-phosphate, so gluconeogenesis pathway is Composed of 11 reactions, but 7 of them are common with glycolysis, only four reactions of gluconeogenesis are unique for gluconeogenesis, that's why in order to study gluconeogenesis you must understand glycolysis.
In the first reaction of gluconeogenesis, pyruvate is caroxylated to oxaloacetate which is a new intermediate, then oxaloacetate is converted to phosphoenolpyruvate which is a common intermediate between glycolysis and gluconeogenesis, the reaction of converting fructose 1,6-bisphosphate to fructose 6-phosphate is catalyzed by a different enzyme in gluconeogenesis rather than glycolysis' PFK, that is also applied to the reaction of dephosphorylating glucose.





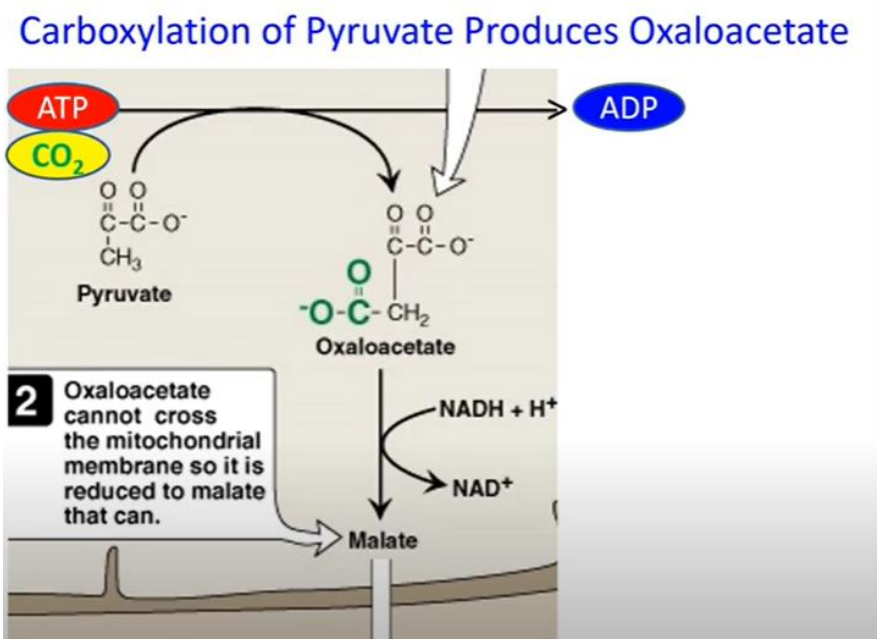
What was mentioned in the lecture:

The last reaction of the gluconeogenic pathway is the reverse of the first reaction in glycolytic pathway, the first reaction in glycolysis in formation of glucose 6-phosphate while the last reaction of gluconeogenesis is hydrolysis of glucose 6-phosphate, the reaction of glucose + ATP → glucose 6-phosphate + ADP is irreversible, therefore it is highly exergonic having a large negative ΔG, therefore, we cannot go in the reverse direction, this reaction is actually a combination of two reactions (steps):, firstly phosphorylating of glucose using an inorganic phosphate which an endergonic step, and then hydrolysis of ATP producing ADP and inorganic phosphate which is the exergonic step, therefore, the first step (glucose + P_i → glucose 6- phosphate + H_2O) is endergonic, therefore, it cannot happen in the forward direction without being accompanied with hydrolysis of ATP (at the expense of ATP), but, the back direction (glucose 6-phosphate + H_2O → glucose +P_i) must be exergonic and favorable form a thermodynamic point of view (the forward direction is endergonic that has a positive ΔG, then the reverse direction must be exergonic and has a negative ΔG), therefore, the first step is reversible, all what you is an enzyme for this to happen, this new enzyme is called glucose 6-phosphatase (any enzyme that hydrolyses phosphate groups is called phosphatase), notice that this occurs without (ADP→ ATP), this is how this reaction is done is the reversible direction in gluconeogenesis. Glucose 6-phosphatase enzyme present in liver cells.



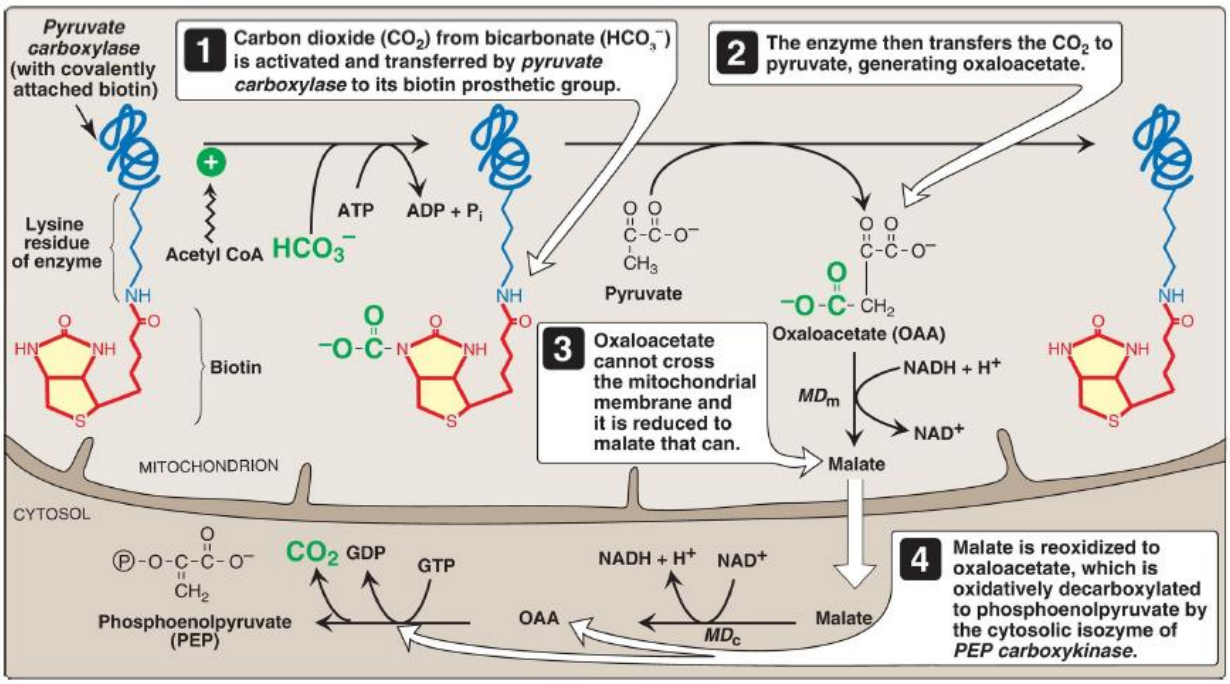
What was mentioned in the lecture:

What is applied for reversing the rection of phosphorylating glucose applies also here (the same logic applies), all what you need is a different enzyme, and in this case it is another phosphatase called fructose 1,6-bisphosphatase which hydrolyses the phosphate group of carbon #1 , this is how we procced the irreversible third reaction of glycolysis in the reverse direction.



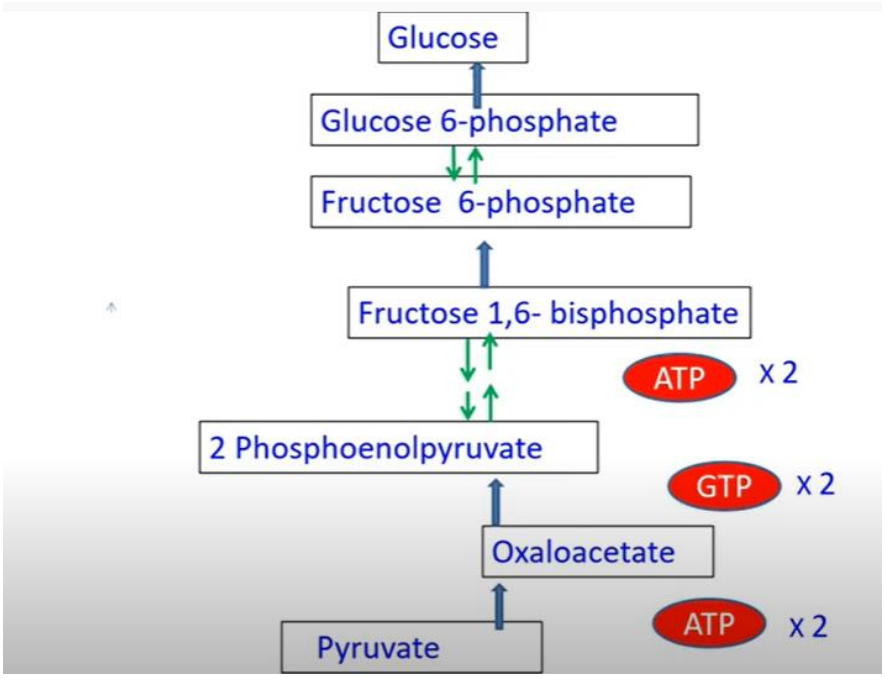
What was mentioned in the lecture:

The last reaction that we must reverse is conversion of phosphoenolpyruvate to pyruvate, this reaction is extremely exergonic (it has a large negative ΔG, equals -7.5 kcal), how can we reverse this reaction? Firstly, we carboxylate pyruvate to oxaloacetate, (we've talked about this reaction previously in TCA cycle), carboxylation of pyruvate requires energy (carboxylation is always endergonic but decarboxylation is always endergonic), the source of carboxyl group in this case is CO_2 or HCO_3^- (they can be used interchangeably), and this carboxyl group is attached to carbon #3 of pyruvate, remember that always carboxylation require a coenzyme that is biotin (biotin is derived from vitamin B_7). (to be continued)



What was mentioned in the lecture:

In conversion of pyruvate to oxaloacetate, biotin functions as a carrier of activated carboxyl groups, to be added to pyruvate, a carboxyl group must be activated, it is activated by being carried on biotin, in the first step, ATP is used to attach CO_2 to biotin by a covalent bond forming carboxybiotin, biotin is also covalently attached to pyruvate kinase enzyme through a lysine residue, then the carboxyl group is transferred to pyruvate forming oxaloacetate so biotin returns to its original form and activated again, the carboxylation of pyruvate occurs in the mitochondrial matrix, and the resulting oxaloacetate cannot penetrate the inner mitochondrial membrane per se, therefore, oxaloacetate is reduced into malate (this is actually the opposite of the last reaction of TCA cycle, this reaction requires consuming of NADH to NAD^+) (remember that we have a malate-oxaloacetate mitochondrial shuttling system, the same idea is applied here) malate can cross the inner mitochondrial membrane freely also (because the inner mitochondrial membrane possess a carrier for malate but not oxaloacetate), one in the cytosol, malate is again oxidized to oxaloacetate (compensating the lost NADH), once we have oxaloacetate in the cytosol, it can be converted now to phosphoenolpyruvate, that is done by acquiring a phosphate group whose source is now GTP which will be converted to GDP, and by liberating a carboxyl group which is an exergonic process, if we considered GTP equal to ATP, then how many ATP molecules are consumed in converting pyruvate to phosphoenolpyruvate, we need two ATP molecules: one that is required for carboxylation of pyruvate, and another one that is required for phosphorylating of oxaloacetate.
So far, two enzymes are required for converting pyruvate to phosphoenolpyruvate (in addition to malate dehydrogenase which converts oxaloacetate to malate and vice versa), which are pyruvate carboxylase and phosphoenolpyruvate carboxykinase which oxidatively decarboxylates and phosphorylates oxaloacetate using GTP.
so far, we can understand that gluconeogenesis happens firstly by converting back two molecules pyruvate into two molecules of phosphoenolpyruvate, then (after 6 steps which are actually just reverse of glycolysis steps), we'll end up with a fructose 1,6-bisphosphate molecule whose one phosphate group is hydrolyzed forming fructose 6-phosphate which will be isomerized into glucose 6-phosphate whose phosphate group will be hydrolyzed to glucose, now we'll review gluconeogenesis from an energy point of view.



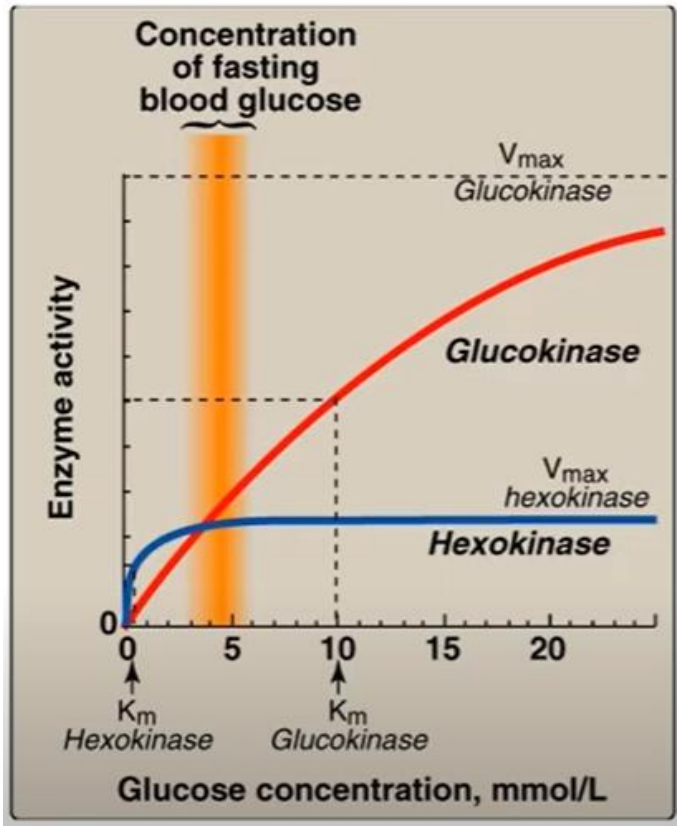
What was mentioned in the lecture:

Let's review gluconeogenesis from an energy point of view, we need two ATP molecules to convert a pyruvate molecule to phosphoenolpyruvate (one for carboxylation on pyruvate to oxaloacetate and another one (actually it is a GTP) in phosphorylation of oxaloacetate), and because we need two molecules of pyruvate to generate a molecule of glucose, we need actually four, we need two more ATP molecules when we convert 2 molecules of 3-phosphoglycerate to 1,3-bisphosphoglycerate (when we doing that, we are actually reversing a reversible step of glycolysis, which is 1,3-bisphosphoglycerate + ADP → ATP + 3- phosphoglycerate, remember that this is also an ATP-generating step, and therefore we must consume ADP when we reverse it), dephosphorylation of fructose 1,6-bisphosphate and glucose 6-phosphate doesn't consume or generate any ATP, therefore the net is six molecules, we need 6 ATP molecules to convert two molecules of pyruvate into one molecule of glucose.
Glucose is called (grape sugar) in Arabic, it is abundant in its free form in grapes, fructose is called fruit sugar because it is abundant in fruits, pyruvate gives sour taste to unripe grapes حصرم, our liver converts pyruvate to glucose just as a grape tree converts pyruvate to glucose in grapes.

Regulation of glycolysis and gluconeogenesis

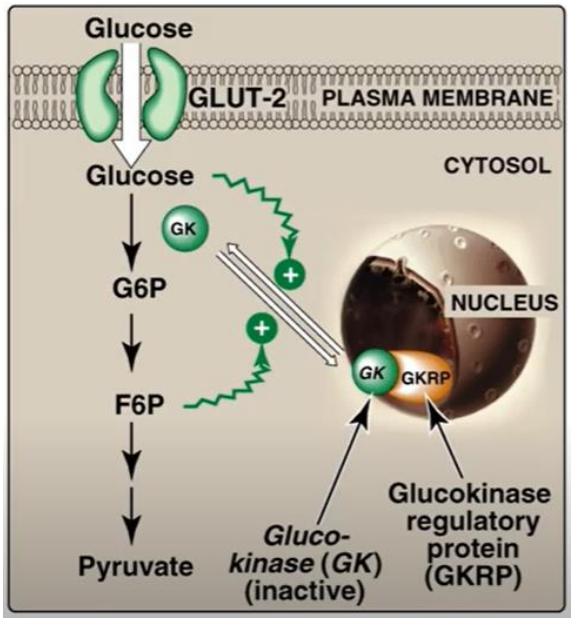
What was mentioned in the lecture:

Gluconeogenesis of a glucose molecules requires 6 ATP molecules while glycolysis of a glucose molecule gives net 2 ATP molecules, this is good example that emphasizes that synthesis always require more energy to overcome energy barriers of endergonic steps (irreversible reactions). Building up is always harder than breaking down, so if you imagine that glycolysis and gluconeogenesis occur at the same time in the same cell, that just would be wasting of energy, it would be a futile cycle, meaning that, that would be useless at all but wasting of energy, so the two pathways must be regulated, gluconeogenesis and glycolysis mustn't occur simultaneously.
Gluconeogenesis and glycolysis are regulated through regulating of the irreversible steps, not the reversible ones, we have three irreversible steps, therefore those three must be regulated, because once an irreversible step has taken place, we cannot come back and the product will be committed to the whole pathway, so glycolysis and gluconeogenesis are regulated through these three steps: glucose ↔ glucose 6-phosphate, fructose 6-phosphate ↔ fructose 1,6-bisphosphate, and phosphoenolpyruvate ↔ pyruvate, the enzymes that catalyze those reactions in glycolysis should be regulated in gluconeogenesis and vice versa.



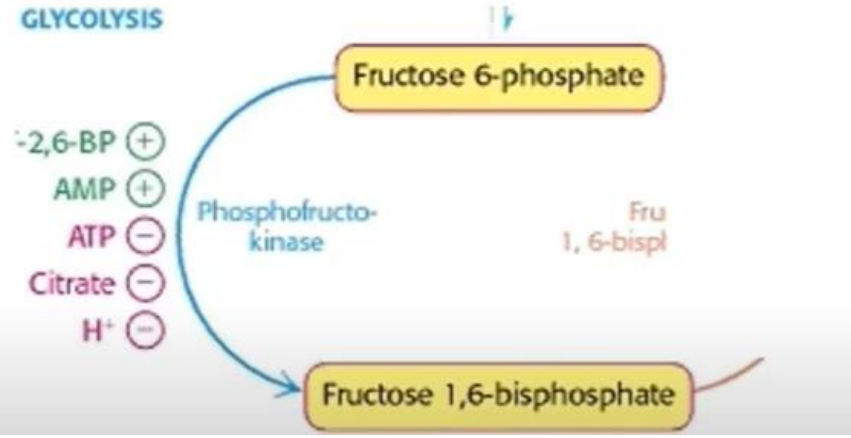
What was mentioned in the lecture:

Let's firstly review the regulation of hexokinase or glucokinase, remember that glucokinase functions in the liver while hexokinase functions in other tissues (especially the brain), let's firstly compare between those two isoforms, hexokinase has low K_m (higher affinity), while glucokinase has higher V_{MAX} (higher capacity), regarding K_m, you can notice that we need 10 mM of glucose to reach half V_{max} of liver's glucokinase, but we need a very little amount of glucose to reach half V_{max} of other tissues' hexokinase, it is like hexokinase can functions at very low glucose concentrations, while the liver's glucokinase needs a high concentration of glucose to function, this is a kind of regulation, the gluconeogenetic organ which is the liver, do not phosphorylates glucose unless at high concentrations (when we do not need gluconeogenesis), this is an example of regulation by having different isoforms that act differently, also the liver is the organ responsible for storage of glucose (either as glycogen or fat), it is not efficient if we stored resources that we need immediately, this is the first way of regulation.



What was mentioned in the lecture:

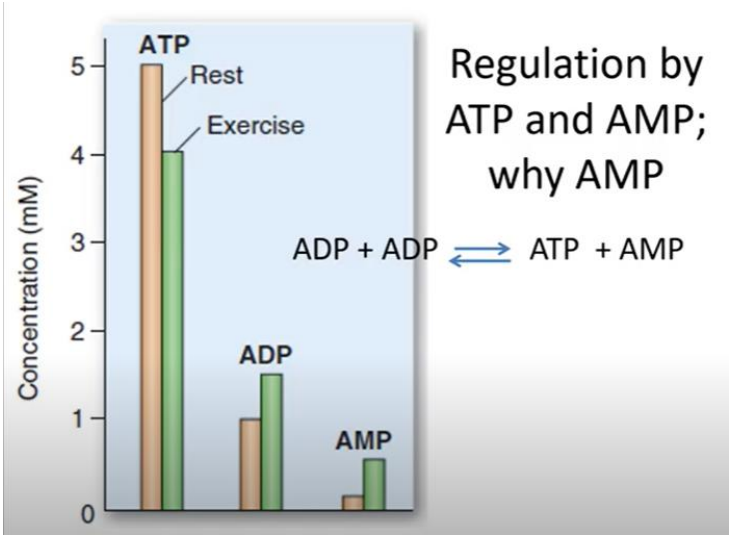
Another way of regulation is through binding of liver's glucokinase to a regulatory protein, once glucokinase binds to a regulatory protein, the complex is transferred to the nucleus where it remains inactive, when blood glucose is high, that will be reflected in the cytoplasm of liver cells, high concentration (level) of glucose in the cytoplasm causes activation of the hexokinase by transferring it again to the cytoplasm where it is converted again to the active form, also, high levels of fructose 6-phosphate will inhibit glucokinase by potentiating its binding to GKRP when gluconeogenesis in underway, always and always, we mustn't allow glucose 6-phosphate or fructose 6-phoshate to accumulate in a cell, that will consume a lot of inorganic phosphate (that will trap the phosphates), leaving only a little amount for oxidative phosphorylation, that is another way of regulation.



What was mentioned in the lecture:

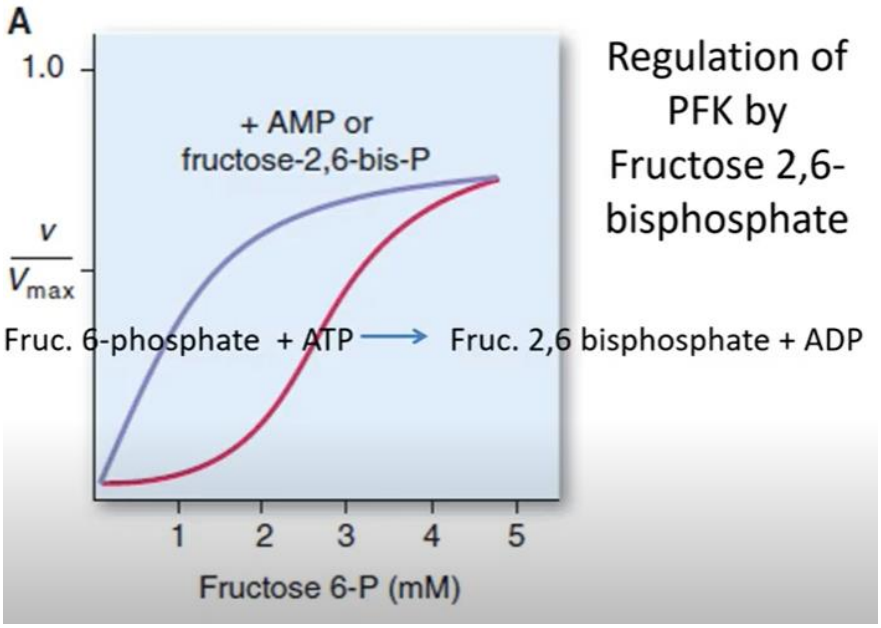
The important enzyme of regulating glycolysis is phosphofructokinase, because it is one that catalyzes the committed step of glycolysis, committed step means that once fructose 1,6-bisphosphate is produced, it can undergo glycolysis and not any other pathway, this enzyme is strictly regulated ,it is allosterically activated by a molecule called fructose 2,6-bisphosphate and by AMP, but it is inhibited by

ATP, citrate and H^+, it is inhibited by ATP even though it uses ATP as a substrate, when ATP is present in large amount in the cell, it binds an allosteric site on PFK, this step is the key step of glycolysis and glycolysis is an ATP-generating process, why to produce more ATP if ATP is high? Citrate is also an indicative of high amounts of building blocks, it indicates that the cell has excess energy it if it is not consumed in the TCA cycle, the end products of glycolysis are pyruvate and lactate and they're both acids, meaning that they increase the concentration of H^+, acids concentration shouldn't increase to a high extent in the cell, that's why H^+ is also an inhibitor for PFK, AMP sends a message that the level of energy in the cell is very low, that's why AMP activates glycolysis, fructose 2,6-bisphosphate is also an activator.



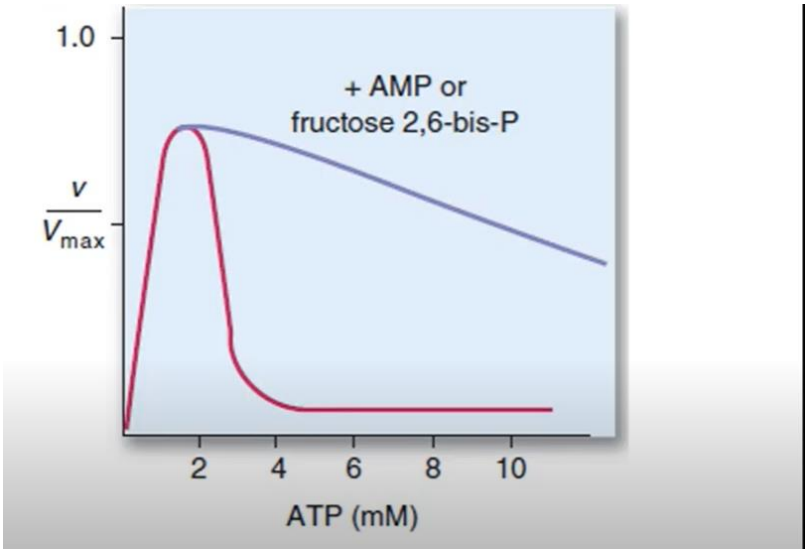
What was mentioned in the lecture:

We've just said that ATP inhibits glycolysis while AMP activates it, ADP cannot be used a source of energy like ATP, because enzymes specific for ATP cannot use ADP although ADP has high energy phosphate groups, when we do not have ATP -especially in muscular tissue- ADP molecule donate phosphate groups to each other, forming ATP and AMP, when this reaction occur, it indicates that the level of energy in cell is very very low, the plot above depicts levels of ATP, ADP and AMP in cases of rest and exercise, ATP level decreases only slightly between rest and exercise and so is ADP, the level of AMP increases greatly during exercise, that's why AMP is better to be used as an activator for glycolysis because it increases too much when a cell is really exhausted.



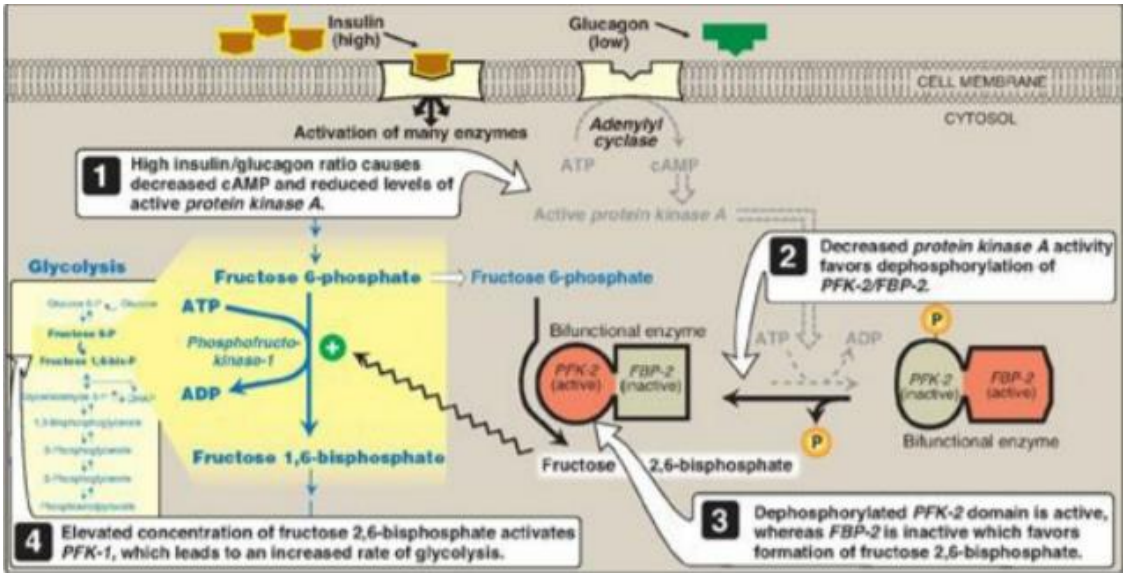
What was mentioned in the lecture:

Fructose 2,6-bisphosphate resembles fructose 1,6-bisphosphate, fructose 2,6-bisphosphate is produced in similar way to that of fructose 1,6-bisphosphate, by phosphorylation of fructose 6-phosphate using ATP, the major function of this molecule is to stimulate glycolysis.
Notice the effect of fructose 2,6-bisphosphate on the PFK enzyme, the curve in the figure depicts the activity of PFK as a function of the concentration of fructose 6-phosphate (the substrate), as any enzyme, the velocity increases as we are increasing the concentration of the substrate, the sigmoidal shape pf the pink curve indicates that this is an allosteric enzyme, in the presence of fructose 2,6-bisphosphate, the velocity increases greatly, AMP and fructose 2,6-bisphosphate are activators of glycolysis.



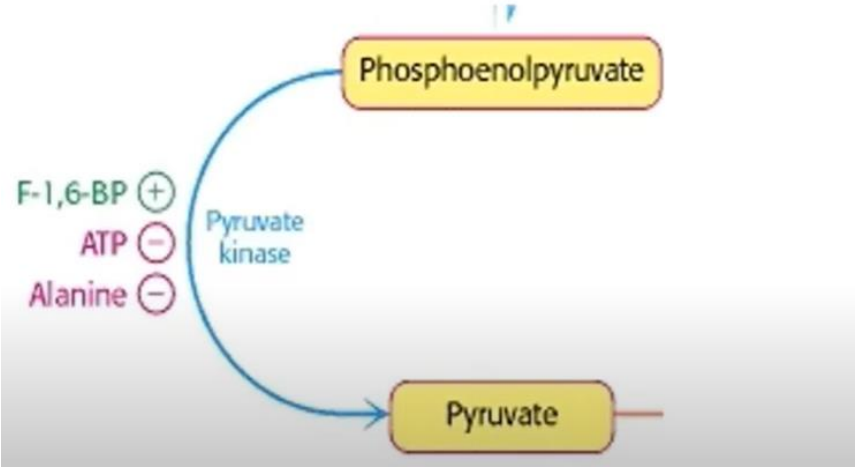
What was mentioned in the lecture:

This curve depicts the velocity of phosphofructokinase as a function of ATP concentration, the same applies here, PKF is activated by both AMP and fructose 2,6-phosphate. Notice that ATP is an activator at low concentration, but an inhibitor at high concentrations, ATP has an inhibitory effect on PFK at concentrations higher than 2 mM, but the activation effect of fructose 2,6-phosphate and AMP overcomes the inhibitory effect by ATP.



What was mentioned in the lecture:

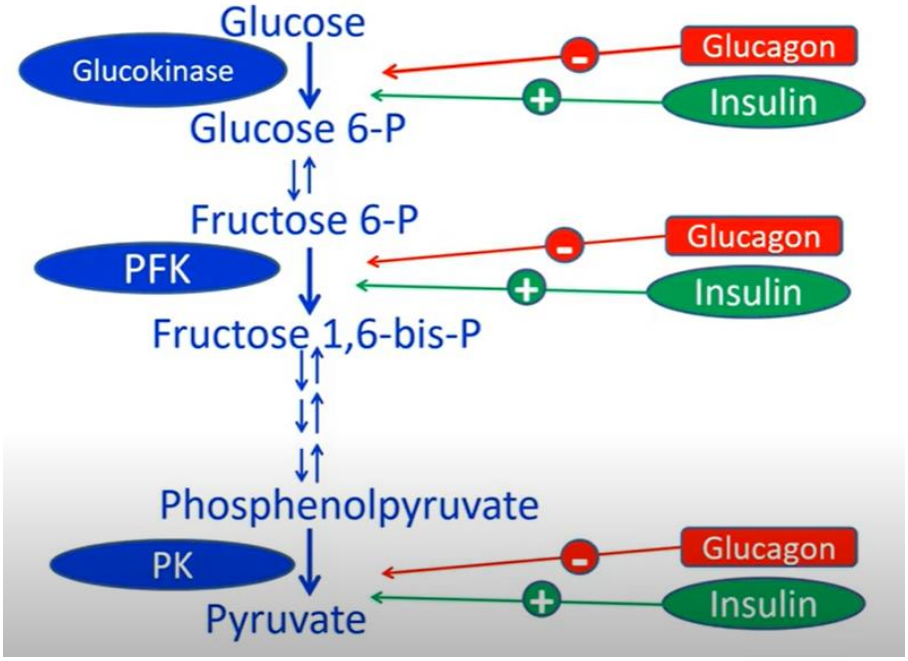
Fructose 2,6-bisphosphate (the activator) is produced through phosphorylation of fructose 6-phosphate by an enzyme called phosphofructokinase-2, (phosphofructokinase-1 is the enzyme that produce fructose 1,6-bisphosphate in glycolysis), phosphofructokinase-2 is a bifunctional enzyme, it has two catalytic domains, one with a kinase activity and the other with a phosphatase activity, phosphofructokinase-2 is regulated through covalent modification (phosphorylation), phosphorylation of this enzymes causes the kinase domain to become inactive (no longer able to add a phosphate group to fructose 6-phosphate) and the phosphatase domain to become active.
Remember this always: phosphorylation enzymes involved in metabolism of glucose always spares glucose (decrease consumption of glucose).
Phosphofructokinase-2 is phosphorylated or dephosphorylated in response to glucagon, glucagon is a hormone that is secreted from the pancreas, it indicates low blood glucose, when blood glucose is low, we should decrease consumption of glucose, glucagon binds to a G-protein coupled receptor causing activation of adenylyl cyclase which produce cyclic AMP form ATP, cAMP (the second messenger) is turn activates protein kinase A (cAMP-dependent protein kinase), which phosphorylated many proteins, including phosphofructokinase-2.



What was mentioned in the lecture:

Pyruvate kinase (the enzyme that catalyzes the irreversible last step of glycolysis), is also regulated , it is activated by fructose 1,6-phosphate (an intermediate exist up in the pathway), it is feedforward mechanism, a lot of fructose 1,6-phosphate will till pyruvate kinase that it is slow, (you are slow!! a lot of intermediates have accumulated!!), ATP inhibits pyruvate kinase, while alanine, which indicates high level of pyruvate in the cell also inhibits pyruvate kinase.

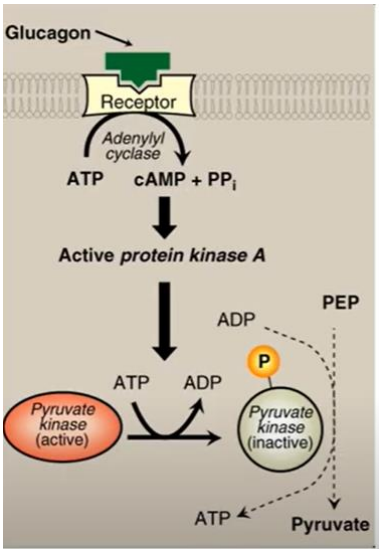
Glycolysis is regulated at the first step, third step, and the last steps which are the irreversible steps.



What was mentioned in the lecture:

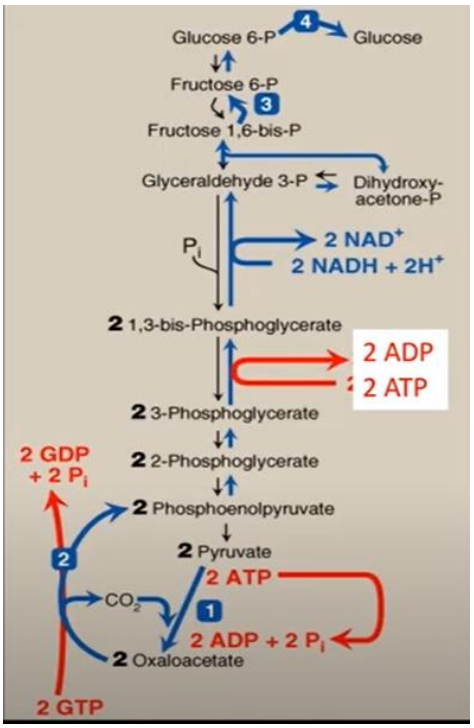
The three enzymes that catalyze the three irreversible steps of glycolysis are also regulated by hormones, glucagon is an inhibitor for these three enzymes, while insulin is an activator for glucokinase, phosphofructokinase, and pyruvate kinase, those hormones regulated those enzymes, they regulate the amount of these enzymes, by regulation the transcription of the genes that code for those enzymes, so these hormones act on the DNA, if we imagine that a person eats too much carbohydrates, his body will convert excess into fatty acids, but we must stimulate glycolysis in that case because pyruvate is the intermediate that is converted to fatty acids, therefore, we must induce those enzymes, that is carried by insulin.

Therefore, the amount of these enzymes can be induced because of the continuous feeding state, in which insulin is here all time because food is here all time.



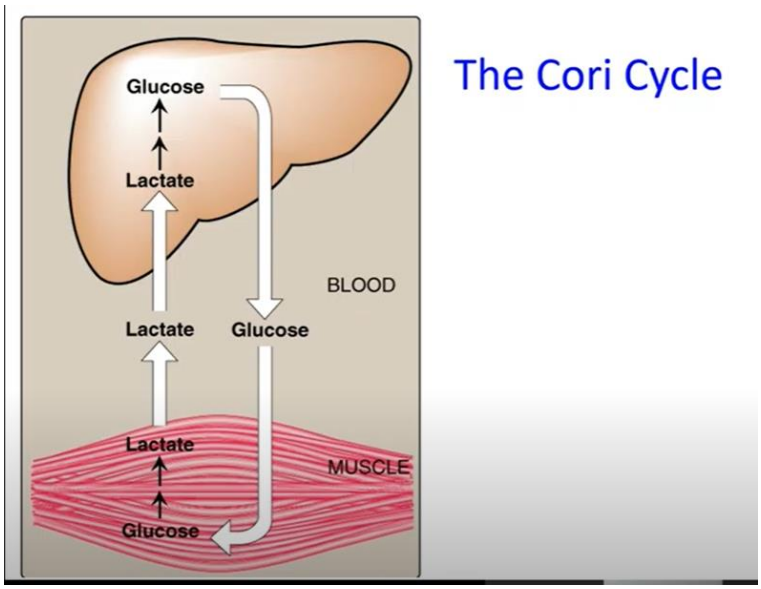
What was mentioned in the lecture:

This figure depicts how pyruvate kinase is regulated through glucagon, as any enzyme involved in glucose metabolism, phosphorylation pf pyruvate kinase will inactivate it (to spare glucose).



What was mentioned in the lecture:

This figure depicts glycolysis and gluconeogenesis at the same time, remember that glycolysis and gluconeogenesis mustn't occur in the same time in the same tissue, that will be a waste of energy.

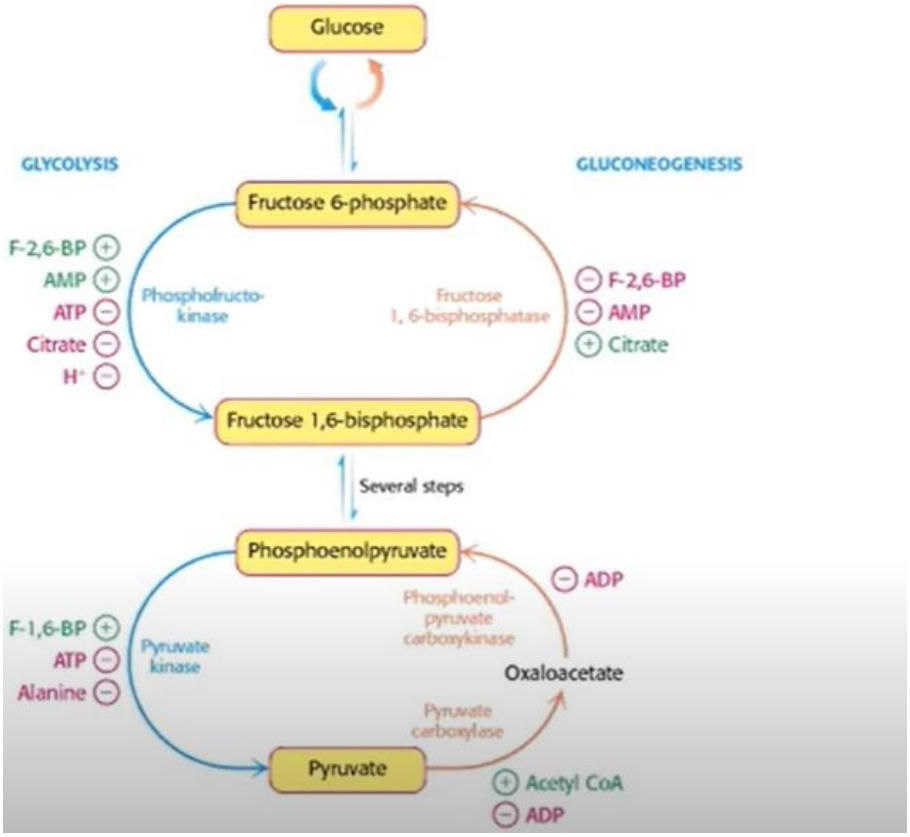


What was mentioned in the lecture:

Gluconeogenesis happens in the liver where lactate is converted into glucose, then glucose is released to the blood stream to reach the muscular tissues, in which glycolysis happen intensively and glucose is broken down to lactate, which in turn released again to plasma to reach the liver again and to be reconverted into glucose, so gluconeogenesis and glycolysis can happen at the same time, in this cycle, 2 ATP molecules are produced in the muscle for every glucose molecule broken down, while 6 ATP molecules are consumed in the liver in synthesizing one molecule of glucose, this is wasteful, but can happen in severe exercising, because muscles then will have inadequate oxygen supply, although wasteful, this cycle can be lifesaving, like, if you are running form a lion, would you think about ATP? 😂

This cycle is called Cori cycle, Cori cycle is glycolysis in the muscle and gluconeogenesis in the liver.

In this cycle, the liver carries some of the load, we don't want lactate to accumulate in the blood stream causing lactic acidosis.



What was mentioned in the lecture:

Regarding the regulation of gluconeogenesis, we can say that every thing in it is the opposite if that of regulation of glycolysis, fructose 1,6-bisphosphatase is inhibited by fructose 2,6-bisphosphate (opposite to activation of phosphofructokinase, the molecule that activates glycolysis inhibits gluconeogenesis, citrate also inhibits fructose 1,6-bisphosphatase but activates phosphofructokinase, it is reciprocal regulation.

The last step pf glycolysis (conversion of phosphoenolpyruvate to pyruvate) is activated -as we said- by fructose 1,6-bisphosphate, but inhibited by a high ratio of ATP/ADP and alanine, but, the first step of gluconeogenesis (conversion of pyruvate to oxaloacetate and then to phosphoenolpyruvate) is inhibited by a low ratio of ATP/ADP, if ADP is low, then this is not an appropriate time to synthesize gluconeogenesis, acetyl CoA is an activator of pyruvate carboxylase, because acetyl CoA requires oxaloacetate to enter Krebs cycle.