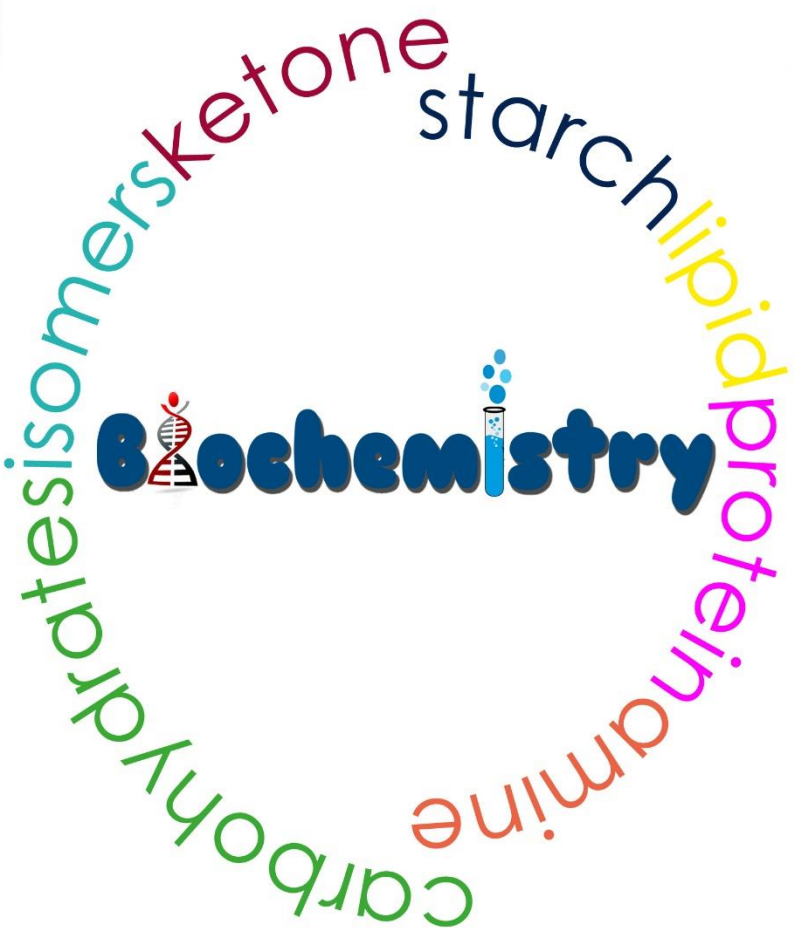

◉Sheet ○Slides

Subject :	**Gluconeogenesis**
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Number :	**13**

Introduction

As we know, glucose synthesis is essential for survival; ultimately because the brain cannot use anything other than glucose as its source of energy. Almost 1/3 of the entire energy requirement is by the brain, where it needs 120 g of glucose per day.

Free glucose in ECF	20 gr "≡80 cal"
Glucose as glycogen in liver	~75 gr "10% of liver mass" Function: to maintain blood glucose level "quick response", and it is enough to maintain it for 16 – 20 hours of fasting
Glucose as glycogen in muscles	~400 gr Function: muscle use only
Brain use of glucose "per day"	~120 gr
Fat mass "for 70 kg man"	~15 kg "≡130,000kcal" Enough to supply energy for 60 - 90 days but with the intake of fluids, vitamins and minerals
[ATP] in the cell	~5mM; degraded and synthesized constantly 'recycled'
[creatine phosphate]	~2mM; recycled
For resting muscles or with moderate exercise, the main energy source is fats	
80% Post-absorptive glucose is utilized by the brain and the red blood cells	

The amount of blood glucose in the body is limited yet the demand is very high. For example, 20 g of glucose in the extracellular fluid, 75 g converted to liver glycogen and 400 g for muscle glycogen (meaning only muscles can make use of these 400).

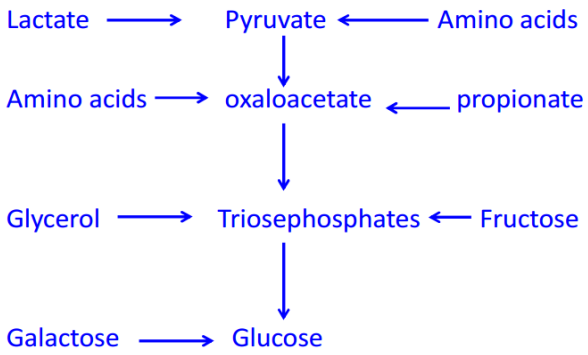
Since the body is dependent on glucose, yet the reserve is very limited, the only solution is to increase the production of glucose via **gluconeogenesis.**

The literal meaning of gluconeogenesis is **"generating new glucose" from non-carbohydrate sources**. For example, a 70 kg man has about 15 kg of fats, and fats can be broken down into glycerol and fatty acids if no glucose is available. Unfortunately, fatty acids cannot be

converted into glucose (because they are converted into acetyl coA; which cannot be converted to pyruvate since pyruvate dehydrogenation reaction is irreversible) but glycerol can. Utilization of fatty acids is therefore increased 4 to 5 times in prolonged fasting states in order to decrease the body's demand on glucose due to its limited availability.

Most tissues will increase fatty acids intake which can be converted into ketone bodies and be used as a source of energy even for the brain. Keep in mind that this only occurs in cases of prolonged fasting where glucose would have already been used up and finished.

Gluconeogenesis precursors



* Gluconeogenesis occurs mainly in the liver using some precursors such as:

1. From tissues that do not oxidize glucose completely (ex. RBCs) , producing lactate.

2. Muscle amino acids: degradation of muscle proteins gives us a variety of amino acids, including alanine, which is then transferred to the liver.

3. Adipose tissues give us glycerol.

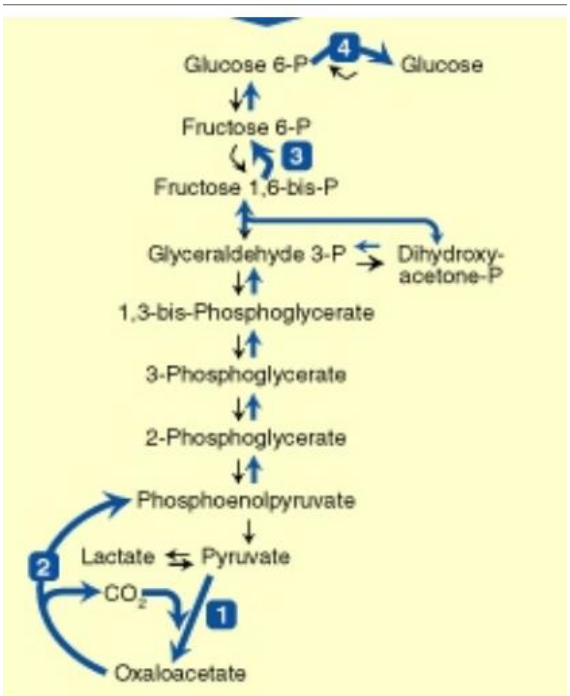
4. Propionate: from degradation of odd-numbered fatty acids. It is converted into oxaloacetate.

5. Other sugars

In general, gluconeogenesis precursors include all the compounds of glycolysis and their precursors, all the compounds of citric acid cycle and their precursors, glucogenic amino acids, lactate and glycerol. These compounds are then converted to glucose in the liver (by using their carbon atoms to build glucose molecules), then glucose is transported by the plasma to reach peripheral tissues, including the brain, where it is used as the source of energy. The kidneys participate in gluconeogenesis in prolonged fasting.

Gluconeogenesis typical steps

We are familiar with the 10 steps of glycolysis, and we know that the overall reaction is exergonic. Separately, 7 of these steps have delta G values near zero and can therefore proceed in both directions whereas the remaining 3 (and an intermediate one, labeled reaction 2 in this diagram, giving a total of 4) are HIGHLY exergonic and are hence irreversible. The irreversible steps in glycolysis are called the *typical glycolytic steps*, and their bypasses in gluconeogenesis are called the *typical gluconeogenesis steps*.



In retrospect, gluconeogenesis can be easily defined as almost the reverse of glycolysis. It is needed to maintain glucose levels in the blood in certain "fasting" or "starving" states (meaning after all glucose and glycogen stores have been used up).

But what mechanisms do our bodies use to try and reverse these 4 irreversible reactions in times of fasting or starvation?

As we found out, gluconeogenesis is an anabolic process because we are trying to build glucose, and therefore it requires energy which is provided by fats breakdown. So the body can easily reverse the 7 reversible reactions of glycolysis to result in the formation of glucose rather than the breaking down of it, but what about these 3 "roadblocks" that we need to overcome for the reaction to be reversed successfully? Thankfully, our bodies designed 4 unique reaction pathways to reverse these reactions.

- Step One

The first roadblock we need to overcome is converting pyruvate into phosphoenolpyruvate. How?

- By the carboxylation of pyruvate into oxaloacetate.

What does the reaction require?

- Energy in the form of ATP and GTP, CO2, pyruvate carboxylase and a coenzyme known as biotin (vitamin B group).

Steps of the reaction:

1. CO2 is activated and transferred to pyruvate by pyruvate carboxylase (biotin is bound to lysyl residue) producing oxaloacetate. An ATP molecule is used per pyruvate molecule. This reaction is allosterically activated by acetyl coA; which is highly produced in cases of hypoglycemia by breaking down fats. So, acetyl coA activates gluconeogenesis in hypoglycemia to produce more glucose and transport it to the blood. (Acetyl coA also inhibits glycolysis by the inhibition of pyruvate dehydrogenase enzyme.)

2. Oxaloacetate is reduced to malate by NADH since malate can cross the inner mitochondrial membrane, while oxaloacetate cannot.

After malate crosses the IMM and leaves the mitochondrion, it is converted back to oxaloacetate.

3. In the cytosol, the oxaloacetate is converted into phosphoenolpyruvate (decarboxylation reaction) by PEP carboxykinase. This reaction also requires a source of energy, usually as GTP.

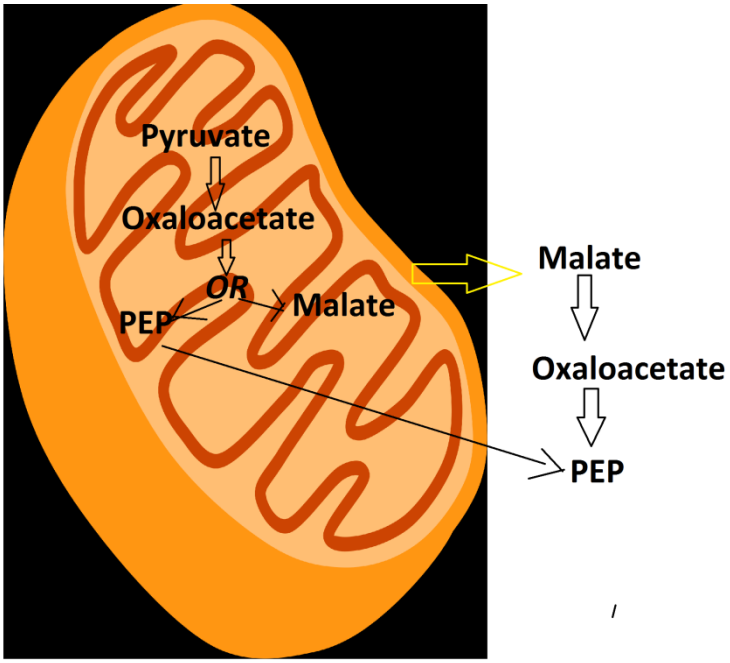
4. CO2 is then released (same CO2 that was used to carboxylate pyruvate, since it was only needed to raise the energy level of pyruvate).

Contributing to the spontaneity of the two-step (carboxylation then decarboxylation) pathway are the following:

- Free energy of cleavage of one **~P** bond of **ATP** is conserved in the carboxylation reaction. **Spontaneous decarboxylation** contributes to spontaneity of the 2nd reaction (Phosphoenolpyruvate synthesis).
- Cleavage of a second **~P** bond of **GTP** also contributes to driving synthesis of PEP.

Note: oxaloacetate, being a keto acid, is the key reason behind utilizing the conversion of amino acids into glucose.

Note: after having the pyruvate converted into oxaloacetate, it may be converted into PEP by PEP carboxykinase inside the mitochondrion, and then it can be transported to the cytosol via transporters.



- Step Two

Second roadblock we need to overcome is the conversion of fructose-1,6-bisphosphate into fructose-6-phosphate. How?

- Hydrolysis of fructose-1,6-bisphosphate by fructose-1,6-bisphosphatase.

(In glycolysis, we use phosphofructokinase, and the reverse of a kinase is ALWAYS a phosphatase)

- This reaction is favorable since the hydrolysis of a phosphate is always an exergonic reaction.

Note: kinetics and thermodynamics are separate entities.

- Allosteric and hormonal regulations "later in this sheet" are well seen in this step.

- Step Three

Third and final roadblock: converting glucose-6-phosphate back into glucose.

In glycolysis, if the phosphate is added directly to glucose to produce glucose-6-phosphate and water, this reaction would have a positive delta G value so we can't just bring glucose and phosphate together and expect them to bind spontaneously. So what actually happens? ATP hydrolysis (which produces energy) is coupled with the phosphorylation reaction. This coupling reaction is catalyzed by the enzyme hexokinase. These two reaction have a negative overall delta G value and are irreversible. So how can we reverse this reaction?

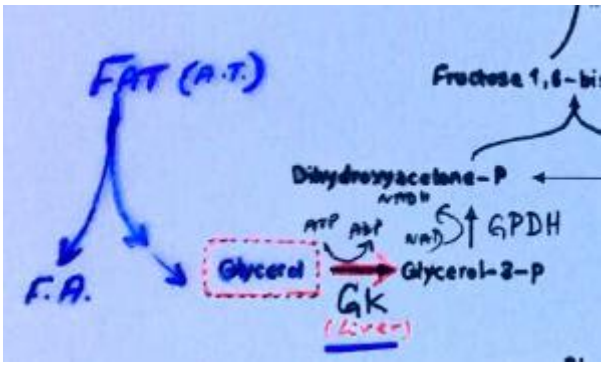
Simple hydrolysis of glucose-6-phosphate (which has a negative delta G value, as all hydrolysis reactions do), but this time using a different enzyme known as glucose-6-phosphatase. This enzyme is found in the liver but not in the muscles, that's why muscles cannot produce glucose and release it in the blood stream; since glucose 6-phosphate is trapped in the cell.

This reaction happens in the endoplasmic reticulum. First, glucose 6-P transferase transports glucose 6-P into the ER. Inside the ER, glucose 6-

phosphatase hydrolyses it into glucose which is transported into the cytosol and then to the blood.

Clinical fact: some individuals lack the glucose-6-phosphatase enzyme entirely, meaning not only can they not go through the above reaction but also they can't break down glycogen stored elsewhere in the body leaving them highly hypoglycemic, which is considered a life threatening condition.

When glycerol is produced from fats, it is phosphorylated by glycerol kinase to produce glycerol 3-phosphate, which is the substrate of the enzyme glycerol phosphate dehydrogenase that turns it into dihydroxiacetone -P.



Bioenergetics and regulation of gluconeogenesis:

Glycolysis & gluconeogenesis pathways are **both spontaneous**. If both pathways were simultaneously active within a cell it would constitute a "**futile cycle**" that would waste energy. Overall, each pathway may be summarized as follows (ignoring water & protons):

Glycolysis:
glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 ATP

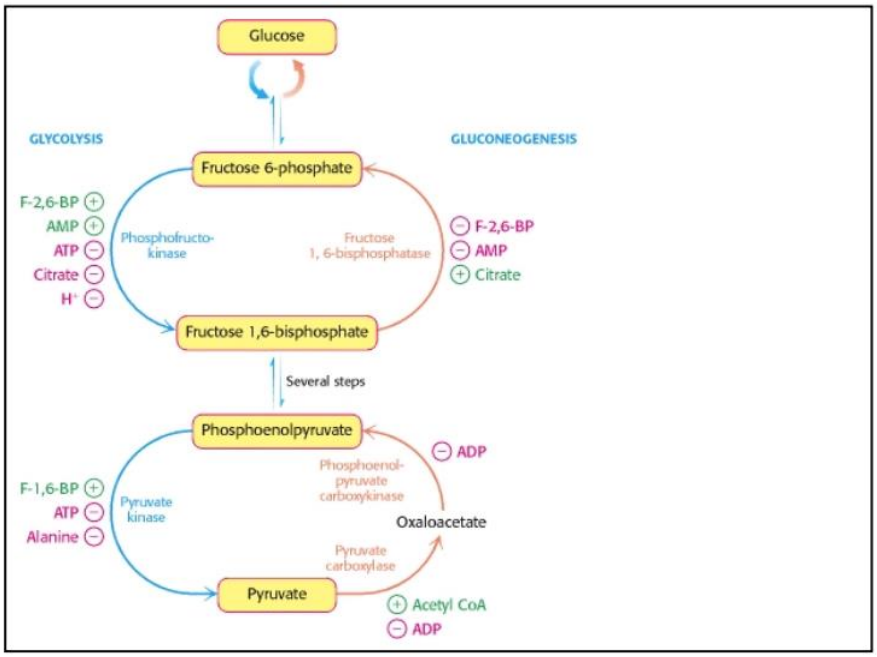
Gluconeogenesis:
2 pyruvate + 2 NADH + 4 ATP + 2 GTP → glucose + 2 NAD^+ + 4 ADP + 2 GDP + 6 P_i

Glycolysis yields 2 ~P bonds of ATP.
Gluconeogenesis expends 6 ~P bonds of ATP and GTP.
A **futile cycle** consisting of both pathways would waste **4 ~P bonds per cycle**.

To prevent this waste, glycolysis and gluconeogenesis pathways are **reciprocally regulated**. How?

If glycolysis is active, gluconeogenesis should be stopped. Therefore, a signal that activates glycolysis should inhibit gluconeogenesis.

For example, in the conversion of fructose-1,6-bisphosphate, fructose-2,6-bisphosphate and AMP are activators whereas in the reverse reaction, they are inhibitors.



- Hormonal regulation:

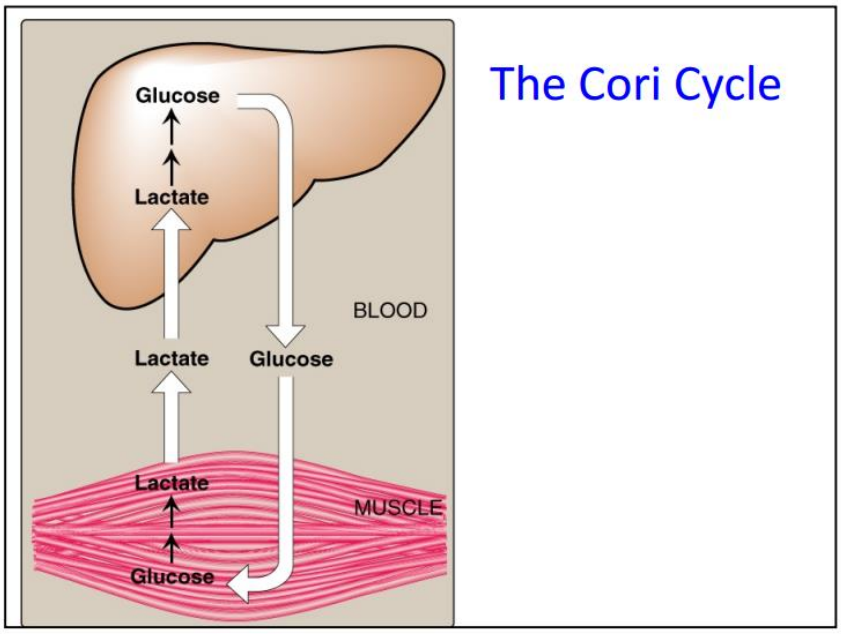
  1- Hypoglycemia: high glucagon concentrations. This leads to an increase in cAMP production, giving rise to the activation of protein kinase A which phosphorylates the bifunctional enzyme that has PhosphoFructoKinase-2 'inactivated' and FructoseBisPhosphatase-2 'activated. This leads to the lessening of F-2,6-BP amounts; causing the removal of the inhibition on glyconeogenesis and so activates it. Also, it leads to the removal of the activation on the glycolysis and so inhibits it.

  2- Hyperglycemia: high insulin concentration. Production of cAMP is not activated. The bifuctional enzyme has an activated PFK-2 domain and an inactivated FBP-2. This leads to the increase of the production of F-2,6-BP which inhibits gluconeogenesis and activates glycolysis.

The Cori Cycle:

Glucose in muscles is converted into lactate in the case of excessive physical activity, and this lactate is carried to the liver where it is converted back into glucose by gluconeogenesis. Ultimately, the produced glucose is then returned back to the muscle tissues for energy production and the cycle repeats.

Notice that the ultimate source of energy is *fats*; the lactate produced in the muscles is transported to the liver; where fat is burnt to produce ATP which is used to make glucose from lactate 'gluconeogenesis'. Glucose is then transported to the muscles.



In conclusion, what is the role of gluconeogenesis?

- During fed state, glucose is available.
- During short fasting, glycogen stores are broken down to produce glucose.
- During prolonged fasting or starvation, the body depends solely on gluconeogenesis to produce glucose for energy.

Note: gluconeogenesis mainly depends on amino acids extracted from body proteins to form keto acids and pyruvate.

Quick recap of important points:

Gluconeogenesis occurs mainly in the liver. Synthesis of glucose from pyruvate utilizes many of the same enzymes as **glycolysis**, except for three reactions of glycolysis that have such a large negative delta G in the forward direction that they are essentially **irreversible.**

Hexokinase or Glucokinase (Glycolysis) catalyzes:
glucose + ATP→ glucose-6-phosphate + ADP

Glucose-6-phosphatase (Gluconeogenesis) catalyzes:
glucose-6-phosphate + H_2O→ glucose + P_i

Phosphofructokinase (Glycolysis) catalyzes:
fructose-6-phosphate + ATP⟶ fructose-1,6-bisphosphate + ADP

Fructose-1,6-bisphosphatase (Gluconeogenesis) catalyzes:
fructose-1,6-bisphosphate + H_2O⟶ fructose-6-phosphate + P_i

Pyruvate Kinase (last step of Glycolysis) catalyzes:
phosphoenolpyruvate + ADP⟶ pyruvate + ATP

The **two enzymes** that catalyze the reactions for bypass of the Pyruvate Kinase reaction are the following:

(a) Pyruvate Carboxylase (Gluconeogenesis) catalyzes:
pyruvate + HCO_3^- + ATP⟶oxaloacetate + ADP + P_i

(b) PEP Carboxykinase (Gluconeogenesis) catalyzes:
oxaloacetate + GTP⟶ phosphoenolpyruvate + GDP + CO_2

THE END.