Fatty Acid Biosynthesis

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Fatty Acid Biosynthesis

- Synthesis takes place in the cytosol
- Intermediates covalently linked to acyl carrier protein
- Activation of each acetyl CoA.
- acetyl CoA + CO_2 ® Malonyl CoA
- Four-step repeating cycle, extension by 2-carbons / cycle
  - Condensation
  - Reduction
  - Dehydration
  - reduction

Fatty acid synthesis

- The enzymes of fatty acid synthesis are packaged together in a complex called as fatty acid synthase (FAS).
- The product of FAS action is palmitic acid. (16:0).
- Modifications of this primary FA leads to other longer (and shorter) FA and unsaturated FA.
- The fatty acid molecule is synthesized 2 carbons at a time
- FA synthesis begins from the methyl end and proceeds toward the carboxylic acid end. Thus, C16 and C15 are added first and C2 and C1 are added last.
- C15 and C16 are derived directly from acetylCoA. For further step-wise 2-carbon extensions, acetylCoA is first activated to malonyl CoA, a 3-carbon compound, by the addition of a CO_2.

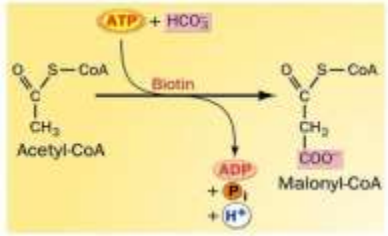
Citrate Shuttle

- FAs are synthesized in the cytoplasm from acetylCoA
- AcetylCoA generated from pyruvate by the action of PDH and by β-oxidation of fatty acids is in the mitochondria.
- For fatty acid biosynthesis, acetylCoA has to be transported from the mitochondria to the cytoplasm. This is done via a shuttle system called the **Citrate Shuttle**.
- AcetylCoA reacts with oxaloacetate to give citrate. A **tricarboxylate translocase** transports citrate from mitochondria to cytosol.
- In the cytosol, citrate is cleaved back to oxaloacetate and acetylCoA. This reaction is catalyzed by ATP-citrate lyase and requires the hydrolysis of one molecule of ATP.

Citrate Shuttle (regeneration of pyruvate)

- Oxaloacetate is converted back to pyruvate for re-entry into mitochondria
- Step 1. Oxaloacetate + NADH + H^+ ⓪ malate + NAD^+. Reverse of the TA cycle reaction. Catalyzed by cytosolic malate dehydrogenase.
- Step 2. Malate + $NADP^+$ ⓪ pyruvate + CO_2 + NADPH. Catalyzed by malic enzyme
- Pyruvate translocase transports pyruvate into mitochondria.
- Pyruvate is converted to oxaloacetate by pyruvate carboxylase with coupled hydrolysis of one ATP. Pyruvate + ATP + CO_2 + H_2O ⓪ oxaloacetate + ADP + Pi (reaction of gluconeogenesis)
- Net Reaction: $NADP^+$ + NADH + H^+ + ATP + H_2O ⓪ NADPH + NAD^+ + ADP + Pi
- Thus, transport of acetylCoA to cytosol requires expense of one ATP and conversion of one NADH to NADPH.

Reaction catalyzed by Acetyl CoA Carboxylase



Activation of acetate : Acetyl-CoA to malonyl CoA

MalonylCoA

- Malonyl CoA is synthesized by the action of acetylCoA carboxylase. Biotin is a required cofactor.
- $CH_3COSCoA + CO_2 + ATP \rightarrow OOC\text{-}CH_2\text{-}CO\text{-}SCoA + ADP + Pi$ (enzyme: acetylCoA carboxylase)
- This is an irreversible reaction. AcetylCoA carboxylation is a rate-limiting step of FA biosynthesis.
- AcetylCoA carboxylase is under allosteric regulation. Citrate is a positive effector and palmitoyl CoA is a negative effector.

Fatty Acid Synthase (FAS)

- FAS is a polypeptide chain with multiple domains, each with distinct enzyme activities required for fatty acid biosynthesis.
- ACP: Recall that CoA is used as an activator for β-oxidation. For fatty acid biosynthesis, the activator is a protein called the acyl carrier protein (ACP). It is part of the FAS complex. The acyl groups get anchored to the CoA group of ACP by a thioester linkage
- Condensing enzyme/β-ketoacyl synthase (K-SH). Also part of FAS, CE has a cysteine SH that participates in thioester linkage with the carboxylate group of the fatty acid.
- During FA biosynthesis, the growing FA chain alternates between K-SH and ACP-SH

Step-wise reactions

1. The **acetyl group gets transferred from CoA to ACP** by **acetyl CoA-ACP transacylase**.
2. The **acetyl (acyl) group next gets transferred to the K arm** of FAS complex.
3. Next, the **malonyl group gets transferred from CoA to ACP** by **malonyl CoA ACP transacylase**. This results in both arms of FAS occupied forming acyl-malonyl-ACP.
4. The COO group of malonyl ACP is **removed as CO_2**, the **acetyl group (C16 and C15) gets transferred to the alpha carbon of malonyl ACP**. This results in **3-keto acyl ACP**.

Reactions of FA biosynthesis (continued)

- The 3-keto group is converted to a CH_2 by a series of reactions reverse to FA β-oxidation. Namely,

1. reduction to hydroxyl group. Enz: 3-keto acyl ACP reductase
2. dehydration to form a 2,3 double bond and Enz: 3-hydroxy acyl ACP dehydratase
3. a second reduction to remove the double bond. Enz: Enoyl ACP reductase

- Both reduction reactions require the reduced **cofactor NADPH**. This is **generated by the hexose monophosphate pathway** of phosphogluconate pathway and during the citrate shuttle

Repeat cycles for chain elongation

- The result of the first cycle of fatty acid biosynthesis is a four carbon chain associated to the ACP arm.
- This chain gets transferred to the K arm.
- A new malonyl CoA is introduced on the ACP arm.
- The reactions proceed as before. For each cycle the acyl group transferred to the α-carbon of malonyl CoA is 2-carbons longer the previous cycle.
- At the end of 7 cycles a 16 carbon chain is attached to the ACP arm (palmitoyl ACP).
- The C16 unit is hydrolyzed from ACP yielding free palmitate
- Net reaction: Acetyl CoA + 7 malonyl CoA + 14 NADPH + 14 H^+ → Palmitate + 7 CO_2 + 8 CoA + 14 $NADP^+$ + $6H_2O$

Modification of Palimitic acid

- Palmitic acid is converted to palmityl CoA for modification.
- Fatty acids modification takes place by the action of enzyme systems that are present on the cytoplasmic face of the ER membrane.
- FA longer than palmitic acid are synthesized by an elongation enzyme system. Additional carbons are added in 2-carbon units using malonyl CoA as the donor.
- Unsaturated fatty acids are synthesized by the action of specific enzymes called as fatty acid CoA desaturases. The desaturases are specific for specific positions of the double bond.

Essential Fatty Acids

- Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C9.
- Hence, all fatty acids containing a double bond at positions beyond C9 have to be supplied in the diet. These are called *Essential fatty acids (EFA).*
- Linoleate (18:2 $\Delta^{9,12}$) and Linolenate (18:3 $\Delta^{9,12,15}$) are the two essential fatty acids in mammals.
- Other unsaturated fatty acids such as arachidonic acid (20:4 $\Delta^{5,8,11,14}$) are derived from these two EFA.
- Eicosanoids are derivatives of arachidonic acid and have hormonal and signaling properties. Classified into prostaglandins, thromaoxanes and leukotrienes

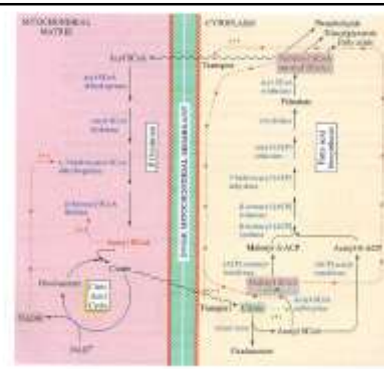
Regulation of FA metabolism

- The two processes of β-oxidation and FA synthesis are coordinately regulated.
- Three hormonal signals determine the state of FA metabolism. Glucagon and epinephrine inhibit FA synthesis and favor oxidation, whereas insulin is anti-lipolytic and stimulates FA biosynthesis.
- The mechanism of hormonal regulation is covalent phosphorylation of acetylCoA carboxylase, the rate-limiting step of FA biosynthesis.
- Acetyl CoA carboxylase is inhibited by phosphorylation. Phosphorylated acetylCoA carboxylase can regain partial activity by allosterically binding citrate.

Allosteric Regulation of FA Metabolism

- Acetyl CoA carboxylase is the rate-limiting step of FA biosynthesis. It is allosterically inhibited by palmitoyl CoA and activated by citrate.
- Palmitoyl CoA also inhibits the citrate shuttle and thus slows down FA biosynthesis.
- Malonyl CoA and acetyl CoA inhibit β-oxidation.
- Acetyl CoA inhibits the final step of β-oxidation catalyzed by 3-ketoacyl thiolase.
- Malonyl CoA inhibits the transport of acylCoA to mitochondria via inhibition of carnitine-acyl transferase I.
- Additionally, ATP and NADH also inhibit β-oxidation.

Co-ordinate regulation of FA metabolism



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Glycolysis v/s Gluconeogenesis

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Introduction

1. Glycolysis

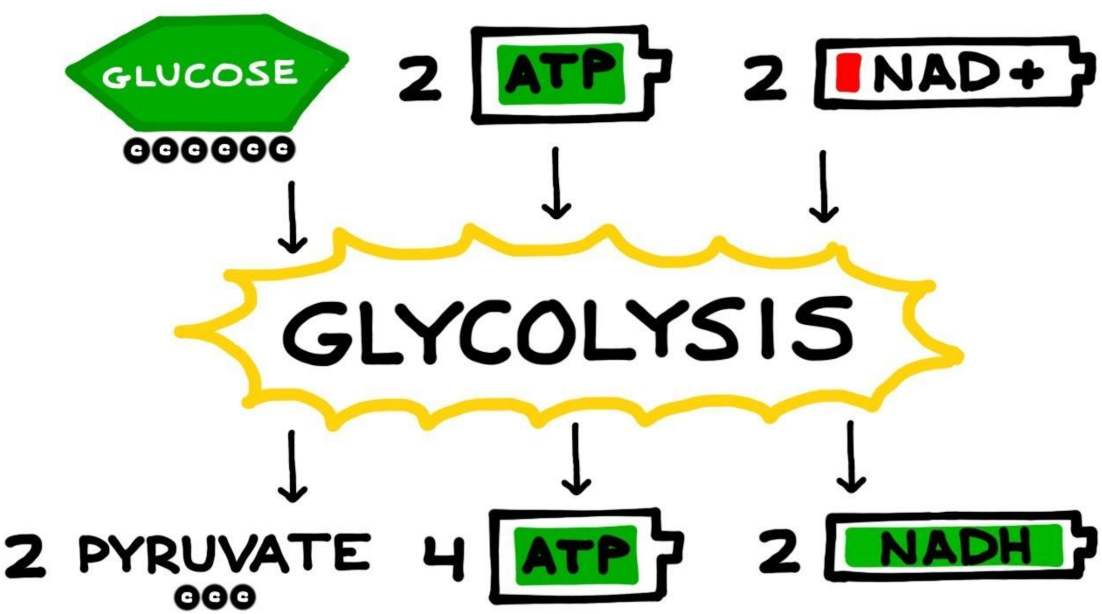
- Exergonic reaction
- 2 ATP are produced
- Raw material = glucose

2. Gluconeogenesis

- Endergonic reaction
- 6 ATP are utilized per 1 glucose molecule
- Raw material = lactate, amino acids (alanine & glycerol)

1. Glycolysis

- Involves the breakdown of glucose into pyruvic acid
- Does not require oxygen
- Occurs in cytoplasm / cytosol



Two parts of glycolysis :

Part 1 (Energy investment phase):

Glucose $\xrightarrow{ATP}$ Glucose co-phosphate $\xrightarrow{ATP}$ Fructose-1,6-phosphate

Part 2 (energy generation phase):

Fructose-1,6-diphosphate $\xrightarrow[-\ 2NADH_2]{}$ $\xrightarrow[-\ ATP]{}$ $\xrightarrow[-\ 2\ ATP]{}$ 2- pyruvic acid

Introduction of Glycolysis

- Glycolysis (from the Greek glykys, meaning sweet, and lysis , meaning splitting) also known as Embden – Meyerhof pathway(EMP).

- This is an oxidative process in which one mole of glucose is partially oxidized into the two moles of pyruvate in a series of enzyme –catalyzed reaction.

- Glycolysis, the major pathway for glucose metabolism,occurs in the cytosol of all cells.

- It is unique pathway occurs aerobically as well as anaerobically and does not involve molecular oxygen.

- The glycolytic sequence of reactions differ from species to species only in the mechanisms of its regulation and in the subsequent metabolic fate of the pyruvate formed.

- In aerobic organisms, glycolysis is the prelude to Citric acid cycle and ETC.

- It is a unique pathway occurs aerobically as well as anaerobically and does not involve molecular oxygen.

Steps of Glycolysis

- **Steps – 1:- (phosphorylation)**

> **Here, the glucose ring is phosphorylated. Phosphorylation is the process of adding a phosphate group to a molecule derived from ATP.**

> **As a result, at this point in glycolysis, 1 molecule of ATP has been consumed.**

> **.The reaction occurs with the help of the enzyme hexokinase , an enzyme that catalyzes the phosphorylation of many six- membered glucose- like ring structure.**

> **The result of this phosphorylation is a molecule called Glucose – 6-phosphate (G6P) , thusly called because the 6 carbon of the glucose acquires the phosphate group.**

➢ Step- 2 (Isomerization)

- The second reaction of glycolysis is the rerrangement of Glucose - 6- phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphste isomerase (phosphoglucose isomerase).

➢ Step-3 : - (phosphorylation)

- Phosphofructokinase, with magnesium as a cofactor , changes fructose 6- phosphate into fructose 1,6-biphosphate.

Step -4 (Aldolase)

- The enzyme Aldolase splits fructose 1,6 bisphosphate into two sugars that are isomers of each other.
- These two sugars are dihydroxyacetone phosphate (DHAP) and Glyceraldehyde 3- phosphate (GAP), only the glyceraldehyde 3-phosphate can proceed immediately through glycolysis.

Step – 5 : (Isomerization)

- Dihydroxyacetone phosphate is isomerized to form glyceraldehyde 3 phosphate.
- The isomerization of these three- carbon phosphorylated sugars is catalyzed by triose phosphate isomerase.

Step-6

- The two molecules of glyceraldehyde 3- phosphate are oxidized.
- Enzyme glyceraldehyde 3- phosphate dehydrogenase catalyzes the conversion of glyceraldehyde 3- phosphate into 1,3- bisphosphoglycerate (1,3- BPG).

Step: - 7

- The transfer of the high- energy phosphate group that was generated in step 6 to ADP form ATP.
- The formation of ATP is referred to as substrate- level phosphorylation because the phosphate donor , 1,3 BPG, is a substrate with high phosphoryl- transfer potential.

Step:-8

- The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2- phosphoglycerate.

Step:- 9

- The removal of water from 2-phosphoglycerate creates a high – energy enol phosphate linkage .
- Enzyme catalyzing this step , enolase , is inhibited by fluoride.

Step:- 10

- The transfer of the high- energy phosphate group that was generated in step 9 to ADP forms ATP.

- This last step in glycolysis is the irreversible transfer of the phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by pyruvate kinase.

- Pyruvate kinase requires either k+ or Mg 2+.

✓ Net reaction :

$$Glucose + 2NAD + 2ADP + 2H_2PO_4 \longrightarrow 2Pyruvate + 2NADH + 2ATP + 2H_2O$$

- **Function :**

- Function of glycolysis is to break down glucose to form NADH and ATP as source of energy to cells.

- As a part of aerobic respiration pyruvate is made available for the citric acid cycle.

- The process results in intermediate compounds, which may be used at various steps for other cellular purposes.

Significance of glycolysis

- Glycolysis is present in nearly all living organisms.Glucose is the source of almost all energy used by cells.
- Glycolysis is an almost universal central pathway of glucose catabolism occuring in the cytoplasm of all the tissues of biological systems leading to generation of energy in the form of ATP of vital activities.
- It is the pathway through which the largest flux of carbon occurs in most cells.

- Some plant tissues which are modified for the storage of starch such as potato tubers and some plants adapted to growth in inundated water such as water cress derive most of their energy from glycolysis.

- Many types of anaerobic microorganisms are entirely dependent on glycolysis.

- Mammalian tissues such as renal medulla and brain solely dependent on glycolysis for major sources of metabolic energy.

Gluconeogenesis

- **Defination :**
- **Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors.**
- **Gluconeogenesis is a universal pathway,found in all organism in all animals , plants , fungi, and microorganism.**
- **Precursors of gluconeogenesis:**
- **Lactate, pyruvate, glycerol, some amino acid (termed glucogenic acids).**

Gluconeogenesis cycle

- Gluconeogenesis is begins in the mitochondria with the formation of oxaloacetate by the carboxylation of pyruvate . This reaction also requires 1 molecule of ATP , and is catalyzed by pyruvate carboxylase.

- This enzyme is stimulated by high levels of acetyl CoA and inhibited by high levels of ADP and glucose.

- Oxaloacetate is reduced to malate using NADH , a step required for its transportation out of the mitochondria.

- Malate is oxidized to oxaloacetate using NAD+ in the cytosol, where the remaining steps of gluconeogenesis.

- Oxaloacetate is decarboxylated and then phosphorylated to form phosphoenolpyruvate using the enzyme PEPCK. A molecule of GTP is hydrolyzed to GDP during this reaction.

- The next steps in the reaction are the same as reversed glycolysis. However , fructose 1,6 – bisphosphate converts fructose -6- phosphate , using 1 water molecule and releasing 1 phosphate (in glycolysis, phosphofructokinase 1 converts F6P and ATP to F1,6BP and ADP.

- Glucose 6 –phosphate is formed from fructose – 6 – phosphate by phosphoglucoisomerase.

- The final reaction of gluconeogenesis , the formation of glucose , occurs in the lumen of the endoplasmic reticulum , where glucose – 6- phosphate is hydrolyzed by glucose – 6 –phosphatase to produce glucose and release an inorganic phosphate.

- Like two steps prior, this step is not a simple reversal of glycolysis,in which hexokinase catalyzed the conversion of glucose and ATP into G6P and ADP.

- Glucose is shuttled into the cytoplasm by glucose transporters located in the endoplasmic reticulm' s membrane.

- Net reaction :

- 2 pyruvate + 2 NADH + 4 ATP + 2 GTP + 6H_2O → Glucose + 2NAD+ + 2GDP + 4 ADP

Function :

- It maintain the level of intermediate of the TCA cycle even when fatty acids are the main source of acetyle coA in the tissues.
- In higher animals, gluconeogenesis happens in liver and renal cortex
- It is essentially glycolysis, which is the process of converting glucose into energy, in reverse.

Significance of gluconeogenesis

- Removal of lactic acid.
- Removal of glycerol producted by lipolysis.
- To maintain the amount of glucose.
- It maintains the level of intermediates of the TCA cycle even when fatty acids are the main sources of acetyl coA in the tissues.

Conclusion

- Glycolysis and gluconeogenesis are two processes involved in the glucose metabolism.

- Glucose is the energy source of almost all the life forms on earth.

- Glucose is broken down in order to generate energy in the form of ATP during the process called cellular respiration.

- Glycolysis is the first step of cellular respiration, breaking down 6 carbon glucose into 2 pyruvate molecules each bearing 3 carbon atoms.

- Glycolysis occurs in the cytoplasm of almost cells. Gluconeogenesis occurs in the mitochondria and cytoplasm.

- Glycolysis is an exergonic reaction where two ATPs are produced. Gluconeogenesis is an endergonic reaction where 6 ATPs are utilized per 1 glucose molecule.

- The raw material of glycolysis is glucose. The raw material of gluconeogenesis is lactate, amino acids like alanine and glycerol.

Glycolysis Net Reaction:

Glucose + 2 ADP + 2 NAD^+ + 2 Pi → 2 Pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H_2O

Gluconeogenesis Net Reaction:

2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 6 H_2O → Glucose + 4 ADP + 2 GDP+ 2 NAD^+ + 6 Pi

Thank you