METABOLISM

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Recall from the previous lectures:

- Gluconeogenesis is the pathway in which glucose is synthesized from several noncarbohydrate precursors under fasting conditions (glycogen stores are depleted). Gluconeogenesis supplies tissues that are exclusively dependent on glucose with glucose for their energy requirements and also maintains blood glucose levels at fasting blood sugar.
- It's the opposite of glycolysis. Recall that glycolysis has 10 steps, 7 of which are reversible (reversed by the same enzymes that catalyse them) and 3 of which are irreversible and require other enzymes to reverse them in gluconeogenesis.
- The 3 irreversible steps are reversed in gluconeogenesis as:

    1. **From pyruvate to phosphoenolpyruvate (PEP):** Occurs in two steps (therefore, gluconeogenesis is composed of 11 steps unlike glycolysis):

       Pyruvate $\xrightarrow{\textit{Pyruvate carboxylase}}$ oxaloacetate (in the mitochondria)
       Oxaloacetate → malate (to leave the mitochondria) → oxaloacetate (in the cytosol)
       Oxaloacetate $\xrightarrow{\textit{PEP carboxykinase}}$ phosphoenolpyruvate

    2. **From fructose-1,6-bisphosphate to fructose-6-phosphate:** by the enzyme *fructose 1,6-bisphosphatase.*

    3. **From glucose-6-phosphate to glucose.**

The first two steps were covered in the previous sheet. Now starting our lecture with the last irreversible step:

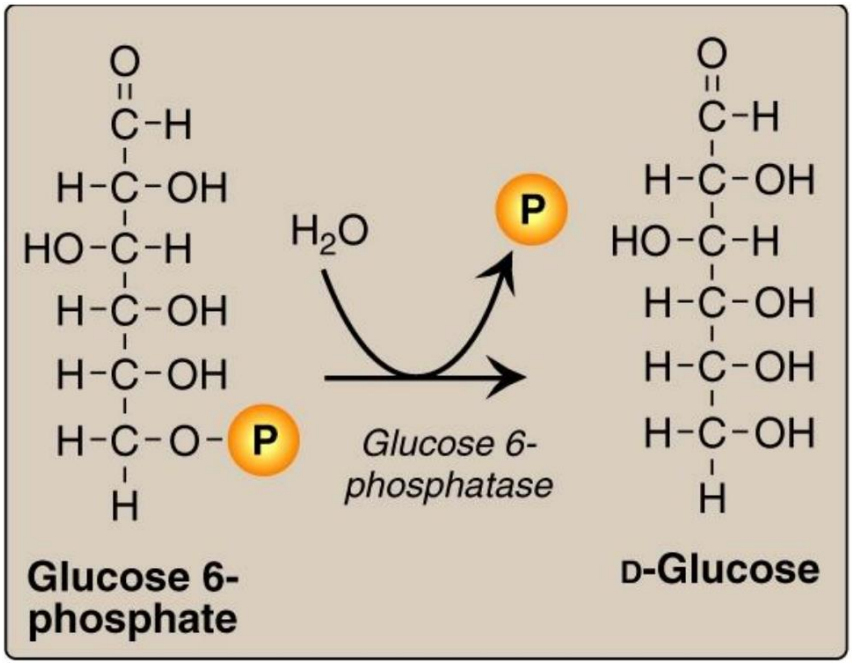
3. From glucose-6-phosphate to glucose:

- Bypasses the irreversible hexokinase/glucokinase reaction.
- Only in liver and kidney (gluconeogenic tissues); recall that glycolysis takes place in all body cells for energy generation, but gluconeogenesis is carried out by specific gluconeogenic tissues (the liver is the primary organ that produces free glucose from glucose 6-phosphate).
- Takes place in the ER by the aid of two proteins:

    1. **Glucose 6-phosphate translocase** which transfers G-6-P molecules across the ER membrane. G-6-P can't be transported by GLUTs as they are not able to recognize it and bind to it.

2. **Glucose 6-phosphatase** in the ER membrane too. Which dephosphorylates glucose 6-phosphate molecules forming free glucose molecules.

- Free glucose molecules are then transferred to the cytosol through **GLUT-7** then transported outside of cells by other GLUTs like GLUT-2, GLUT-3 (brain), and other GLUTs **but not GLUT-4,** GLUT-4 is insulin sensitive and upregulated at high concentrations of glucose not in the state of starvation when gluconeogenesis takes place. Glucose molecules are then taken by the blood stream to the tissues that are exclusively dependent on them and they also maintain blood glucose levels.



- Keep in mind: Muscles lack glucose 6-phosphatase, and therefore muscle glycogen cannot be used to maintain blood glucose levels.

Formation vs. Hydrolysis of Glucose 6- phosphate (energetics):

Formation:

Glc. + Pi ⟶ Glc. 6-phosphate + H2O **ΔG = +ve**

ATP + H2O ⟶ ADP + Pi **ΔG = -ve**

Phosphorylation of glucose is an energy requiring reaction (has a positive ΔG) and it gets coupled to an energy releasing reaction (has a negative ΔG) which is the hydrolysis of ATP. The sum of both reactions has a negative ΔG so the reaction runs in glycolysis as the following:

Glc. + ATP $\xrightarrow{\textit{hexokinase}}$ Glc. 6-phosphate + ADP **ΔG = -ve**

Hydrolysis:

Glc. 6-phosphate + H2O $\xrightarrow{phosphatase}$ Glc. + Pi **ΔG = -ve**

Dephosphorylation of glucose 6-phosphate is a spontaneous reaction (negative ΔG).

Energy requirements of gluconeogenesis:

Gluconeogenesis is an anabolic reaction that requires energy, it builds glucose from smaller molecules.

Trace down the glycolytic pathway in this figure. Notice how the first step (glucose → glucose 6-P) as well as the third step (fructose 6-P → fructose 1,6-bis-P) utilized a net of **2ATP** molecules, 1 in each. ATP molecules are produced in the following steps:

2 1,3-Bisphosphoglycerate→**2** 3-phosphoglycerate **(2ATP)**

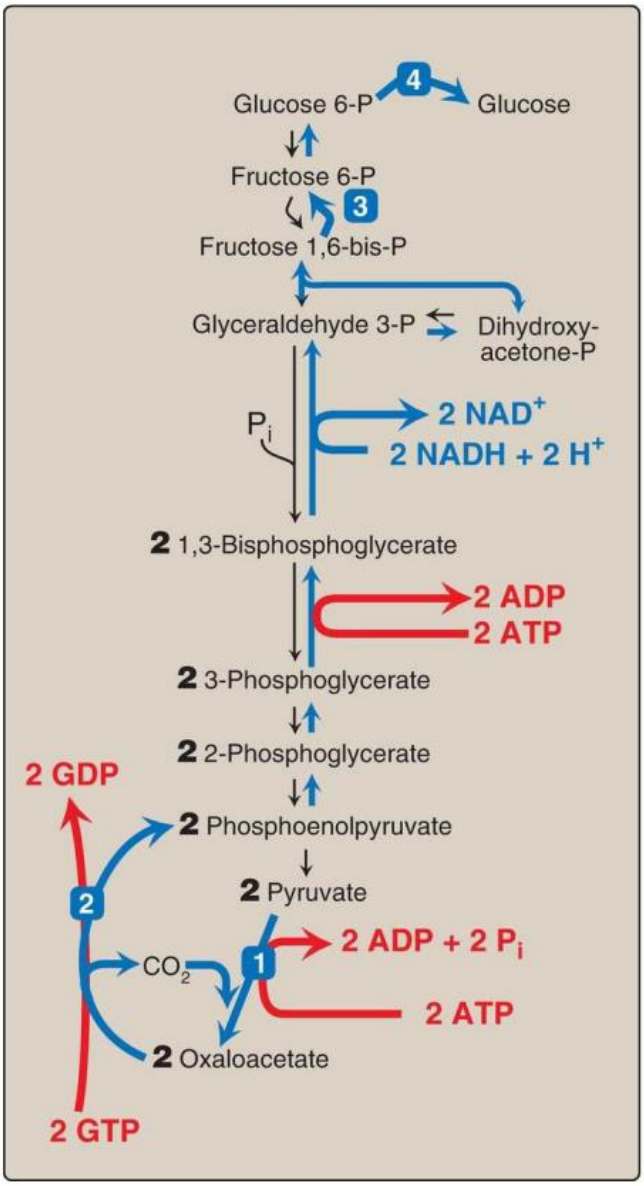
2 PEP → **2** pyruvate **(2ATP)**

As well as **2 NADH** molecules produced in the sixth step.

Let's compare that to what happens in gluconeogenesis:

When pyruvate is converted to oxaloacetate through a **carboxylation** reaction that requires energy as we all know, **2ATP** molecules are used (we need 2 pyruvate molecules to synthesize glucose). Then in the next reaction which is the decarboxylation and phosphorylation of OAA to PEP energy was utilized in the form of **2GTP** molecules. And the conversion of 1,3-bisphosphoglycerate to GA3P **doesn't yield 2NADHs** like the reverse reaction in glycolysis but rather oxidize **2NADH** molecules to **2NAD$^+$**.

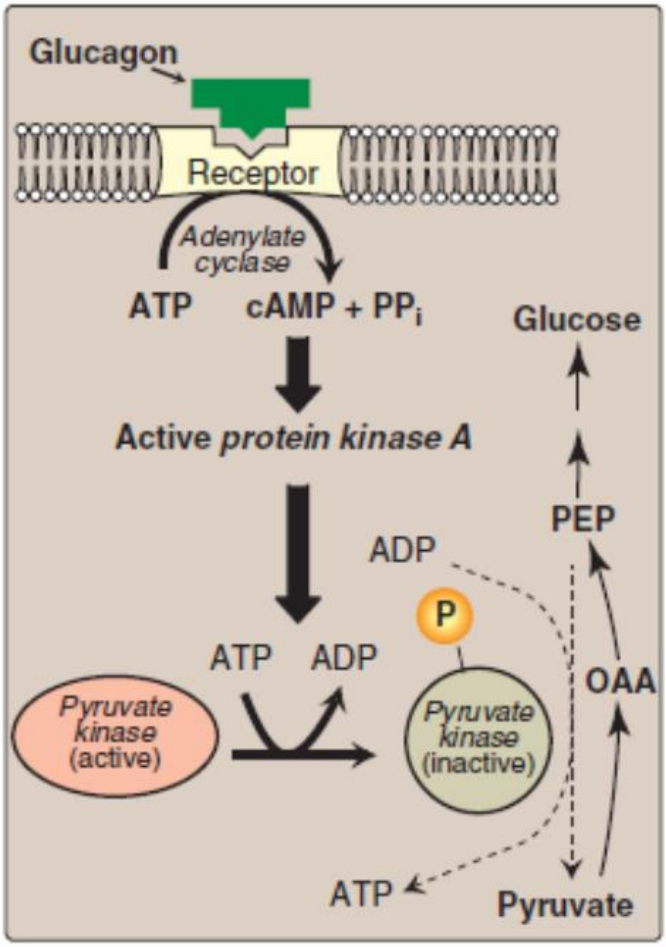
Net molecules USED : 4ATPs, 2GTPs, 2NADH.



In spite of starvation, energy is used up to synthesize glucose molecules which indicates the importance of gluconeogenesis to glucose dependent tissues. We have other sources of energy that supply the other tissues like lipids which produce high energy.

Regulation of gluconeogenesis:

1. **The circulating level of glucagon**



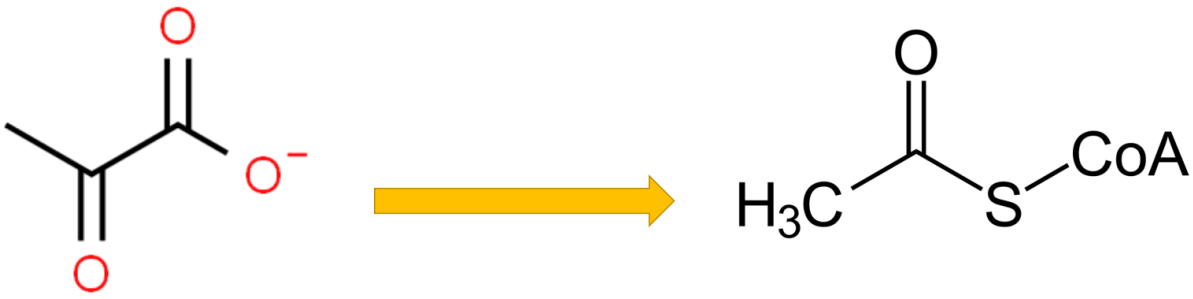
- Glucagon is secreted in high concentrations in the state of starvation. It binds to its **GPCR** initiating a series of changes that activate **adenylyl cyclase** and increase **cAMP** concentration which leads to the activation of **protein kinase A.** Some of the targets of protein kinase A are **pyruvate kinase** which is inactivated when phosphorylated and the enzyme complex **PFK-2/FBPase-2** (the kinase is inhibited when phosphorylated and the phosphatase is activated) reducing the amount of **fructose-2,6-bisphosphate** which inhibits the enzyme PFK-1 but normally activates it at high concentrations. Therefore, glycolysis is inhibited. However, fructose-2,6-bisphosphate is an inhibitor of gluconeogenesis so it only makes sense for the resultant low levels of it to do the opposite and activate gluconeogenesis by activating the enzyme **fructose 1,6- bisphosphatase.**

- Glucagon increases the transcription of the gene for PEP-carboxykinase by activating certain transcription factors. PEP-carboxykinase is the second enzyme used in gluconeogenesis which catalyses the conversion of OAA to PEP and glucagon increases the expression of its gene.

2. The availability of gluconeogenic substrates (lactate, glycerol, glucogenic amino acid,etc).

3. Slow adaptive changes in enzyme activity due to an alteration in the rate of enzyme synthesis or degradation, or both. Control of gene expression whether activation or inhibition of certain genes takes a long time so these adaptive changes are slow compared to allosteric regulation for example. (Activation can produce molecules that inhibit other molecules, so activation of a gene simply refers its increased expression and gives no information about the changes it can induce provided that the gene wasn't specified).

After Glycolysis: From Pyruvate to Acetyl-CoA



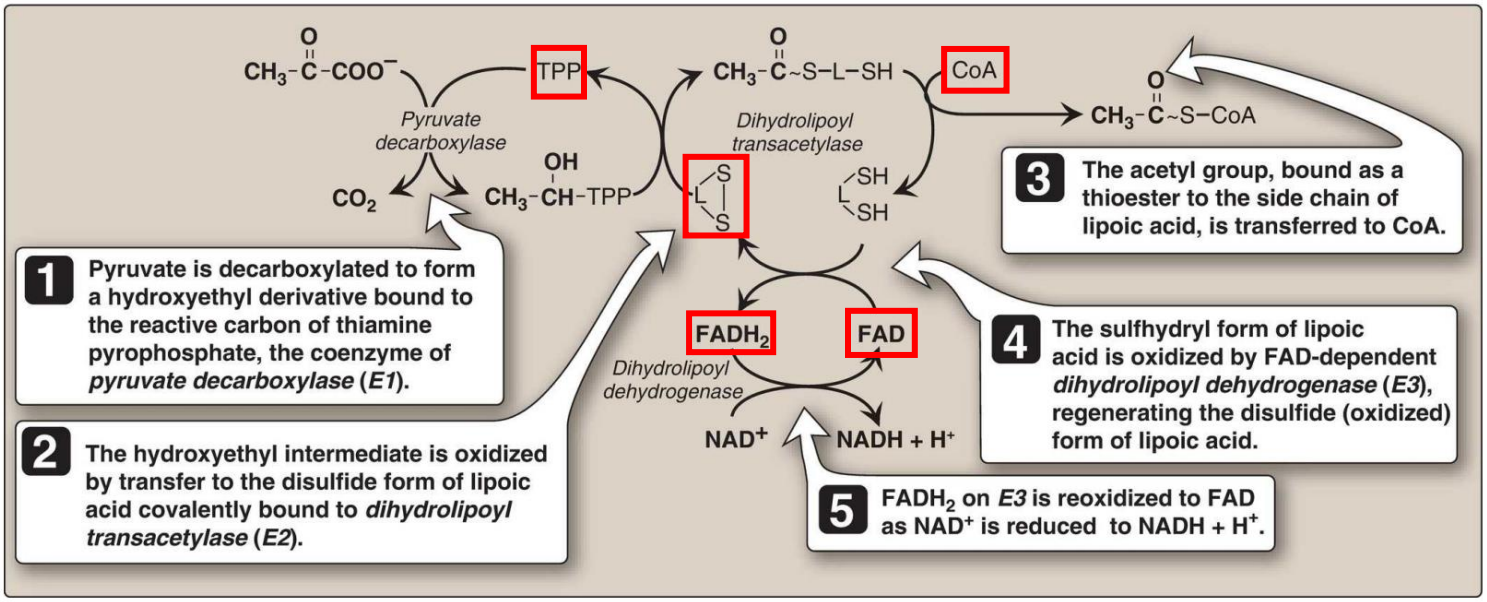
Oxidative decarboxylation of pyruvate

Pyruvate is produced in the cytosol and needs to be transported to the mitochondria (where TCA cycle occurs) by a **specific pyruvate transporter.**

Pyruvate contains 3 carbons while acetyl-CoA has 2 so a decarboxylation reaction is needed to reduce the number of carbons by 1 and a transfer reaction is needed to add the CoA. These changes are induced by the enzyme that catalyses this transformation of pyruvate to acetyl-CoA.

Once in the matrix, pyruvate is converted to acetyl CoA by the ***pyruvate dehydrogenase (PDH) complex***, which is a multienzyme complex made of 3 enzymes, E1 **(decarboxylase)**, **dihydrolipoyl transacetylase** (E2), and **dihydrolipoyl dehydrogenase** (E3) as well as many coenzymes that play major roles in this complex.

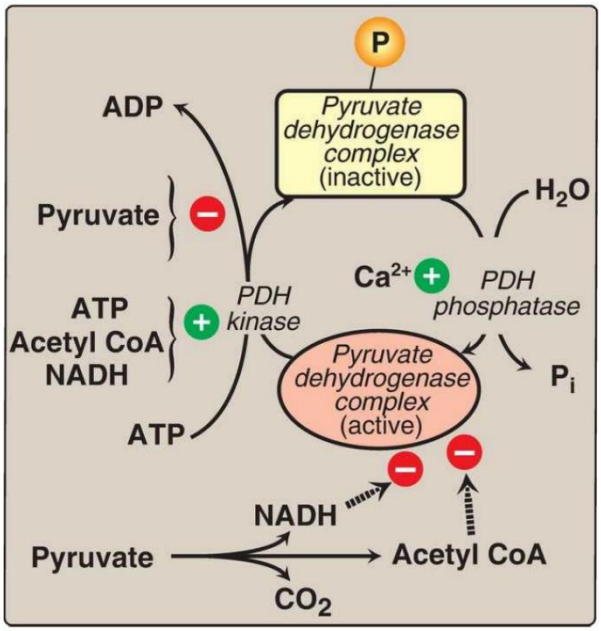
the following figure explains the steps of the reaction:



TPP: thiamine pyrophosphate

Keep in mind that coenzymes must return to their original form so additional reactions may take place.

Regulation of PDH Complex:



The complex contains two tightly bound regulatory enzymes, **PDH kinase** and **PDH phosphatase**. PDH kinase phosphorylates the PDH complex and inactivates it while PDH phosphatase dephosphorylates the PDH complex and activates it.

These two enzymes are regulated by their own allosteric regulators:

PDH kinase:

Pyruvate is an inhibitor of this enzyme which activates the reaction by inhibiting the kinase and activating the synthesis of acetyl-CoA.

Acetyl-CoA is an activator because it's the product of this reaction so increased amounts of it need to inhibit the reaction by phosphorylating the PDH complex through the activation of the kinase.

ATP is an activator; it indicates a high energy state so no need for more acetyl-CoA production and carrying on with Kreb's cycle.

NADH is an activator. High NADH/NAD+ inhibits Kreb's cycle. Therefore, there's no need to synthesize more acetyl-CoA that cannot go into the TCA cycle.

PDH kinase activators lead to the phosphorylation of the PDH complex and its inactivation → inhibition of the formation of acetyl-CoA.

PDH kinase inhibitors lead to the dephosphorylation of the PDH complex and its activation → activation of the formation of acetyl-CoA.

PDH phosphatase:

Ca^{2+} activates this enzyme (especially in muscles) because it indicates high activity and high energy demand. More acetyl-CoA is needed to get into Kreb's cycle and generate energy.

Activation of PDH phosphatase leads to dephosphorylation of the PDH complex and its activation → activation of the formation of acetyl-CoA.

Allosteric regulators of the PDH complex itself:

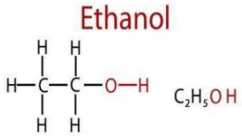
Acetyl-CoA: feedback inhibition.

NADH inhibits the reaction through the kinase as well as the PDH complex itself.

Clinical Application: PDH deficiency:

- Rare deficiency in the **E1** component of the PDH complex
- However, **the most common** biochemical cause of congenital lactic acidosis.
- This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactic acid via lactate dehydrogenase.
- Affected tissues: brain, relies on the TCA cycle for most of its energy, and is particularly sensitive to acidosis.
- Symptoms are variable and include neurodegeneration, muscle spasticity and, in the neonatal onset form, early death.
- X-linked dominant. One abnormal gene is enough to have the disease, equal susceptibility in males and females.
- No proven treatment.
- To improve the situation:
    1. **Dietary restriction of carbohydrate.** Acetyl-CoA can be produced from other sources to run the Kreb's cycle but in the presence of carbohydrates these carbohydrates are used up first to synthesize acetyl-CoA then lipids and other sources.
    2. **Supplementation with TPP may reduce symptoms in select patients.** Deficiencies are not necessarily 100%. In the presence of traces of functional enzymes addition of more coenzyme would further activate those enzymes and improve the situation.

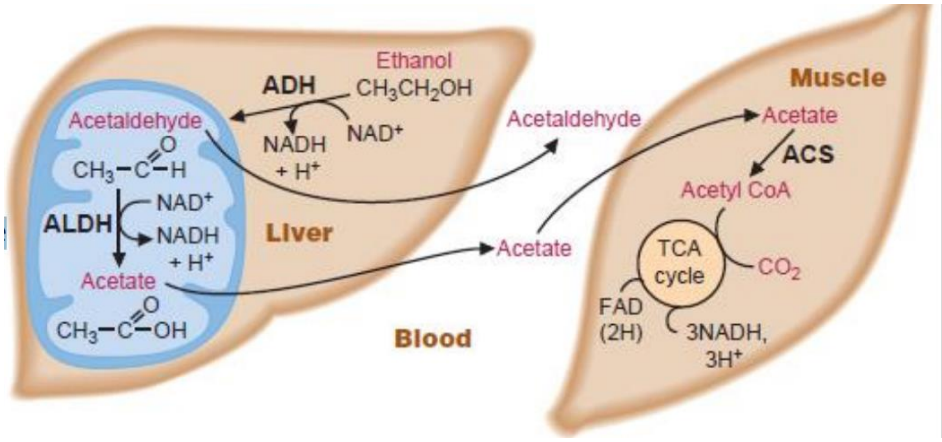
Alcohol Metabolism



Metabolism of Alcohol:

When alcohol is ingested, a small amount is immediately metabolized in the stomach.

Most of the remaining alcohol is subsequently absorbed from the gastrointestinal tract, primarily the stomach and upper small intestine.



Following its absorption, the majority of ethanol is metabolized in the liver. That's why liver cirrhosis is common among alcoholics.

How is ethanol metabolized in the liver?

1.In the cytosol of hepatocytes an enzyme called ***alcohol dehydrogenase ADH*** catalyses the conversion of ethanol to acetaldehyde.

2. acetaldehyde is toxic and is further converted by the enzyme ***acetaldehyde dehydrogenase ALDH*** to acetate (carboxylic acid) in the mitochondria.

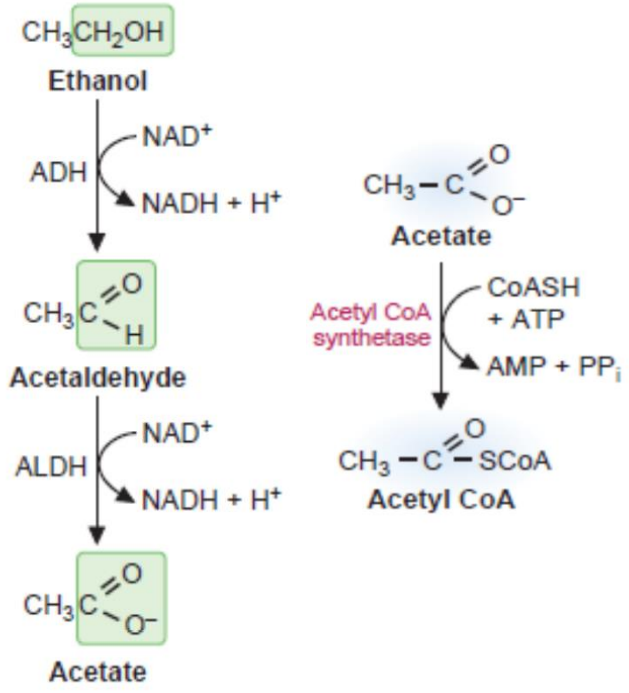
3.Acetate is taken up by the muscles and converted to acetyl-CoA by the enzyme ***acetyl-CoA synthetase ACS*** which requires energy.

4.Acetyl-CoA enters Kreb's cycle and supplies muscles with energy.

NAD+ is reduced to NADH in the mitochondria which increases NADH/NAD+ and inhibits kreb's cycle so the hepatocytes activity is highly affected.

What happens when a high amount of ethanol is metabolized?

- **High NADH/NAD+ (inhibition of kreb's cycle)**
- **Inhibition of FA oxidation (decreased acetyl-CoA and inhibition of kreb's cycle)**
- **Lactic acidosis (anaerobic respiration)**
- **Inhibition of gluconeogenesis (inhibition of the degradation of TG into fatty acids and glycerol which as a precursor for gluconeogenesis)**



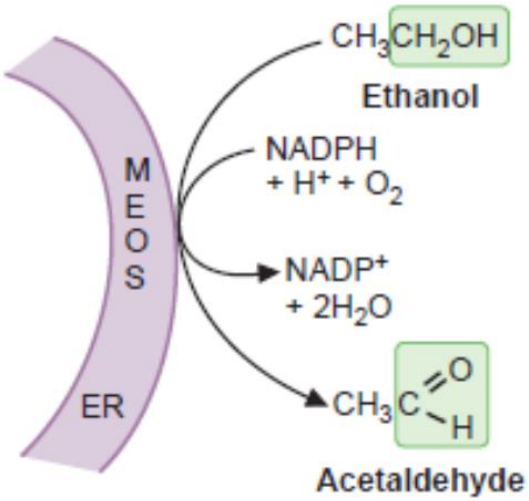
Additional information from the book about the inhibition of gluconeogenesis:

The abundance of NADH favors the reduction of pyruvate to lactate and of oxaloacetate (OAA) to malate. Recall that pyruvate and OAA are substrates in the synthesis of glucose. Thus, the ethanol-mediated increase in NADH causes these gluconeogenic precursors to be diverted into alternate pathways resulting in the decreased synthesis of glucose. This can precipitate hypoglycemia, particularly in individuals who have depleted their stores of liver glycogen.

That was the major pathway of ethanol metabolism. Here are the minor pathways:

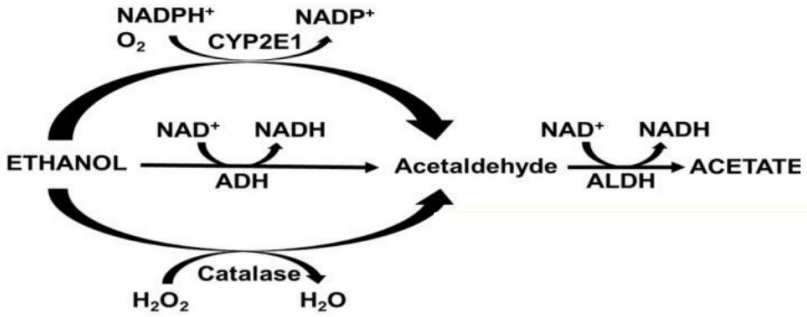
MEOS: Microsomal Ethanol Oxidizing System:

- An alternative pathway for ethanol metabolism.
- 10-20% of the ingested ethanol.
- Involves primarily the **cytochrome P450 2E1 (CYP2E1)**
- CYP2E1 is associated with NADPH-cytochrome P450 reductase in the
- High Km for ethanol
- Inducible by ethanol
- CYP2E1 is a major contributor of oxidative stress in the hepatocytes by generating several reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), hydroxyethyl radical (HER·), hydroxyl radical (OH−) and superoxide (O2 −)



Metabolism of Alcohol Metabolism of Alcohol-Catalase:

- **The peroxisomal catalase** converts H2O2 to oxygen and water (peroxisomes reduce oxidative stress; hypoxia/ROS)
- It can also oxidize ethanol to acetaldehyde.
- Is not a key pathway for ethanol elimination.
- Catalase is ubiquitously expressed in almost all tissues
- Catalase is also expressed by colonic floras which may lead to acetaldehyde production in the lower gastrointestinal tract.
- Catalase activity relies on the cellular level of H2O2.
- Has the least contribution to ethanol metabolism.



Catalase carries both reactions at the same

Ethanol Metabolism Application:

- ADH has 5 classes or isoenzymes
- Different isoforms are expressed in different tissues such as liver, lung, stomach and esophagus. People with different races inherit different sets of ADH isoenzymes, for example African Americans have an isoform with a high maximal velocity resulting in fast ethanol metabolism unlike southeast Asians that lack the efficient forms of these enzymes.

GOOD LUCK