بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Final 6

Degradation of Fatty acids pt.1

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NST

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وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: يدل الاسمان على الرحمة الشاملة لجميع الخلائق بإيجادهم وإمدادهم، وعلى الرحمة الخاصة بالمؤمنين في الدنيا والآخرة.

الورود: ورد اسم الرحمن (٥٧) مرة، أما اسم الرحيم فورد (١٢٣) مرة.

الشاهد: ﴿ٱلرَّحۡمَٰنُ ① عَلَّمَ ٱلۡقُرۡءَانَ﴾ [الرحمن:١-٢]، ﴿إِنَّ ٱللَّهَ غَفُورٞ رَّحِيمٞ﴾ [المزمل:٢٠]

اضغط هنا لشرحٍ أكثر تفصيلًا

Degradation of fatty acids

Dr. Diala Abu-Hassan

Lippincott's Biochemistry, Ch. 16

Why FAT not carbohydrates?

Fatty acids are the main source of energy in fasting conditions or ketogenic diets. The activation and mediation of the fatty acid degradation pathway is carried out by hormonal changes. As discussed before, the storage of fats is more preferrable than carbohydrates because of the following reasons:
More reduced:

9 kcal per gram compared with

4 kcal per gram of carbohydrates

- Hydrophobic:
  Can be stored without H_2O
  Carbohydrates are hydrophilic
  1 gram carbohydrates : 2 grams H_2O

Triacylglycerol (TAG) or FAT is the major energy reserve in the body

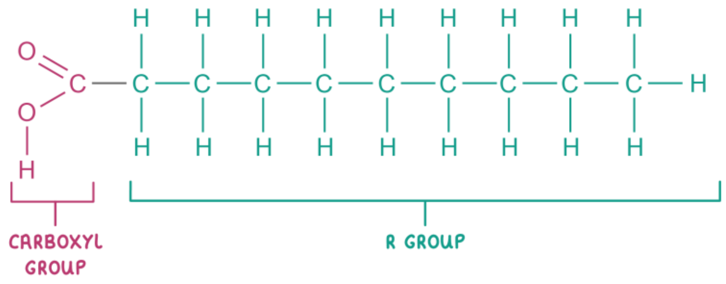
It is more efficient to store energy in the form of TAG

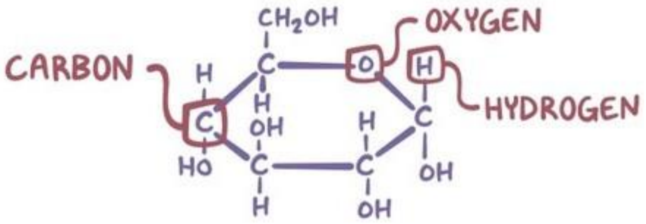
Ketogenic diets are diets that entail very low consumption of carbohydrates. Therefore, fatty acids are utilized as the main sources of energy.

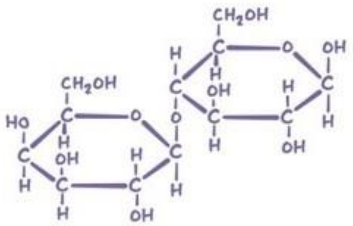
Fatty acids are hydrophobic in nature. Despite having some polar groups in their structure, their size is rather small compared to the hydrophobic, nonpolar chain. Accordingly, fatty acid storage is more efficient because it doesn't attract water into the cell.

On the other hand, carbohydrates attract water into the cells, causing cellular swelling, a gel-like structure, and increased cell volume. This all goes to prove that the storage of fatty acids is more efficient that the storage of carbs.

) Remember carbs contain hydroxyl groups that can form H-bonds with water, causing the cell to swell up. Check out the structures of these molecules)







FATTY ACID as FUELS

The major fuel used by tissues; however, **glucose** is the major *circulating* fuel.

Fuel type	**Amount used/kcal/12 hours (gram)**
Fatty acids	60 (540) 60 grams * 9 kcal/gram
Glucose	70 (280) 70 grams * 4 kcal/gram

Even though more glucose was used, the energy extracted from fatty acids was almost double that of the glucose.

The release of fatty acids from TAG

To activate the FA degradation pathway, the stored fatty acids must be obtained the TAG in adipose tissues to be oxidized and form energy.
This release is mediated via hormones, mainly glucagon (fasting state), or epinephrine (fight or flight).

Hormonal Regulation

Both hormones (EP & glucagon) have receptors on adipocytes. They bind to GPCR and activate the receptor, then the alpha subunit, adenylyl cyclase which catalyzes the conversion of **ATP to cAMP.**

PKA phosphorylates many substrates. Among these substrates is **hormone-sensitive lipase (HSL)**, an enzyme present in *adipocytes*. It hydrolyzes ester bonds in TAGs and requires hormonal activation. Phosphorylation by PKA activates this enzyme. It acts on **diacyl glycerol** only. **Adipose triglyceride lipase (ATGL)** is the enzyme that acts on **triacyl glycerols**, it is also located in the adipocytes. ATGL removes the first fatty acid, then HSL removes the second, and MAGL (monoacylglycerol lipase) removes the third fatty acid, forming 3 free fatty acids and a glycerol molecule.

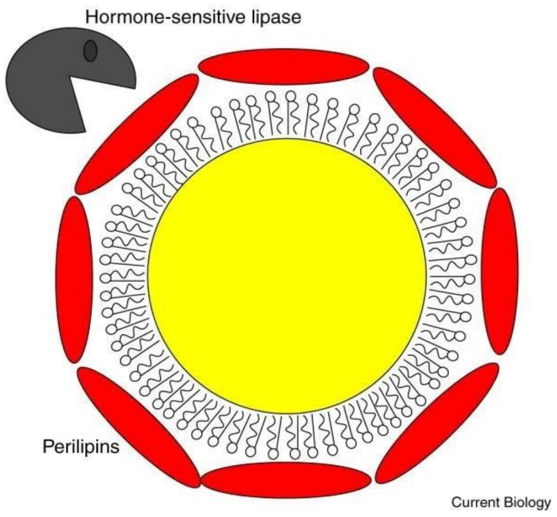
If fatty acids are long they will move in the bloodstream on albumin, but if fatty acids are short they will move freely in the bloodstream.

Glycerol will move freely in the bloodstream and will be uptaken by gluconeogenesis organs like liver and kidneys.

Check out the picture 2 slides over :)

Hormonal Regulation

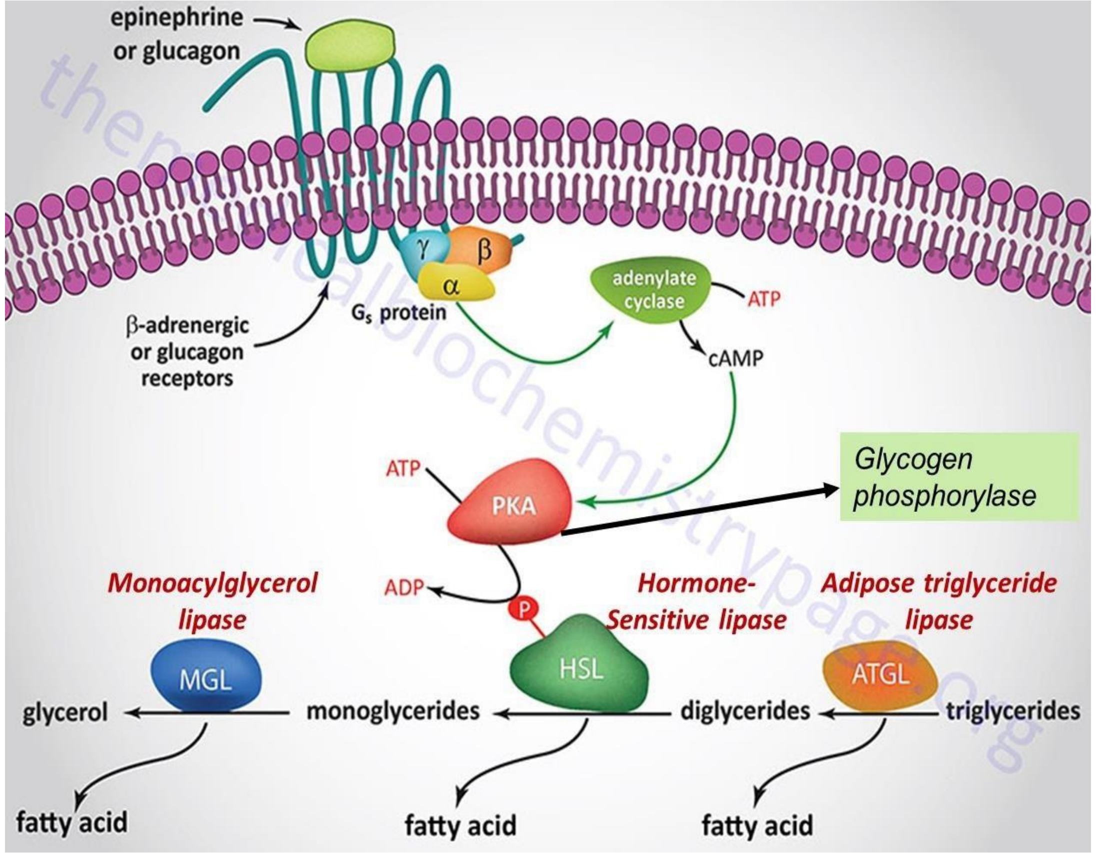
TAG in adipocytes are sequestered by a protein called perilipin. This protein is necessary to isolate the TAG from the cytosol and its aqueous environment. Direct contact with the aqueous environment is unstable for the TAG, can cause premature lipolysis therefore, an emulsifier is crucial for the protection of the TAG. Perilipin is phosphorylated by PKA, which induces its dissociation from the lipid droplet, allowing for HSL to easily access the TAG and initiate lipolysis. Unphosphorylated perilipin has negative charges on its surface from amino acid residues, once it is phosphorylated by PKA, the phosphate group adds additional negative charges to the perilipin. These negative charges start to repel from each other, causing the dissociation of the perilipin due to weakening of its attachment to the lipid droplet, exposing the TAG to HSL.



Perilipin (in red) coats fat droplets blocking HSL. It is phosphorylated by PKA releasing it.

Hormonal Regulation

The β-oxidation of fatty acids doesn't take place in adipocytes, but in cells that need fatty acids as a source of energy. Inhibition of fatty acid synthetic pathways is also necessary; running two contradictory pathways simultaneously wastes energy. One regulatory mechanism is the compartmentalization of the opposite pathways: β-oxidation of fatty acids occurs in the mitochondria, whereas FA synthesis occurs in the cytosol.

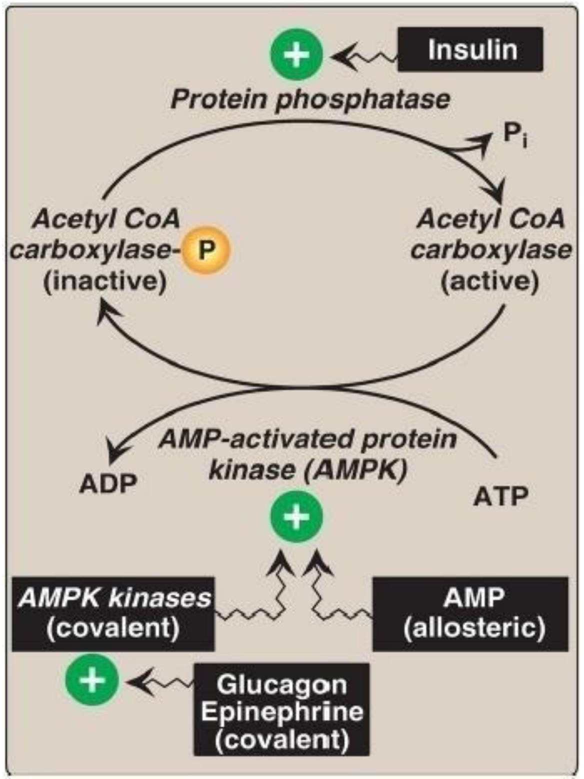


Hormonal Regulation

EP and glucagon can also activate AMPKK (AMP activated-protein kinase kinase), the kinases that phosphorylate AMPK, activating it. Active AMPK phosphorylates acetyl CoA carboxylase-- adds a carboxyl group to acetyl CoA to form malonyl CoA (3 carbons).

High [EP or glucagon] inhibit the synthesis of fatty acids. In turn, the phosphorylation of acetyl CoA carboxylase inhibits it. However, under well-fed states, insulin activates phosphatases to remove the phosphate group from acetyl CoA carboxylase. The ACC is now active and so is the fatty acid synthetic pathway.

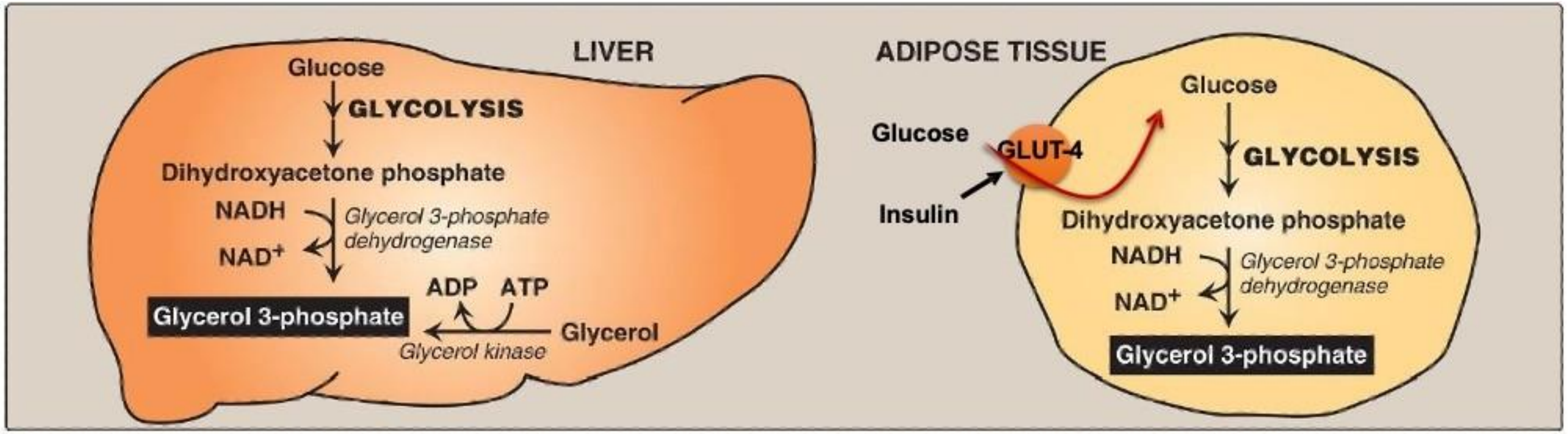
In general, insulin activate the enzymes inactivated by glucagon and inactivate the enzymes activated by glucagon



Acetyl CoA carboxylase (important for fatty acid synthesis) is inhibited by the same signaling pathway of glucagon or epinephrine.

Glycerol in liver and adipose tissues

The products generated from the degradation of TAGs and the hydrolysis of the ester bonds between fatty acids and glycerols in the adipocytes **are free fatty acids and glycerols.** The **free fatty acids** exit adipocytes through different mechanisms depending on the length of the hydrocarbon chain. If the fatty acid chain is short or medium, they can enter the bloodstream without a carrier. On the other hand, long fatty acids, are mostly the ones that get degraded to provide energy, do require albumin as a carrier to be distributed to different cells.



Glycerol in liver and adipose tissues

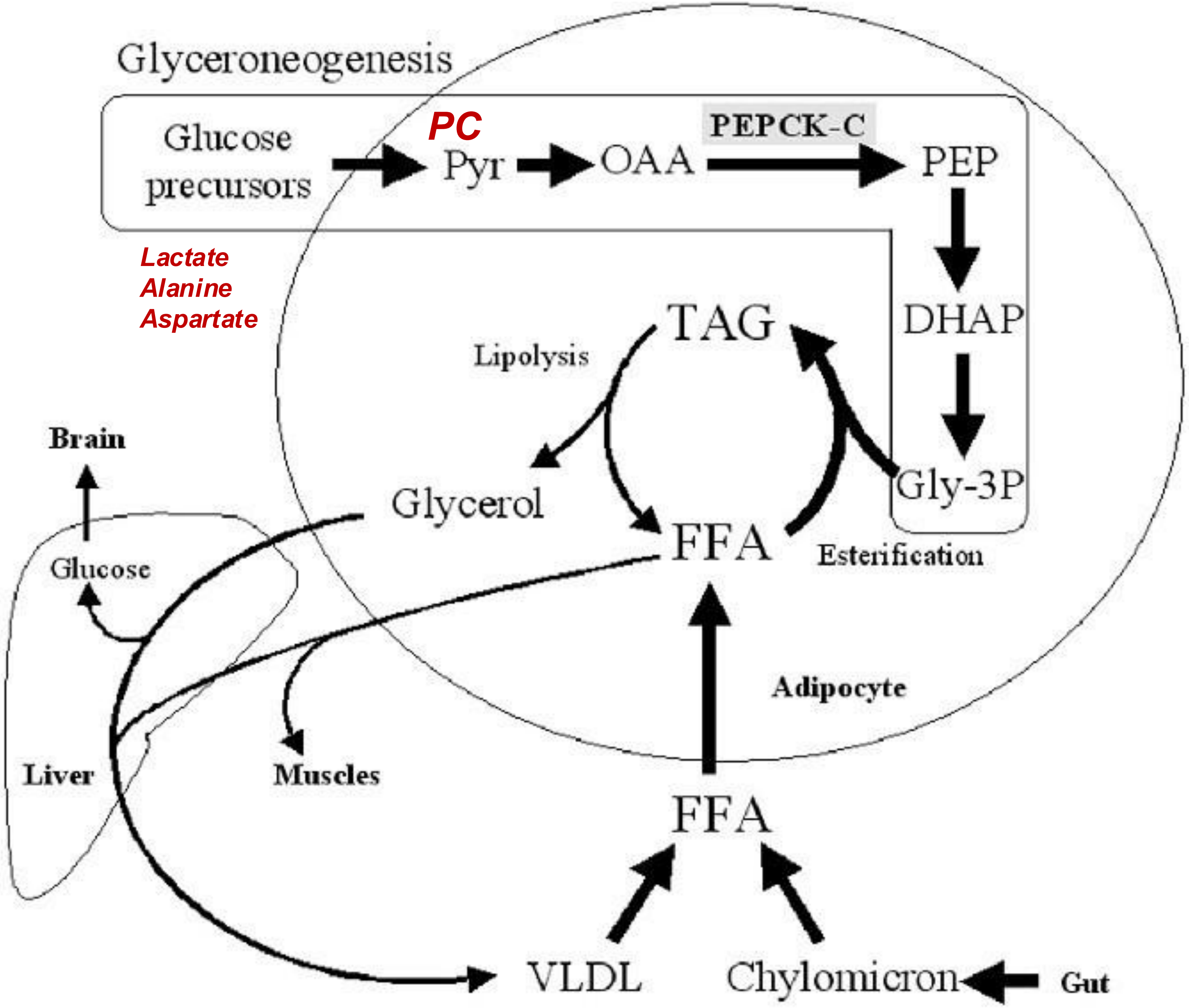
Glycerol molecules are used by cells that perform gluconeogenesis (hepatocytes and kidney cells). Glycerol is phosphorylated by glycerol kinase to glycerol-3-phosphate, then oxidized to DHAP by G3P dehydrogenase, which can be used in the gluconeogenesis pathway.

In adipocytes, insulin (well-fed state) induces the expression of GLUT-4 resulting in high [glucose], activation of glycolysis, formation of DHAP. DHAP is then reduced to G3P and is used for TAG synthesis. This is why overeaters gain weight. Excessive [sugar] in our diet can promote the utilization of G3P for TAG synthesis. As TAG increase in adipocytes, body weight also increases. In young people, the number of adipocytes can increase, so more lipids can be stored. Then, this number becomes fixed, but the capacity of the adipocytes increases, allowing for more lipid storage. In overly obese people, an "overspill" of lipids can occur where stored lipids exceed the capacity of adipocytes in the body (this will be discussed later, Insha'Allah).

Glyceroneogenesis

The synthesis of glycerol molecules out of non-glucose precursors, such as lactate and amino acids (aspartate, alanine). Synthesis of glycerol takes place in adipocytes and sometimes in hepatocytes. Once they enter, they can be converted to pyruvate and then via pyruvate carboxylase it forms oxaloacetate, then PEP through PEP carboxykinase (recall gluconeogenesis). Glyceroneogenesis follows the same pathway as gluconeogenesis until the formation of DHAP. DHAP is then reduced to form G3P which can be combined with fatty acids to from TAG. Arising issues in this pathway's regulation can cause type 2 diabetes. This is because if these precursors aren't utilized to form G3P and combine with fatty acids to be stored as TAG, consumed fatty acids linger in the bloodstream as well as glucose.

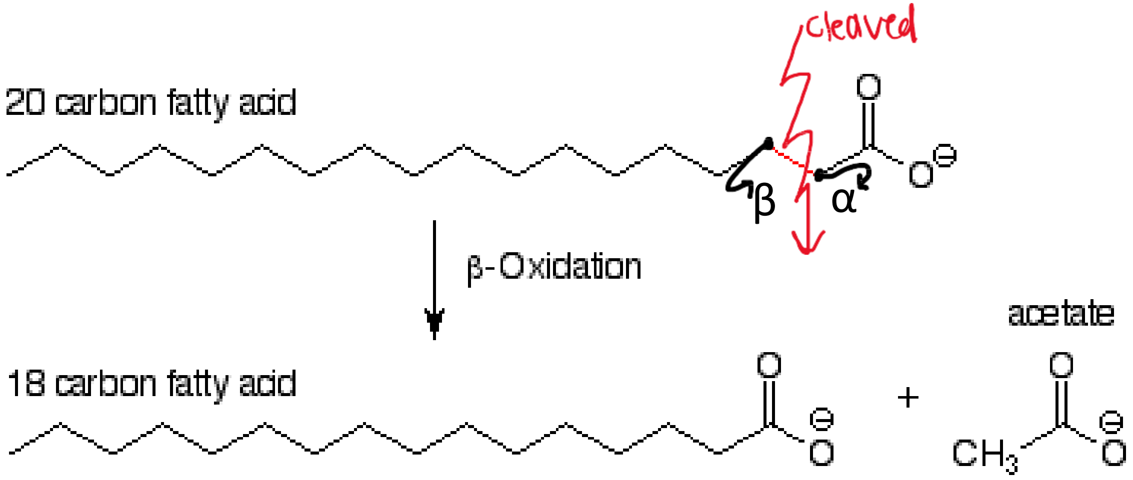
- ***Purpose: regulating the levels of FAs in blood.***
- ***In liver and adipose tissue***
- ***Glycerol leaves the adipocytes into the liver.***
- ***Failure in regulating glyceroneogenesis may lead to Type 2 diabetes due to excess fatty acids and glucose in the blood***



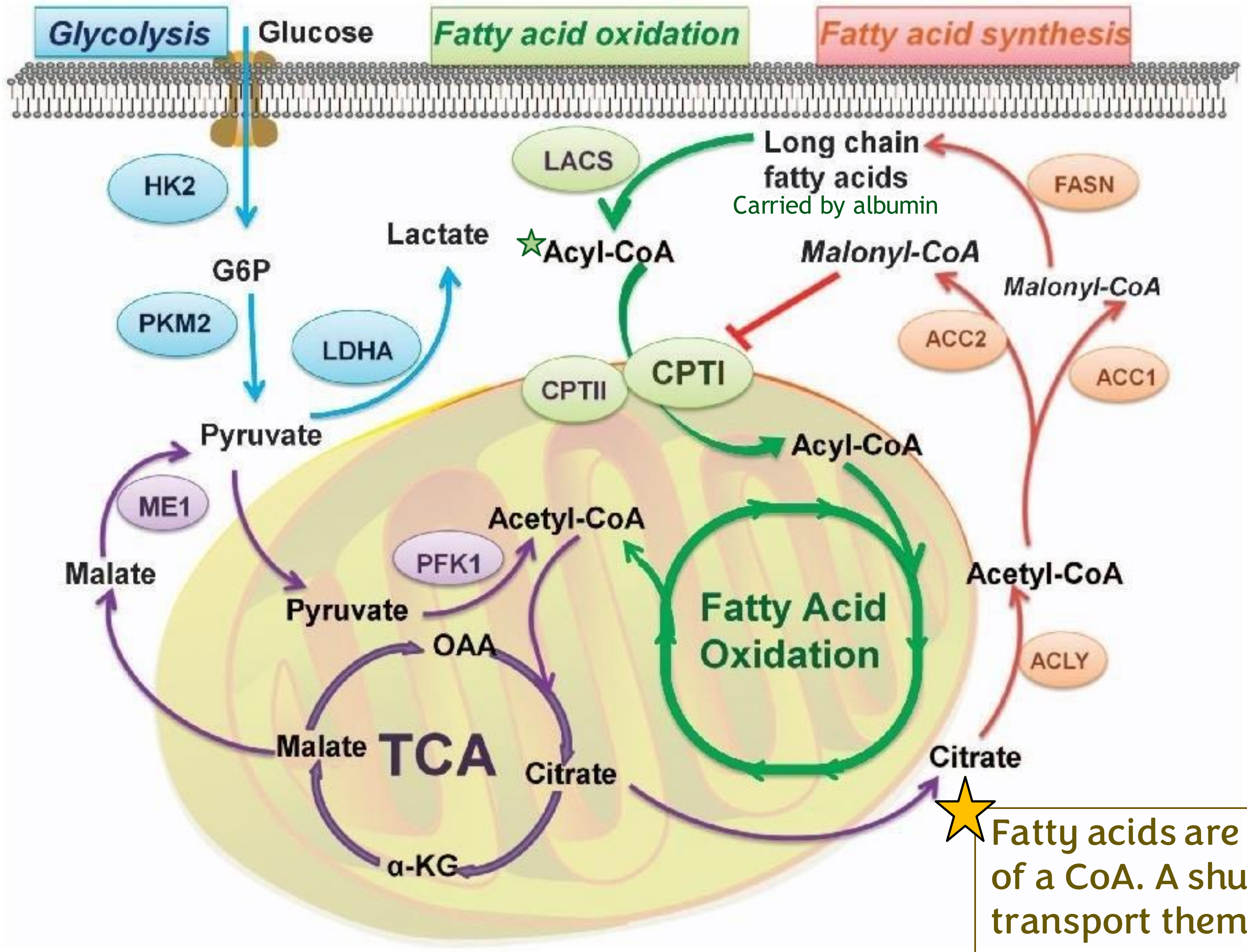
PC: Pyruvate carboxylase
PEPCK: phosphoenolpyruvate ***carboxykinase***

β-oxidation of Fatty acids

We mentioned earlier that short and medium length fatty acids don't require carrier to transport across the bloodstream, and that long fatty acids require albumin. Short and medium fatty acids enter the cells through the plasma membrane. Once inside the cytosol, fatty acids must be transported inside the mitochondria, where β-oxidation takes place. β-oxidation is named this because each cycle of fatty acid degradation cleaves two carbon atoms, forming acetyl CoA. *Cleavage occurs at the second (β) carbon attached to the carboxyl group. It is a spiral pathway and an example of spatial regulation (compartmentalization).*



β-oxidation of Fatty acids



Fatty acids produce more acetyl CoA than glucose, but they will not produce more energy than glucose because oxaloacetate is the limiting factor

Fatty acids are activated by the addition of a CoA. A shuttle system is used to transport them to the mitochondria.

β Oxidation of Fatty Acids (overview)

$CH_3\,(CH_2)_n\text{-}CH_2\text{-}CH_2\text{-}\,COO^-$

Structure of a fatty acid when it diffuses through the plasma membrane.

↓

$CH_3\,(CH_2)_n\,\text{-}CH_2\text{-}CH_2\text{-}CO\,\sim CoA$

The fatty acid is then activated by the addition of a CoA. This activation marks the fatty acid's destination.

↓
↓
↓

$$\begin{array}{c} \quad\quad\quad\quad\quad O \\ \quad\quad\quad\quad\quad || \\ CH_3\,(CH_2)_n\,\text{-}C\text{-}CH_2\text{-}CO\,\sim CoA \end{array}$$

Before the cleavage of the β carbon, a new carbonyl group (C=O) must be formed for the CoA molecule to bind to. The fatty acid undergoes a series of reactions to produce the carbonyl group, one of which is oxidation (addition of O & removal of H). Now, we can cleave the CH3-CO~CoA, and add a CoA to the remaining fatty acid chain.

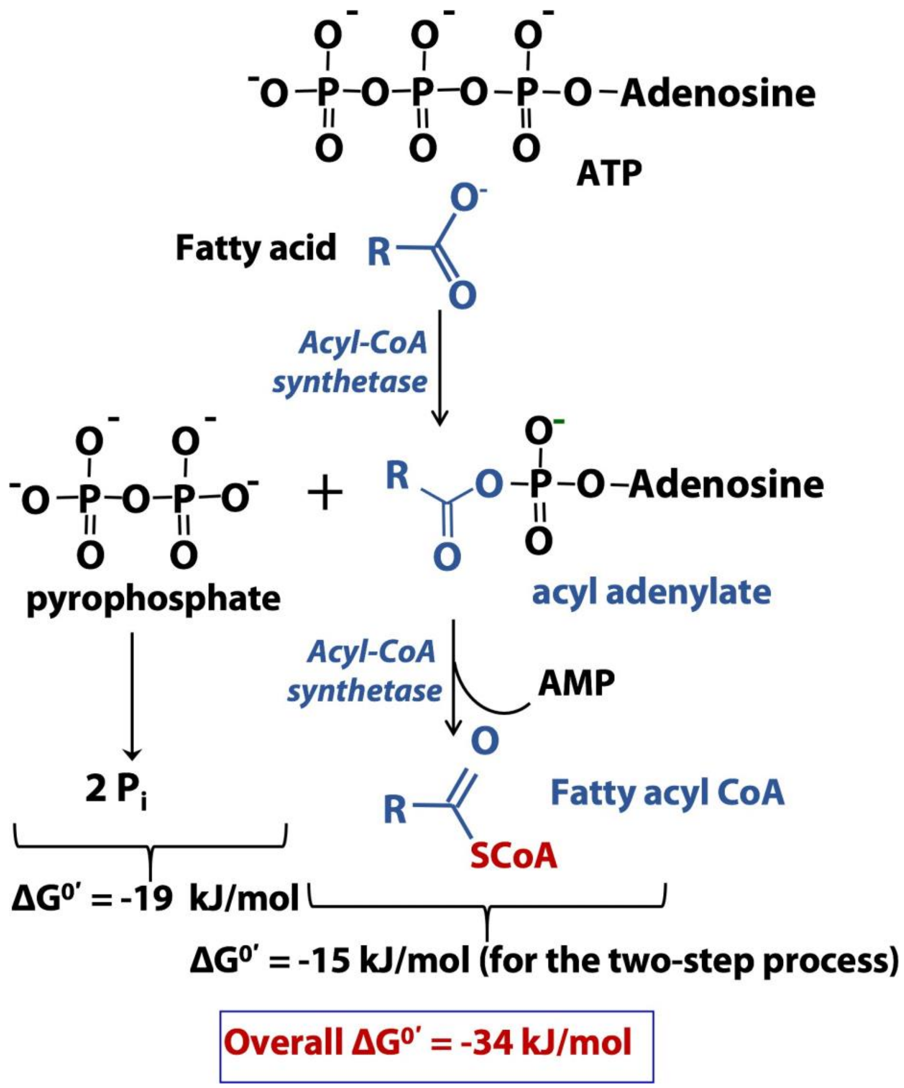
↓

$CH_3\,(CH_2)_n\,\text{-}CO\,\sim CoA \quad + \quad CH_3\text{-}CO\,\sim CoA$

This repeats again and again until we have oxidized the entirety of the fatty acid chain.

Activation of Fatty Acids

β-oxidation is enzyme-catalyzed. This pathway requires CoA, which is a high-energy group. Joining its bond with another molecule releases energy (TCA step #4). Conversely, the addition of a CoA requires energy, which cannot be supplied solely by the pathway's energy. Therefore, ATP is needed and **thiokinase** is used to catalyze this addition. **Thiokinase** is also known as acyl CoA synthetase, synthetases normally require energy. Fatty acids + CoA + ATP produce a fatty acyl CoA + AMP + release of pyrophosphate. Pyrophosphates are then converted to two inorganic phosphates. **Long chain fatty acids** cannot fully enter through the mitochondrial membrane, so their oxidation takes place on the outer mitochondrial membrane. **Short and medium** fatty acids can readily diffuse through the mitochondrial membranes via passive diffusion to the mitochondrial matrix. Once in the mitochondrial matrix, a CoA is added to the fatty acid, thus it's activated. **Short and medium** FAs degrade faster than long chain FAs. Their absorption and digestion from food intake is faster and reaches cells faster. They can also diffuse through both the plasma and mitochondrial membranes. Since they're relatively shorter, the duration of their degradation is also shorter. Disregard the adenosine.



Activation of Fatty Acids

2 ATP equivalents are needed

Thiokinase
(Acyl CoA Synthetase)

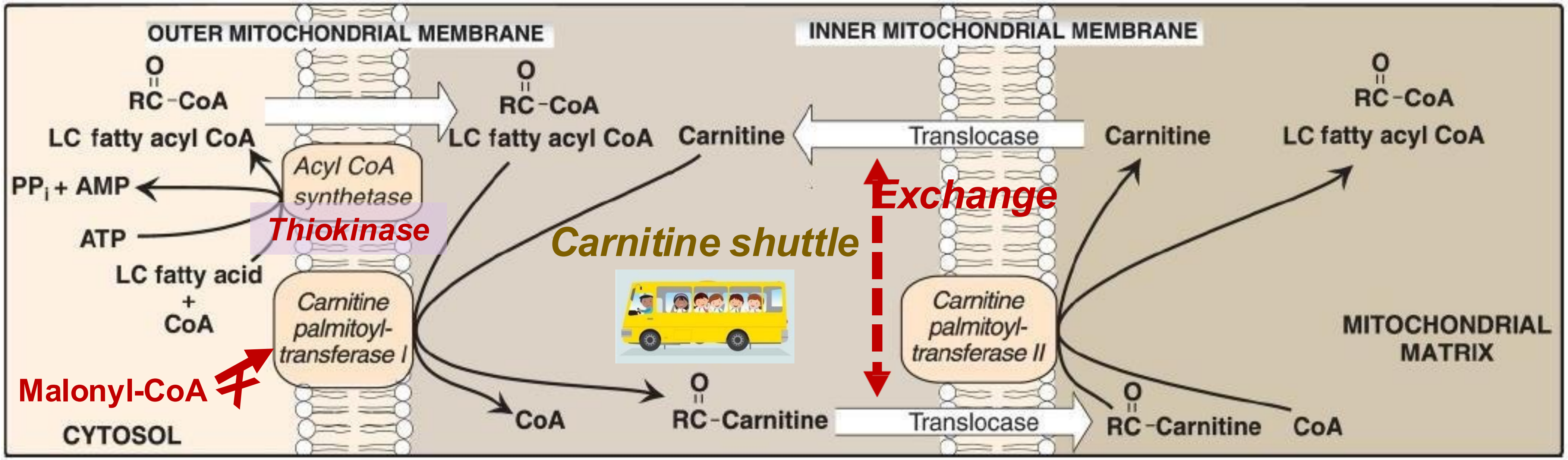
$$FA + HSCoA + ATP \rightleftharpoons FA{\sim}CoA + AMP + PP_i$$

$$PP_i + H_2O \longrightarrow 2\ P_i$$

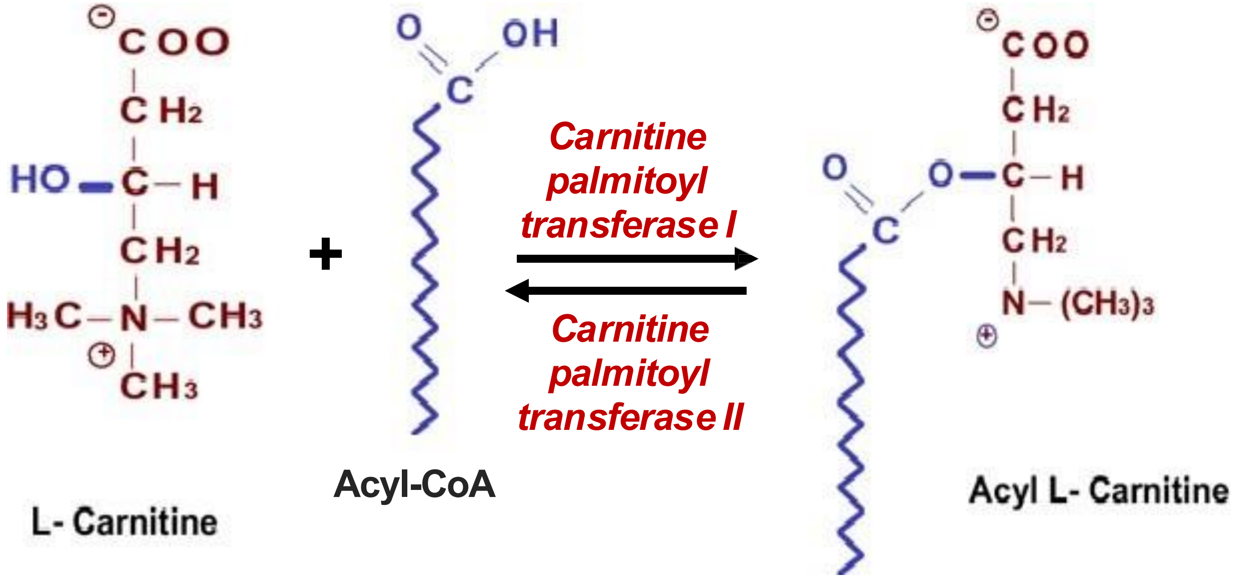
$$FA + HSCoA + ATP \longrightarrow FA{\sim}CoA + AMP + 2\ P_i$$

Location:
LCFA: outer mitochondrial membrane
Short and medium chain FA: mitochondrial matrix

Transport of LCFA



The transport system consists of:
1. A carrier molecule (carnitine)
2. Two enzymes
3. Membrane transport protein (translocase)

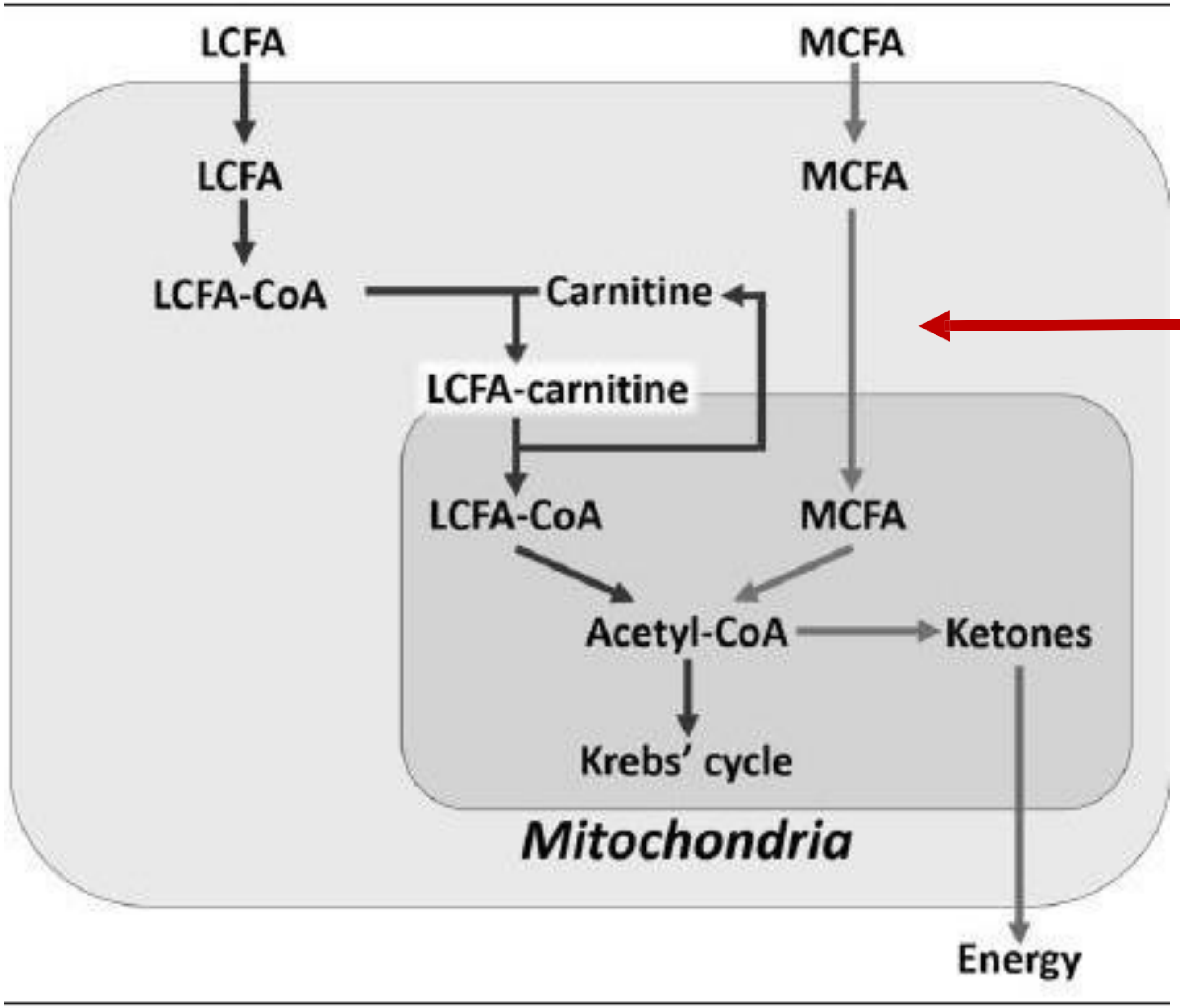


Transport of LCFA

Long-chain fatty acids are activated by CoA by thiokinase on the outer mitochondrial membrane. OMM is permeable, so the long-chain fatty acyl CoA can easily cross it. However, the IMM isn't, so the LCFA CoA stays within the intermembranous space. There are shuttling systems that function to serve this exact purpose. One of these shuttling systems is carnitine, which is present in the intermembranous space. A transferase in this shuttling system replaces the CoA with the carnitine producing a **long fatty acyl carnitine.** The LFAC can move to the mitochondrial matrix through the membrane via translocase enzymes, also a part of the shuttling system. However, LFAC must be reverted to its original form-- LCFA CoA, for oxidation. This is catalyzed by the second enzyme in the mitochondrial membrane, carnitine-palmitoyl transferase II. In conclusion, this shuttling system is composed of **carnitine, translocase, and carnitine-palmitoyl transferases I & II.** Translocase can transport LCFA carnitine into the mitochondrial matrix and also transport the carnitine back to the intermembranous space. Carnitine-palmitoyl transferase I is **inhibited by malonyl-CoA**, which is crucial for fatty acid synthesis. This is so the fatty acids that were just synthesized can't enter the mitochondria to be oxidized.

Transport of SCFAs and MCFAs

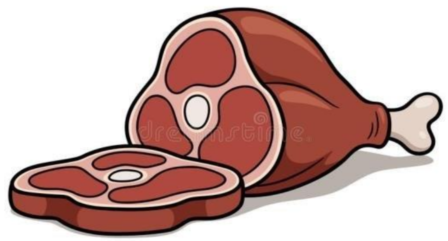
After their diffusion, MCFA will be combined with a CoA group to produce Acetyl-CoA eventually through the β-oxidization process discussed previously.



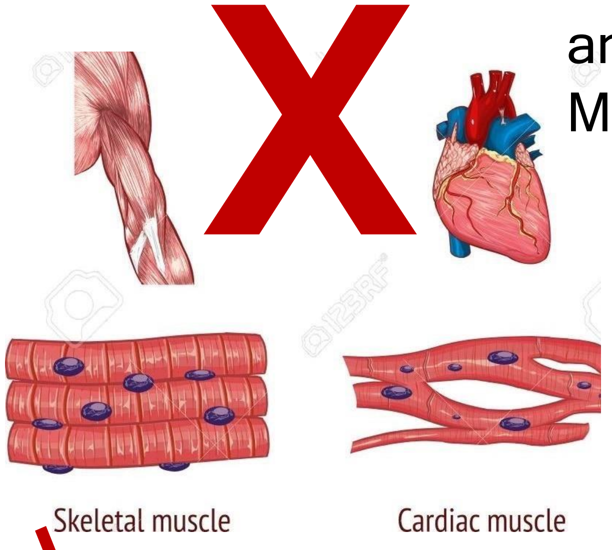
Note: No regulation of entry like that of CPTI by malonyl CoA

Medium chain fatty acids can diffuse through the plasma and Mitochondrial membranes .Faster absorption and oxidation.

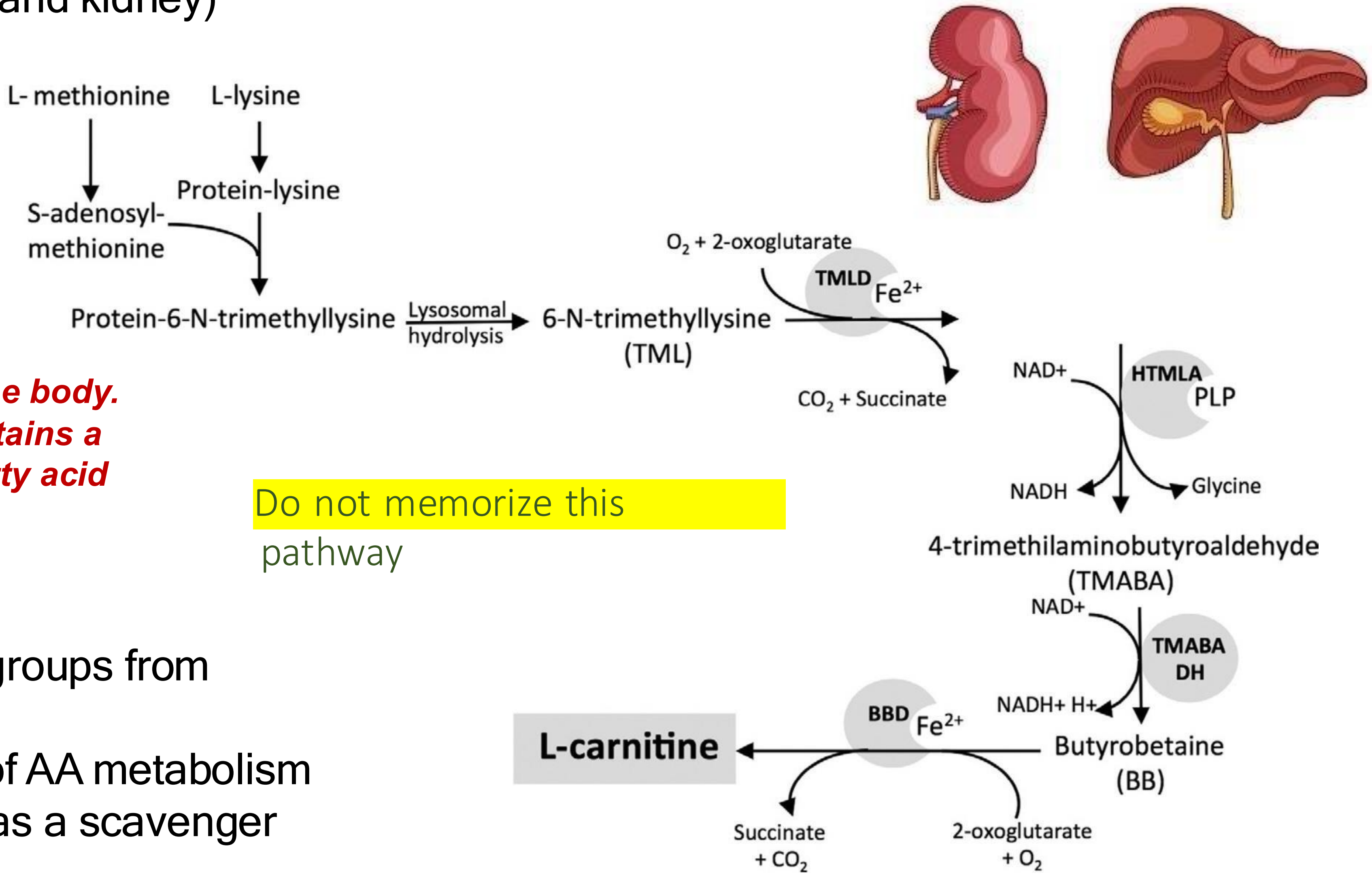
Application: Carnitine sources



Source: meat product and plant product (both grains and legumes) and synthesis in the body from Lys and Met (liver and kidney)



contains ~97% of all carnitine in the body. No ACC1, no FA synthesis but contains a mitochondrial ACC2 to regulate fatty acid degradation.



Do not memorize this pathway

Other functions:

- Export of branched chain acyl groups from mitochondria
-Binding to acyl groups derived of AA metabolism and their execration functioning as a scavenger

Application: Carnitine sources

The primary function of carnitine in β-oxidation is to facilitate the transport of **long- chain fatty acids (LCFAs)** into the mitochondria for oxidation. The LCFAs previously discussed were either saturated or unsaturated.

Carnitine also plays a role in the **export of branched-chain fatty acids** (those with hydrocarbon branches, not other functional groups like hydroxyl groups etc) from the mitochondria.

Additionally, during the **degradation of amino acids**, various **acyl groups**, particularly **acetyl-CoA** and **acetoacetyl-CoA**, can be produced. Carnitine acts as a **scavenger**, aiding in the **excretion** of these molecules.

Although carnitine is primarily synthesized in the liver and kidneys, about 97% of the body's carnitine resides in **skeletal and cardiac muscle cells**, which are highly dependent on fatty acid oxidation for energy. These muscle cells **lack the ACC1 (Acetyl-CoA Carboxylase 1) enzyme**, which is involved in **fatty acid synthesis**, but they **express ACC2 which is found in the mitochondria,** an isoform that **regulates fatty acid oxidation** by producing regulatory molecules (malonyl-CoA).

Application: Carnitine deficiencies

- **Primary carnitine deficiency** A direct deficiency in carnitine due to genetic mutations affecting carnitine transport or synthesis.
  - Defects in a membrane transporter: No uptake of carnitine by cardiac and skeletal muscles and the kidneys, causing carnitine to be excreted.
    - Treatment: carnitine supplementation.
- **Secondary carnitine deficiency** A deficiency caused by other underlying conditions such as metabolic disorders, certain medications, or diseases that impair carnitine absorption, increase its excretion, or disrupt its utilization.
  - Taking valproic acid (antiseizure drug) → decreased renal reabsorption
  - Defective fatty acid oxidation → acyl-carnitines accumulate → urine
  - Liver diseases → decreased carnitine synthesis
  - Transferase Deficiencies:
  - CPT-I deficiency: affects liver; no use of LCFA, no energy for glucose synthesis during fasting → severe hypoglycemia, coma, and death
  - CPT-II deficiency: affects liver, cardiac muscle, and skeletal muscle
    - Treatment: avoidance of fasting and adopting a diet high in carbohydrates and low in fat but supplemented with medium-chain TAG.

Application: Carnitine deficiencies

Primary deficiency:

- e.g. if the translocase that moves carnitine was mutated, absent, etc. It would interfere with the movement of carnitine, especially in FA oxidation-dependent cells (cardiac and skeletal muscle)
- Carnitine excretion (both diet-aquired and body-synthesized) will occur since carnitine is present but ineffective (can't move)
- Giving a carnitine supplement will overflood the area with carnitine which might result in better transport, hence improving the deficiency.

Application: Carnitine deficiencies

Secondary deficiency: (indirect)

- **Valproic acid** decreases renal reabsorption of carnitine, leading to increased urinary excretion through the renal system, ultimately causing a carnitine deficiency.
- If **β-oxidation** is defective, activated fatty acids (acyl-CoA) can still enter the mitochondria due to a functional carnitine transport system. However, since fatty acids cannot undergo proper oxidation, they will accumulate inside the mitochondria.

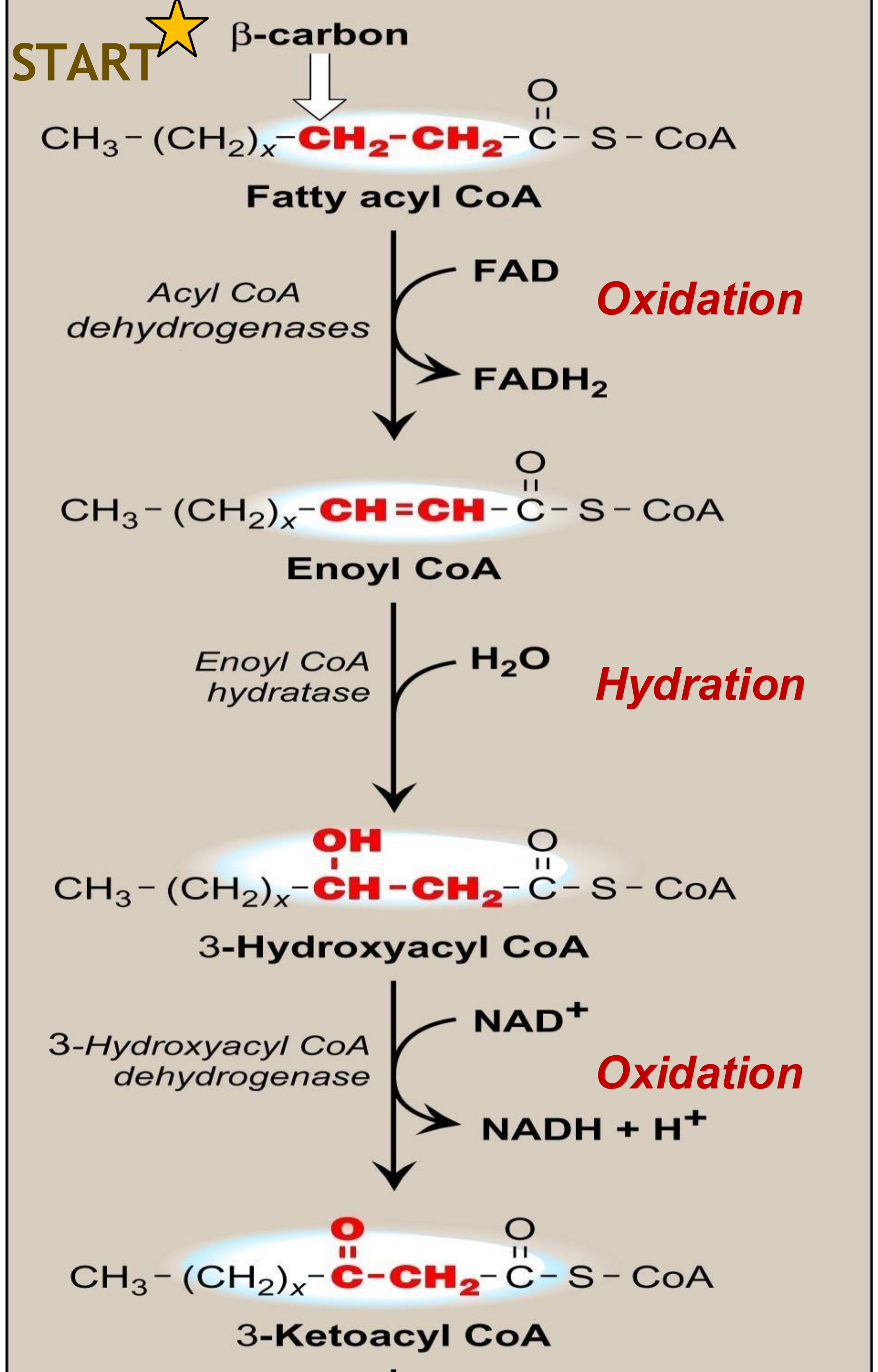
  In this case, carnitine appears deficient, not because of an actual lack of carnitine, but because its normal role in facilitating fatty acid transport becomes ineffective due to the metabolic block. Additionally, acyl-carnitine (a fatty acid-bound form of carnitine) will accumulate. This triggers increased urinary excretion of acyl-carnitine, reducing free carnitine levels and causing a secondary carnitine deficiency.
- Since the liver synthesizes carnitine, any liver-related issues can impair its production

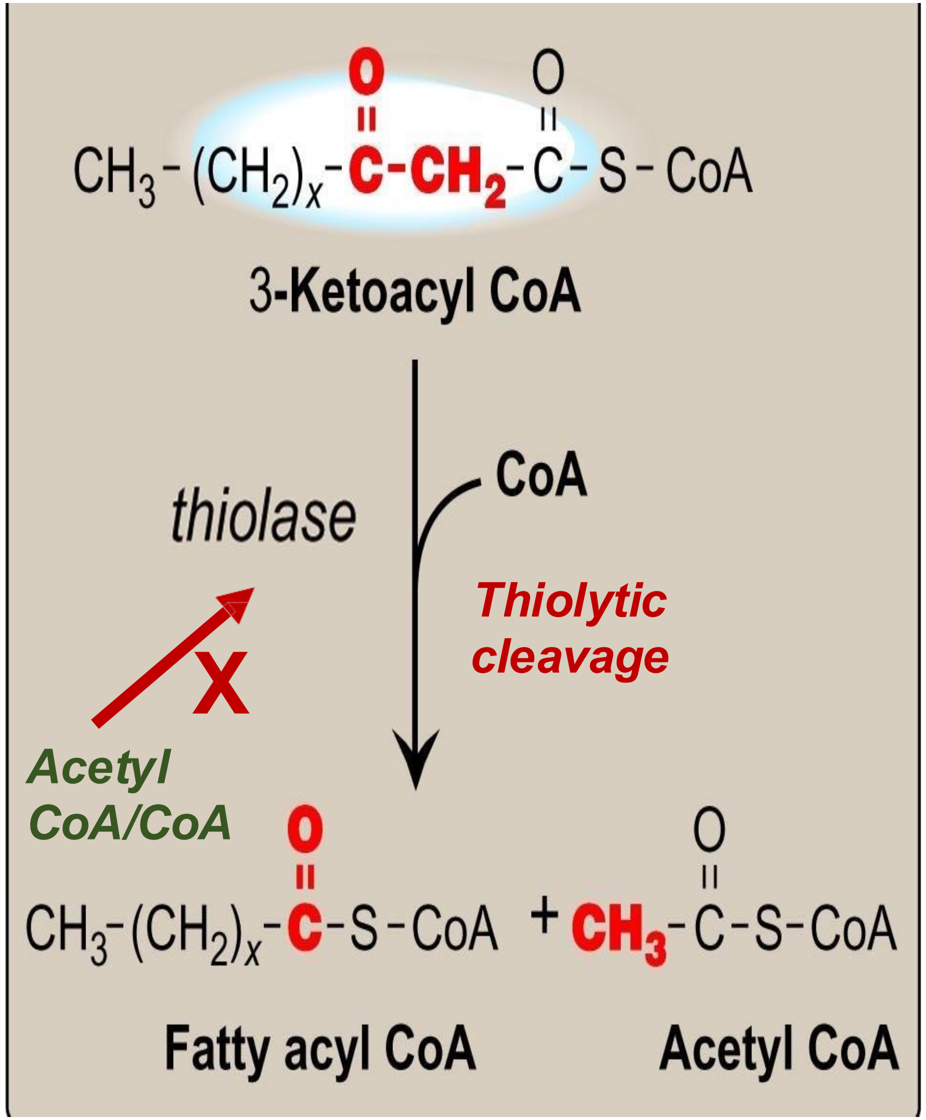
Application: Carnitine deficiencies

Secondary deficiency: (indirect)

- **Transferase Deficiencies:**
- Normally CPT-1 functions in forming Acyl-Carnitine
- If **CPT-1** (Carnitine Palmyotransferase-1 on OMM) is deficient, FA can't bind carnitine. As a result, LCFA won't be oxidized, leading to a reduced amount of energy, severe hypoglycemia because cells will resort to using glucose even during gluconeogenesis, due to the inability to degrade FA for energy. This leads to coma and death.
- Normally CPT-2 functions in forming LFAC (removing carnitine from acyl [LCFA] and adding CoA)
- If **CPT-2** (IMM) is deficient, carnitine cannot be separated from FA upon entering the mitochondrial matrix. As a result, LCFA will not bind to CoA to become LFAC, thus interfering with b-oxidation as well since FAs will not reconvert into the activated form, leading to reduction of the availability of energy under fasting conditions for dynamic cells that depend on b-oxidation like cardiac and skeletal muscles
- To reduce problems associated with these deficiencies: avoid being in fasting conditions or in ketogenic diets which obligate the body to depend on FA oxidation for energy, so they must have small-portioned high-carb low-lipid/protein frequent meals.
- These people can only benefit from SCFA and MCFA (they aren't common in diet, and aren't that effective), which could be taken as supplements to compensate.

β-Oxidation of fatty acids





Number of cycles: (n/2)-1

β-Oxidation of fatty acids

LFACs are now ready to enter β-Oxidation reactions as they're bound to CoA.

in C 3

A carbonyl group must be made so the cleavage of the β carbon can occur.

The first step is to create a double bond, by an oxidation reaction mediated by Acyl-CoA Dehydrogenase, it removes a hydrogen atom off the β carbon and the α carbon adjacent to it

These hydrogens will go to FAD reducing it to $FADH_2$ which will be oxidized again in the ETC since both processes happen in the mitochondria.

The second step is to add an Oxygen atom, by a hydration reaction mediated by Enoyl-CoA hydratase which will add an H_2O molecule and produce an alcohol group.

Again, another Oxidation reaction to create a double bond, mediated by 3-Hydroxyacyl CoA Dehydrogenase, which will remove a hydrogen atom off the β carbon and the hydroxyl group on it, creating the carbonyl group (ketone) that's needed to drive the cleavage.

These hydrogens will go to NAD^+ reducing it to ($NADH + H^+$) which will be oxidized again in the ETC since both processes happen in the mitochondria.

β-Oxidation of fatty acids

Now that the carbonyl group is present, 3-ketoacyl CoA will undergo thiolytic cleavage by thiolase enzyme, since CoA will be cleaved alongside the first two carbons to produce acetyl CoA, and the lost CoA must then be replaced.

Remember that CoA has a thiol group in it :)

This cleavage will produce a fatty acid that is 2 carbons less in length (leftover FA)

Thiolase is inhibited by high concentration of acetyl CoA, since the CoA levels will be depleted as they are consumed by acetyl attachement rather than being free to be used in this reaction.

This pathway is considered a spiral pathway, as the steps will be repeated until the FA is completely oxidized.

For even-number fatty acids, the reaction will end in the last cycle producing two acetyl-CoA molecules.

Energy Yield from FA Oxidation

This FA has n=16 carbons, divide n by 2 (bcz 2 carbons= 1ACoA released per cycle) and subtract 1 (bcz the cycle directly produces 2 ACoA in the last cycle instead of 1) So it needs 7 cycles to complete oxidation.

$CH_3\text{-}(CH_2)_{14}\text{-}CO\text{-}CoA$

6 Acetyl CoA

6 $FADH_2$

6 NADH

7H2O

7Co

$CH_3\text{-}CH_2CH_2\text{-}CO\text{-}CoA$

$CH_3\text{-}CO\text{-}CoA$ + $CH_3\text{-}CO\text{-}CoA$ + $FADH_2$ + NADH

This shows the last cycle directly producing: 2 ACoA, 1FADH, 1NADH2

not all ACOA will necessarily enter krebs cycle!!

- ✓ Oxidation of C 16 FATTY ACID

  7 $FADH_2$ X 2 → 14 ATP

  7 NADH X 3 → 21 ATP

  8 Acetyl CoA → 96 ATP

  1AcoA = 12 ATP (approx.)

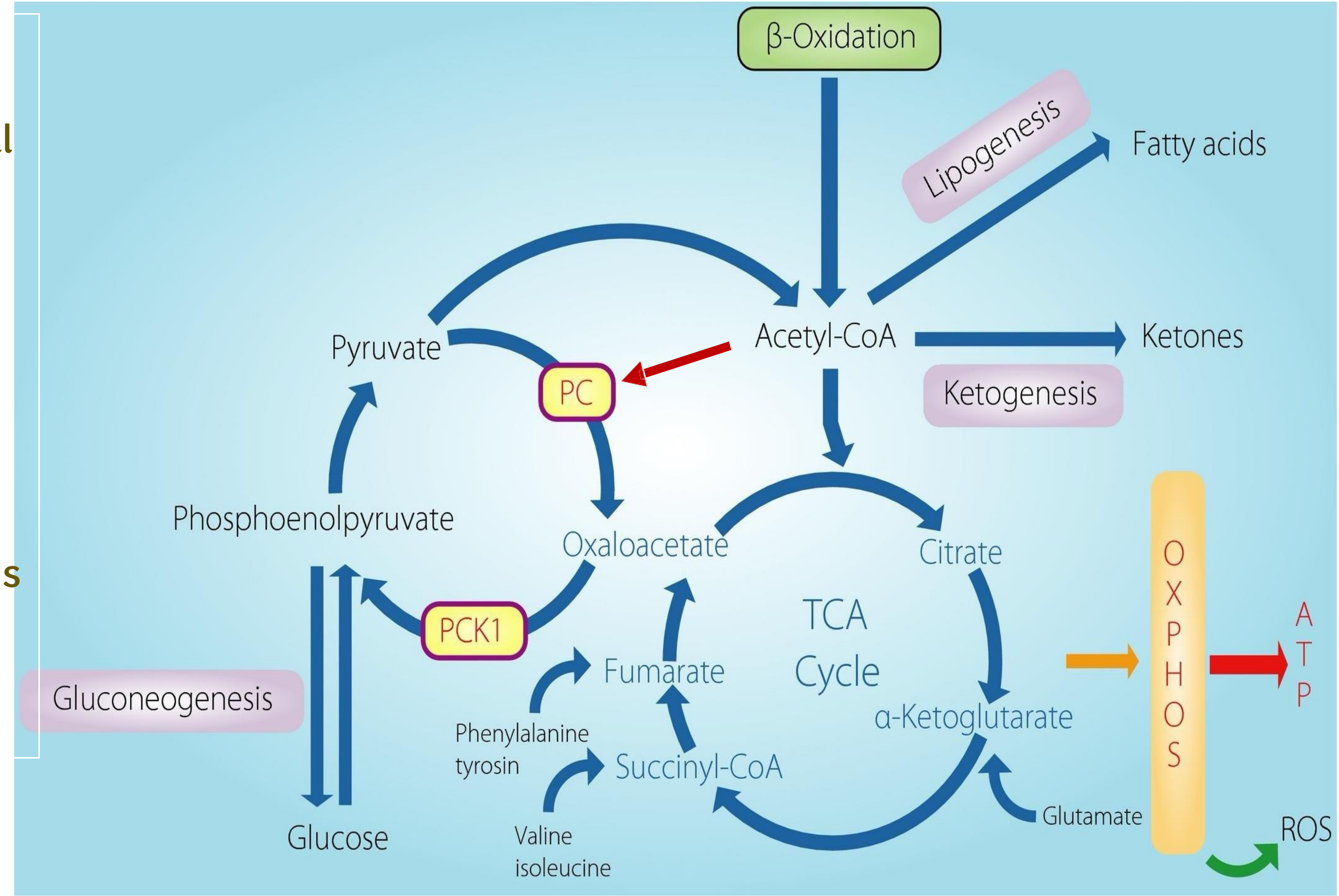
- ✓ Activation of the Acid consumes 2 ATP
- ✓ Net 129 ATP mole per mole of C16 Fatty Acid

Induction of gluconeogenesis and fates of acetyl CoA

Of course, the numbers presented in the previous slide are unrealistic, as not all ACoA will go into TCA cycle and produce energy.

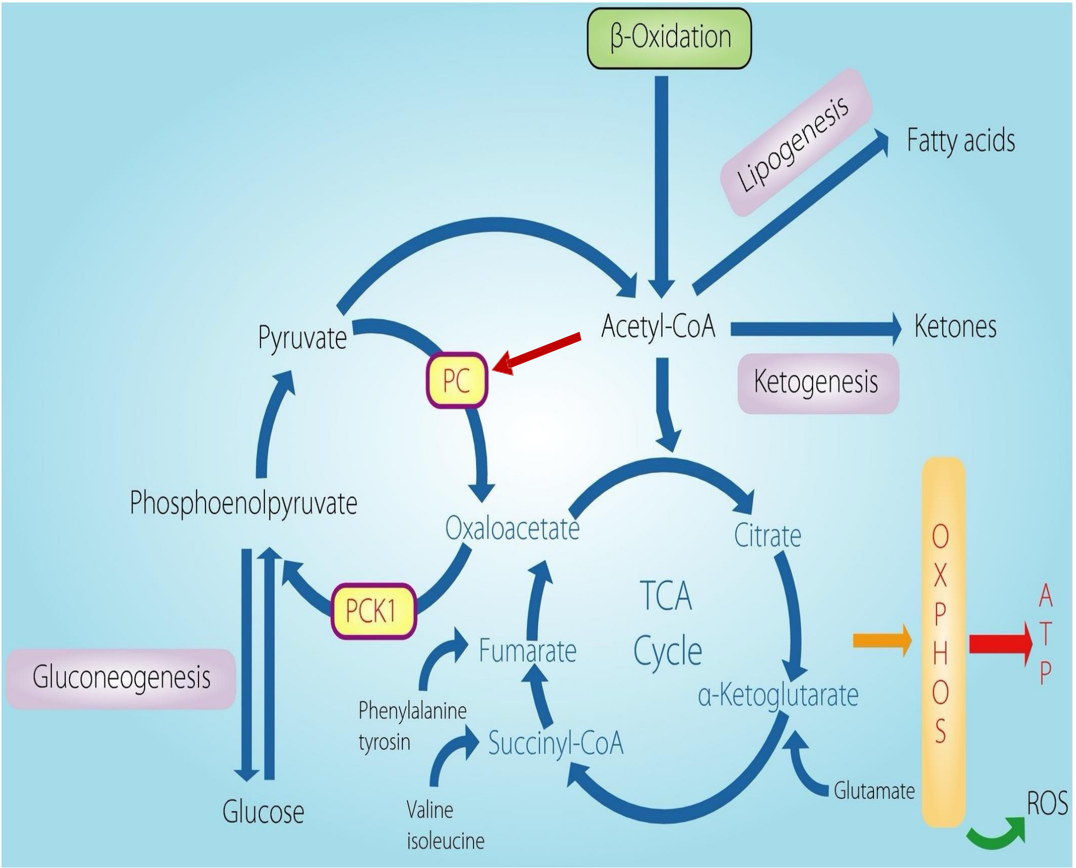
)reason explained in the next slide)

Since β-Oxidation occurs under fasting conditions, another pathway that will be active is gluconeogenesis in hepatocytes and kidney cells to provide energy for the cells that depend on glucose and to maintain glucose levels in the bloodstream.



Induction of gluconeogenesis and fates of acetyl CoA

- Gluconeogenesis consumes some of OAA (since it's a gluconeogenic intermediate).
- OAA will be distributed, a portion to TCA cycle and the other goes to gluconeogenesis.
- Therefore, **OAA's availability** is a limits the ability to use ACoA generated by β-Oxidation in TCA cycle
- This is why some of the ACoA is redirected towards another process which is **ketogenesis,** an alternative pathway.
- It produces ketone bodies, that despite their various chemical structures all contain a ketone group.
- These ketone bodies, at some point, will be used to provide energy for the brain cells, they are the only alternative to sugar in its absence.



- During prolonged fasting, the brain increasingly relies on ketone bodies for energy. Their utilization depends on their concentration in the blood—the higher the concentration, the more likely they are to cross the Blood-Brain Barrier (BBB), where they can be metabolized by brain cells to provide energy for neurons.

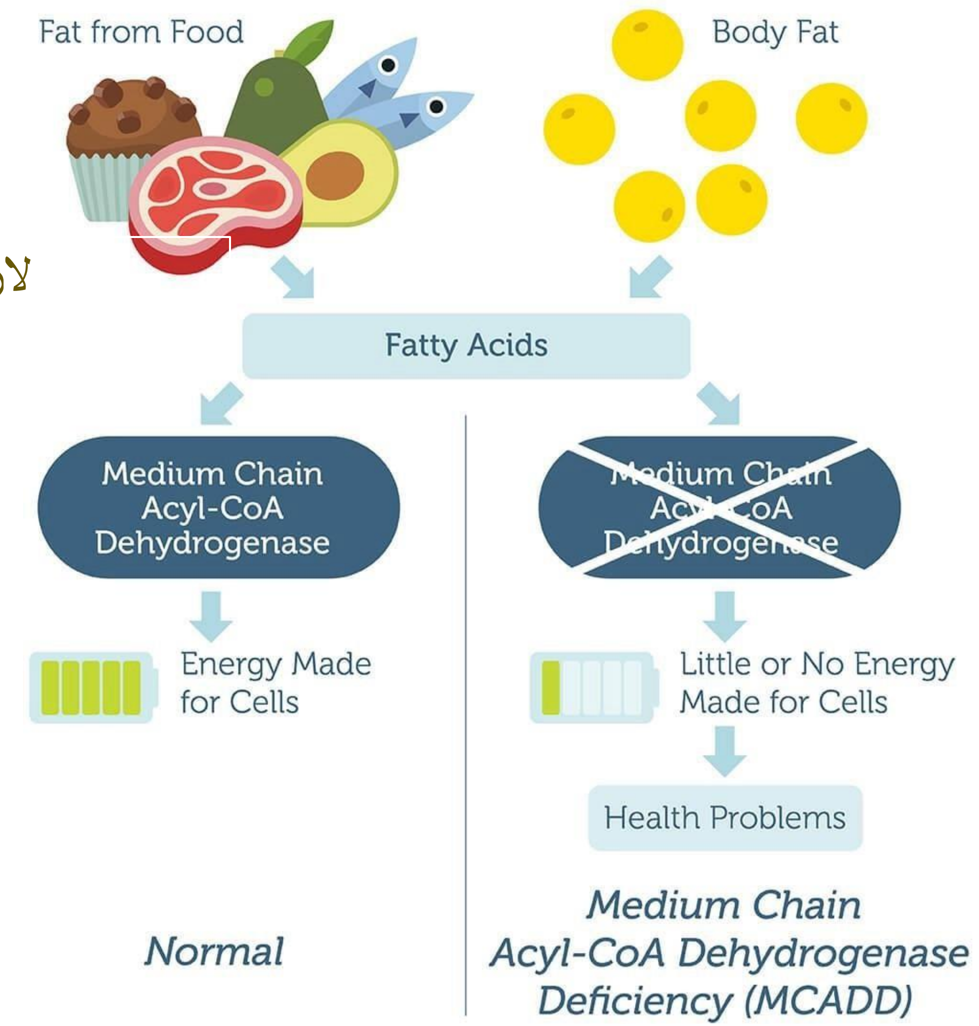
Application: MCAD deficiency

Explanation in the next slide :)

- There are 4 isozymes of fatty acyl CoA dehydrogenase
- for SCFA, **MCFA**, LCFA, and VLCFA
- Medium-chain fatty acyl CoA dehydrogenase (MCAD) genetic deficiency,
  - An autosomal-recessive disorder لازم يكون نسختين غير طبيعيات
  - Most common inborn error of β-oxidation (1:14,000 births worldwide)
  - Higher incidence among Caucasians of Northern European descent
  - Decreased ability to oxidize MCFAs (lack of energy)
  - Severe hypoglycemia and hypoketonemia
  - Treatment: avoidance of fasting

    Regular and frequent meals and snacks

    Diet high in carbohydrates and low in fat



Application: MCAD deficiency

The amount of glycerol will decrease and lead to hypoglycemia

- In breast milk, the majority of lipids are SCFA and MCFA which are essential in the neuronal and brain development of infants after birth since they are easily diffused and oxidized, so they are considered a faster energy source than LCFA.
- MCAD is the isoform of ACD enzyme that is most affected by mutations, this is why this specific deficiency is quite common.
- As a result of this deficiency, MCFA's oxidation will be compromised, so affected individuals are more susceptible to hypoglycemia and hypoketonemia, because MCFA are considered a fast energy source & now that they can't be utilized, the body will resort to glucose, depleting its levels in the bloodstream. Also, they are normally used to produce ketone bodies, but this mechanism will be stopped as well.
- This case is not severe, just needs a few dietary adjustments and depend on sugar.

يعني الطفل المصاب مش لازم يدخل بحالة fasting

NOTES REGARDING THE PREVIOUS SLIDES

Last time, we discussed the process of beta-oxidation , which refers to the degradation of fatty acids under fasting conditions or during ketogenic diet.

- Fasting Conditions and Hormonal Changes

Under fasting conditions , hormonal changes play a key role. Specifically , there is an increase in glucagon levels , often accompanied by elevated epinephrine. These hormones bind to receptors on adipocytes , triggering the activation of lipolysis. This leads to the hydrolysis of triacylglycerols into fatty acids and glycerol. Fatty acids are then released into the bloodstream , taken up by cells , and oxidized through beta-oxidation to produce acetyl-CoA molecules. Meanwhile , the glycerol is utilized in gluconeogenesis by hepatocytes and kidney cells.

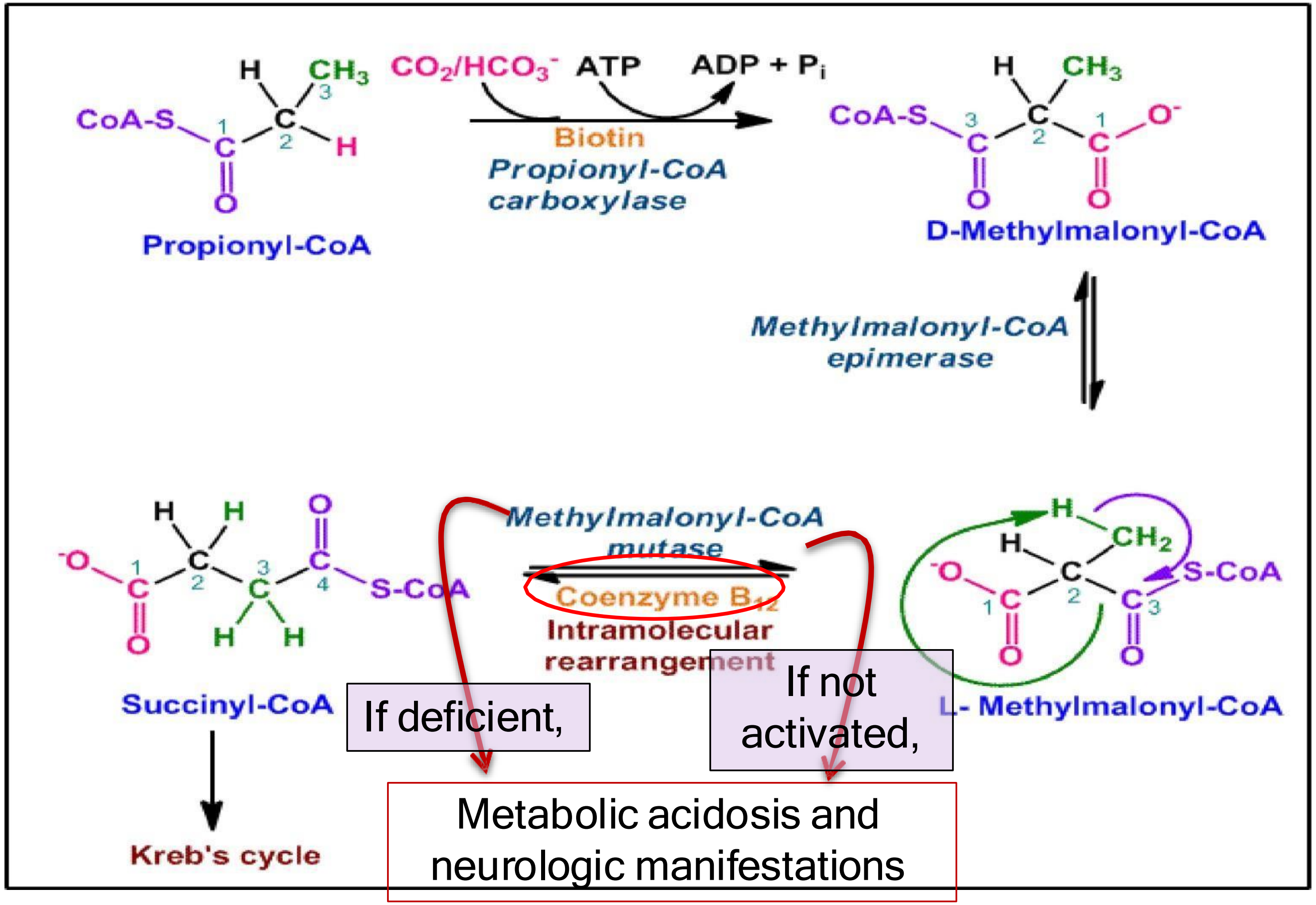
- Beta-Oxidation

Beta-oxidation derives its name because it involves the cleavage of fatty acid chains at the beta-carbon (the second carbon from the carboxyl group). This results in the sequential production of acetyl-CoA molecules. This pathway occurs in the mitochondria , so fatty acids must first be transported into the mitochondria to undergo degradation into acetyl-CoA.

In the previous lecture, we focused exclusively on saturated fatty acids with an even number of carbons. We have not yet addressed how beta-oxidation handles unsaturated fatty acids (whether mono- or polyunsaturated) or those with an odd number of carbons.

Oxidation of odd-numbered FAs

- Starts as cycles of beta-oxidation producing acetyl-CoA and propionyl-CoA



Oxidation of odd-numbered FAs / 1

Odd-chain fatty acids are found in various sources , including certain fish, algae, etc . Their oxidation begins similarly to the oxidation of even-chain fatty acids. Through beta-oxidation , two carbons are removed at a time , producing acetyl-CoA molecules.

- The Unique Challenge of Odd-Chain Fatty Acid Oxidation

In the final step of beta-oxidation for odd-chain fatty acids , a five-carbon molecule remains. This molecule undergoes
cleavage , producing :

- A two-carbon molecule in the form of acetyl-CoA (no issues here).
- A three-carbon molecule called propionyl-CoA , which requires special handling to be metabolized efficiently.

- Conversion of Propionyl-CoA to Succinyl-CoA

To make propionyl-CoA usable for energy production , it undergoes a series of steps :

1) Carboxylation of Propionyl-CoA

- An additional carbon atom is added to propionyl-CoA in the form of bicarbonate (HCO_3^-) or (CO_2(
- This reaction is catalyzed by propionyl-CoA carboxylase , an enzyme that requires biotin as a coenzyme and ATP as an energy source.
- The product of this reaction is D-methylmalonyl-CoA.

TO BE CONTINUED

Oxidation of odd-numbered FAs / 2

2) Epimerization to L-Methylmalonyl-CoA

· The D-methylmalonyl-CoA is converted into L-methylmalonyl-CoA by the enzyme methylmalonyl-CoA epimerase. This step involves reorienting the methyl group to produce the L-enantiomer.

3) Isomerization to Succinyl-CoA

- The L-methylmalonyl-CoA is rearranged (by transferring the branch – intramolecular rearrangement) to form succinyl-CoA , a linear four-carbon molecule that is an intermediate in the Krebs cycle (TCA cycle).
- This reaction is catalyzed by methylmalonyl-CoA mutase and requires vitamin B12 (cobalamin) as a coenzyme.

▪ Significance of Vitamin B12

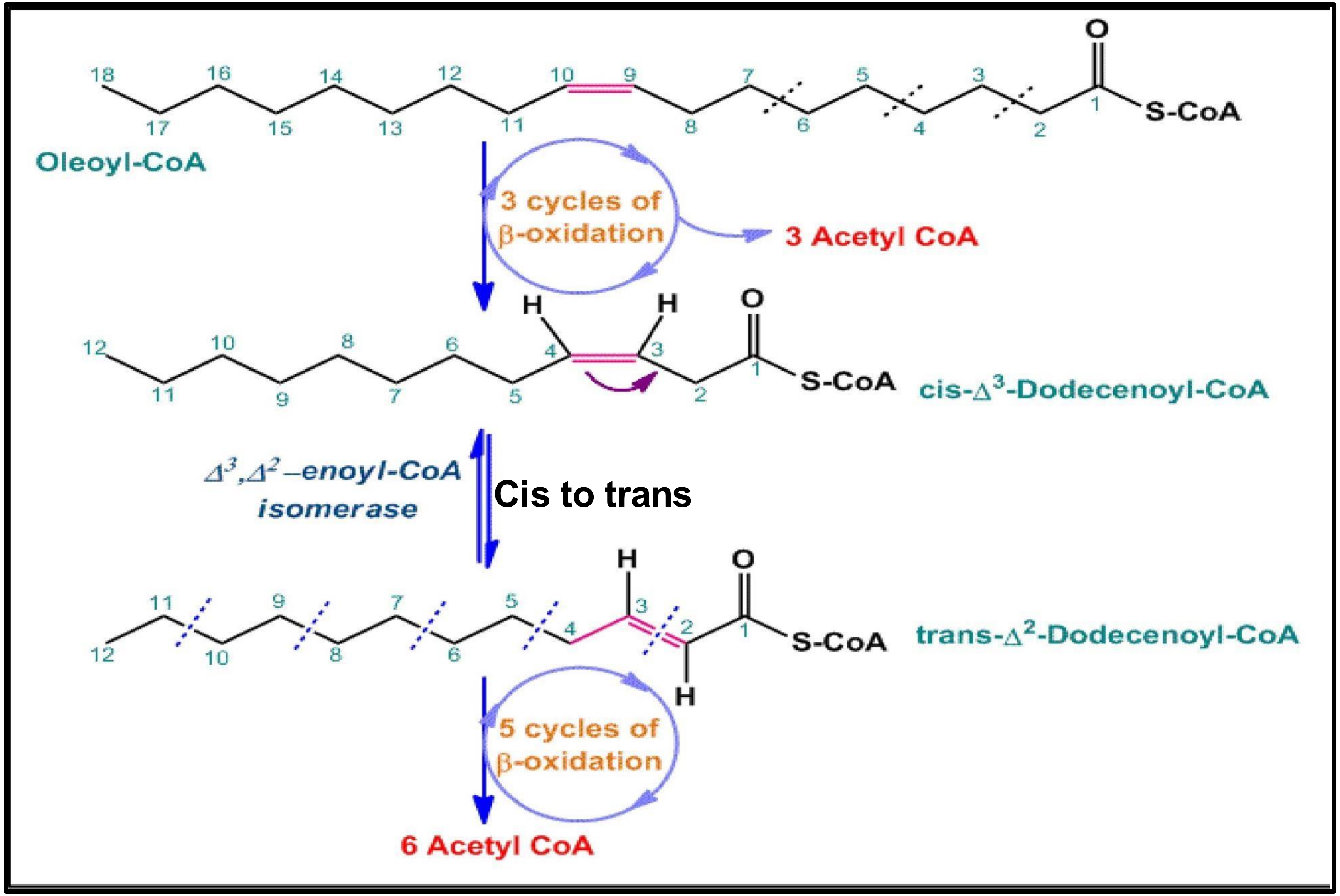
- Vitamin B12 is essential for only two reactions in our metabolism course , one of which is the conversion of odd-chain fatty acids into usable energy intermediates.
- By completing these steps , the three-carbon propionyl-CoA is effectively converted into succinyl-CoA , allowing it to enter the Krebs cycle and contribute to energy production.

Deficiency or Inactivation of Coenzyme B12 :

If Methylmalonyl-CoA mutase or Coenzyme B12 is deficient or inactive , the conversion to Succinyl-CoA is impaired , which leads to the accumulation of Methylmalonyl-CoA and related metabolites in the body , which causes :

- Metabolic acidosis : Excess acid in the bloodstream due to the buildup of organic acids.
- Neurologic manifestations : Potentially severe neurological issues , such as developmental delay , seizures , or intellectual disabilities.

Monounsaturated fatty acid β-oxidation



Monounsaturated fatty acid β-oxidation

Oleic acid, a monounsaturated fatty acid with 18 carbons and a single double bond between carbons 9 and 10 , undergoes beta-oxidation for energy production. Here's how the process proceeds:

- Initial Rounds of Beta-Oxidation :
- In the first cycle , beta-oxidation cleaves carbons 1and 2 , producing the first acetyl-CoA.
- In the second cycle , carbons 3 and 4 are cleaved , generating the second acetyl-CoA.
- In the third cycle , carbons 5 and 6 are cleaved , producing the third acetyl-CoA.

- Encountering the Double Bond :
- At this stage , further cleavage would encounter the double bond between carbons 9 and 10. To proceed , the double bond must first be addressed.
- The enzyme enoyl-CoA isomerase shifts the double bond from its current position (between carbons 3 and 4 in the new molecule) to a new position (between carbons 2 and 3) , placing it at the beta-oxidation cleavage site (isomerization)
- Continuation of Beta-Oxidation :

Then , beta-oxidation continues , generating additional acetyl-CoA molecules until the fatty acid is fully oxidized.

In the ß-oxidation of mono-unsaturated fatty acids, the double bond is first shifted to the correct position through isomerization during the early cycles .B-oxidation continues normally until the cycle in which the double bond becomes located between C2 and C.3 Only at this specific cycle is the first oxidation step skipped, because the presence of the double bond makes the FAD-dependent dehydrogenation unnecessary. Therefore, this skip happens once per molecule, and the energy yield decreases by the equivalent of 1 $FADH_2$.

Polyunsaturated fatty acid β-oxidation / 1

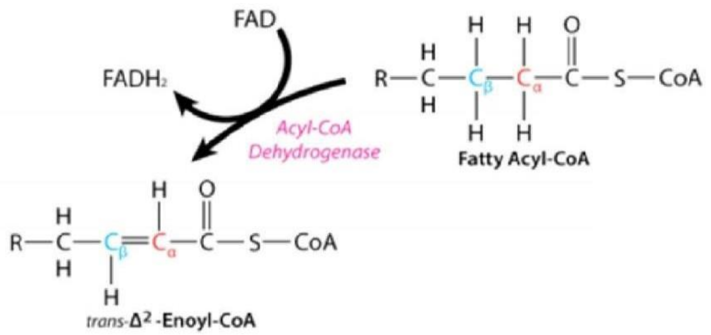
Polyunsaturated FA will also need an *NADPH-dependent 2,4-dienoyl CoA reductase* in addition to the *isomerase.*

→ loss of electrons

For polyunsaturated fatty acids , which contain multiple double bonds , their processing during beta- oxidation involves two main strategies :

- Isomerization : **Similar to monounsaturated fatty acids , some double bonds can be shifted to the cleavage site using** enoyl-CoA isomerase **, allowing beta-oxidation to proceed normally.**

- Reduction **: For certain double bonds that cannot be directly isomerized , the enzyme** 2,4-dienoyl-CoA reductase **reduces them to a single double bond , to processed further in the beta-oxidation cycle.**



But this reaction is skipped resulting in one less $FADH_2$ → loss of electrons

- The molecule shown is a saturated fatty acid attached to Coenzyme A (CoA) through a thioester bond.

Cα (alpha-carbon) : The carbon directly attached to the carbonyl group (C=O).
Cβ (beta-carbon) : The carbon next to the alpha-carbon.

- The enzyme Acyl-CoA Dehydrogenase catalyzes the oxidation of the Cα-Cβ bond in the fatty acyl-CoA molecule.
- This step removes two hydrogen atoms , creating a trans double bond between the alpha and beta carbons.
- During this reaction , FAD is reduced to $FADH_2$ as it accepts the two hydrogen atoms removed from the fatty acyl-CoA.
- The product of this reaction is trans-Δ^2-enoyl-CoA , which now contains a double bond between the alpha and beta carbons.
- $FADH_2$ is generated and will later contribute to ATP production in the electron transport chain.

In saturated fatty acids , during β-oxidation , a double bond must be introduced before cleaving the acetyl-CoA units. This is necessary because the cleavage process must occur adjacent to a carbonyl group. Initially , the fatty acid contains only one carbonyl group , which is removed in the first cycle of β-oxidation. To enable further processing , a double bond is formed by oxidation of the Cα-Cβ bond , followed by the addition of a water molecule and a subsequent oxidation step to generate a new carbonyl group.

In unsaturated fatty acids, the presence of a pre-existing double bond eliminates the need to create one during a specific cycle of β-oxidation. Instead, the double bond is simply rearranged (isomerized) to the correct position. This isomerization step does not require energy and does not produce energy carriers like FADH2. Consequently , one cycle of β-oxidation for unsaturated fatty acids generates NADH but not FADH2, leading to reduced ATP production compared to
saturated fatty acids of the same carbon length.

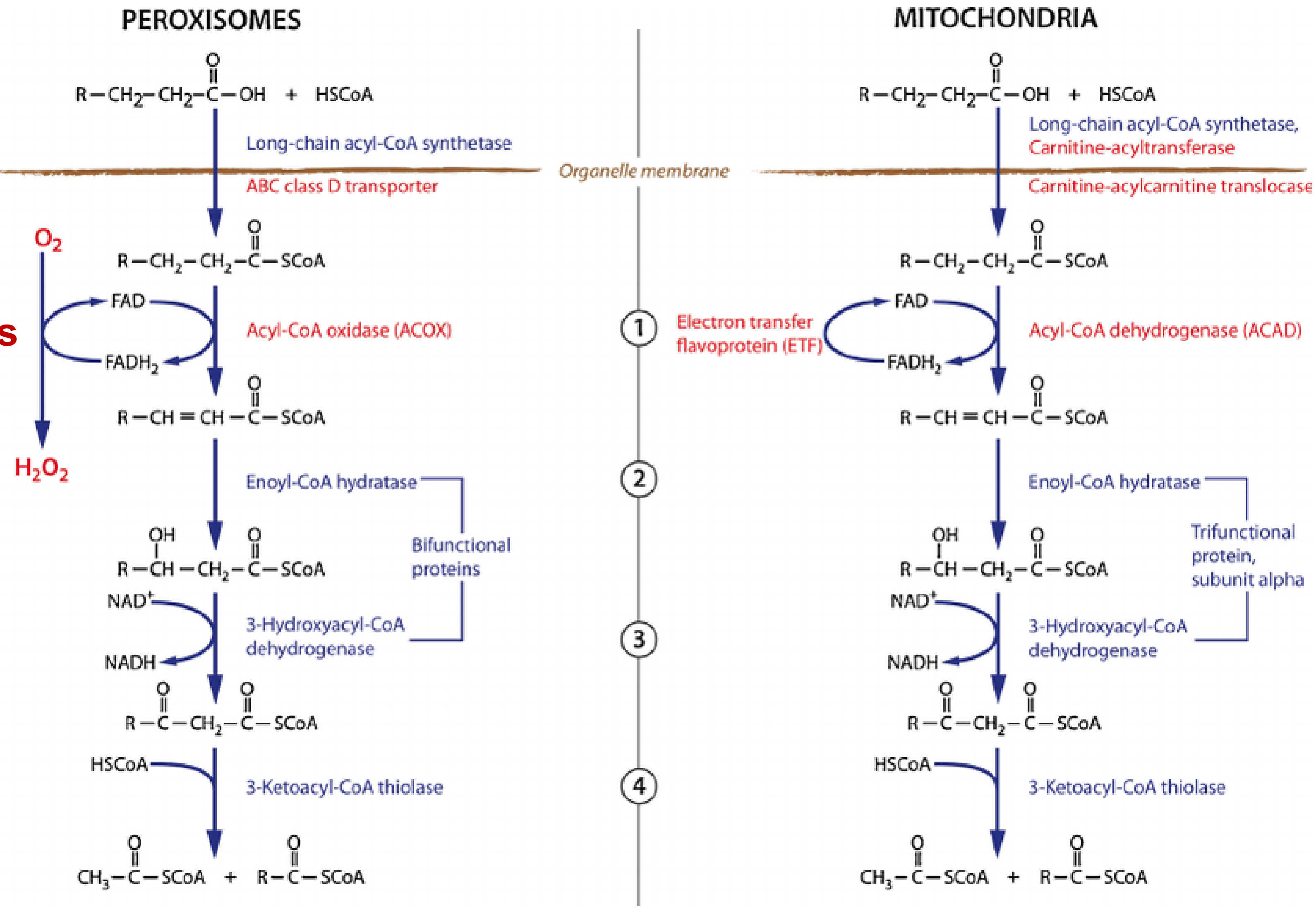
For polyunsaturated fatty acids, the absence of FADH2 production occurs multiple times due to the repeated presence of double bonds. This results in a greater loss of electrons , fewer electron carriers , and ultimately less energy production compared to saturated fatty acids with an equivalent number of carbons.

Peroxisomal β-oxidation

VLCFA ≥22

FAD Containing acyl CoA Oxidase

Loss of electrons



Peroxisomal β-oxidation / 1

Beta oxidation in the mitochondria can metabolize short- , medium- , and long-chain fatty acids. However, very long-chain fatty acids (VLCFAs) undergo beta oxidation in peroxisomes rather than mitochondria due to differences in their transport and enzymatic systems.

- Transport of VLCFAs into Peroxisomes
- VLCFAs cross the peroxisomal membrane through a specialized transport system distinct from that of mitochondria. In peroxisomes , the ABC class D transporter facilitates the entry of VLCFAs in an inactive form , accompanied by CoA (coenzyme A). Activation of VLCFAs through CoA addition occurs inside the peroxisome.
- In general, fatty acids must be linked to CoA to participate in metabolic reactions, as this activates the acyl chains for subsequent processing.

- Beta Oxidation in Peroxisomes

Once activated, the beta oxidation pathway in peroxisomes proceeds similarly to that in mitochondria but with key differences :

1)Oxidation :

- In mitochondria , acyl-CoA dehydrogenase catalyzes the first oxidation step, reducing FAD to $FADH_2$, which enters the electron transport chain to produce energy.
- In peroxisomes , the enzyme acyl-CoA oxidase performs this step. Instead of contributing $FADH_2$ to the electron transport chain , $FADH_2$ is re-oxidized to FAD, reducing oxygen to hydrogen peroxide (H_2O_2) , a reactive oxygen species.
- This distinction results in less energy production during beta oxidation in peroxisomes compared to mitochondria.

Peroxisomal β-oxidation / 2

2) Hydration :
The enzyme enoyl-CoA hydratase creates an alcohol group .

3) Second Oxidation :
3-Hydroxyacyl-CoA dehydrogenase catalyzes the oxidation of the alcohol group , reducing NAD to NADH. This step is similar to the process in mitochondria.

4) Cleavage :
3-Ketoacyl-CoA thiolase cleaves the fatty acid chain, adding another CoA molecule and producing a shortened fatty acyl-CoA chain.

- Structural and Functional Differences between mitochondrial and peroxisomal oxidation
- Transport Process
- The hydratase and 3-hydroxacyle dehydrogenase enzymes in peroxisomes are bifunctional proteins , whereas in the mitochondria they are trifunctional proteins.
- The overall process is less energy-efficient in peroxisomes due to the generation of hydrogen peroxide instead of ATP from $FADH_2$.

Diseases Associated with Peroxisomal Beta Oxidation

- **Zellweger syndrome : a peroxisomal biogenesis disorder**

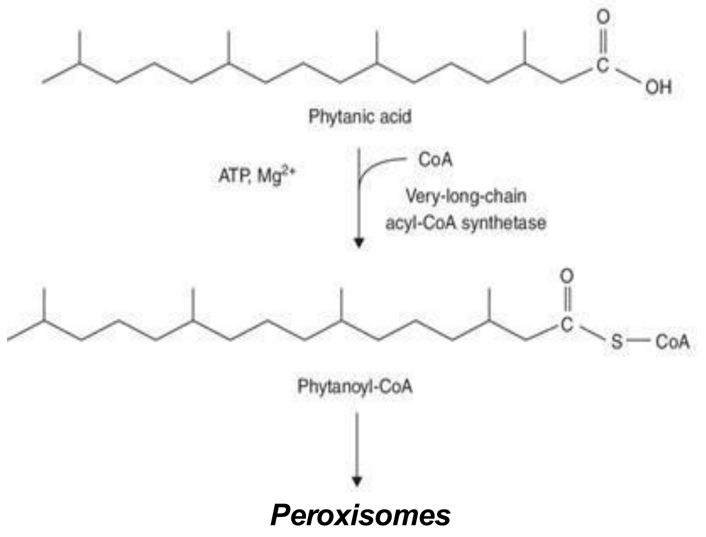
A disorder caused by defective peroxisome (which is a dynamic organelle) biogenesis. As a result, peroxisomal functions , including beta oxidation and detoxification of oxidative stress, are impaired.

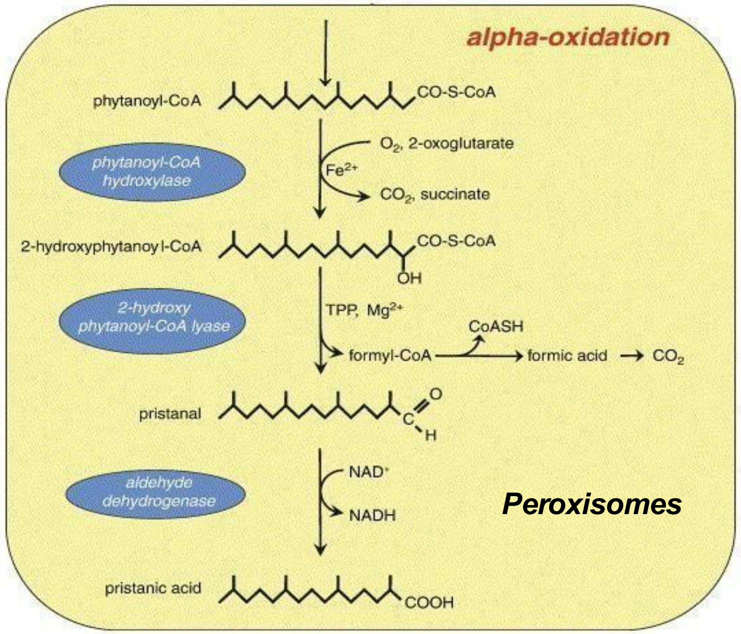
- **X-linked adrenoleukodystrophy: dysfunctional transport VLCFA across the peroxisomal membrane (Accumulation of VLCFAs)**

A genetic condition characterized by impaired transport of VLCFAs into peroxisomes due to defective ABC class D transporters. This leads to the accumulation of VLCFAs .

Peroxisomal α-oxidation of branched chain FAs

- Phytanic acid is a breakdown product of Chlorophyll.
- It is activated by CoA , transported into peroxisome , hydroxylated by phytanoyl CoA α-hydroxylase (PhyH) and carbon 1 is released as CO2 (unlike beta oxidation) .
- When fully degraded , it generates formyl-CoA , propionyl-CoA , acetyl-CoA , and 2-methyl-propionyl- CoA.





Refsum disease is an autosomal recessive disorder caused by a deficiency of peroxisomal PhyH.

PLEASE SEE NEXT SLIDE

Peroxisomal α-oxidation of branched chain FAs

This pathway is used for branched-chain fatty acids like phytanic acid , which is a product of chlorophyll breakdown. Although phytanic acid is saturated , it contains branches that make it unsuitable for beta-oxidation.

- Activation:

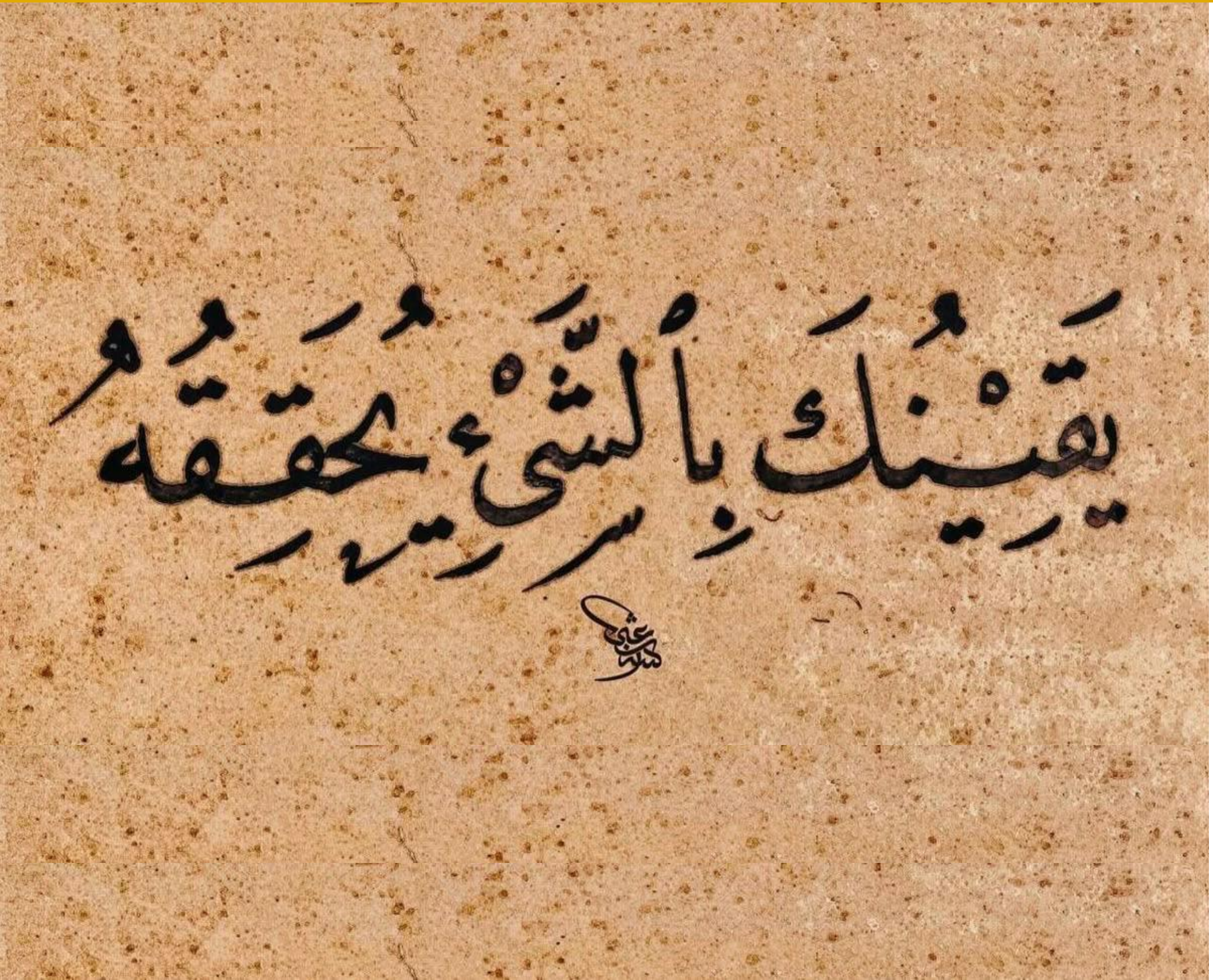
Initially , very-long-chain acyl-CoA synthetase activates phytanic acid outside the peroxisome by attaching a CoA group , forming phytanoyl-CoA , which is now active and ready for peroxisomal metabolism.

- Alpha-Oxidation Process in Peroxisomes :

- Phytanoyl-CoA hydroxylase introduces a hydroxyl (-OH) group on the alpha-carbon
- This facilitates the cleavage of the carbonyl group with CoA by 2-hydroxy phytanoyl-CoA lyase , releasing it as formyl-CoA , which is further broken down (by removing CoA) to formic acid and then CO_2.
- The remaining hydroxyl group is oxidized to form an aldehyde via 2-hydroxy phytanoyl-CoA lyase , resulting in the production of pristanal.
- Pristanal undergoes further oxidation to a carboxyl group using aldehyde dehydrogenase , with NAD^+ reduced to NADH , forming pristanic acid.

- The cycle repeats until the fatty acid is completely metabolized. However, due to the branches , subsequent cycles may produce varying products , including formyl-CoA , acetyl-CoA , propionyl-CoA , or methyl-propionyl-CoA , depending on the structure of the acid.

رسالة من الفريق العلمي:



يقولُ اللهُ عزَّ وجلَّ : أنا عند ظنِّ عبدي بي فليظُنَّ بي ما شاء

يعني: إن ظنّ بالله خيرًا فله، وإن ظنّ به سوى ذلك فله، وحسن الظنّ بالله عزّ وجلّ يكون بفعل ما يوجب فضل الله ورجاءه، فيعمل الصالحات، ويحسن الظنّ بأنّ الله تعالى يقبله، فالله سبحانه عند منتهى أمل العبد به، وعلى قدر ظنّ واعتقاد العبد فيه، ويكون عطاء الله وجزاؤه من جنس ما يظنّه العبد في الله ثوابًا أو عقابًا، خيرًا أو شرًّا، فمن ظنّ بالله أمرًا عظيمًا وجده وأعطاه الله إياه، والله لا يتعاظمه شيء، أمّا أن يحسن الظن وهو لا يعمل، فهذا من باب التمنّي على الله، ومن أتبع نفسه هواها، وتمنّى على الله الأماني فهو عاجز.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide **43**	Last paragraph (2nd box)	Last paragraph was corrected as it was reversed
V1 → V2			