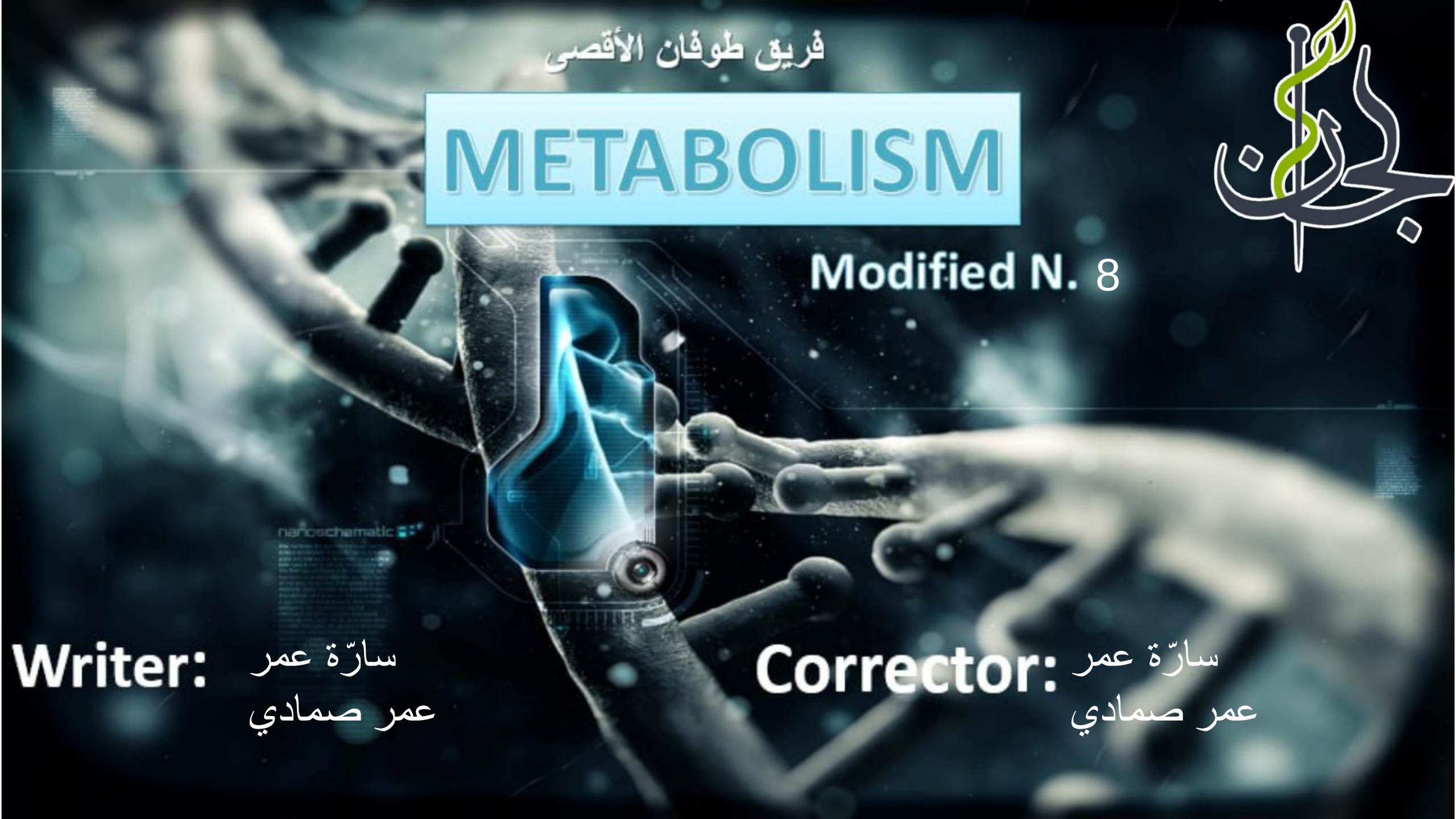
فريق طوفان الأقصى

METABOLISM

Modified N. 8



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عمر صمادي

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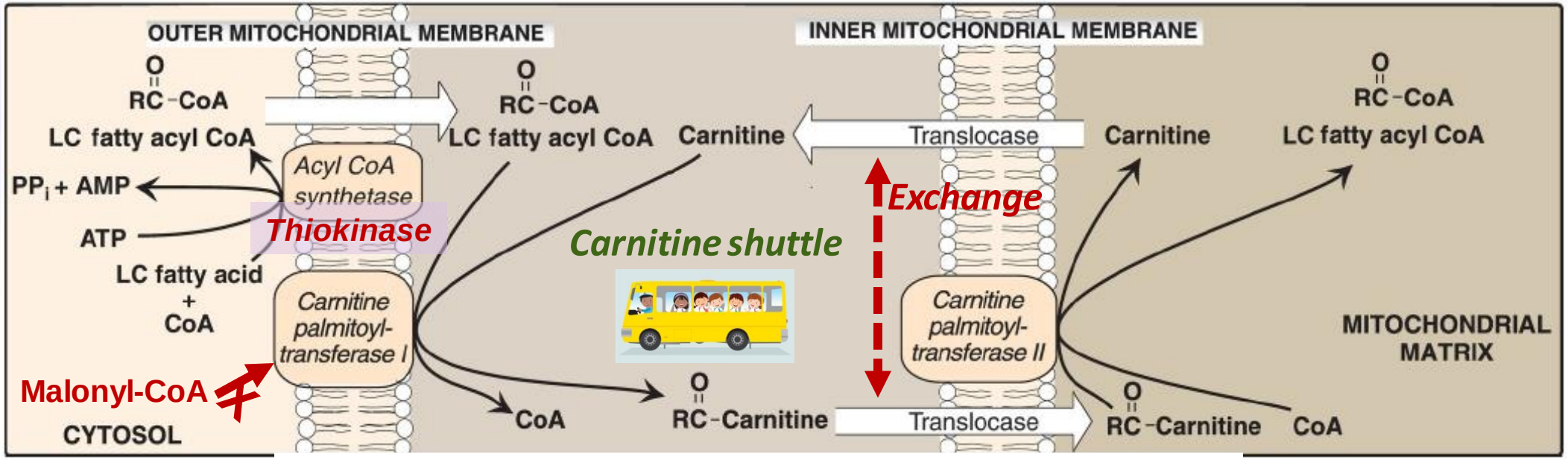
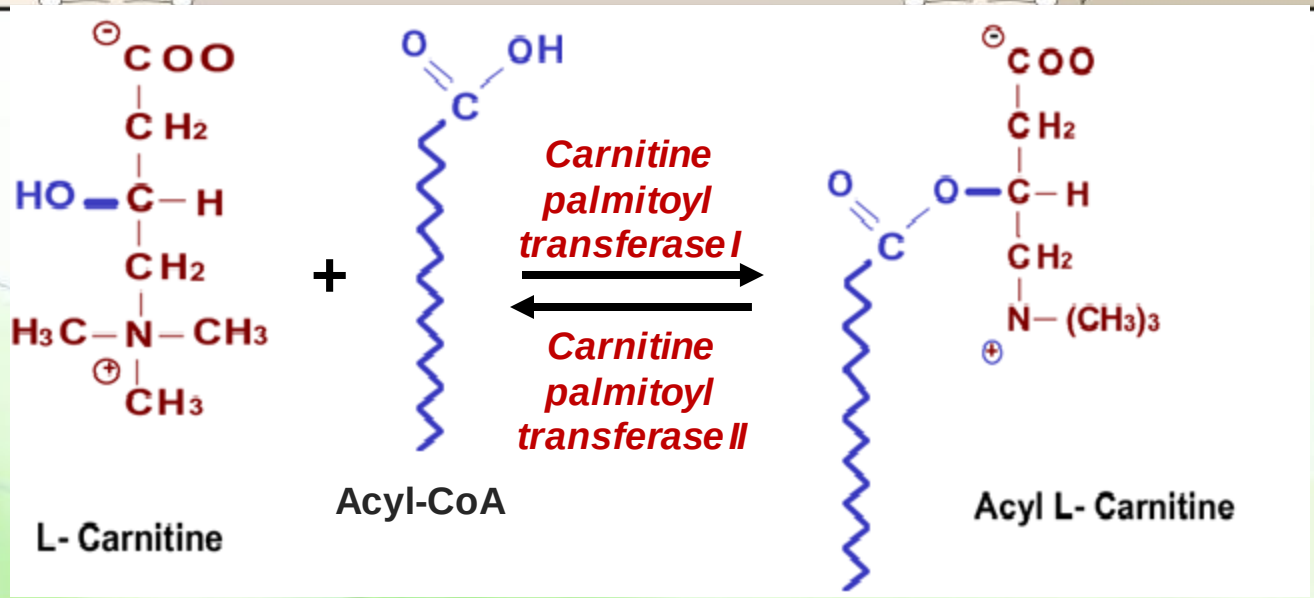
LCFA is mitochondrial





The complement in this slide:

For LCFAs to enter mitochondria, it uses a mechanism called carnitine shuttle.

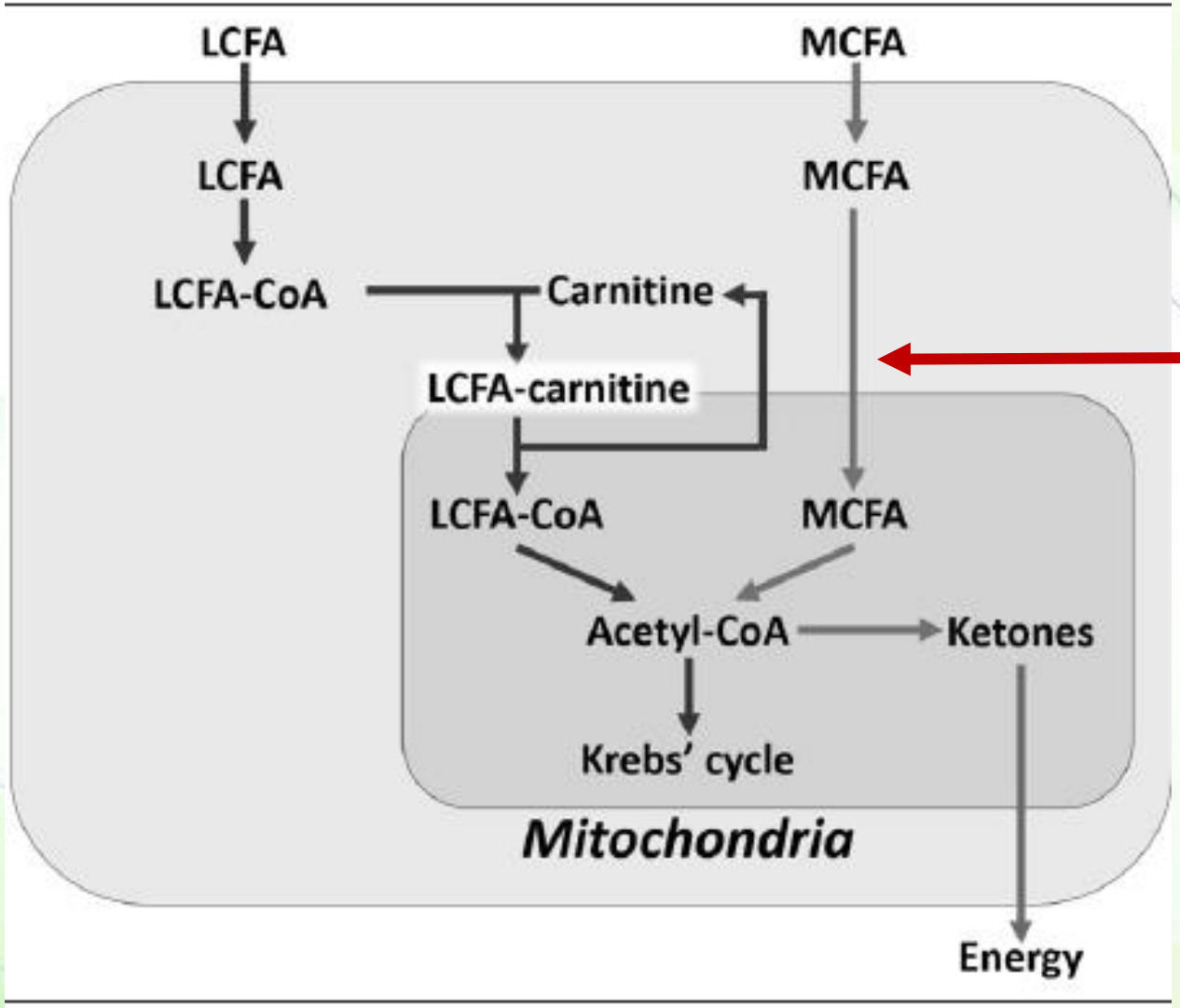
1- First, the FA needs to be activated by attaching CoA to the FA, and this reaction is coupled with ATP —> AMP + ppi, ppi is further hydrolised to 2 pi molecules, to decrease the concentration of ppi and drive the equilibrium towards AMP + ppi.

2- Secondly, the activated FA enters the intermembrane space, but it can't go any further, so the CoA is replaced with **carnitine**.

3- After that, the FA enters the mitochondrial matrix and gets its CoA back.

SCFAs and MCFAs

- **NOTE:** Only LCFAs need carnitine shuttle mechanism.
- **NOTE:** MCFAs and SCFAs don't need this mechanism.
- **NOTE:** VLCFAs are treated differently, which we will discuss later.



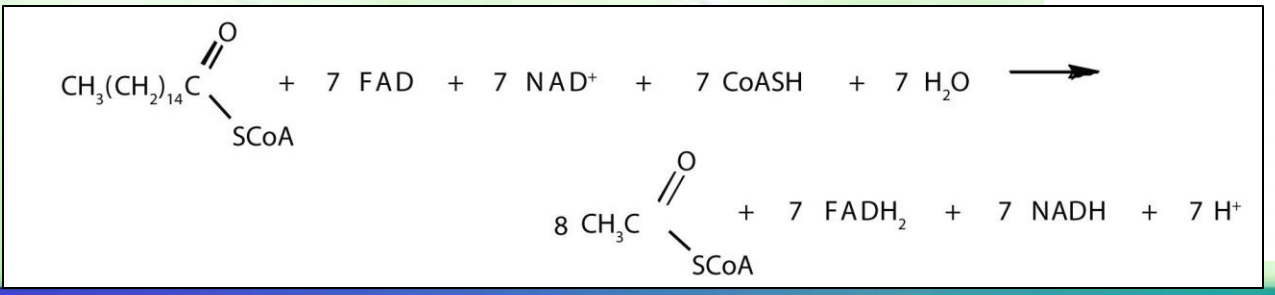
Note: No regulation of entry like that of CPTI by malonyl CoA

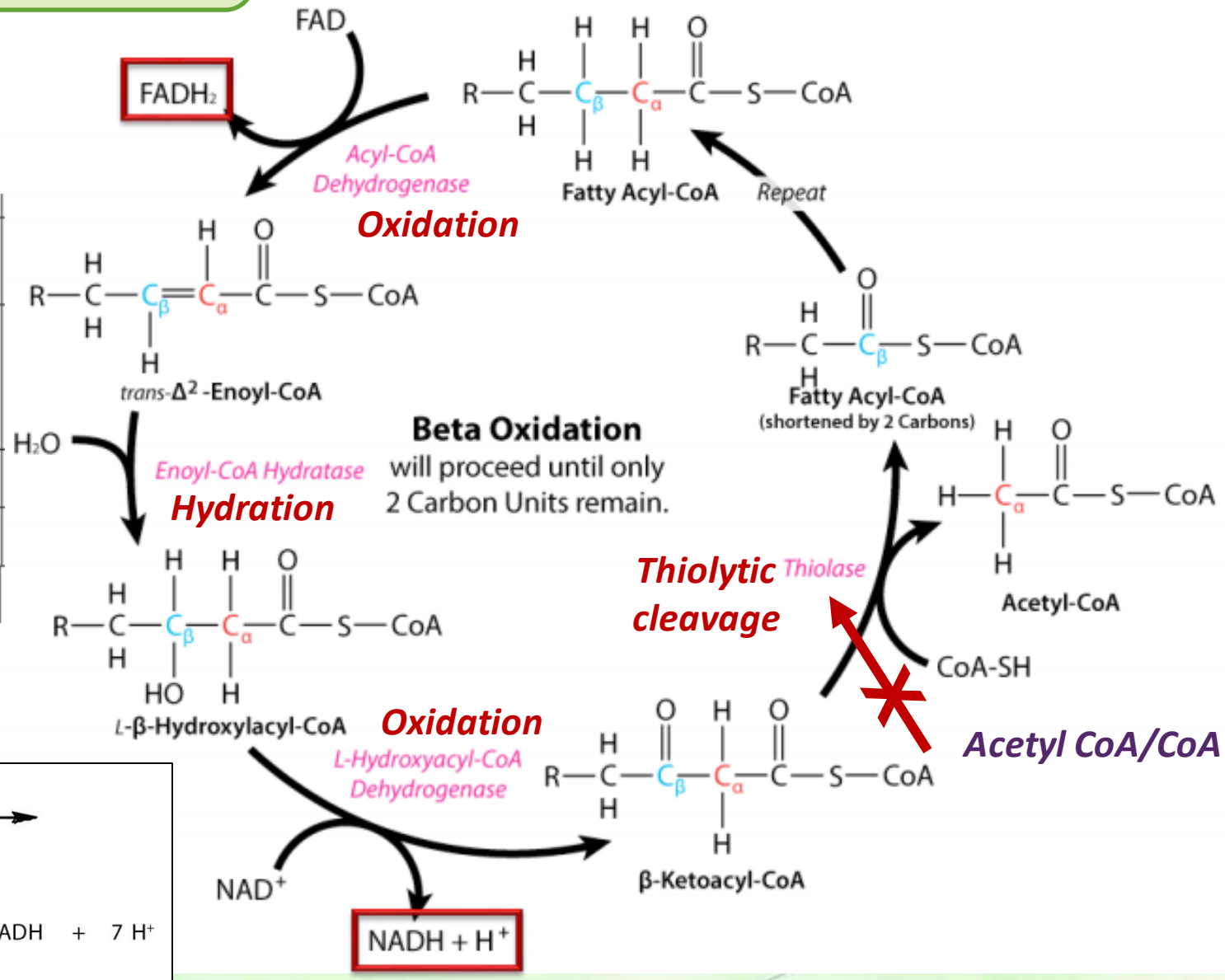
- **NOTE:** All FAs are "activated", it's just that MCFAs and SCFAs are activated after entering the mitochondria.

NOTE: it's called beta oxidation because the beta carbon is modified, the bond between beta carbon and alpha carbon is hydrolysed

- **Number of cycles: (n/2)-1**

Mechanism	ATP Yield
1. β-oxidation 7 cycles 7 $FADH_2$ and 7 NADH are generated when oxidized by ETC	7$FADH_2$- 7 x 1.5 = 10.5 7 NADH- 7 x 2.5 = 17.5
2. From 8 acetyl CoA 1 acetyl CoA =3 NADH + 1 $FADH_2$ + 1 GTP Oxidized by citric acid cycle, each acetyl CoA provides ATP	10 x 8 = 80
Total Energy from one mole of palmitoyl CoA	**108**
Energy utilized for activation (Formation of Palmitoyl CoA)	2
Net yield of oxidation of one mole of palmitate	108-2 =**106**





The complement in this slide:

Beta and alpha carbons are saturated and not reactive so we need to make them reactive.
Fatty acid breakdown is the total opposite of fatty acid synthesis ,
Fatty acid synthesis reactions were : reduction —> dehydration —> reduction
While fatty acid breakdown is : oxidation —> hydration —> oxidation.

In the first oxidation, FAD takes electrons from beta and alpha carbons forming a double bond between them.

In the hydration we add water making a hydroxyl group on the beta carbon.

In the second oxidation NAD+ takes 2 electrons turning the hydroxyl group into a carbonyl group marking a ketone.

The bond between beta and alpha carbon is weak, so it’s cleaved by thiolytic cleavage making an acetyl-CoA molecule.

These reactions are repeated on the fatty acid molecule making an acetyl-CoA each turn, until the fatty acid is done.

The complement in this slide:

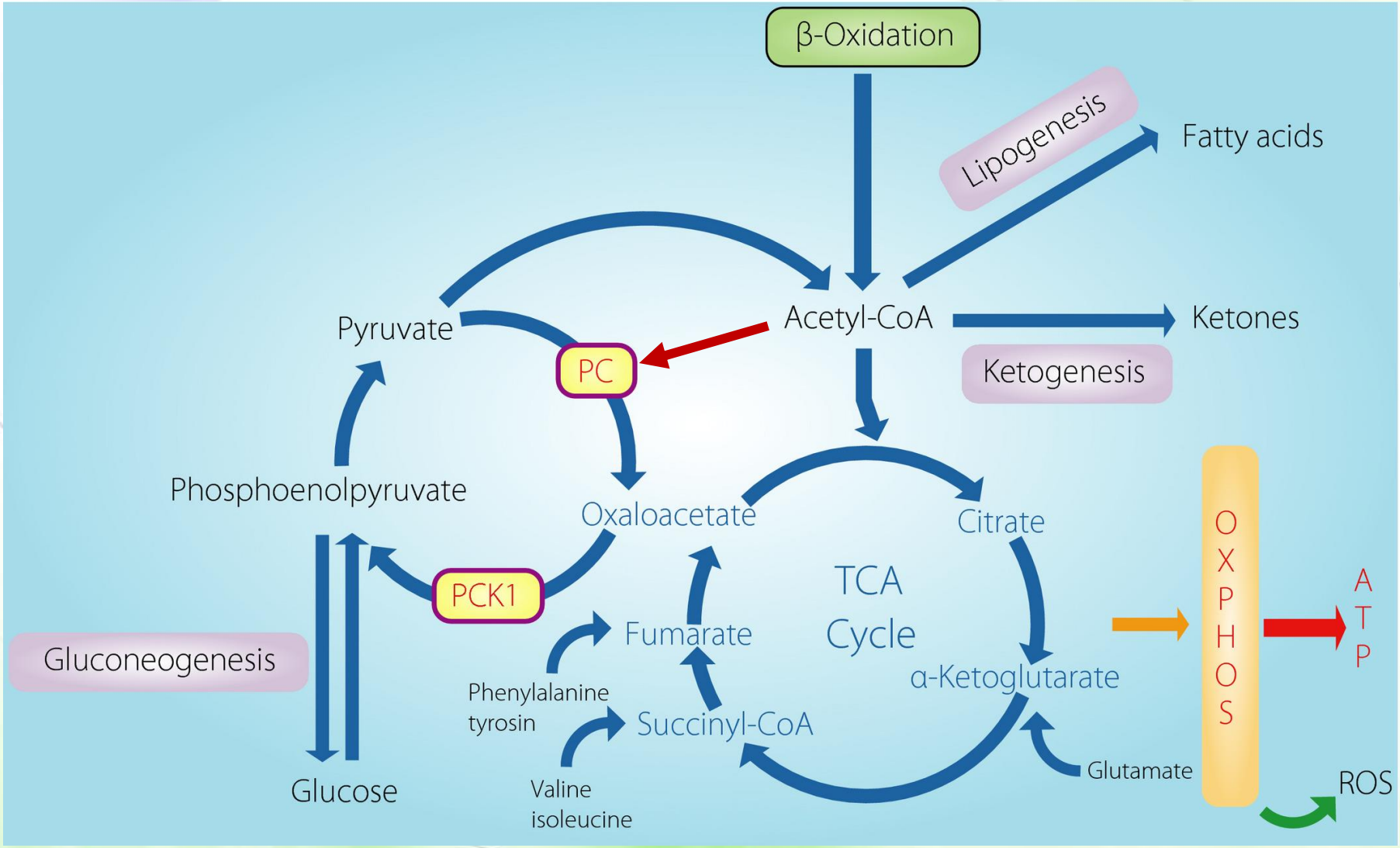
To calculate how many ATP molecules a fatty acid makes :

1- count the number of acetyl-CoA made from the fatty acid = number of carbons of the fatty acid / 2. (for example, a 16 carbon fatty acid makes 8 acetyl-CoA)

2- count the FADH and NADH made from beta oxidation and Krebs cycle, notice that the last acetyl-CoA form beta oxidation does not use FAD or NAD+. (so a 16 carbon fatty acid uses 7 NAD+ and 7 FAD in beta oxidation)

3- calculate the ATP made minus the 2 phosphate groups used in the activation of the fatty acid at the beginning.

Induction of gluconeogenesis and fates of acetyl CoA

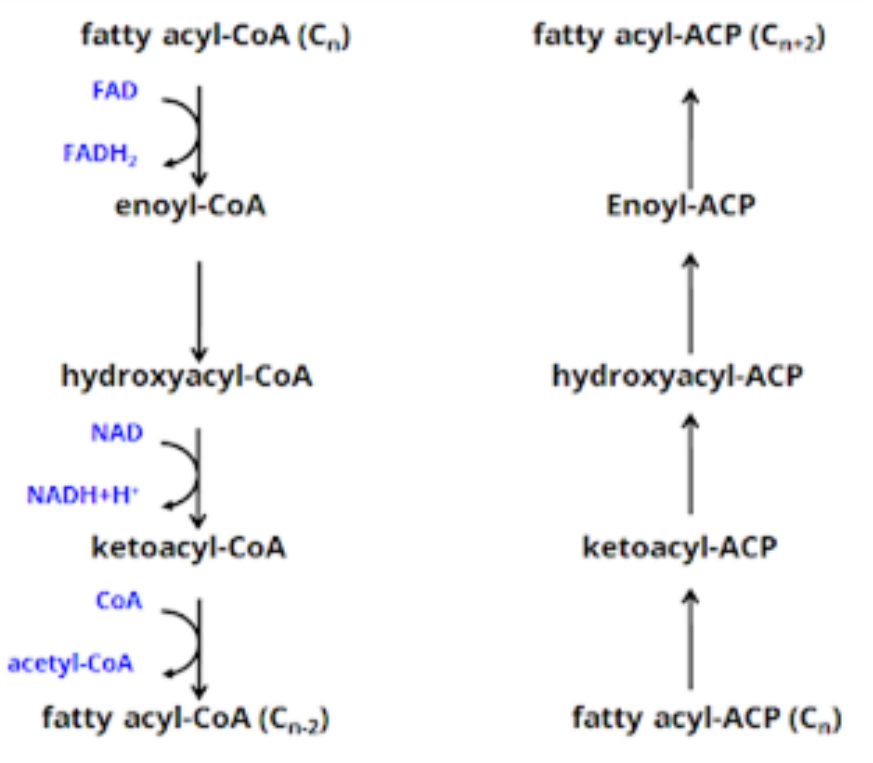


- **NOTE:** when there are increased amount of acetyl-CoA and very low amount of oxaloacetate (because of gluconeogenisis), acetyl-CoA is converted to ketone bodies, which we will discuss later.

Synthesis vs. degradation

NOTE: read only

VARIABLE	SYNTHESIS	DEGRADATION
Greatest flux through pathway	After carbohydrate-rich meal	In starvation
Hormonal state favoring pathway	High insulin/glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (mitochondria to cytosol)	Carnitine (cytosol to mitochondria)
Phosphopantetheine-containing active carriers	Acyl carrier protein domain, coenzyme A	Coenzyme A
Oxidation/reduction coenzymes	NADPH (reduction)	NAD^+, FAD (oxidation)
Two-carbon donor/product	Malonyl CoA: donor of one acetyl group	Acetyl CoA: product of β-oxidation
Activator	Citrate	—
Inhibitor	Palmitoyl CoA (inhibits *acetyl CoA carboxylase*)	Malonyl CoA (inhibits *carnitine palmitoyltransferase-I*)
Product of pathway	Palmitate	Acetyl CoA
Repetitive four-step process	Condensation, reduction dehydration, reduction	Dehydrogenation, hydration dehydrogenation, thiolysis

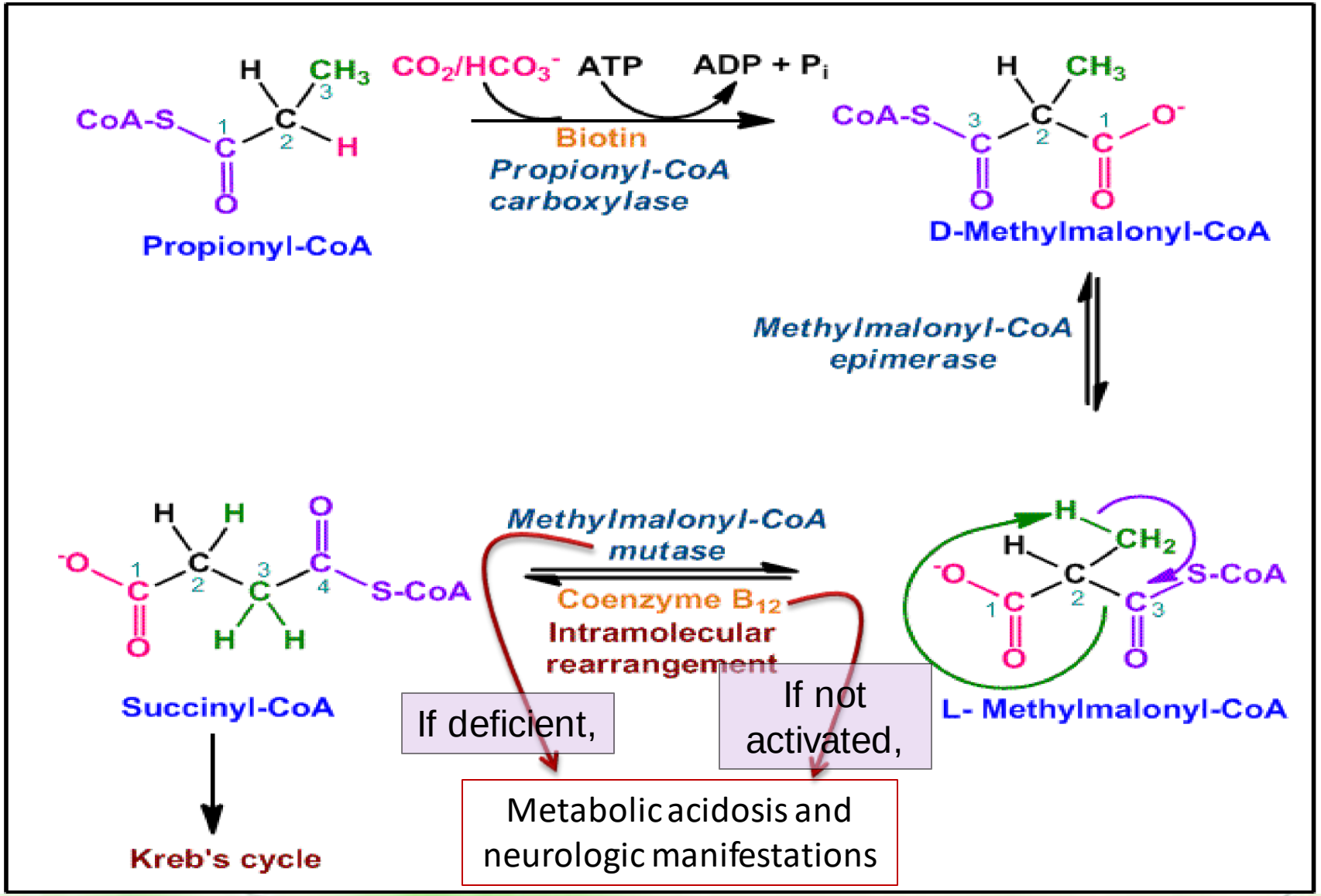


MCAD deficiency

- There are 4 isozymes of fatty acyl CoA dehydrogenase for SCFA, MCFA, LCFA, and VLCFA.
- Medium-chain fatty acyl CoA dehydrogenase (MCAD) deficiency,
  - An autosomal-recessive disorder
  - 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

NOTE: MCAD deficiency is prevalent in Northern Europe, this condition is autosomal recessive.

Oxidation of odd-numbered FAs



Note: Loss of electrons

The complement in this slide:

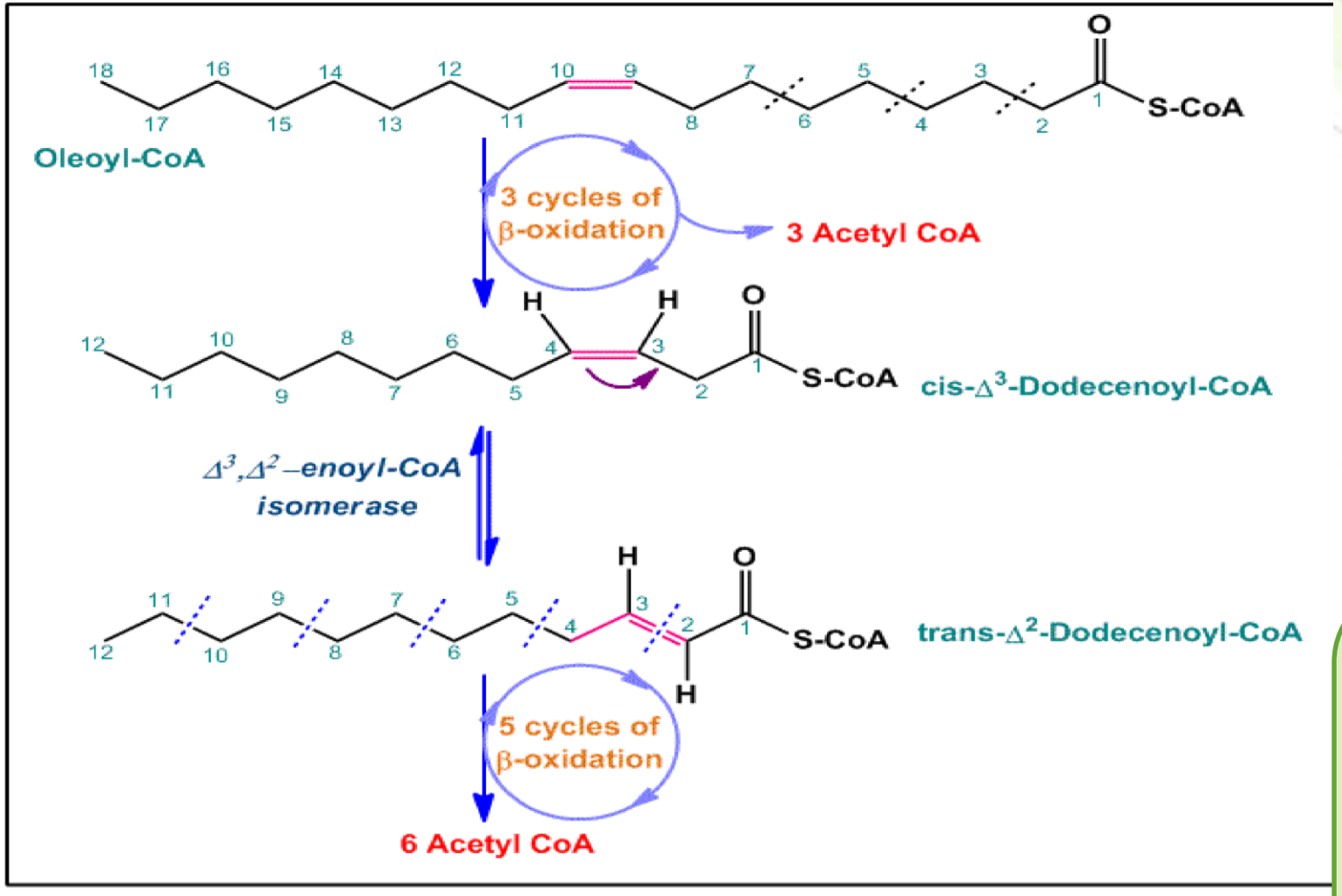
In case of odd numbered FA, after multiple beta oxidation reactions and making many acetyl-CoAs, we will end up with a 3 carbon molecule called propionyl-CoA instead of acetyl-CoA.

To make use of this molecule, it is first carboxylated (with the help of biotin) to D-methylmalonyl-CoA, then its epimerised to L-methylmalonyl-CoA, after that it's turned into succinyl-CoA with the help of coenzyme B12, succinyl-CoA is an intermediate of Krebs cycle and it's then used there.

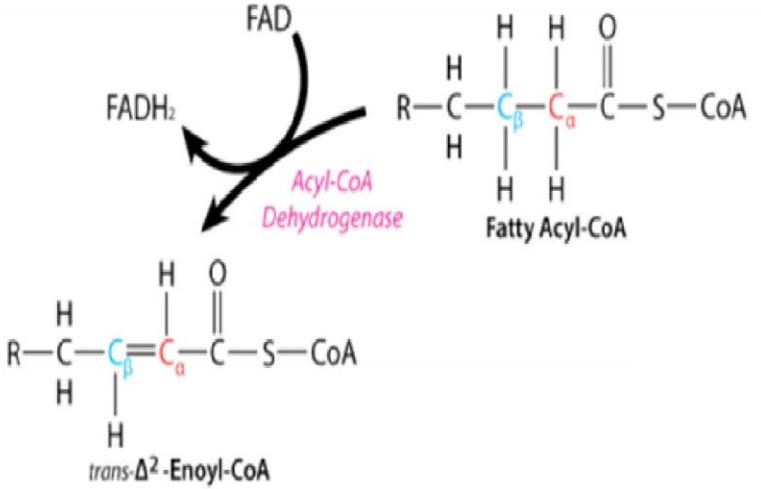
Because Krebs cycle is not started from the beginning, it makes less energy, therefore there is a loss of electrons.

People with B12 deficiency may have neurological manifestations and metabolic acidosis because of the accumulation of L-methylmalonyl-CoA.

Monounsaturated fatty acid β-oxidation



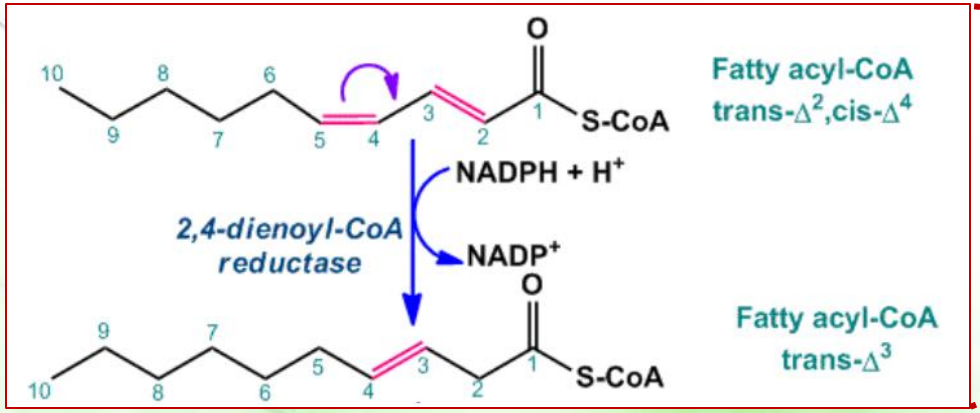
But this reaction is skipped resulting in one less $FADH_2$ → loss of electrons

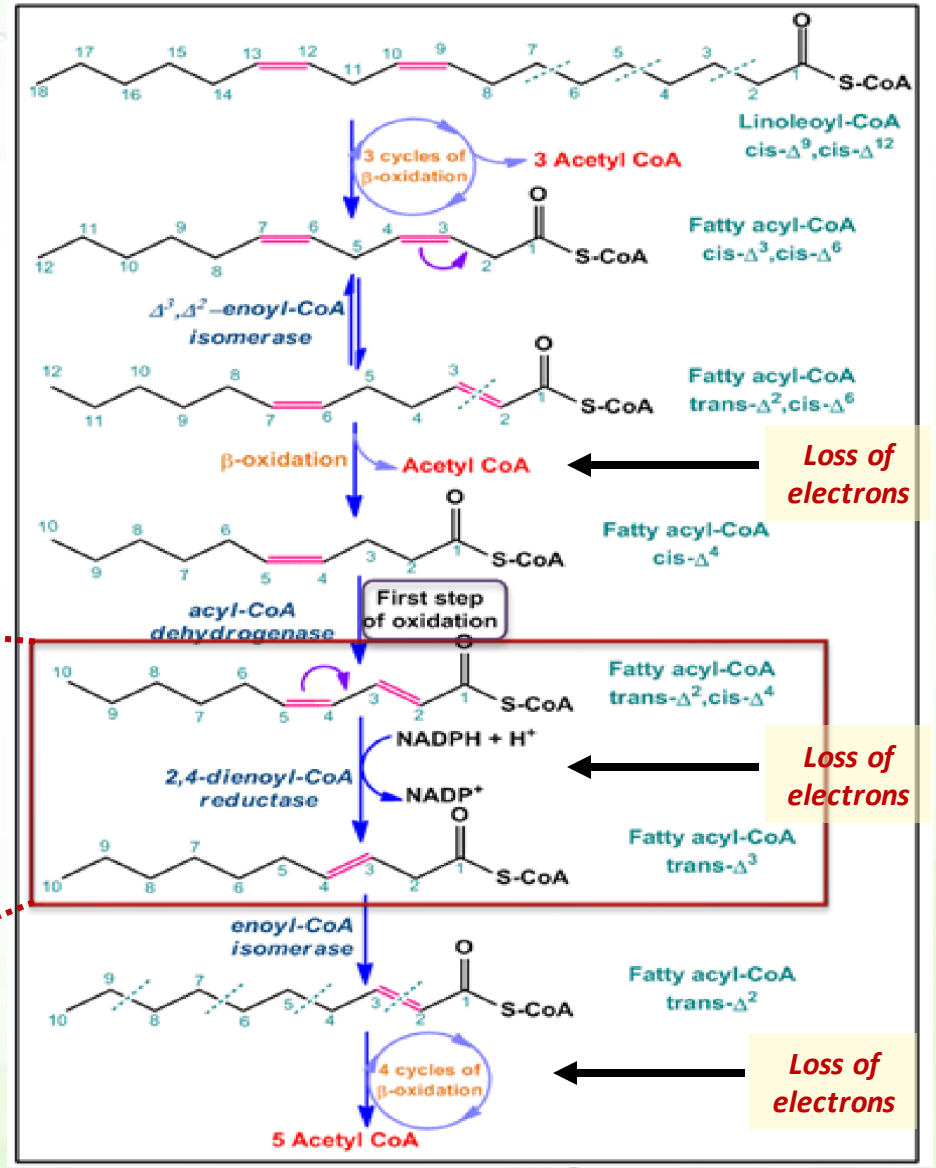


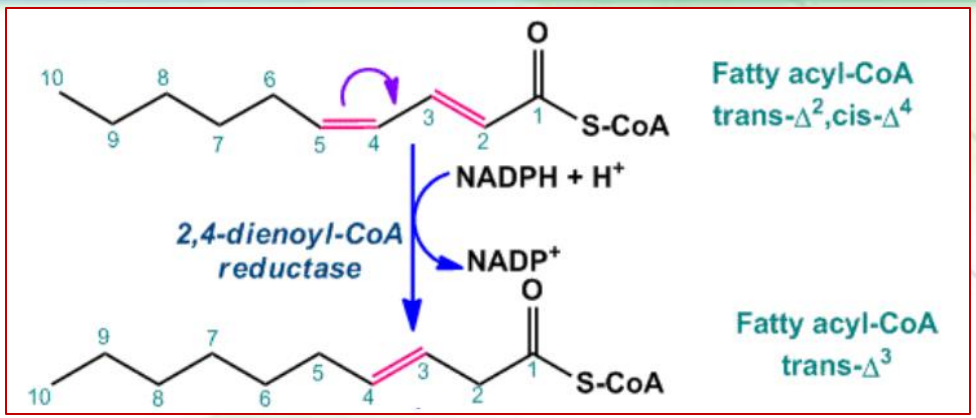
NOTE: in case of monounsaturated FA, when beta oxidation reaches the double bond, it's basically identical to the first intermediate of beta oxidation, so the first step is simply skipped, but also there is loss of electrons, therefore loss of energy.

Polyunsaturated fatty acid β-oxidation

- Oxidation of a double bond at an even-numbered carbon, such as 18:2(9,12) (linoleic acid), requires an *NADPH-dependent 2,4-dienoyl CoA reductase* in addition to the *isomerase.*
- Note: loss of electrons



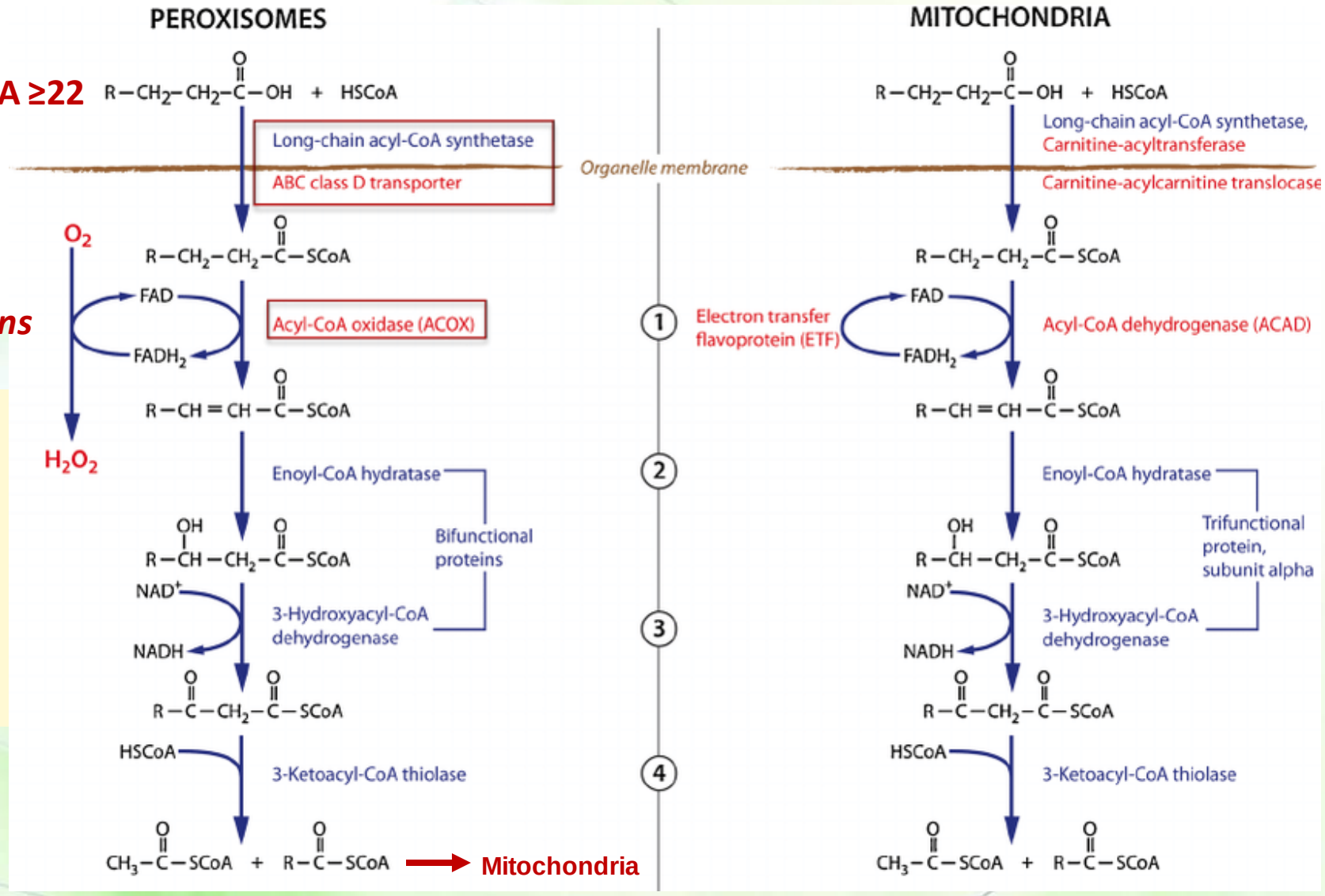




- **NOTE:** in case of polyunsaturated FA, a reductase enzyme comes to two adjacent double bonds, reduces them, then forms a double bond in between, the the double bond is treated the same way as in monounsaturated FA.

But in polyunsaturated FA there is loss of electrons twice, first is in the reduction using the reductase, and the second is by skipping the step.

Peroxisomal β-oxidation



Note: Loss of electrons

- **Zellweger syndrome: a peroxisomal biogenesis disorder**
- **X-linked adrenoleukodystrophy: dysfunctional transport VLCFA across the peroxisomal membrane**

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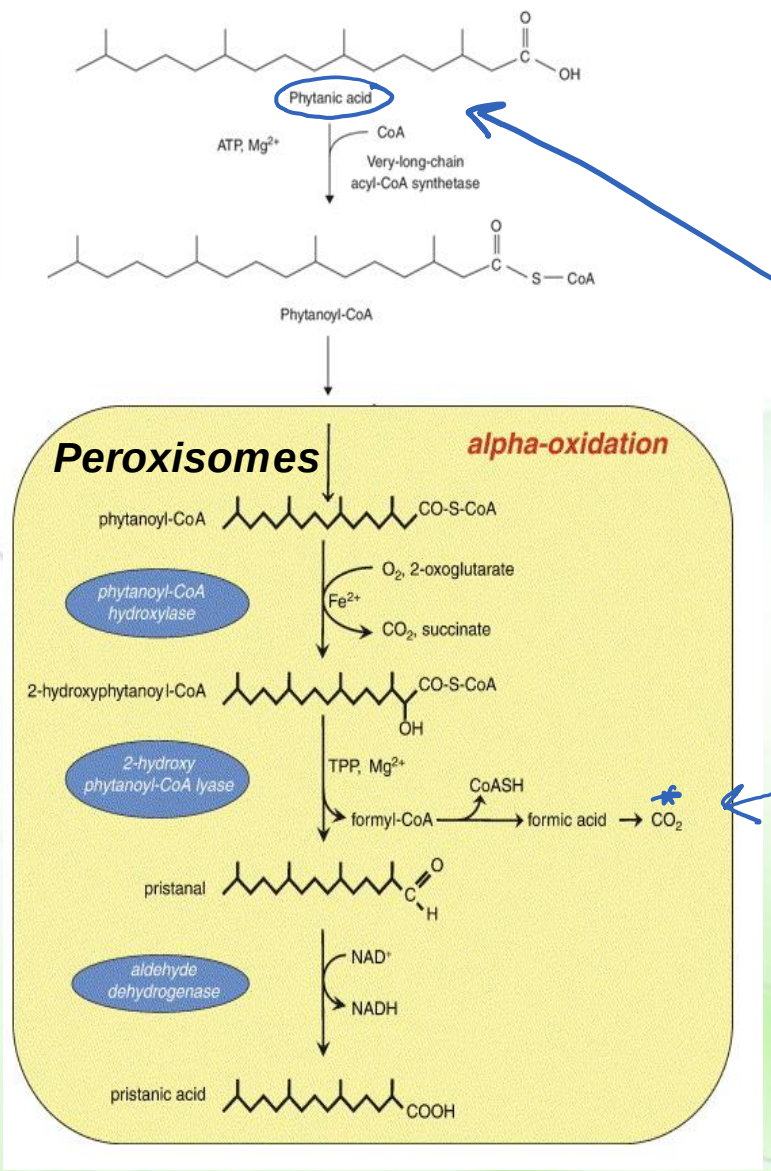
Accumulation of VLCFAs

The complement in this slide:

VLCFAs are shortened before entering the mitochondria for beta oxidation.
They first enter a peroxisome and they are introduced to an oxidase enzyme called acetyl-CoA oxidase enzyme, this enzyme does the same as acetyl-CoA dehydrogenase that is present in the mitochondrial beta oxidation, but it regenerates FAD by using the produced FADHs to make H2O2. The rest reactions are the same as normal beta oxidation, and they are repeated until the chain is LCFA.
After that it enters the mitochondria for normal beta oxidation.

There are some diseases associated with peroxisomal beta oxidation like zellweger syndrome that has a problem with peroxisomes overall, and X-linked adrenoleukodystrophy that has a problem with VLCFA transporters in peroxisomes.

Peroxisomal α-oxidation



NOTE:

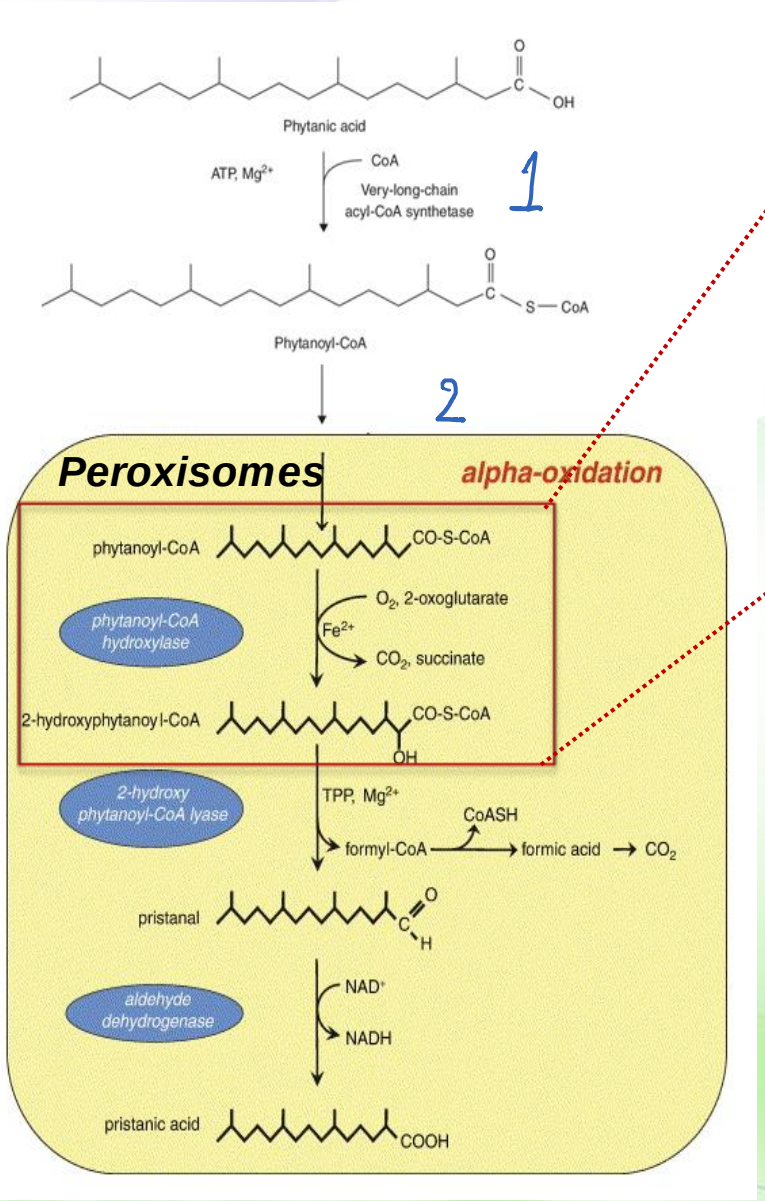
-peroxisomal alpha oxidation in peroxisome : Peroxisomal Alpha oxidation is basically the metabolism of chlorophyll, if you eat green leaves there is **chlorophyll** inside them.

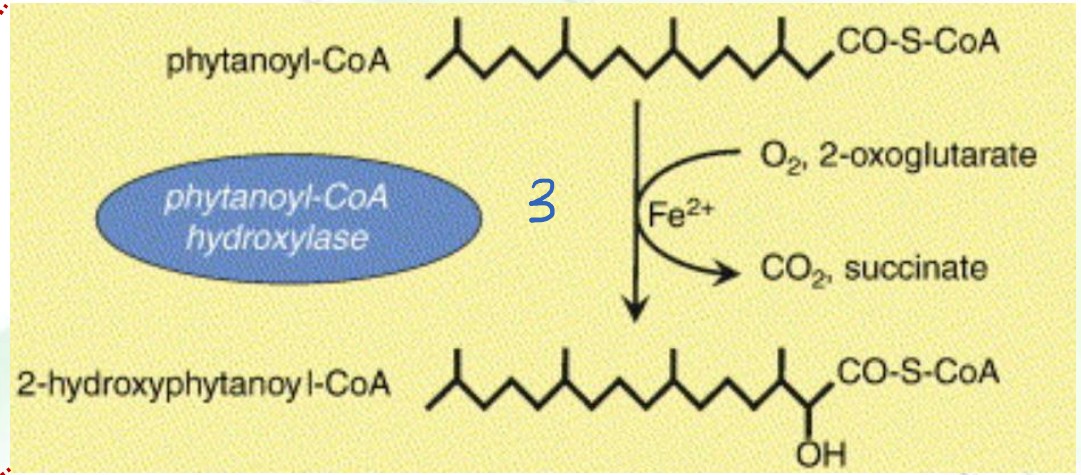
- **phytanic acid is** The product of breaking down of chlorophyll (intermediate of Chlorophyll metabolism.
- **phytanic acid is branched fatty acid** (there are methyl groups associated with more than carbon).

NOTE:

we call it alpha oxidation because we break exactly before alpha carbon (between alpha c and the first c) (releasing the first carbon as CO2) .

Peroxisomal α-oxidation

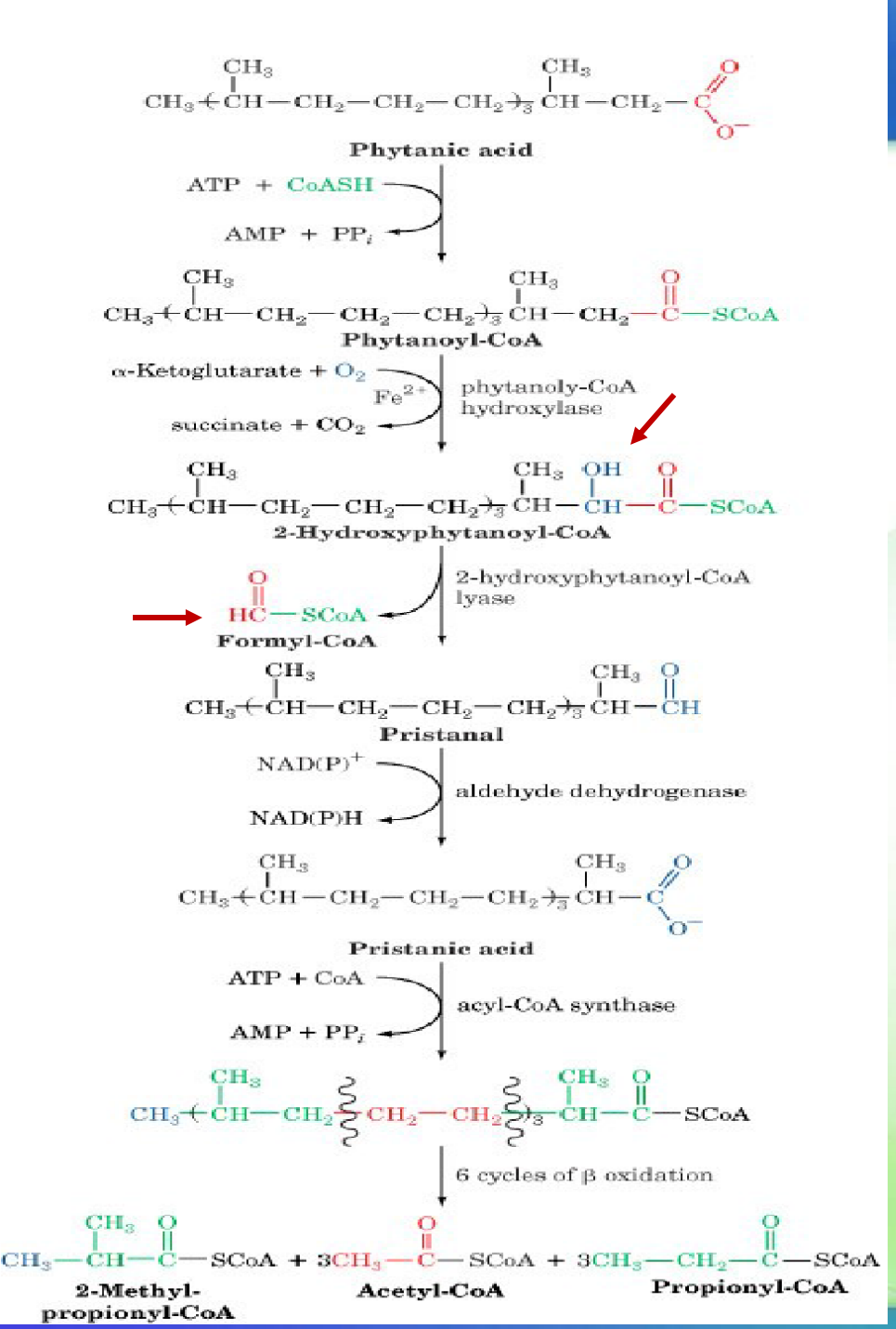




- Phytanic acid is a breakdown product of Chlorophyll.
- .1It is activated by CoA,
- 2transported into peroxisome,
- 3**hydroxylated** by **phytanoyl CoA α-hydroxylase (PhyH)** _Important reaction in phetanic acid metabolism_ and carbon 1 is released as CO_2.

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

Peroxisomal α-oxidation



NOTE:

This is the remaining pathway for hydrolyzing phetanoic acid.

Don't memorize the enzymes and intermediates

- When fully degraded, it generates 4 byproducts

[1]formyl-CoA,

[2]propionyl-CoA,

[3]acetyl-CoA,

[4]2-methyl-propionyl-CoA.

RECALL:

Propionyl CoA (it follows the metabolism of odd numbered fatty acids and gets converted to succinyl CoA)

ω-Oxidation

- **NOTE**: -we have something that is called " Omega oxidation "
  It is related to oxidation that starts from omega carbon which is the last carbon in the fatty acid chain. And that's why we say omega 3 omega 6, we are talking about methyl group.

- What happens is that there is a conversion of the methyl group to carboxyl group or addition of carboxyl group on the methyl group , the molecule is a dicarboxylic molecule which means that the fatty acid would have a carboxyl group on both ends. So oxidation can start from omega carbon same way as beta oxidation mono decarboxylic fatty acids .

- ω-Oxidation is a minor pathway of the SER
- It generates dicarboxylic acids.
- It is upregulated in certain conditions such as MCAD deficiency.

- RECAP:
- beta oxidation→mitochondria , peroxisome
- Alpha oxidation→ peroxisome
- Omega oxidation for dicarboxylic acids→ SER

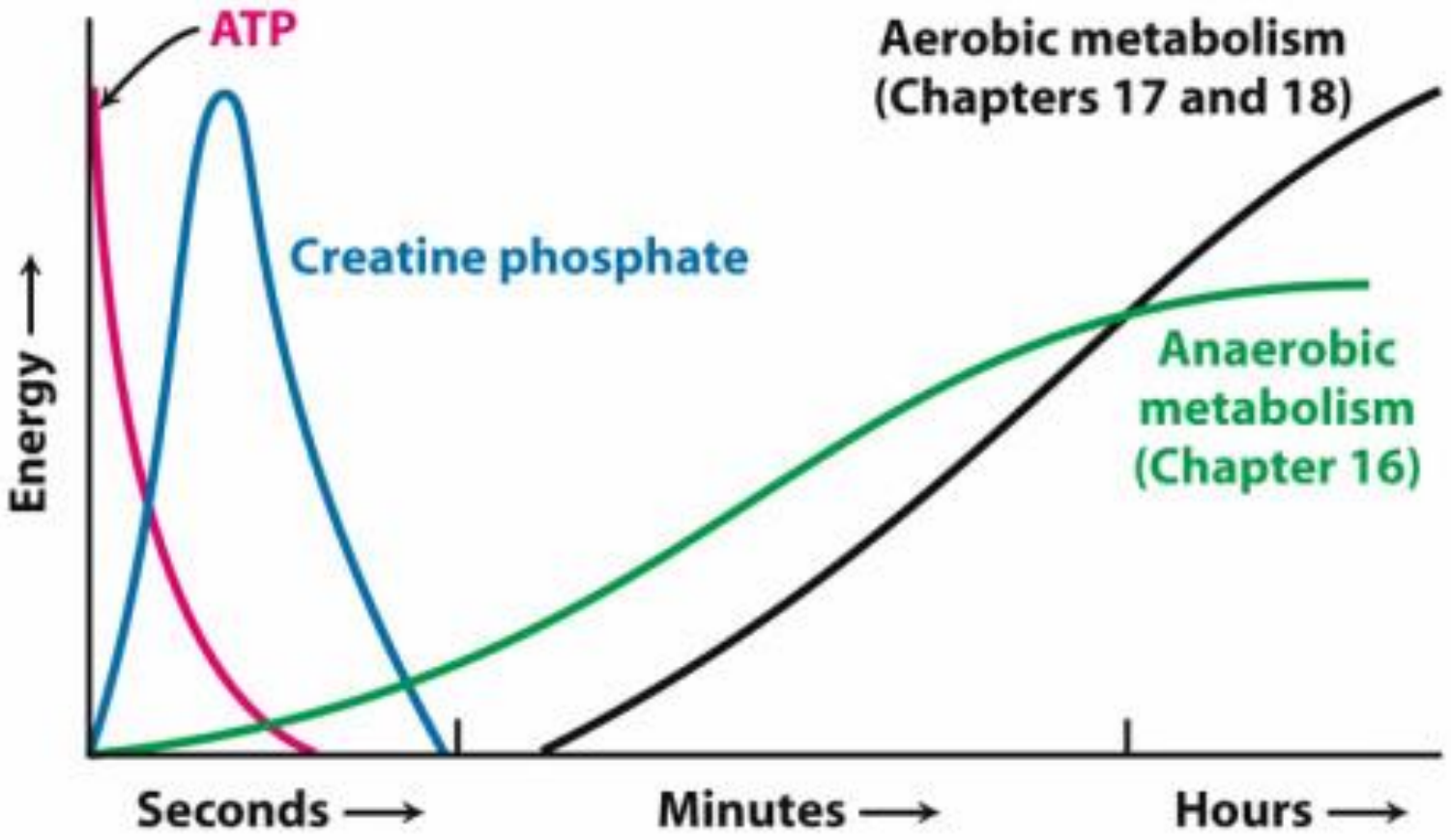
Lipids and energy

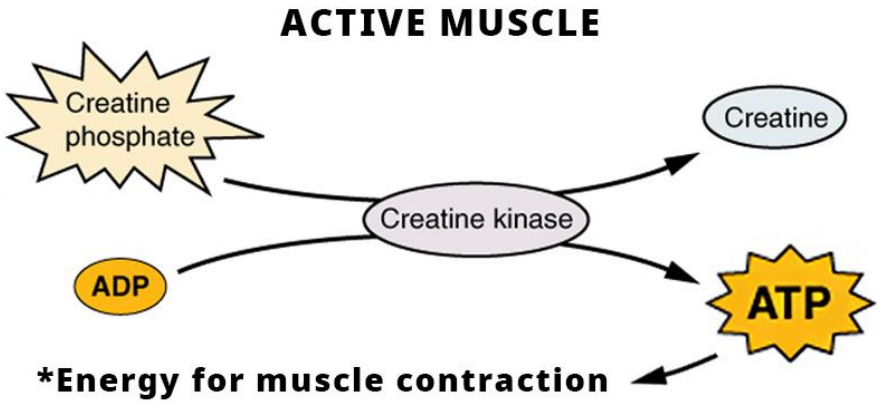
NOTE: comparison between lipids metabolism and carbohydrates metabolism in terms of reactions and amount of energy produced .

- TAGs are the body's major fuel storage reserve.
- The complete oxidation of fatty acids to CO_2 and H_2O generates 9 kcal/g of fat (as compared to 4 kcal/g protein or carbohydrate). Why?

	carbohydrates	lipids
Stored as...?	Starch - plants Glycogen - animals	Fats & oils (plants Fat (animals)
Long/short term storage?	Starch: long-term Gylcogen: short-term	Long term
Ease of digestion/ release of energy?	Easy to release energy	Harder to release energy (needs more oxygen)
Energy per gram?	17kJ/g	38kJ/g
Solubility in water? (and consequence)	Soluble	Not soluble
Use of oxygen in metabolism? (and consequence)	Needs less oxygen, useful for high-demand activity	Needs more oxygen, less efficient to release energy

Exercise and sources of energy





- **NOTE:**
- the body switches between the four sources of energy
- ATP → creatinine → anaerobic → aerobic

The complement in this slide:

in Case if you are running, actually muscles don't know how much you are going to run,

First source of energy : muscles do is using the **ATP molecules that are already found** in the muscles ,which ends in **5 seconds**

2nd source: creatine phosphate , muscles that have this molecule (creatine phosphate) gives phosphate to the ADP to become ATP which is used for muscle movement (mobility) which ends in **30 seconds** then...

3rd source: the body goes to the **Anaerobic metabolism** to produce some ATP (glycolysis) , for **minutes** . .... etatcal →etavoryp→esoculg

4th source : Aerobic metabolism (needs oxygen) to produce more ATP and the Aerobic metabolism is important for runners to run long distance for long time without exhausting and this is why runners exercise more and more to have more oxygen to make Aerobic metabolism to produce more ATP to run long distance for long time .

- **NOTE:**
- Short distance runners metabolism depends on creatinine and ATP
- Marathon runners exercises depends on breathing exercises. So they can get enough oxygen for aerobic metabolism. If not, lactatic acid will accumulate and they would have muscle cramps due to ATP deficiency.

- **Note:.**

  FA break down depends on the presence of oxygen , if there is no oxygen it goes into anaerobic metabolism which is basically Glucose to lactic acid , so if a Person wants to lose wight he/she has to make aerobic metabolism , so he must make exercise to have enough Oxygen in the body to make oxidation for FA and if he have lack of oxygen in the body or in the cells specifically we will go into anaerobic metabolism as we mentioned

Metabolism of lipids IV:

Ketone bodies

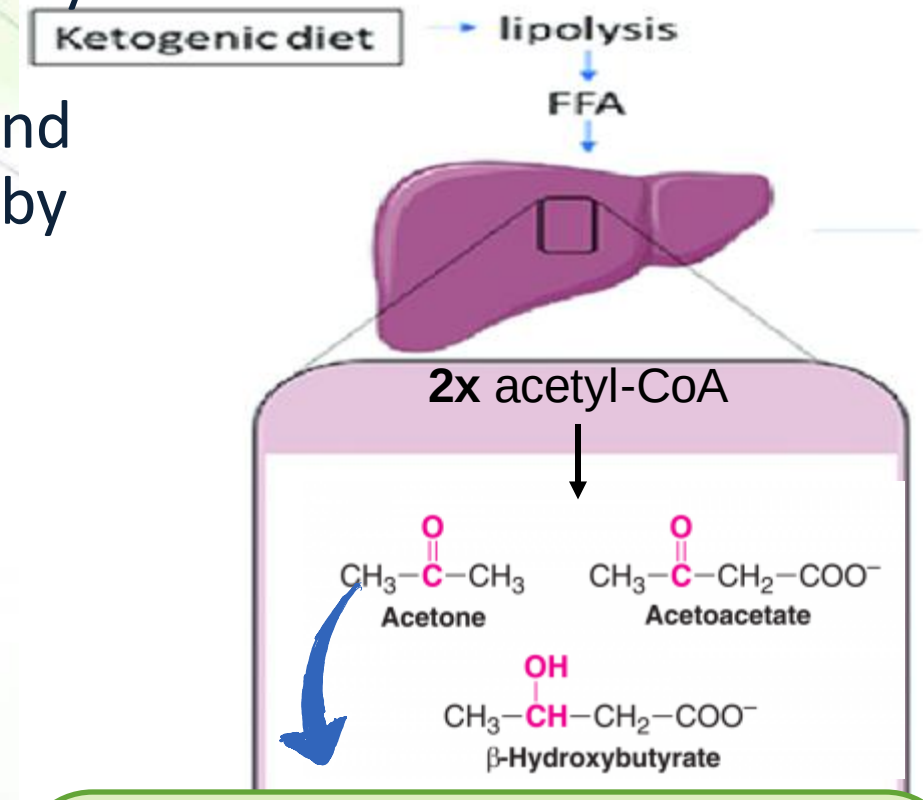
Prof. Mamoun Ahram

Resources

- This lecture
- Lippincott's Biochemistry, Ch. 16
- Diabetic, alcoholic and starvation ketoacidosis
  - https://derangedphysiology.com/main/cicm-primary-exam/required-reading/acid-base-physiology/acid-base-disturbances/Chapter%20617/diabetic-alcoholic-and-starvation-ketoacidosis
- Deep Dive – Alcoholic Ketoacidosis
  - https://aomcfoamed.com/2020/01/14/deep-dive-alcoholic-ketoacidosis/
- Alcoholic Ketoacidosis: Mind the Gap, Give Patients What They Need
  - https://www.emra.org/emresident/article/alcoholic-ketoacidosis/

What are ketone bodies?

- Ketone bodies are produced from condensation of 2 acetyl-CoA in the liver releasing it in blood stream for other tissues (e.g. muscle, heart, brain, …etc., but not RBC and liver) to use as a source of energy in case of **starvation** by re-forming acetyl CoA ,entering the krebs cycle.
- They are [1]acetoacetate,[2] 3-hydroxybutyrate (AKA β-hydroxybutyrate), and [3]acetone (volatile)
- Advantages:
  - Soluble (no carrier is needed)
  - Fast
  - Spare glucose



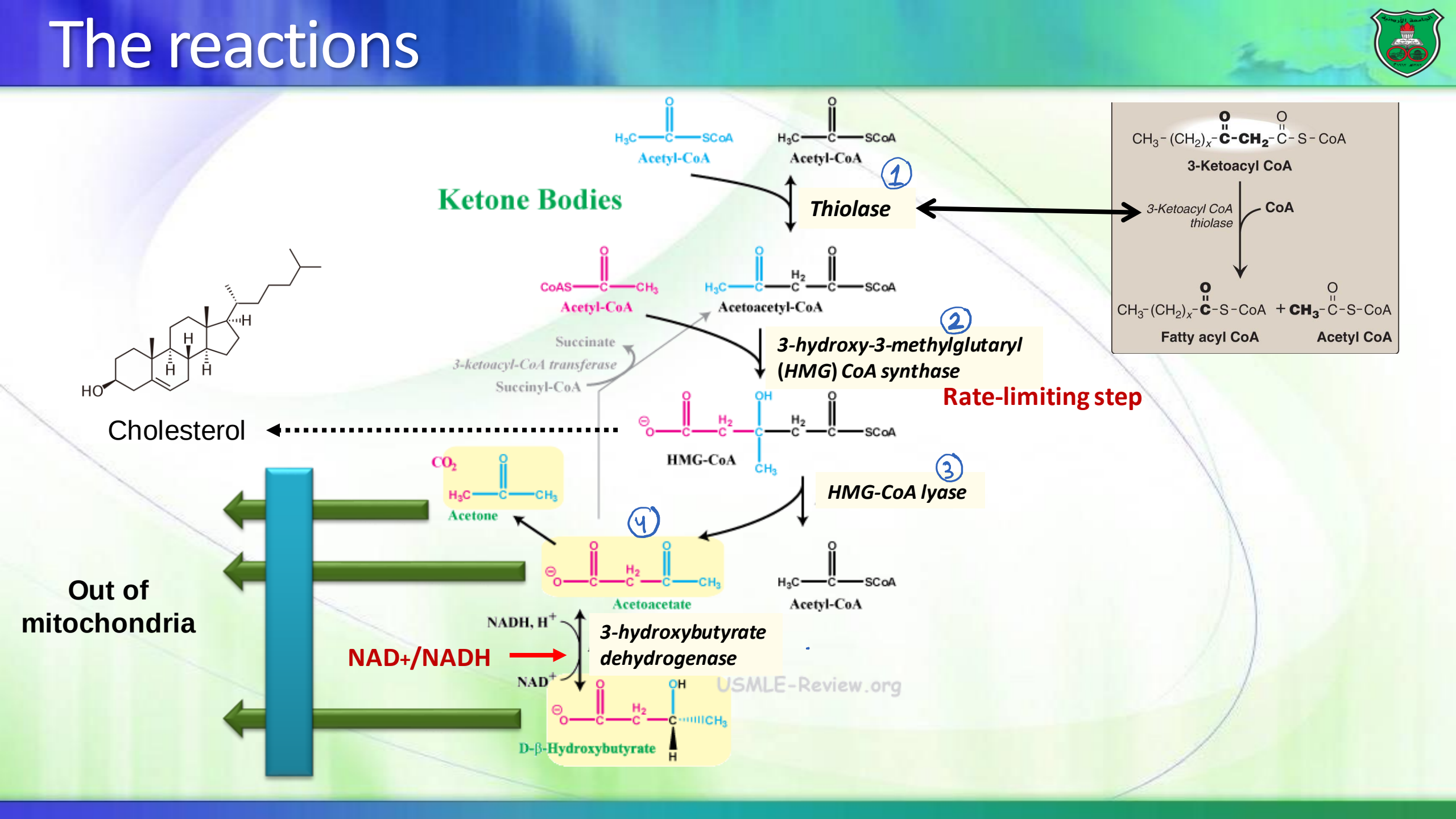
ketone bodies is responsible in generating

- **At wake-up time: 3-4% of energy**
- **Prolonged fasting: 30-40% of energy**

The longer the starvation period the greater the dependence on ketone bodies

NOTE: acetone is (volatile) Wich means it can be transported from the liver to the blood and then to the lung, then it is released causing acetone-like odor(fruity) in fasting and diabetic people

The reactions



- **The complement in this slide: REACTIONS in mitochondria**

1. 2 acetyl CoA which will **condense** by thiolase to form acetoacetyl Co-A(**reversible reaction** catalyzed by **Thiolase,** occurs in the **mitochondria matrix**)
2. another acetyl CoA enter and condense with acetoacetyl CoA by enzyme **3-hydroxy-3-methylglutaryl (HMG) CoA synthase** producing HMG CoA, which will produce ketone bodies (**rate limiting step**) or it could be converted to cholesterol.
3. release acetyl CoA by **HMG CoA lyase** to produce ketone bodies.
4. ketone bodies that are produced:

- acetoacetate
- acetone: it produced by breaking up of acetoacetate
- D-beta-hydroxybutyrate: it produced by conversion of acetoacetate. There ia an equilibrium between these two ketone bodies

These ketone bodies will leave mitochondria to the blood, they are hydrophilic doesn't need carrier molecule, acetone goes to lungs out of the body.
Acetoacetate and D-beta-hydroxybutyrate go to skeletal muscles, cardiac tissues and different tissues they are utilized

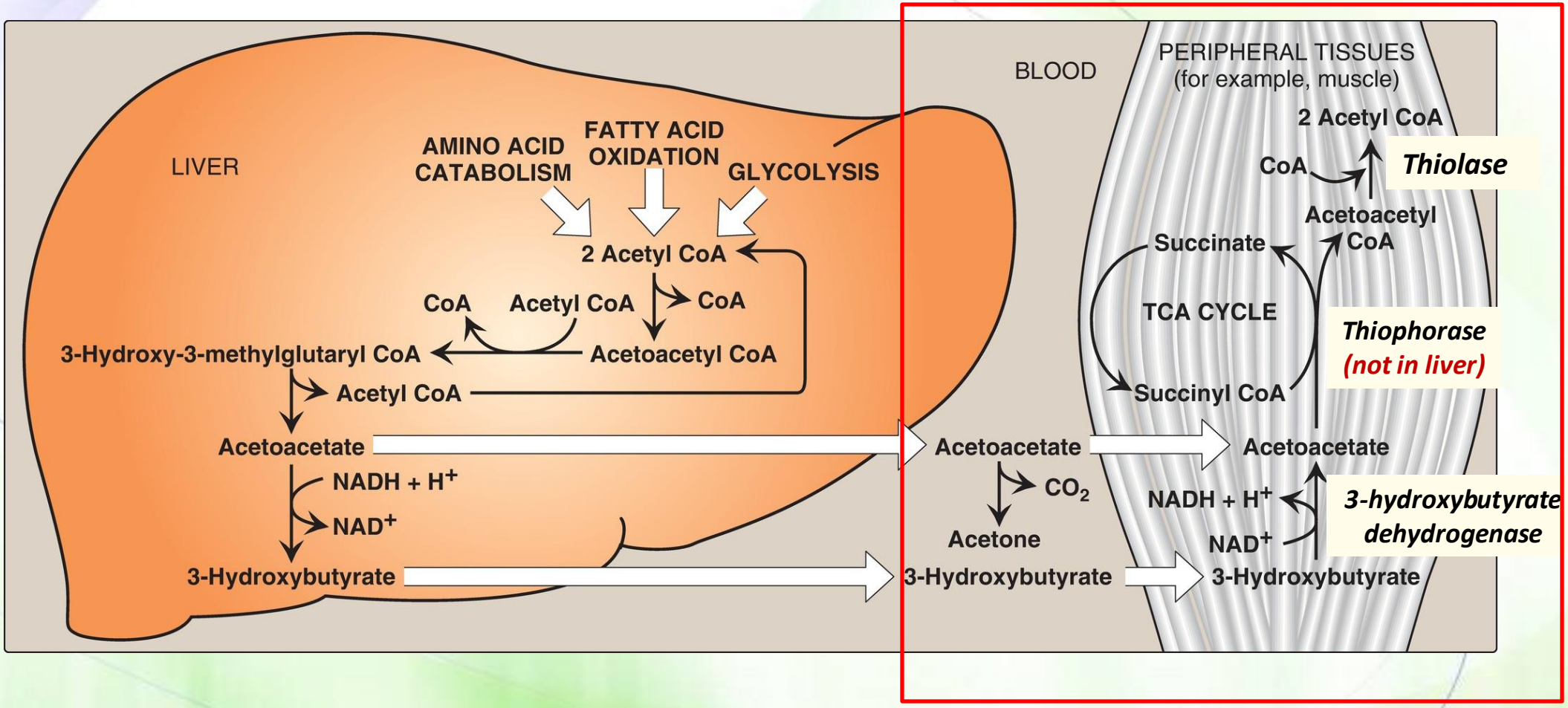
- **Note:** the reaction that happen by 3-hydroxy-3-methylglutaryl (HMG)CoA synthase is the rate limiting step in the process, it is very important because it is used in the formation of cholesterol, we will talk about that in details.

- Note that thiolase is the same enzyme that catalyse the breaking down of 3-ketoacyl into fatty acyl CoA in beta-oxidation.

- **NOTE:**
- Glutaryl=five carbon molecule. Methyl+glutaryl = six carbon molecule

- The energy is provided by the Coenzyme A ;due the energy rich bond between CoA and the molecule, so when it is hydrolyzed the energy would be used in condensation.

Use of ketone bodies



The complement in this slide:

How do the peripheral tissues utilize these ketone bodies?

So in liver again there is the formation of these ketone bodies, then they go to the blood and then

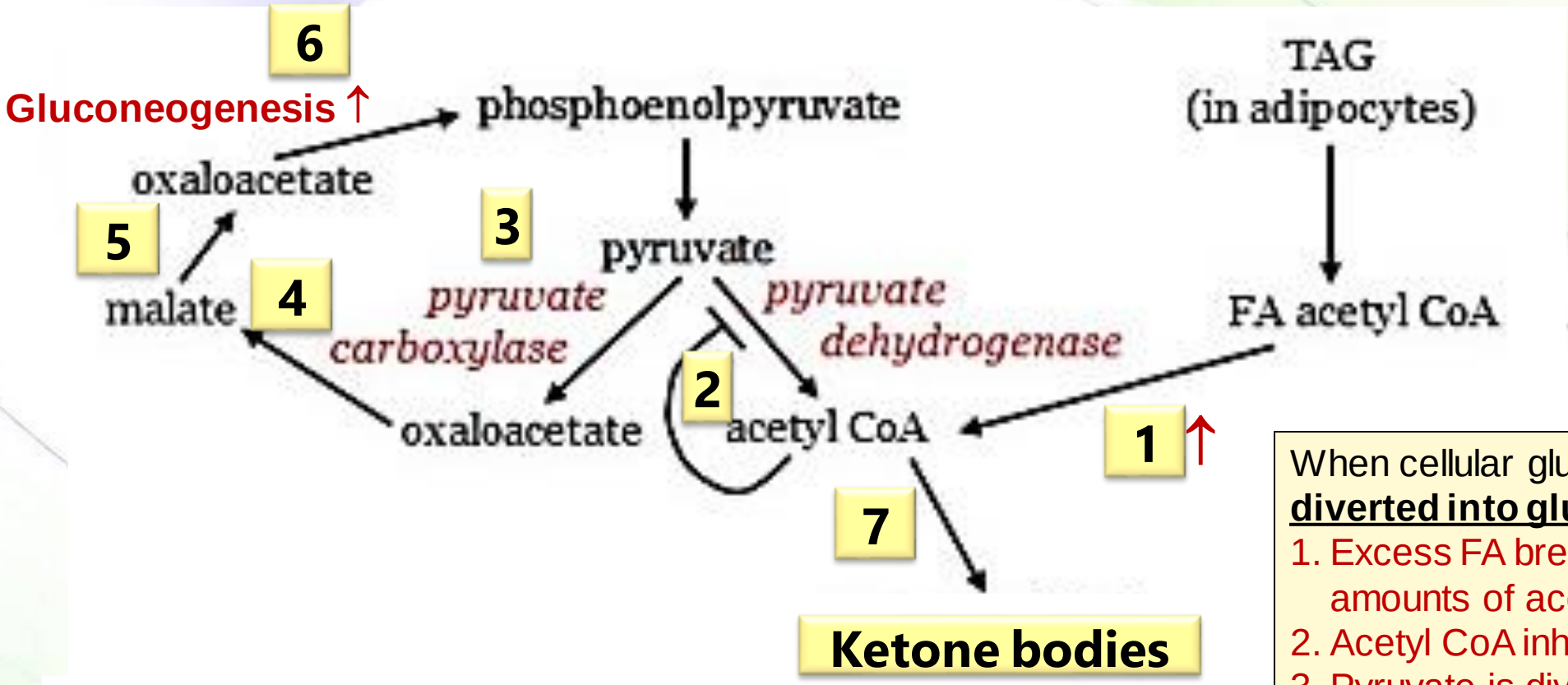
1. they are taken up by hydroxybutyrate it is converted into acetoacetate.
2. Acetoacetate → acetoacetyl CoA, this process is catalyzed by **Thiophorase**.
3. Now acetoacetyl CoA is converted into 2 Acetyl CoA by **Thiolase**
4. Acetyl CoA can get into the Kreb's cycle forming GTP, NADH and FADH2;ketone bodies are quick sources of energy

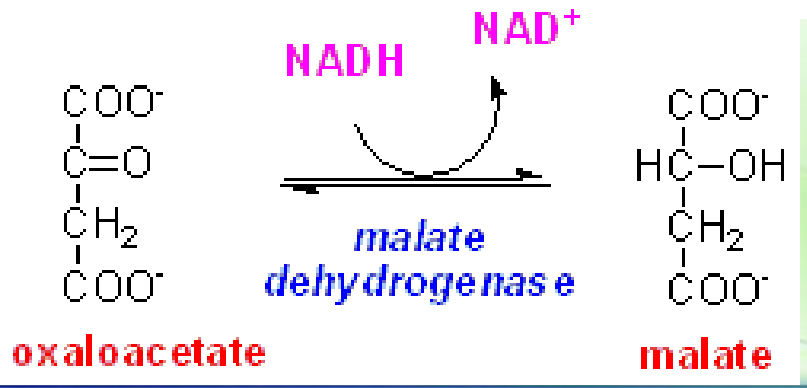
There are 2 types of cells that can NOT utilize ketone bodies:

1.RBCs, they don't have mitochondria.

2. liver, because liver cells don't have thiophorase enzyme, so they stick in the point where they have just acetoacetate and hydroxybutyrate.(exam question!!!!)

Under glucose-poor condition,





When cellular glucose is low, **oxaloacetate is diverted into gluconeogenesis**. In addition,

1. Excess FA breakdown produces large amounts of acetyl CoA.
2. Acetyl CoA inhibits pyruvate dehydrogenase.
3. Pyruvate is diverted toward oxaloacetate by pyruvate carboxylase.
4. Oxaloacetate is converted to malate,
5. and then back to oxaloacetate in the cytosol
6. Gluconeogenesis is activated and **oxaloacetate is depleted**.
7. **Acetyl CoA is diverted into ketogenesis**

The complement in this slide:

During extend starvation duration , glucose storage in the liver is depleted, so the body depends on fatty acids and ketone bodies.

So when there is no enough sugars → low [pyrovate]→ Gluconeogenesis is activated step 2 in the picture, pyruvate will convert to oxaloacetate.Then, oxaloacetate is converted to malate,step 7, which will leave mitochondria to cytosol and will be converted to oxaloacetate,step5, then oxaloacetate converts to PEP activating "gluconeogenesis".

Also, the body will depend on Fatty acids (earobic metabolism) Triacylglyceride→ FA acetyl coA → beta oxidation→ increase [acetyl CoA] ,can not enter Krebs cycle, because oxaloacetate is consumed in Gluconeogenesis→ SO THIS IS THE TIME TO FORM KETONE BODIES

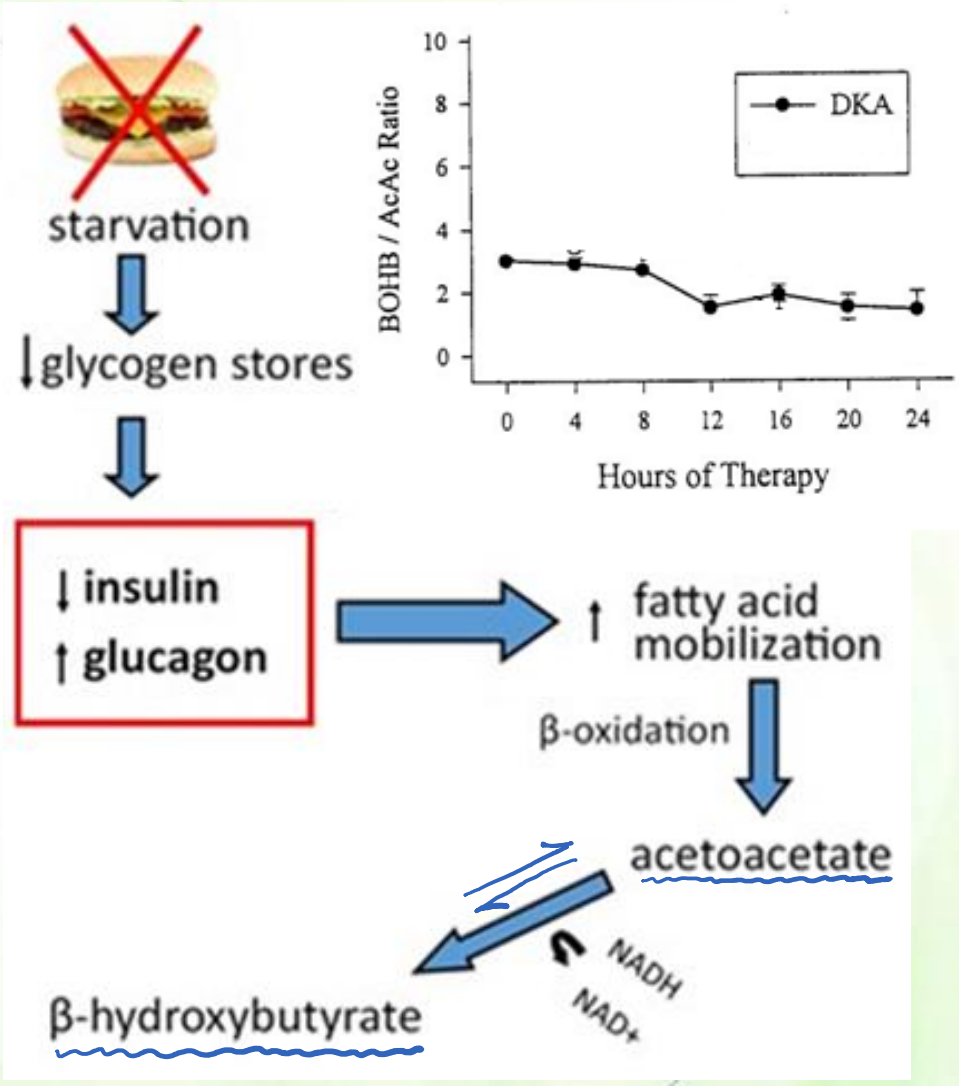
That what happens in **liver** during Starvation

Diabetic ketoacidosis

- **Normally,**
  - **Levels of ketone bodies: <3 mg/dl**
  - **NAD+:NADH is 10:1**
  - **3HB:AcAc is ~1:1**

NOTE: These numbers are important

- Under uncontrolled diabetes,
  - Levels of ketone bodies: 90 mg/dl and urinary excretion of ketone bodies may be 5,000 mg/24 hours.
- The end-results:
  - Acidemia (ketoacidosis)
  - Dehydration
  - Fruity odor of breath



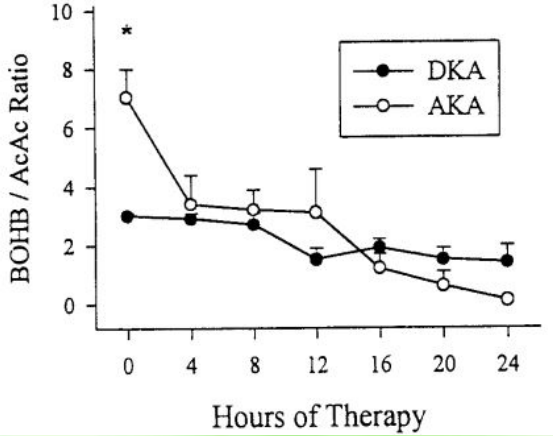
- **NOTE:the ratio hydroxybutyrate :** acetoacetate is 1:1 the reaction is in equilibrium , bidirectional. Conversion between acetoacetate and D-beta-hydroxybutyrate depends on NAD+/NADH,when we have high level of NADH the reaction will go towards D- beta-hydroxybutyrate, and vice versa.

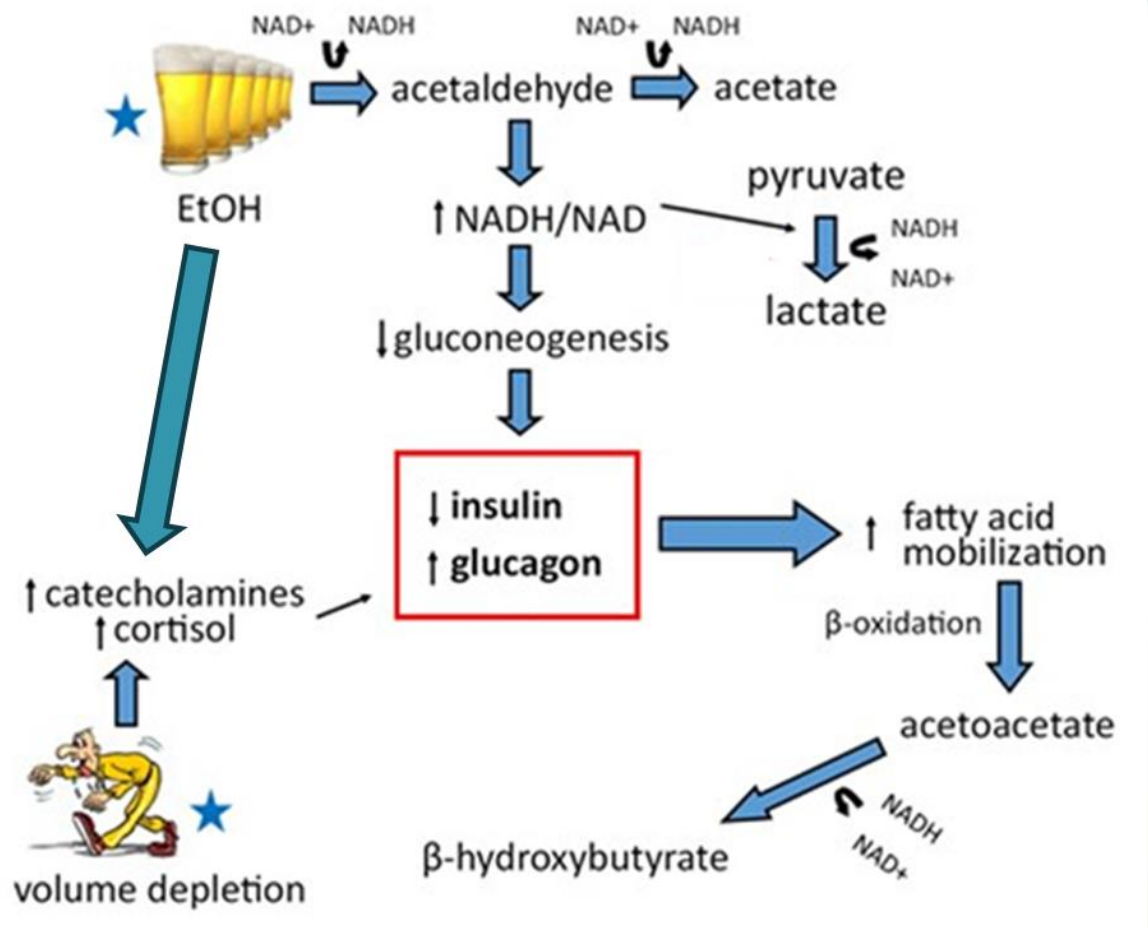
- **The complement in this slide:**

Normally,Levels of ketone bodies: <3 mg/dl , however, uncontrolled diabetes, the cells can't get glucose which seems like starvation, all the glucose in the blood → however, low [insulin] & high [glucagon] → high beta oxidation → increase ketone bodies level → diabetic ketoacidosis which could be fatal, as the PH of the blood will decrease dramatically..

Alcoholic ketoacidosis

- There is also,
  - Acidemia (ketoacidosis)
- But,
  - 3HB:Ac is ~3:1
    - *The ratio gets back to 1:1 after a few hours*
  - *Gluconeogenesis is suppressed.*
  - *Pyruvate is converted to lactate leading to hypovolemia, heart failure, and sepsis.*





Alcoholic ketoacidosis

The complement in this slide:

- Consuming excessive alcohol elevates ketone bodies, leading to alcoholic acidosis.

[1]a.This is triggered by ethanol's stimulation of catecholamines and cortisol,[1]b. in addition to starvation and dehydration (as intoxicated individuals often neglect food and water intake).

[2]Catecholamines and cortisol further activate glucagon, promoting beta oxidation and raising ketone levels, ultimately causing ketoacidosis.

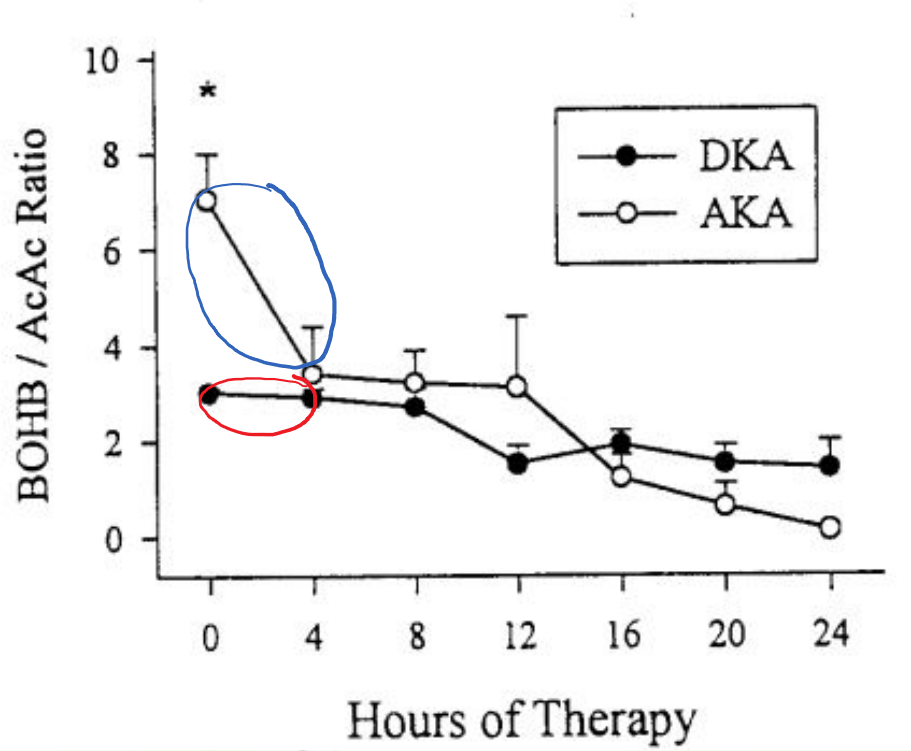
NOTE:

It's important to note that alcoholic and diabetic ketoacidosis are some how different.

In alcoholic ketoacidosis : normally the NAD+/ NADH ratio is 10:1 and 3HB:AcAc is approximately 1:1, alcoholic people get rid of ethanol by converting it into acetyl CoA -which will be converted toacetete,this reaction produces NADH, so the equilibrium will shift towards 3HB causing the increase of the 3HB: AcAc However, after few hours ratio will go back to normal

3HB:Ac is higher than 1:1 it could be 3:1 or 5: 1and so on,

the ratio of NAD+:NADH could be 1:1



NOTE:

Diabitic kitoacidosis (DKA) 3HB :AcAc ~1:1 constant

Alcoholic ketoacidosis (AKA) 3HB:AcAc ~ higher than 1:1

Eventually after few hours it will return to 1:1

NOTE:

Alcoholic ketoacidosis (AKA) produces less acitone ketone bodies than Diabitic kitoacidosis (DKA)

That why drunk people dont have fruity odor incontrast to Diabitic people