We have said before that fatty acids are oxidized by B-oxidation pathway, in which B-carbon is oxidized into a carbonyl carbon (ketone group), into two carbons unit (acetyl CoA). It's cleaved in each cycle of B-oxidation. This process occurs in mitochondria in which fatty acids are completely converted into acetyl CoA with generation of NADH and FADH2 in each cycle of this pathway. Remember that the number of cycles= (n/2) -1 "n= number of carbons in the fatty acid".

*Fatty acids differ according to their chain length:

- Short-chain fatty acids which have 4-6 carbons.
- Medium-chain fatty acids which have 8-10 carbons.
- Long-chain fatty acids which have 16-18 carbons commonly.

#B-oxidation in peroxisomes:

Very long- chain fatty acids (VLCFAS) of 20 carbons or more which cannot be oxidized in mitochondria, they undergo B-oxidation in peroxisomes, in a mechanism similar to that in mitochondria but with different enzymes because they are very long fatty acids, it occurs by the following steps:

1) Dehydrogenation of VLCFA: removal of two hydrogen atoms, one atom from B-carbon and another atom from a-carbon, so double bond is formed. It is catalyzed by ((FAD-containing acyl CoA oxidase)), which has a tightly bound FAD molecule which is converted to FADH2 upon dehydrogenation of VLCFA >>> how this FADH2 molecule is oxidized?! By transferring e's to o2 and reducing it into H2O or H2O2 rather than transferring these e's into another electron carrier molecules, why?? Because there's no ETC in peroxisomes as in mitochondria to reoxidise the electron carriers (FADH2, NADH).

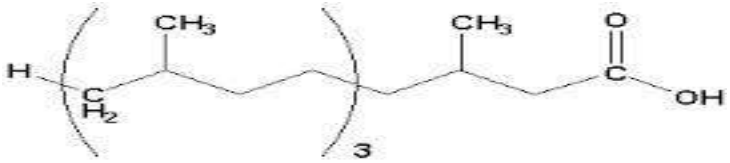
**This step is similar to the dehydrogenation step in B-oxidation in the mitochondria which is catalyzed by a different enzyme ((acyl CoA dehydrogenase)), but both enzymes are oxidoreductases. Then what is the difference?? electron are added to electron carriers(FAD, NAD+,NADP+) not to an oxygen molecule , BECAUSE THERE'S ETC in mitochondria to reoxidise these electron carriers again by Co-Q or other complexes .

Now... does this peroxisomal oxidation continue all the way until the fatty acid is converted completely to acetyl Co-A??

>>No , it's only until the chain is decreased in length to 16 or 18 carbons, then the fatty acid will be transported again to mitochondria to continue oxidation pathway.

#a-oxidation in peroxisomes:

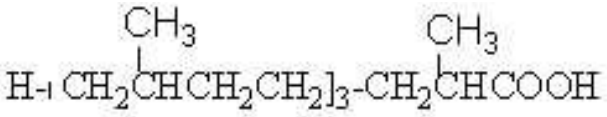
It's occurred by modifications of the original pathway, let's take an example of a fatty acid with modifications which is the phytanic acid:



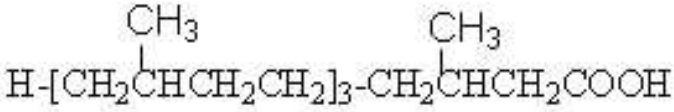
Phytanic Acid

It contains 4 methyl groups branched from the main chain, the first methyl group is on B-carbon, so it cannot be oxidized to a carboxylic acid "it already has a methyl group". How does the cell handle such a situation?!

1) By introduction of OH group on a-carbon, it's now hydroxylated.
2) Fatty acid undergoes oxidative decarboxylation: oxidation by converting OH into COOH, and decarboxylation by removing COOH as CO2 "COOH which already exist in the fatty acid".
   >>> Now the branch is on a- carbon and B-carbon can be oxidized. Just for explanation: in this picture we can see the new fatty acid (pristanic acid*not required to memorize*) which can undergo oxidation as usual.



Pristanic acid



Phytanic acid

*Where can we find this kind of fatty acids in diets??

>>> It's found in food derived from leafy vegetables, this fatty acid is associated with chlorophyll which is found in leaves, remember its called phytanic acid.

It's a minor constituent, and then what is the significant of this pathway?! In fact, it has a clinical significance, in genetic disease where the enzyme which adds OH is deficient or missing then we can't precede the oxidation of this fatty acid as usual, so it will accumulate in brain and nerves causing death of nervous system cells which leads to CNS manifestations. It can cause mental retardation. The management in this case is to remove this type of fatty acids from diet and avoid eating food containing it, because we can't replace the enzyme or take bills of it. Such a disease will be discovered later on when an irreversible damage is occurred but we can still protect the second and the third children if diagnosed early enough.

#ketone bodies:

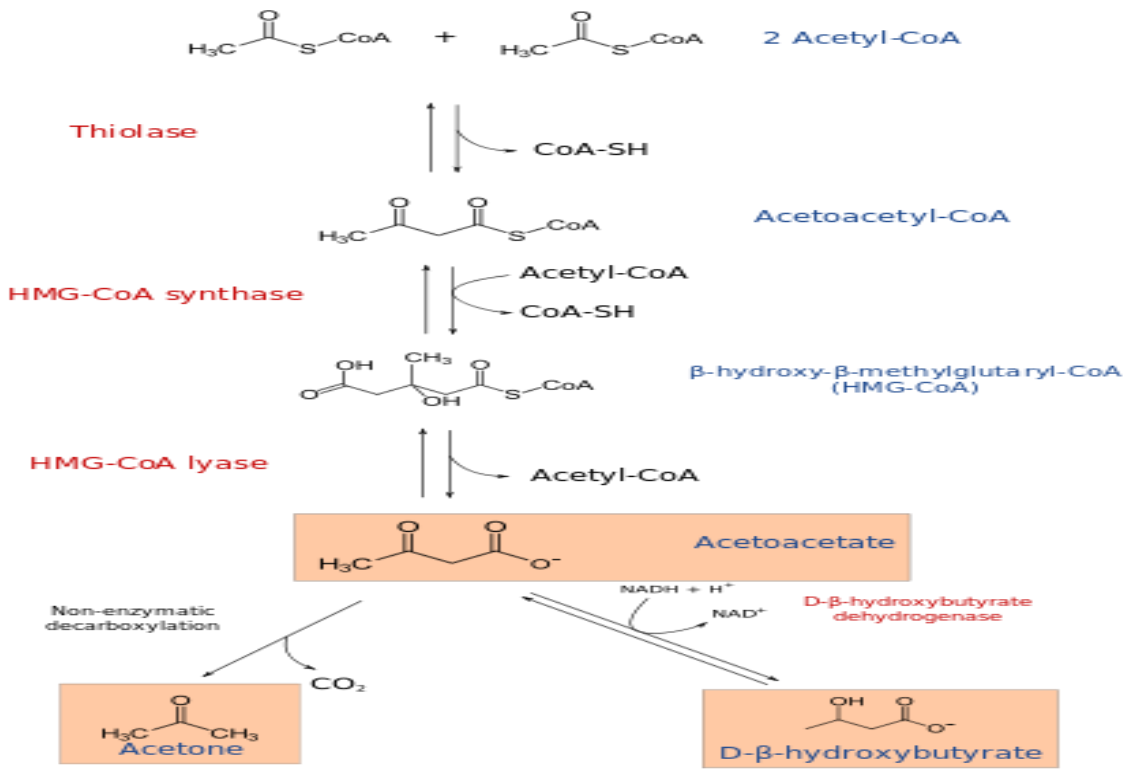
They are synthesized in liver from acetyl CoA as a starting material (precursor). They are3 compounds: acetoacetate, 3-hydroxybutyrate, and acetone. They are synthesized all the time but at a low rate in the normal conditions. During fasting" when ketone bodies is needed to provide energy "and uncontrolled diabetes they are synthesized at a high rate. We mean by uncontrolled diabetes the one in which patients don't take the treatment, especially if the patient was young, and if he has diabetes type 1 which depend on insulin as a treatment, if he doesn't take insulin then he has uncontrolled diabetes.

***Pathways for synthesis:**

1) When fatty acyl CoA doesn't complete the pathway to its end, it forms acetoacetyl CoA. It occurs in mitochondrial matrix.

2) By condensation of two acetyl CoA molecules forming acetoacetyl CoA by reversal of the thiolase reaction (the very last reaction in degradation of a fatty acid which cleaves the last two acetyl CoA).

after forming acetoacetyl CoA which is **first step** in synthesis of ketone bodies, the **second step** is combining a third molecule of acetyl CoA with acetoacetyl CoA to produce HMG CoA by ((HMG CoA synthase)) .we get 3-hydroxy-3-methylglutaryl CoA (HMG CoA). it's a 5-carbon fatty acid which have 2 carboxylic groups (exactly as glutamate and a-ketogluterate which we've taken before) from which it took the "glutaryl" part of the name. It also has two branches on carbon 3 "methyl and hydroxyl" which explains the first part of the name. We have to memorize this compound.



The **third step** is cleavage of acetyl CoA *but it's not reversal of the* previous step because we don't cleave the acetyl CoA which is introduced in the previous step, we cleave the acetyl CoA that was already in the acetoacetyl CoA compound. Which means that there's an acetyl CoA which is condensed and another one which is removed. This step is catalyzed by ((HMG CoA lyase)). It produces acetoacetate" the first ketone body" which is an acidic molecule.

*how can we describe the structural features of acetoacetate compound?!

>>> By determining the functional groups and number of carbons: it's a 4-carbon molecule which has a carboxyl group and a ketone group on carbon 3. We can recognize its common name by splitting it into two parts; each one represents an acetic acid, so it constitutes of two acetic acids together which is called acetoacetate.

So the net reaction:

2 acetyl CoA ----------→ acetoacetate + 2 CoA

""The substrate which is produced in one step and then used in the next step, like the third acetyl CoA in this pathway, is removed from the net reaction, they both cancel each other. ""

acetoacetate then can either form :

1) 3-hydroxybutyrate by reduction of ketone group in acetoacetate by ((NADH requiring enzyme)) into OH group. It's is also an acidic molecule.
2) Or it can be spontaneously decarboxylated to form acetone by losing the carboxyl group as CO2. Spontaneously means it's not catalyzed by an enzyme. Then how it can lose the carboxyl group easily without an enzyme?!
   >>> Due to the presence of a ketone group which make it easy for the molecule to lose COOH. If the molecule was butyric acid (carboxylic acid without a ketone group) then it won't lose COOH spontaneously.

Remember that we consider all those three compounds as ketone bodies.

*the purpose of synthesis of ketone bodies:

- **In liver**: is to get CoA not to produce acetoacetate which is a waste product for liver, it'll be eliminated to blood.
- **In tissues**: they provide energy to the peripheral tissues during fasting like muscles and brain as we'll see later.

*Why do we need to produce CoA?!

>>> in the pathway of B-oxidation of palmitic acid (16 c) we need 8 CoA , 7 NAD+ , and 7 FAD, with production on net 8 acetyl CoA. The fate of both NADH and FADH2 "which is formed upon reduction of

both NAD+ and FAD" is to be reoxidized again in ETC to give energy ,which means that B-oxidation by itself is a source of energy by feeding into both ETC and TCA cycle by acetyl CoA. the fate of acetyl CoA is entering TCA cycle to be combined with OAA to produce citrate as we all know, then in this first step of TCA cycle, CoA is produced. This CoA is going to be used in continuation of B-oxidation. Because the pool of CoA molecules is limited and it's found only in a catalytic amount, then we should regenerate it to use it again.

 # During fasting after the third or fourth day:

As we knew increasing numbers of acetyl CoA during fasting inhibits pyruvate DH, so it inhibits TCA cycle, and activates pyruvate carboxylase which starts gluconeogenesis. Gluconeogenesis starts from OAA so its level decreased but be careful: the decreasing level of OAA is due to consuming it in large amounts in gluconeogenesis, not due to inhibition of TCA cycle. OAA is not produced by TCA cycle; it produces OAA at the end but then consuming it at the beginning of the cycle. So without it there's no TCA cycle, and when its level decreases then TCA cycle will stop "OAA is only found at catalytic amount".

Here is the problem... When TCA cycle stops during fasting, then from where can we synthesize CoA?!

>>> We can get it from ketone bodies synthesis in liver; they go then to plasma, finally to tissues to be used there.

So the purpose of ketone bodies synthesis is to regenerate CoA which allows B-oxidation of fatty acids to continue. We can also get some energy by this pathway which is better than nothing. So if you're asked to calculate the number of ATP molecules which is produced by liver during fasting, then you should only calculate those produced from fatty acids. Energy is only produced by B-oxidation not by TCA cycle during fasting.

***Lipolysis** is enhanced by glucagon, norepinephrine and epinephrine through hormone sensitive lipases (HSL). So during fasting: insulin level is low but glucagon level is high, which enhances production of fatty acids by lipolysis. This will increase the hepatic output of ketone bodies, and when there's an overproduction of these molecules then the PH will decrease" remember that two of the ketone bodies are acids: acetoacetate and 3-hydroxybutyrate". So lung starts to compensate, increasing O2 by breathing faster, making alkalosis thus increasing PH.

*Ketone bodies are excreted in urine as sodium salts (Na hydroxy butarate) not as hydroxybutarate alone, it should be associated with Na. when increasing excretions in urine as Na salts due to increasing production of ketone bodies ,then loss of Na ions is followed by loss of water which leads to dehydration.

*** In uncontrolled diabetes** liver makes gluconeogenesis even though the blood glucose level is high. Why? Because of the high level of glucagon and the low level of insulin. Liver doesn't sense glucose level in blood but senses the amount of glucagon and insulin, so it makes glucose even though the blood glucose is high .Due to this high level of glucose in blood then our Body starts to get rid of the excessive

glucose through urination, which will lead to dehydration and thirst in diabetics. If this condition persists it will lead to unconsciousness and coma, which is a medical emergency.

**before start using insulin as a therapeutic replacement in diabetics, type I diabetes was fatal due to ketoacidosis. The patient is seen very dehydrated , has high levels of ketone bodies and glucose , very dry tongue , might become unconscious and finally there is a smell of acetone in his breath due to high levels of ketones . This ACETONE BREATH is used in diagnosis of type I diabetes.

**before giving insulin, we should start with rehydration.

using of ketone bodies as an energy source by muscles:

When 3-hydroxybuterate goes from blood to muscles it is converted into Acetoacetate that can be used as a source of energy.

1) The first step is adding CoA to Acetoacetate becoming Acetoacetyl CoA. CoA is taken from SuccinylCoA converting it to Succinate.
2) Acetoacetyl CoA is converted into Acetyl-coA which is a source of energy in citric acid cycle.

*from where do we get OAA to start TCA cycle? We said it's low in liver! Actually OAA is only low in liver, but in muscles, level of OAA is still sufficient to keep the cycle going, because muscles don't make glucose by gluconeogenesis as liver.

#using of ketone bodies as an energy source by brain:

In normal situations Brain only uses glucose for energy, but in Prolonged fasting Brain adapts to use ketone bodies for energy.

In the 2nd and 3rd days of fasting the source of energy is gluconeogenesis; amino acids that are obtained by turnover of muscle proteins (as albumin, prealbumin, and globulin) are used in gluconeogenesis to produce glucose. But this can't stay so long, so Brain starts using Ketone bodies for energy and thus muscle proteins are spared. We still use them for gluconeogenesis but at a much lower rate. This would make glucose level above zero, and the major source is the ketone bodies, which makes their level in blood greatly increased in the fourth day. Also, the rate of consuming fatty acids is twice as much.

The difference in metabolism in brain in the 3^{rd} day and the 40^{th} day appears clearly in:

- Glucose which was consumed in a rate equals to 100 g/day, but it decreases by 60% to be only 40g/day, but still it's not zero.
- Ketone bodies were consumed in a rate equals to 50 g/day, but it increases to be 100g/day.
- Muscle proteins degradation, which is very important, was in a rate equals to 75 g/day, but it decreases to be nearly the quarter (20 g/day).

All of these changes are adaptation for survival.

Synthesis of fatty acids:

Requires:

1) **Carbon source**: which is *acetyl CoA,* we get it from pyruvate by ((pyruvate DH)) not from B-oxidation. This means that if you eat excess carbohydrates then they'll be converted to fats but fats won't convert to carbohydrates.
2) **Reducing power:** which is NADPH produced by PPP (pentose phosphate pathway). Fatty acids are more reduced than acetyl CoA.
3) **Energy input:** from *ATP*, why do we need ATP??
   We said before that the pathway ((fatty acid---------→ acetyl CoA)) has an overall negative delta G, so it's an exergonic, irreversible pathway.
   Accordingly, the pathway ((acetyl CoA ---------→ fatty acid)) has a positive delta G, but by coupling with a number of ATP molecules, it becomes with an overall negative delta G.
   This confirms the fact that degradation and synthesis of fatty acid cannot happen at the same time, but they are somewhat similar, how?

** In the pathway of degradation ((fatty acid-------→ acetyl CoA)) the sequence of reactions is: oxidation, hydration, oxidation, and then thiolysis.

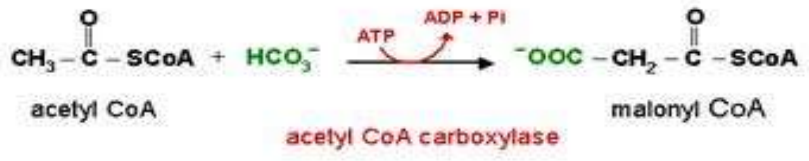
But in the pathway of synthesis ((acyl CoA+ malonyl CoA -------→ fatty acid)) the sequence of reactions is: condensation, reduction, dehydration, and then reduction. They are the obverse of those in the degradation pathway.

**the synthesis pathway occurs in the cytosol, while the degradation pathway occurs in mitochondria.

#how to get malonyl CoA??

malonyl CoA (dicarboxylic acid) is an intermediate donor for acetyl CoA in fatty acid synthesis.

It's synthesized from acetyl CoA by addition of CO2 (bicarbonate) to acetyl CoA which is catalyzed by a biotin- containing enzyme ((acetyl CoA carboxylase. It's an endergonic reaction which requires ATP.



** This is the most important step in this pathway; it's a committed step which means that the product of this step is only used for fatty acid synthesis. So, it should be regulated; it consumes energy and it's irreversible. This reaction is called Saleh Alwakel reaction; as it's discovered by him.

#the remaining series of reactions are catalyzed by fatty acid synthase:

- It's a complex multifunctional enzyme with 7 domains of seven catalytic activities; it catalyzes a multistep reaction, which is more efficient.
- It's a dimer of two identical substrates (chains), they both come together to make an active enzyme, if they're separated then it's no longer active.
- One of the catalytic activities of this enzyme is condensing enzyme (CE), it has SH group which makes a thioester bond.
- One domain of the fatty acid synthesis is linked to phosphopantetheine, a derivative of vitamin pantothenic acid; it has a reactive SH group. It is a component of CoA and a carrier for acyl groups at it's SH group. When this this acyl carrier group (phosphopantotheine) is a part of a protein domain then it's known as an acyl carrier protein (ACP). So, ACP is used to refer to the phosphopantetheine-containing domain of fatty acid synthase. It carries intermediates (acyl, acetyl, and malonyl) during synthesis catalysis; it's like a large CoA.
- CoA is the carrier of intermediates during fatty acid degradation.

#overview:

1) The acetyl group which is linked with CE (acetyl-CE) and malonyl ACP (the malonyl which is linked to ACP) are condensed together to form ketoacyl ACP and CO2 is released, which CO2?! The one which is added by acetyl CoA carboxylase. This CO2 has been added to acetyl CoA to raise the energy level of acetyl making it malonyl (donor of acetyl group) then COOH is released again as CO2 so this is the role of CO2.
2) Then the sequence of reactions: reduction, dehydration, and reduction happens making acyl ACP. This means that the ketone group is reduced to CH2.
3) Transfer of acyl ACP to the CE, and starting another cycle so the synthesis cycle is repeated again and again until acetyl CoA is converted into fatty acid with 16 carbons.

****fatty acids have an even number of carbons because they are synthesized by repeating the addition of 2 carbons at a time, two after two, until it's 16- carbon length.

يقول الشّيخ الغزاليّ - رحمهُ الله - :

"" أنّنا لَوْ واصَلنا الليلَ بالنّهارِ دأباً،،، ثمّ حُرِمْنا عِنايَةِ السَّماءِ ... فلنْ نحْصدَ من تعبنا إلّا بَوارًا ""

خديجة أبوزيد