Biochemistry

Pavel Pestryakov

Novosibirsk State University
Institute of chemical biology and fundamental medicine, SB RAS

+7(913)892-3045
Pavel.pestryakov@niboch.nsc.ru

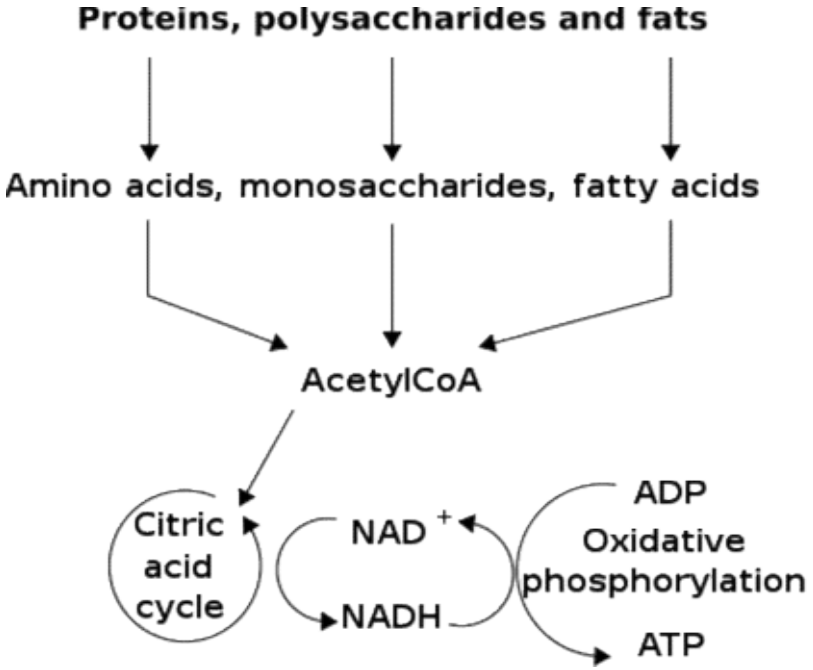
Bioenergetics

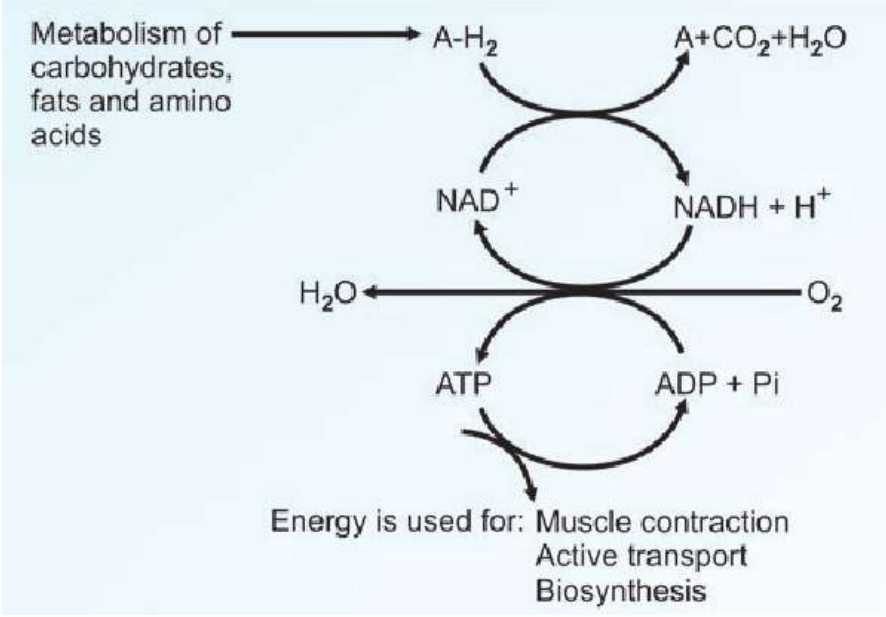
Biological oxidation and electron transfer chain

Metabolism

1. Primary – digestion of macromolecular food into monomers
2. Secondary – catabolization of monomers/derivatives –
   generation of O2 and reduced equivalents NADH, FADH2 (majorly in TCA)

3. Tertiary (**internal respiration**) – reduced equivalents go to electron transfer chain (ETC) and release energy, which is captured into ATP





Bioenergetics

Biological oxidation and electron transfer chain

BIOLOGICAL OXIDATION

The transfer of electrons from the reduced coenzymes through the respiratory chain to oxygen is known as biological oxidation. Energy released during this process is trapped as ATP. This coupling of oxidation with phosphorylation is called oxidative phosphorylation. In the body, this oxidation is carried out by successive steps of dehydrogenations.

$$\tfrac{1}{2}\,O_2 + 2H^+ \rightarrow H_2O \;(E^{\circ}_0 = +0.82)$$
$$NAD^+ + H^+ + 2e \rightarrow NADH \;(E^{\circ}_0 = -0.32)$$

Sums up to

$$\tfrac{1}{2}\,O_2 + NADH + H^+ \rightarrow H_2O + NAD^+;\; E^{\circ}_0 = 1.14\ V$$

Chemically speaking there are two Ox-Red pairs (2 compounds that differ in number of electrons – more electrons – reduced, less electrons – oxidated).

Electrons are transferred between these two pairs. However the energy which can be released is so great that it body cannot utilize it in one chunk. Process goes in steps via ETC and energy is captured by high energy bonds in ATP.

Bioenergetics

Biological oxidation and electron transfer chain: ENZYMES

OXIDOREDUCTASES

1. **Oxidases – enzymes remove hydrogens from substrates to O2 ONLY! (acceptor of hydrogen) with the result of H2O. Ex:** *cytochrome oxidase*

   2AH2 + O2 -> 2A +2H2O

2. **Aerobic dehydrogenases - catalyze the removal of hydrogen from a substrate, but oxygen CAN act as the acceptor with the result (usually) – H2O2 (peroxide) Ex:** flavoproteins Laminoacid-oxidase. (prostetic groups FMN, FAD)

   AH2 + O2 -> A + H2O2

3. **Anaerobic dehydrogenases - catalyze the removal of hydrogen from a substrate, but oxygen CANNOT act as the acceptor. So they use coenzymes as H-acceptors.**
   a) NAD+ - linked (nicotinic acid derivative – vitamin B class) Ex: Isocitrate dehydrogenase, pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase

      AH2 + NAD+ -> A + NADH + H+

   b) NADP+ - linked (resulting NADPH cannot be utilized to ATP, but used in other ways of biosynthesis)
   c) FAD – linked (FAD accepts both H, unlike NAD+) Ex: Succinate dehydrogenase ..

Bioenergetics

Biological oxidation and electron transfer chain: ENZYMES

OXIDOREDUCTASES

4. Hydroperoxidases (peroxidase)

AH2 + H2O2 -> A + 2H2O

5. Oxygenases (mixed function oxidases) – one atom of O2 is incorporated into substrate, second reduced to water. Ex: microsomal P-450 monooxygenase and drug metabolism.

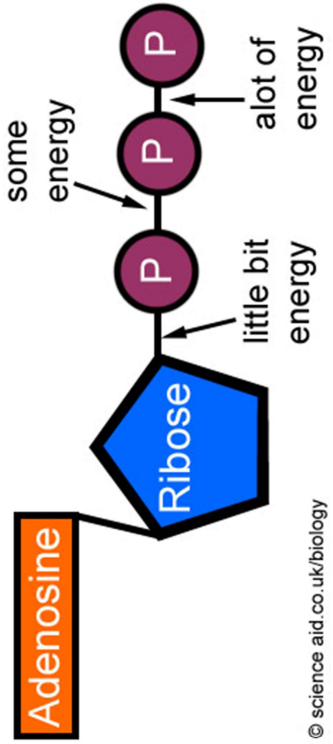
AH2 + O2 + BH -> A + H2O + B-OH

Bioenergetics

Biological oxidation and electron transfer chain: HIGH ENERGY COMPOUNDS

ATP – universal currency

usually energy is released during ATP -> ADP hydrolysis
1/3 of ATP pool is used for Na/K gradient pumps (!)
other – biosynthesis/kinesis/contractions/phosphorylation etc



Chemical FATE of ATP:

- *Glucose + ATP = glucose-6-phosphate + ADP (ATP hydrolysis + phosphorylation)*
- *Pyruvate + CO2 + ATP = oxaloacetate (OAA) + ADP + phosphate (ATP hydrolysis + release of phosphate)*
- *Fatty acid + CoA + ATP = Fatty Acyl-CoA + AMP + diphosphate (hydrolysis + release of ppi)*
- *Ribose-5-phosphate + ATP = Phosphorybosyl pyrophosphate + AMP (hydrolysis + incorporation of pyrophosphate (ppi))*
- *Amino acid + ATP = Amino acyl adenylate + ppi (hydrolysis + incorporation of AMP)*
- *Methionine + ATP = S-adenosyl methionine + ppi + pi (hydrolysis + incorporation of adenosyl)*

rate of consumption of ATP – 3 molecules per second (1.5 kg/day)

Bioenergetics

Biological oxidation and electron transfer chain:

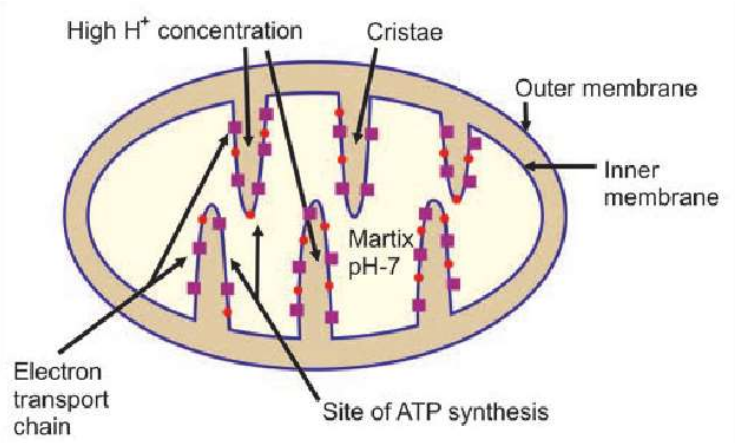
HIGH ENERGY COMPOUNDS

Creatine phosphate

Reservoir for replenishment of ATP.

ADP + creatine phosphate = ATP + creatine (by enzyme Kreatine kinase, Lohmanns reaction)
Replenishments happens in mitochondria especially in myocardium, skeletal muscle, brain!

SITES OF ATP SYNTHESIS



1. Glycolysis – outside mitochondria

* Pyruvate is channeled through outer membrane and is cotransported with H+ through inner membrane

2. PDH complex is located in the matrix
3. TCA is located in soluble matrix
4. Respiratory chain enzymes are located at inner surface of inner membrane (cristae)
5. Creatine kinase – between outer and inner membranes

THE MOST OF METABOLIC PROCESSES IN MITOCHONDRIA DEPEND ON SELECTIVE PERMEABILITY OF INNER MEMBRANE

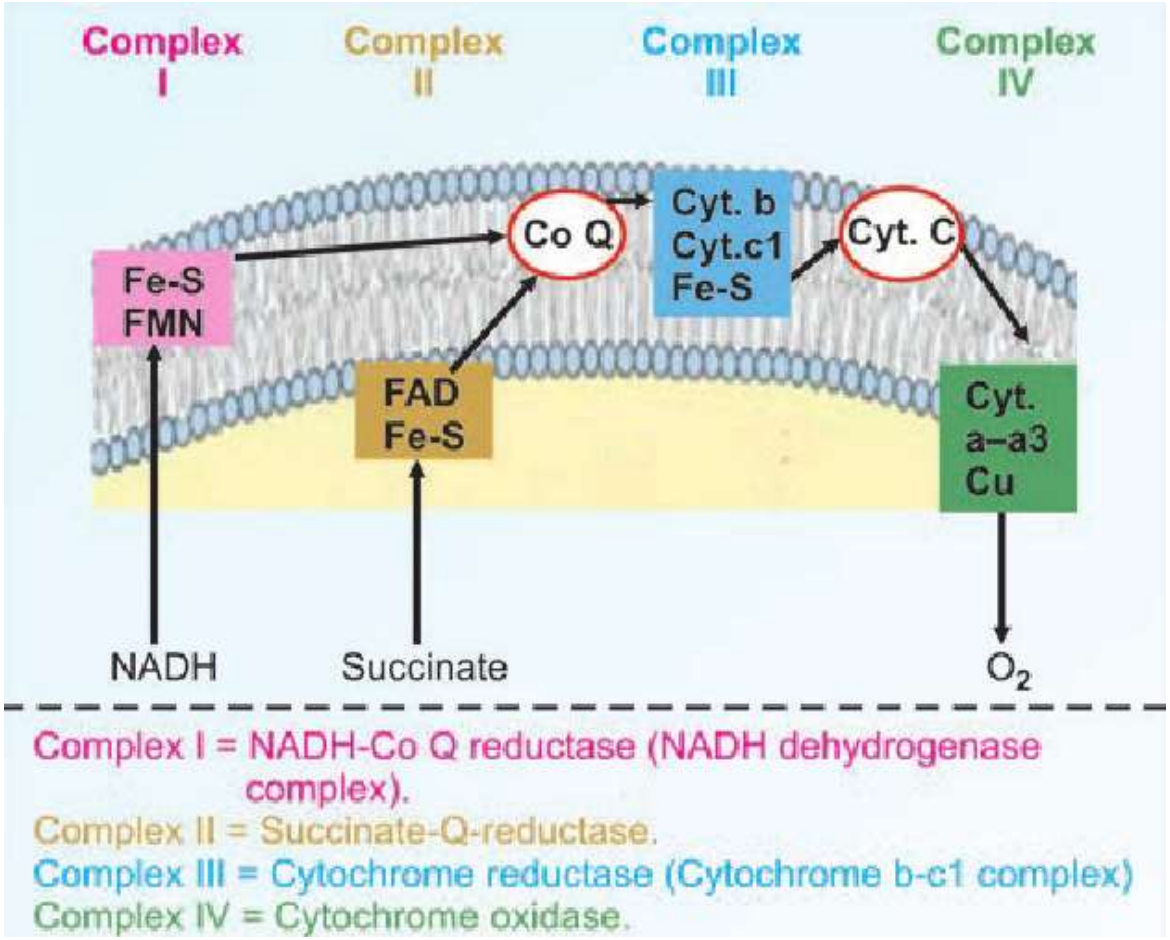
Bioenergetics

Biological oxidation and electron transfer chain:

In ETC electrons are transferred from NADH to some molecules, called electron carriers. There is chain of electron carriers.

4 MULTIPROTEIN COMPLEXES of ETC plus 2 MOBILE CARRIERS

Complexes ***I II III IV*** carriers ***co-enzyme Q (CoQ), cytochrome c***



Bioenergetics

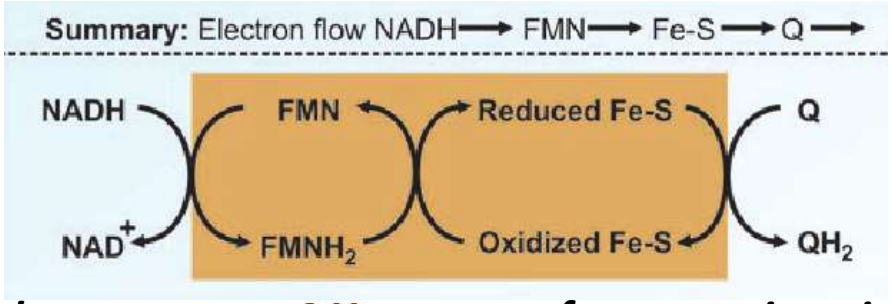
Biological oxidation and electron transfer chain:

***ETCI*: NADH-Q-reductase or NADH-dehydrogenase (FMN-prostetic group protein and Fe-S protein)**

electron flow: ***starts from NADH -> (FMN -> Fe-S) -> CoQ (ubiquinone)***

1st reaction: ***NADH + H+ + FMN -> FMNH2 + NAD+ (2e and H+ is taken by FMN)***

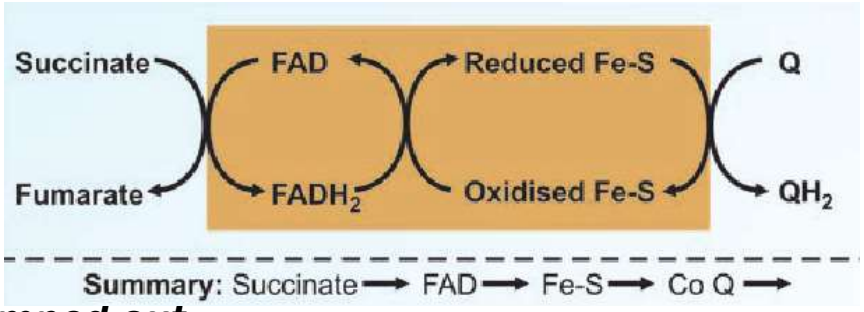
apart of using proton in reaction energy is released during this step.



This energy is used to pump out 4 H+ protons from matrix to inner space

***ETCII*: succinate-Q reductase (Fe-S protein)**

electron flow**: *starts from succinate -> or FADH2 -> (Fe-S) -> CoQ***



No protons are pumped out

CoQ (Coenzyme Q – ubiquinone)

CoQ is reduced to semiquinone (QH) and further to quinol (QH2)

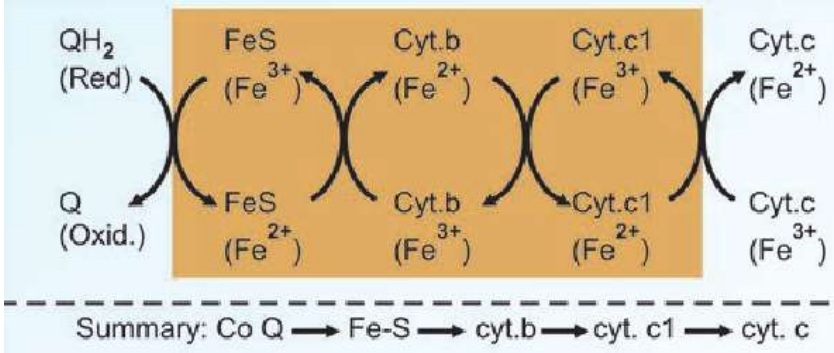
Bioenergetics

Biological oxidation and electron transfer chain:

***ETCIII*: cytochrome-reductase (Fe-S + heme proteins: cytochrome b and c1)**

electron flow: ***from QH2 (quinol) -> (…Fe-heme…) -> Cytochrome C-Red (Fe2+)***

energy is released during this step.



One molecule of QH2 leads to reduction of 2 molecules of CytC. (switch 2e -> 1e)

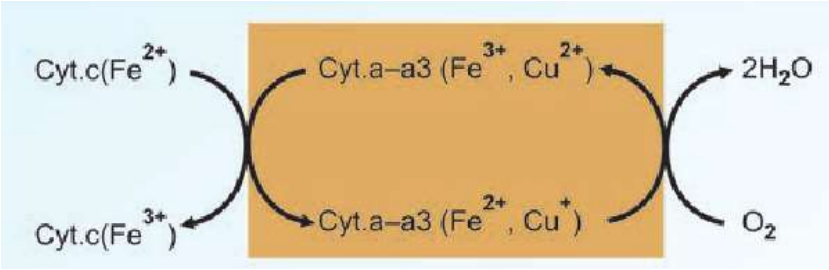
This energy is used to pump out 4 H+ protons from matrix to inner space

***Cytochrome C*: membr. protein (Fe-heme protein) – transfers electron from ETC3 to ETC4**

***ETCIV:* cytochrome oxidase (cytochromes proteins inside)**

electron flow: ***4 electrons are taken from CytochromeC-red and passed to oxygen***

last reaction: ***4H+ + O2 + 4Cyt-C-Red(Fe2+) -> 2H2O + 4Cyt-C-Ox(Fe3+)***



This energy is used to pump out 2 H+ protons from matrix to inner space

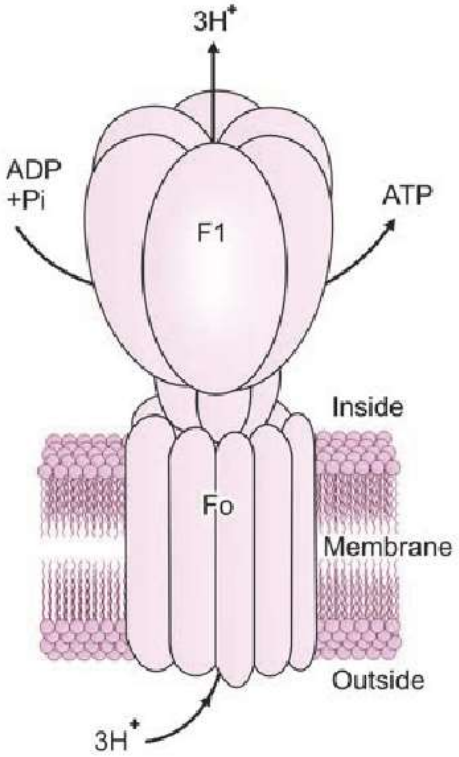
Bioenergetics

Biological oxidation and electron transfer chain:

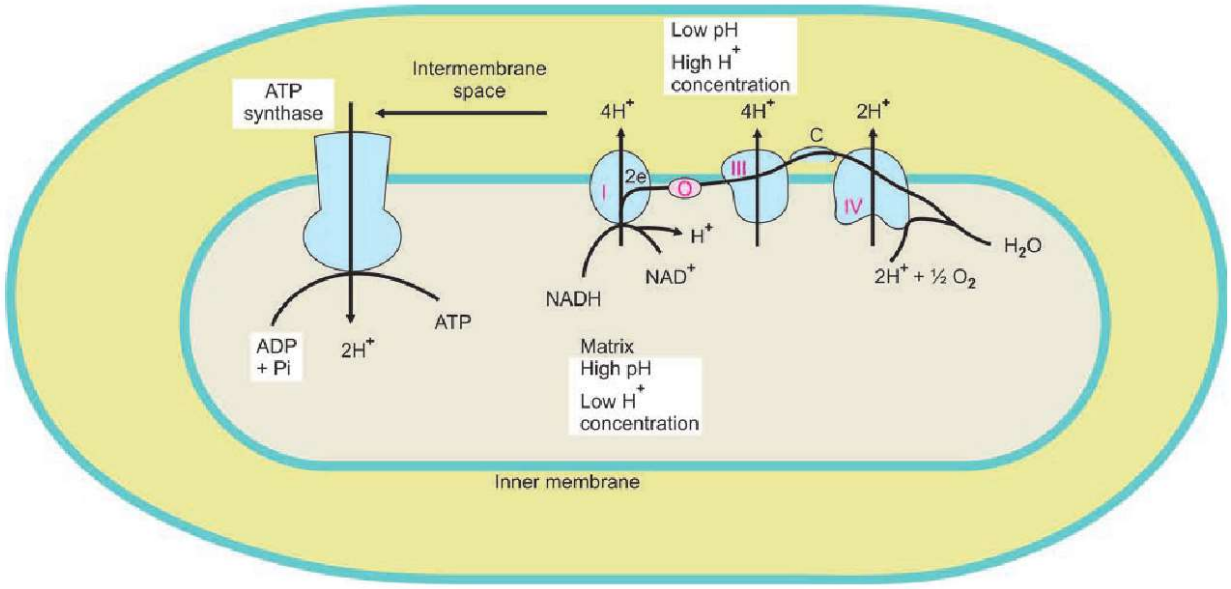
SO FAR in the beginning we have NADH -> NAD+ reaction, at the end O2 -> H2O reaction ENERGY is stored not in chemical bonds but in gradient of H+ (protons) between the matrix and inner space (different pH!) ELECTROCHEMICAL GRADIENT!!! =FORCE

Net effect – 10 protons out from 1 molecule of NADH, if starting from FADH2 – 6 protons

ATP SYNTHESIS (oxidative phosphorylation – theory of Peter Mitchell, Nobel prize 1978)



ATP-synthase : Fo part (proton channel) + F1 (synthesis)



Bioenergetics

Biological oxidation and electron transfer chain:

ATP SYNTHASE (complex V)

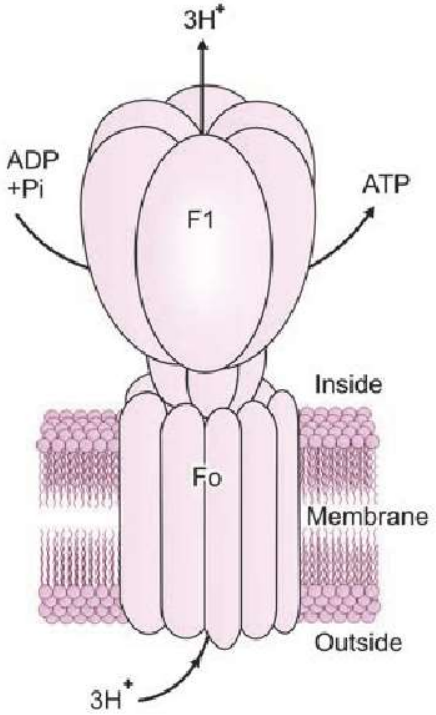
ATP-synthase : Fo part (proton channel) + F1 (synthesis)

F1 in inactive conformation – no binding no reaction

Upon free flow of protons through Fo energy is released

and _can_ change the conformation of active centers in F1

Therefore upon gradual flow – conformation is changed to high affinity binding of ADP and pi (phosphate), and then to catalytically active conformation that forces chemical reaction of ADP + pi -> ATP



REGULATION

Low ATP and high ADP – high rate of synthesis

SOURCE of NADH/FADH2 – citric acid cycle

INHIBITORS

Barbiturates, chrolpromasine – complex I

Carbon monoxide, cyanide, azide – complex IV

UNCOUPLERS – uncouples proton flow and ATP synthesis – leads to dissipation of heat – BROWN ADIPOSE TISSUE in hibernating animals and infants. (**thermogenin** – alternative proton flow through membrane)

Metabolism

Pathways of Metabolsm –

CARBOHYDRATES – mainly around glucose
carbohydrates go through glycolysis pathway, are converted to acetyl-CoA
Further are oxidized in the citric acid cycle
AIM – provision of energy

LIPIDS – mainly around fatty acids
AIM – provision of energy

PROTEINS - amino acds
Can be transaminated and oxidized at carbon skeleton
AIM – building blocks AND energy

Metabolism

LIPIDS metabolism

DURING DIGESTION –

The major dietary lipids are triacyl glycerol, cholesterol and phospholipids. The average normal western food contains about 40-90 g of lipids per day.

DIGESTION IN STOMACH

Enzyme – lingual lipase (from mouth but is active in stomach, optimal pH 2-2.5) **+ gastric lipase**
Substrates – short/medium chain triglicerides (milk, butter)
How much is digested – 30% triglycerides

DIGESTION IN INTESTINES

Enzyme – pancreatic lipase (with coLipase) **+ intestinal lipase + cholesterol esterase + phospholipase A2**
Substrates – long chain triglicerides (pancreatic lipase, intestinal lipase)
medium chain triglicerides (pancreatic lipase, intestinal lipase)
phospholipids with unsaturated fatty acids at posn 2 (phospholipase a2)

Metabolism

LIPIDS metabolism

WHAT IS NEEDED?

Emulsification of lipids to little drops with high surface area.

Bile salts (glycocholate Na + taurocholate Na)
+ mechanical stirring
+ phospholipids

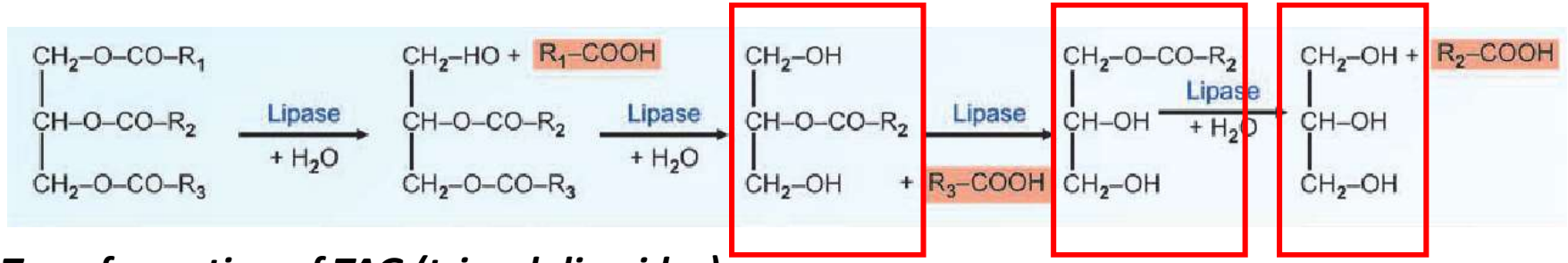
Bile salts and phospholipids have hydrophobic tails that enter fat drops/lipid aggregates and hydrophilic part that faces outside to intestinal environment. Therefore the drops and aggregates are further divided.

Effectively happens in INTESTINES

Also intestinal lipases do not like acidic pH – bile neutralizes pH to pH favourable for pancreatic enzymes

Metabolism

LIPIDS metabolism – RESULT OF DIGESTION



Transformation of TAG (triacylglicerides)

RESULT of ENZYMES ACTION – incomplete!

Monoacylglycerides with FA at 2 position	**78%**
Monoacylglycerides with FA at 1 position	**6%**
Glycerol	**14%**

Transformation of cholesterol esters (by cholesterol esterase)

RESULT of ENZYMES ACTION – incomplete!

Free cholesterol

Transformation of phospholipids (by phospholipase A2)

RESULT of ENZYMES ACTION – incomplete!

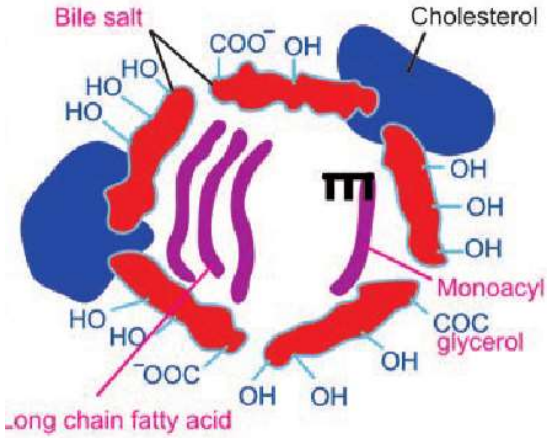
Phospholipid with FA stripped from position 2

Metabolism

LIPIDS metabolism – ABSORBTION

Long chain FA (>14 C atoms) – directly tolymph (NOT TO BLOOD!)

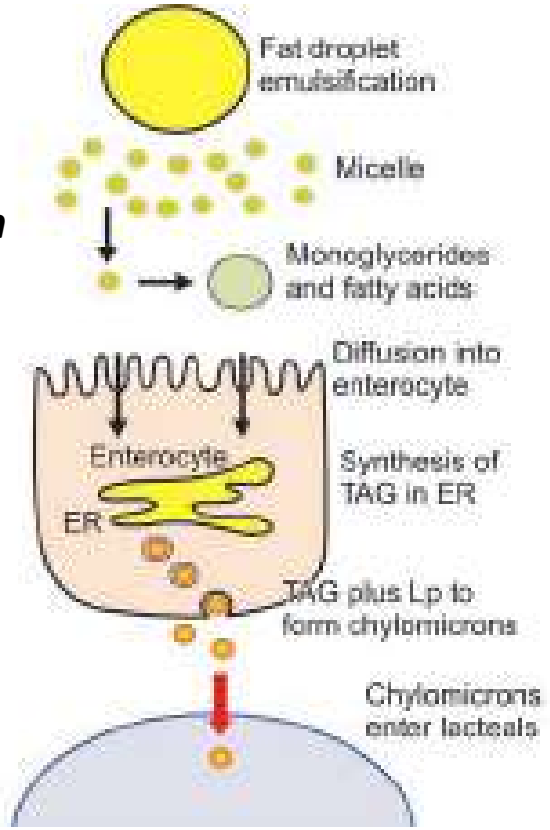
Theory of Bergstrom, Nobel prize 1982



1. Formation of mixed micels

2. Micells are aligned with mucosal cells and passively diffuse into them

3. Long chain FA are activated into fatty Acyl-CoA and are again attached to MAG to form TAG



4. TAG with Lp forms chylomicrons and enter into lymphatics.

5. Short chain fatty acids are directly absorbed into blood capillaries.

6. Glycerol also absorbed from intestinal lumen to blood.

7. Bile acids are reabsorbed into portal vein

Metabolism

LIPIDS metabolism – ABSORBTION

1. **Minor digestion** of triacylglycerols in mouth and stomach by lingual (acid-stable) lipase.
2. **Major digestion** of all lipids in the lumen of the duodenum/jejunum by pancreatic lipolytic enzymes.
3. **Bile acid** facilitated formation of mixed micelles.
4. **Passive absorption** of the products of lipolysis from the mixed micelle into the intestinal epithelial cell.
5. **Reesterification** of 2-monoacylglycerol with free fatty acids inside the intestinal enterocyte.
6. **Assembly** of chylomicrons containing Apo B48, triacylglycerol, cholesterol esters and phospholipids **and export** from intestinal cells to the lymphatics.

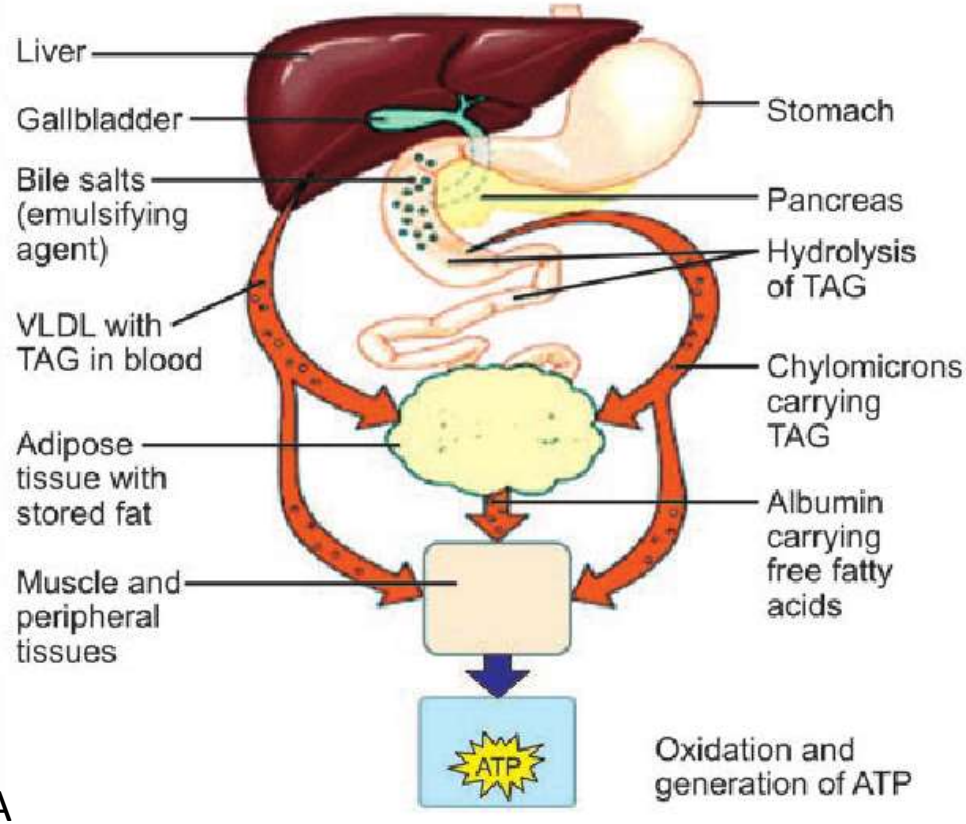
Fate of Chylomicrons

i. The absorbed (exogenous) triglycerides are transported in blood as chylomicrons. They are taken up by adipose tissue and liver.

ii. Liver synthesizes endogenous triglycerides. These are transported as VLDL (very low density lipoproteins) and are deposited in adipose tissue.

iii. During starvation states, triglycerides in adipose tissue are hydrolyzed to produce free FA

General FAT utilization



Metabolism

LIPIDS metabolism – OXIDATION

BETA OXIDATION OF FATTY ACIDS

Summary – oxidation and splitting of 2 carbon atoms sequentially.

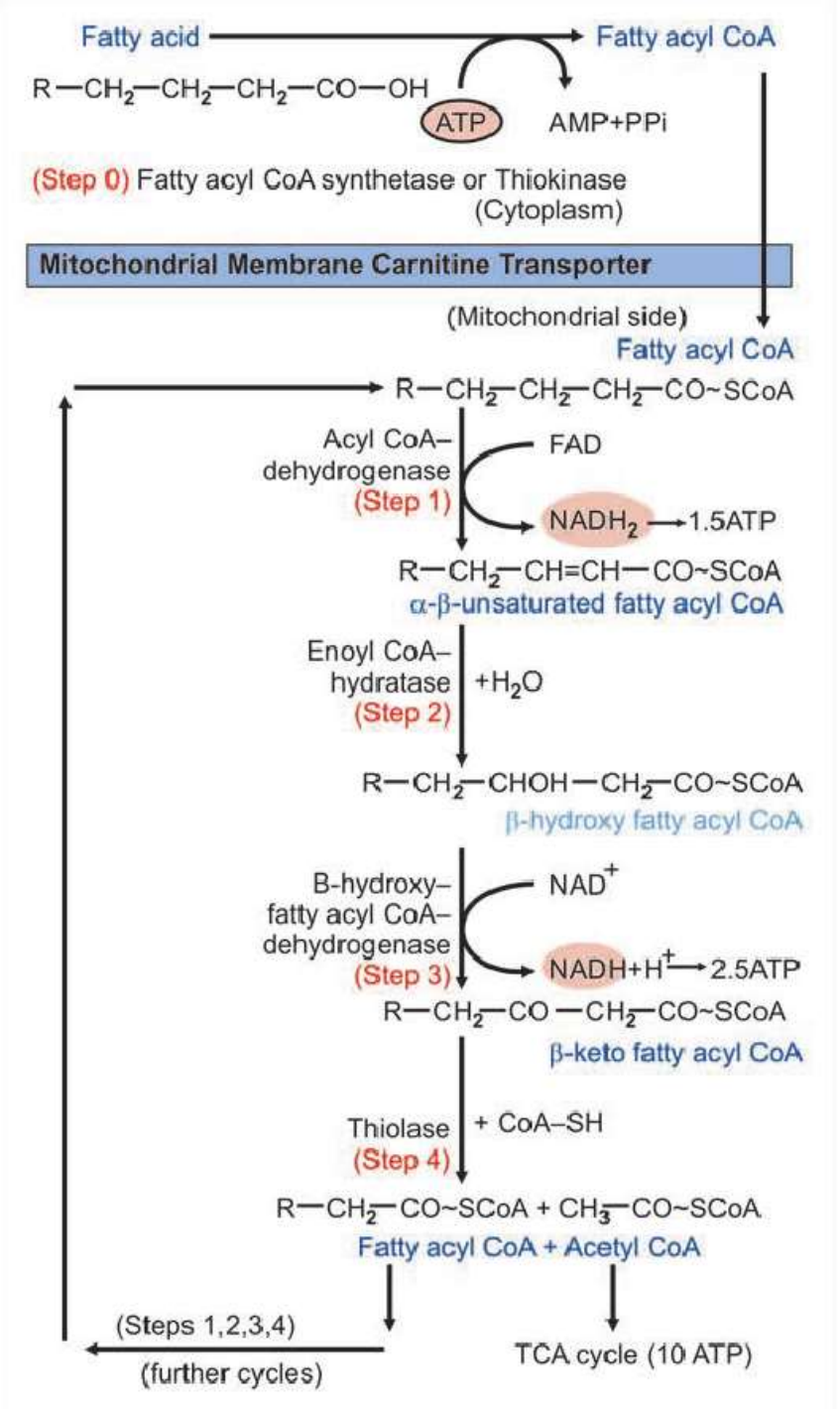
Preparative steps

1. *Activation of FA*

FA is attached to CoA with energy taken from hydrolysis of ATP to AMP and ppi to pi+pi (two high energy bonds are used)

Enzyme – fatty **acyl** CoA synthetase. For FA of different length there are three enzymes.

happens in cytoplasm



Metabolism

LIPIDS metabolism – OXIDATION

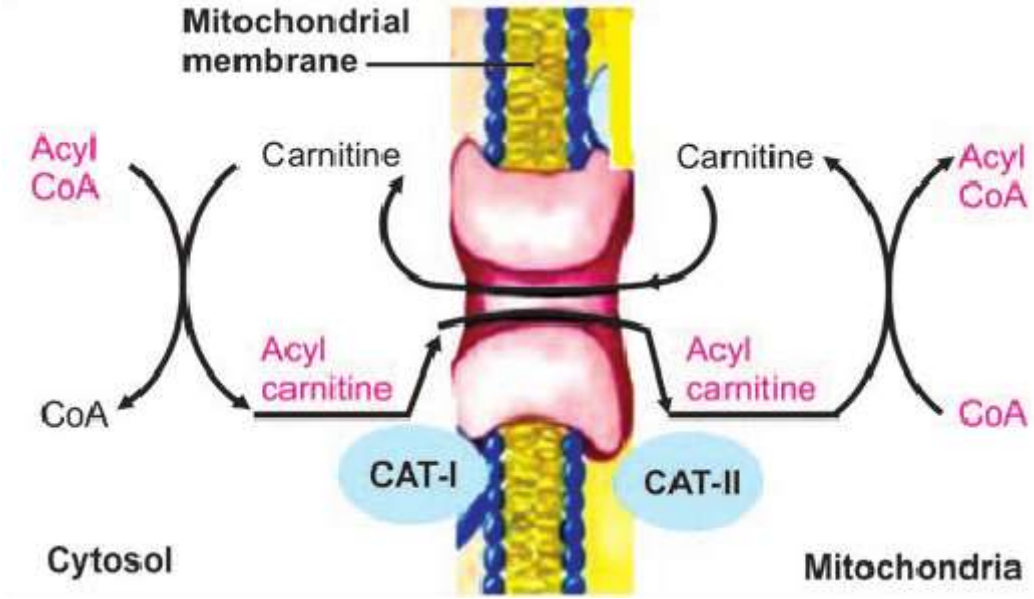
BETA OXIDATION OF FATTY ACIDS

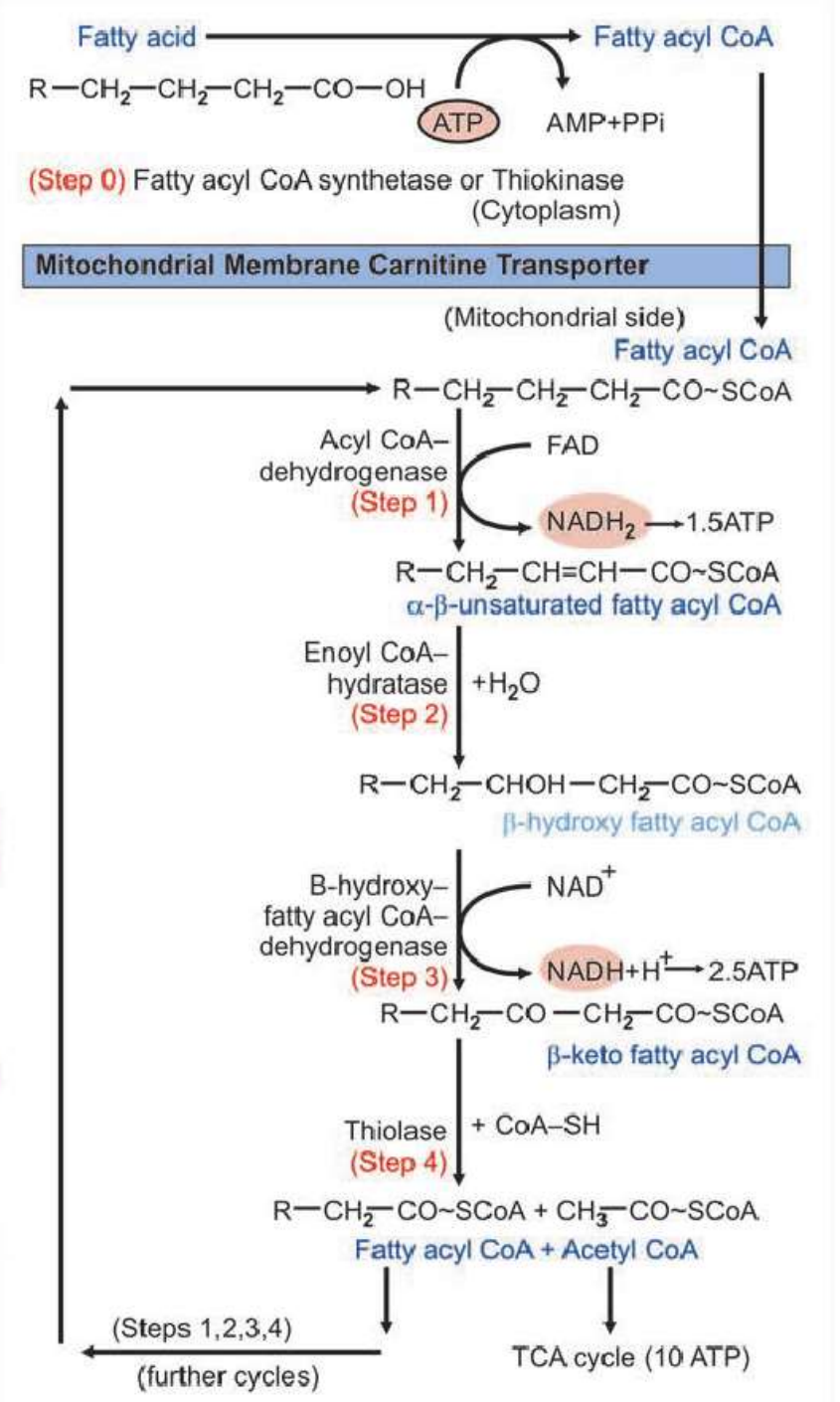
Summary – oxidation and splitting of 2 carbon atoms sequentially.

Preparative steps

2. Transport of long chain Fatty Acyl CoA to inner mitochondria, using carnitine (short and medium are free to pass)

(CH3)3–N+–CH2–CHOH–CH2–COOH





Metabolism

LIPIDS metabolism – OXIDATION

BETA OXIDATION OF FATTY ACIDS

Summary – oxidation and splitting of 2 carbon atoms sequentially.

MAIN steps

Sequentially repeated!

1 round = splittage of one Ac-CoA from Fatty Acyl-CoA

1. Dehydrogenation

(energy is generated by FADH2)

2. Hydration

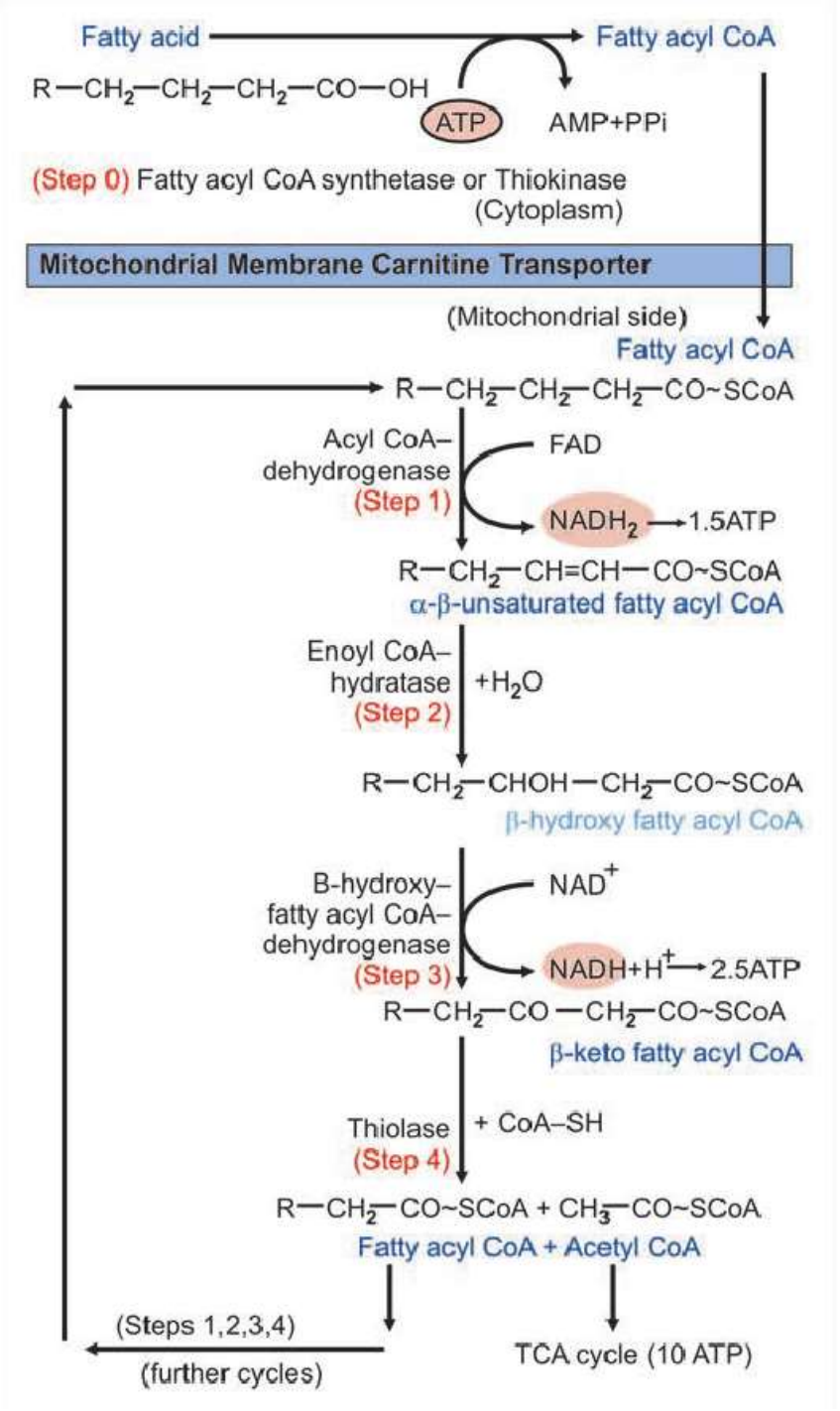
(stereoscopically only L-isomer is formed!)

3. NAD+ dependent dehydrogenase

(FA is oxidized to beta-keto AcylCoA)

4. Cleavage

Splitting of AcCoA and attachment of rest of FA to another CoA



Metabolism

LIPIDS metabolism – OXIDATION

BETA OXIDATION OF FATTY ACIDS

Summary – oxidation and splitting of 2 carbon atoms sequentially.

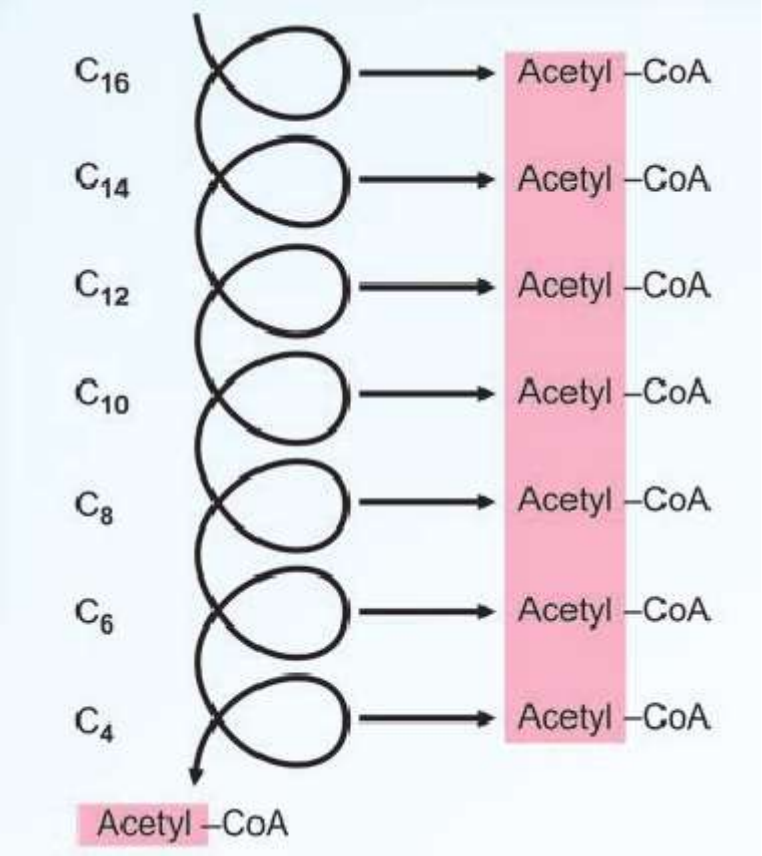
When one molecule of palmitate undergoes beta-oxidation, the net reaction is:

Palmitoyl CoA	→	8 Acetyl CoA
+ 7FAD		+ $7FADH_2$
+ $7NAD^+$		+ 7NADH
+ $7H_2O$		+ $7H^+$
+ 7HSCoA		

Net balance –

8 AcCoA = 96 ATP 7 NADH = 21 ATP

7 FADH2 = 14 ATP **TOTAL = 131 ATP**



PALMITIC ACID oxidation

β-Oxidation of FA with an odd number of carbon Atoms: Fatty acids with an odd number of carbon atoms are oxidised by β-oxidation pathway to produce acetyl-CoA until a 3-carbon residue **propionyl-CoA** is left. Propionyl-CoA is metabolised to succinyl-CoA through methyl malonyl-CoA.

Note:

- *Propionyl-CoA formed from an odd-chain FA is the only part of the FA which is glucogenic,* as it is converted to succinyl-CoA.

Literature biochemistry

1. Lehninger Principles of Biochemistry (Nelson D.L., Cox M.M.)
2. Principles and Techiniques of Biochemistry and Molecular Biology (Wilson K., Walker J.)