10

Biochemistry

Doctor 2017 | Medicine | JU

Sheet ○ Slides

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Doctor

Dr. Nafeth

*Requirement of oxidative phosphorylation:

1- Source and target for electrons(NADH+FADH2 >> O2).

2- Electron carriers.

3- Enzymes, like oxidoreductases and ATP Synthase enzymes.

4- Intact inner mitochondrial membrane, because if there is any hole, proton motive force which drive electron transport will be distracted.

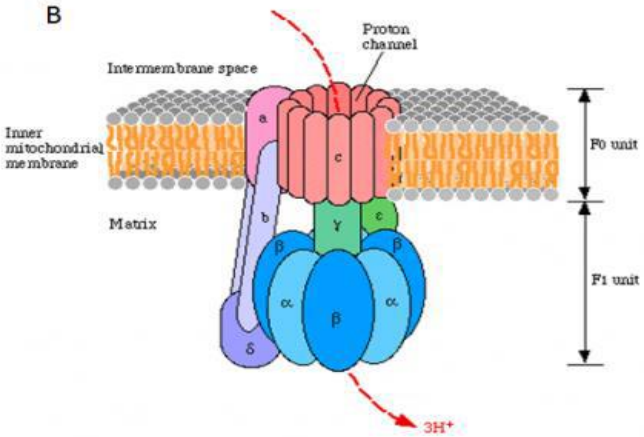
** we have studied the first 4 complexes that facilitate ETC (the doctor said that we don't have to go deep through these complexes, it just important to know

the carriers – the external components- for each complex to be functional)

NOW, lets continue talking about the last complex on the ETC which is:

**ATP Synthase:

as the name implies, it is an enzyme that synthesis ATP, and as any enzyme it can decrease the activation energy and catalyse reactions for both directions (forward & backward), so it also works as ATPase which breaks down the ATP.



** To know how the process goes through this complex, lets know its structure first;

this enzyme consists of **2** portions:

1- **The cytoplasmic side** (outside the mitochondria), which called **F0 Headpiece**>> a cylinder rotates in the membrane with 2 parts:

a- Curved shape (C-shape) part that can't move within the membrane, so the cylinder can move around it !, this part called (**a**)subunit which is fixed within the membrane and consider as the entry point and exit point for protons.

b- The cylinder which is 12 subunits around each other, each one of them called **c** subunit attached to it **gamma** subunit that have these properties:

- Its direction toward inside the matrix.
- Its angled (not straight).

- While **c** subunits moving, it will rotate with it.

2- **Matrix side** (inside the mitochondria) which called **F1 Headpiece**:

- 6 subunits, ordered in a sequence of alpha>> beta >> etc.
- Its shape like a pyramid.
- 3 Alpha subunits are the **structural subunits** which give the shape for the enzyme.
- 3 Beta subunits are the ones which **catalyse** the conversion of ADP + inorganic phosphate to ATP or vice versa.

NOW! What happen exactly??

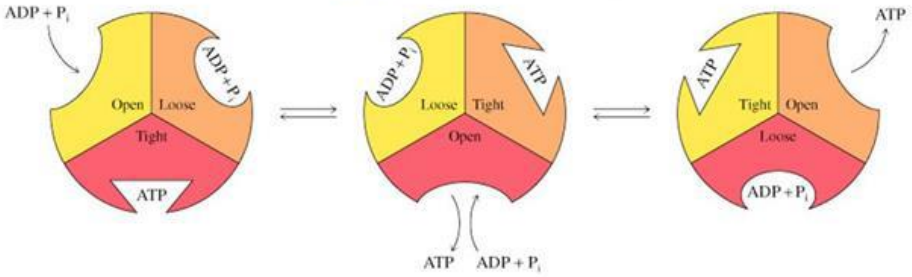
- Protons are outside trying to enter according to their motive force and don't have a straight channel to enter through it.
- The only exit for the protons is the ATP Synthase, specifically **(a)**subunit.
- They enter through it and directly find one of the **c** subunits that has in its structure **a glutamic acid** as an amino acid (negatively charged).
- The proton will attach to the Glutamate and it will be protonated >> loss its charge>> conformational change for the protein>> **c** subunit will move away from the open site>> allow the other proton to come through and attach with other **c** subunit >> conformational change ... etc >> **until full rotation occur**.
- After full rotation: there is another opening in the **(a)** subunit (totally different opening) designed to lose the proton that was attached to it.
- This controlled by the difference of PKa and PH values for each area.
- Rotation of **c** subunit induce the angled gamma subunit that attached to it, to hit **B-subunit** (catalytic ones)>> induces conformational change for protein.
- Betta subunit can undergo 3 conformational changes:

  1) Open 2)loose 3) tight

  At first it is open, Open has high affinity for ADP with in-organic phosphate, when a conformational change is happening, it is turned into loose, which makes ADP and Pi close together, Then another change takes place that makes the subunit tight and generate ATP, and it comes back to open conformation,which has low affinity for ATP, so ATP is released.
  this process based on the concentration of protons (outside higher than inside). If the concentration of the proton **REVERSED**>> PH will change >>
  all the process will be reversed>> **catch ATP and releases ADP**.

Binding Change Mechanism

- Different conformation at 3 catalytic sites
- Conformation changes due to proton influx
- ADP + Pi bind to open-site in exchange for ATP
- Proton driven conformational change (loose site) causes substrates to bind more tightly
- ATP is formed in tight-site.
- Requires influx of four protons to get one ATP



****Energy yield… efficiency and efficacy of ETC**:

In order to know the efficiency of the ETC, you need to calculate the theoretical yield which is the difference of potential energy between the NADH or FADH 2 and O2.

As you know the capacity of energy for NADH ic -53Kcal and -41Kcal for FADH2.

On the other hand, the actual yield is 2.5 ATP(-18 kcal) For NADH, and 1.5 ATP for FADH2(-11 kcal).

- 18/53= 33% efficiency of NADH
- 11/41=26% efficiency of FADH2

- **WE CAN NOTICE THAT ENERGY YIELD IS LOW COMPARED TO CITRIC ACID CYCLE, WHY?**

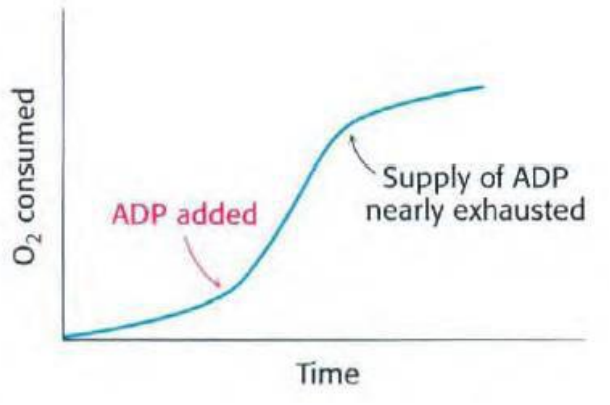
  - Energy is lost as heat by uncoupling proteins + energy is needed to bring inorganic phosphate and coupling it with ADP inside mitochondria and take ATP outside it.
  - Nothing can cross mitochondrial membrane except by using channels.
  - anything against gradient you have to pay energy for it.
  - Delta G is very negative according to the value of reduction potential for electron carriers and enzymes.

- The ETC is never being a reversible process
- ETC is our major source of heat

**** Regulation for oxidative phosphorylation process is effected by:**

1- **The concentration of ADP**: the most important factor controlling the process.
- it's the molecule that control the whole respiratory process>> it's the only allosteric activator for isocitrate dehydrogenase.
-The more O2 is consumed the more efficient and active ETC is.

When you add ADP there's a sharp increase in O_2 consumption and once the ADP supply ends the, rate decreases. This is a physiological way of regulation.



2- regulation by inhibition:

The mechanism of this regulation is inhibiting the complexes as all of the complexes can be inhibited. (also there is a natural inhibitors for these compounds)

By inhibiting enzymes, we stop the electron transport and the pumping of protons will be stopped. Which will result in decreasing the electrochemical gradient across the membrane. This is fatal because we need high electrochemical gradient for the ATP synthesis process to work effectively.

- Examples of inhibitors:

Specific inhibitor	**Target**
Rotenone (insecticide)& Amytal(sedative)	Complex 1
AntimycinA(antibiotic)	Complex 3
Cyanide (CN-), Azide (N3-), (CO)	Complex 4
Oligomycin(antibiotic)	ATP Synthase

- **More explanation:**
  - CO2 is something different than oxygen, they exchange in the lungs,but in different sites, otherwise metabolically in the body they are so different.
  - CO and Cyanide mimic O2, they go parallel with it, they can bind to where oxygen bind (where you find heme) >> myoglobin/hemoglobin/complex 4 in ETC, so when cyanide for example bind to complex **4** it will inhibit the movement of electrons >>So, no proton will be pumped >> no ATP will be synthesized (because it a coupled process).

3- uncoupling inhibition:

- Scientists have worked on producing a chemical that is able to bind with protons and transport them across inner mitochondrial membrane without producing ATP so it can treat obesity**.**

DNP: 2,4-dinitrophenol is a drug designed with benzene ring structure and hydroxyl group so its lipophilic and can cross the membrane>> then go to mitochondria and cross its membrane >> it can lose and gain its H >> when its on the outer surface of the membrane it picks up H >>when it's toward the matrix it gives its H (because the difference in PH)>> so it picks up the proton from the outside and release it toward the matrix.

2,4-Dinitrophenol (DNP)

- this drug returns the protons back to the matrix >> generating more heat and less ATP>> less anabolism and building up process>> many problems by this chemical inhibitor like: eyes bleeding and blindness and some death cases.

****uncoupling protein (UCP):**

Mechanism of uncoupling proteins in the inner membrane of mitochondria:
when the protons pumped toward the inner membranous space>> the uncoupling proteins will take out some of these protons and bring them back toward the matrix>> so part of them is used for heat generation >> adaptive thermogenesis (normal physiological process).

From the slides:

UCP1 (thermogenin): found in Brown adipose tissue, non-shivering thermogenesis. Infants: neck, breast, around kidneys.

Fatty acids directly activates UCP1

UCP2 (most cells)

UCP3 (skeletal muscle)

UCP4, UCP5 (brain)

**uncoupling proteins mutation>> more ATP synthesis>> more fat.

****oxidative phosphorylation diseases:**

- **the 5 complexes in ETC are enzymes (proteins),consist of large amount of polypeptides:**

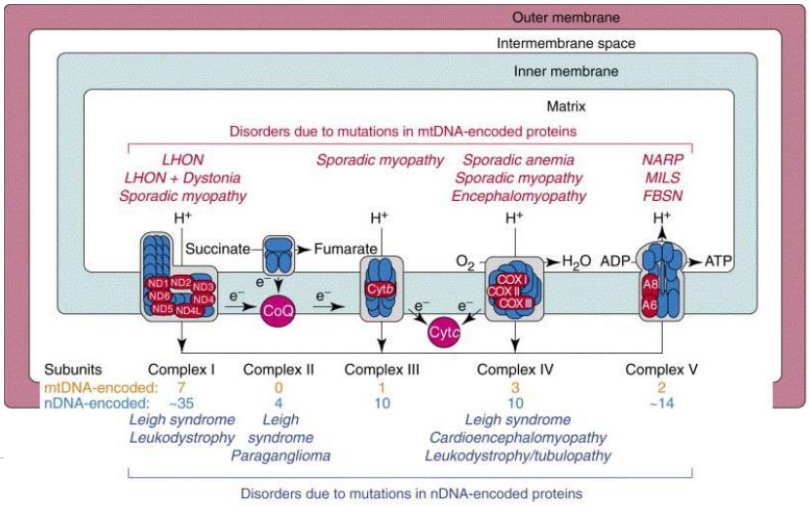
- Mitochondrial DNA works to synthesise part of the complexes polypeptides:

- 7 polypeptides out of 25 for complex1
- 1 subunit from complex 3
- 3 subunits from complex 4
- 2 subunits from F0 portion in ATP Synthase.

- Most of proteins synthesised by nuclear DNA>> translocation to mitochondria.

- So mitochondrial proteins have mixed origin, the mitochondrial DNA make the lowest percentage of them compared to the nuclear DNA.

- The genetic diseases that affect the mitochondria, they might be from mitochondrial origin (from the mother only, it may shows heteroplasmy) or nuclear origin (it will present in all cells of the body).
- inner mitochondrial membrane is filled with proteins so that's why nucleus synthesize higher than 1000 protein.



- **Mitocondrial Shuttling Systems**
- **{ Cytosolic NADH }:**

- Glycolysis is one of the source of NADH from outside the mitochondria

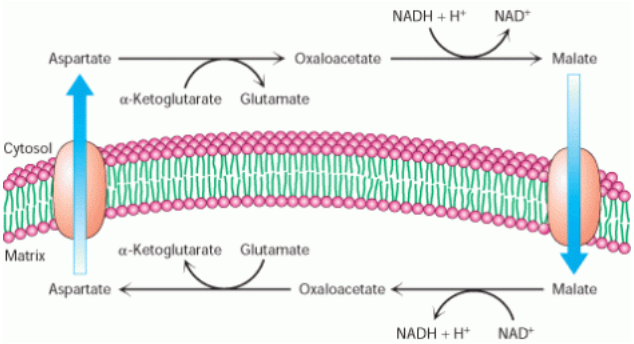
- There should be a way to get the NADH from outside to inside.
- it couldn't enter freely, there must be a specific transporter for it, but there is not (why??)>> according to the electrochemical gradient, NADH prefer to exit the matrix of mitochondria toward the intramembranous space by these transporters, and that will affect the process of ETC and ATP Synthesis.
- so the electrons from the NADH must be used in another form to enter the mitochondria.

NOW , How to translocate the NADH in the cytosol to the mitochondria, to be used as an energy source?

1- Aspartate-Malate shuttle:

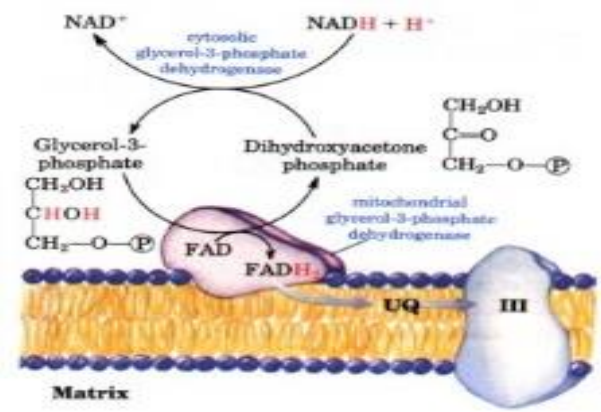
-Aspartate can be converted to oxaloacetate by transaminases.
- Oxaloacetate can be converted to malate (it's the reversible reaction that found in citric acid cycle , where we form NADH) but in this REVERSIBLE reaction, cytosolic NADH is used by malate dehydrogenase enzyme >> malate have transporter (because of gluconeogenesis) so it can cross through the membrane>> malate is inside the matrix >> converted to oxaloacete by malate dehydrogenase enzyme and regenerate the NADH so it can go to complex1>> **generate 10 protons**>> **3ATPs**

**note: malate dehydrogenase enzyme has 2 copies:
- **mitochondrial copy** >> convert malate to oxaloacetate.
- **cytosolic copy** >> convert oxaloacetate to malate.



2- glycerol- 3 phosphate shuttle:

- Glycerol 3-phosphate dehydrogenase enzyme:
- Has mitochondrial and cytosolic copies.
- This enzyme converts dihydroxyacetone phosphate to glycerol 3-phosphate >> this process (converting ketone to alcohol) uses NADH (take its electrons).
- On the outer surface of the inner membrane, there is the mitochondrial copy of this enzyme that switches the reactions and converts alcohol into ketone extracting the electrons.



- by the coenzyme FADH2 that is found inside the enzyme.
- Generate FADH2 >> coenzyme Q take its electron>>complex 3 (4 proton pumped)>>complex 4 (2 protons pumped) >> **generate 6 protons** >> **2ATP**
- NADH from outside the mitochondria has 2 pathways:
  If it comes through aspartate-malate shuttle it will generate 3 ATPs.
  If it comes through the glycerol-3-phosphate shuttle it will generate 2 ATPs.

- **ATP –ADP Translocase Enzyme:**
  - Also known as adenine nucleotide translocase (ANT), is a transporter protein that enables the exchange of cytosolic ADP and mitochondrialATP across the inner mitochondrialmembrane.
  - Free ADP is transported from the cytoplasm to the mitochondrial matrix, while ATP produced from oxidative phosphorylation is transported from the mitochondrial matrix to the cytoplasm, thus providing the cells with its main energy need.
  - This enzyme can undergo conformational changes; inside the mitochondria it has high affinity for ATP, so when it binds to ATP it will Undergo conformational changes and it opens up out, where it will have low affinity for ATP to release it, and high affinity for ADP to move it inside.
  - Notice that; the entry of ADP into the matrix is coupled to the exit of ATP.
  - This protein forms 14% of the proteins found on the inner membrane of mitochondria.
  - The Reaction is endergonic; with 25% of energy spent on this enzyme, that's why the efficiency of ATP synthase is not as much as TCA cycle.

What we have took with doctor Nafeth?

1- Methods to separate the components of blood.

2- plasma and how to extract its proteins.

3- Detect which organ is damaged from the concentrations of plasma.

4-Polymorphism, half-lives and function of plasma proteins.

5-Types of plasma proteins (their properties and clinical disorders).

6-To study a reaction, there are two points of view: kinetic energy, thermodynamically.

7-Factors that affect delta G from the equation >>

$$\Delta \boldsymbol{G} = \Delta \boldsymbol{G}^{\circ} + \boldsymbol{RT} \mathbf{ln} \frac{[\boldsymbol{C}][\boldsymbol{D}]}{[\boldsymbol{A}][\boldsymbol{B}]}$$

8- The properties of a reaction at equilibrium.

9-The importance of mitochondria.

10-The properties of ATP that make it the main source of energy.

11-Biochemical pathways in the body intersect each other to conserve energy.

12-How does the body deal with endergonic reactions.

13-Oxidation reduction reactions.

14-Reduction potential and its relation to delta G>> $\Delta \boldsymbol{G} = -\boldsymbol{nf}\Delta \boldsymbol{E}$

15- Steps of citric acid cycle and the products that come out from it.

16- The deficiencies that affect one of the enzymes in the complex (enzyme 1 and 2) in alpha-ketoglutarate complex

17- Regulation of citric acid cycle.

18- Citric acid cycle intermediates.

19- Reactions that replenish the intermediates of TCA cycle (anaplerotic reactions).

20- Electron transport chain and the sources of electrons.

21-The mechanism of ATP synthase in generating ATP.

22-Regulation of oxidative phosphorylation (by inhibition, uncoupling…).

23- Genetic diseases originated from mitochondrial DNA and nuclear DNA.

24- Mitochondrial shuttling system

good luck and sorry for any mistake.

you were born to be a doctor
you were meant to be here
the moment is yours… ☺