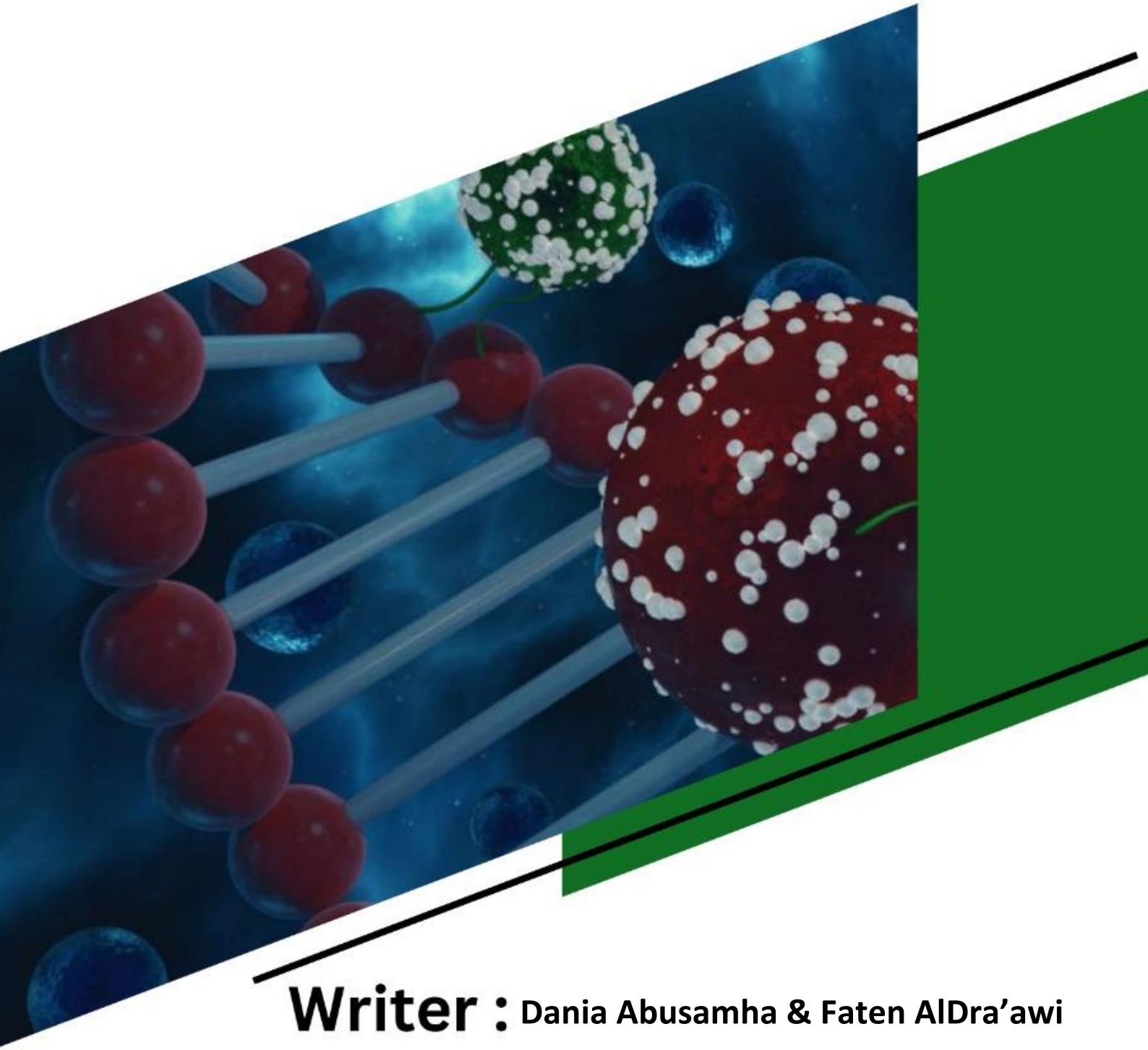
Doctor 021

METABOLISM

Sheet no. 9



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OXIDATIVE PHOSPHORYLATION DISEASES (GENETIC)

As we studied before, Mitochondria has a **small** (16,569 base pair), **double stranded** and a **circular DNA** (like bacteria) which could be affected by mutations.

The 5 complexes in ETC are enzymes (proteins), consist of large number of polypeptides, the Mitochondrial DNA encodes 13 subunits of the complexes' polypeptides:

- **7 subunits of complex I.**
- **1 subunit of complex III.**
- **3 subunits of complex IV.**
- **2 subunits of F0 portion in ATP Synthase.**

Those subunits are important, no OxPhos without them!

Mitochondrial DNA also encodes necessary components for translation of its own mRNA: a large and small rRNA and tRNAs.

Mutations of Mitochondria can happen early in embryogenesis. During embryogenesis, cells divide, each cell is basic unit of a specific tissue. The mother cell cannot control the distribution of mitochondria to daughter cells (they're distributed randomly), so we might end up with a daughter cell with mutated mitochondria while the other is not. So, if a cell with a mutated mitochondria is responsible of making bone tissue, you will find that bone tissue has disease of mitochondrial origin, while the nervous tissue is healthy since cells responsible of it has a clean mitochondrion.

Unlike nuclear mutations, Mitochondrial disease of Mitochondrial DNA origin cannot be expressed by any cell in the body, doctors have to take different biopsies from different tissues to know the extent of effect of that disease on the body.

Since cell division occurs by mitosis, If the mutation occurs the nucleus, it will be present in any cell of the body.

The average number of mitochondria per cell is 2000.

Heteroplasmy: the issue of mitochondria where a tissue could be affected and the other is not.

OxPhos Diseases (Genetic)

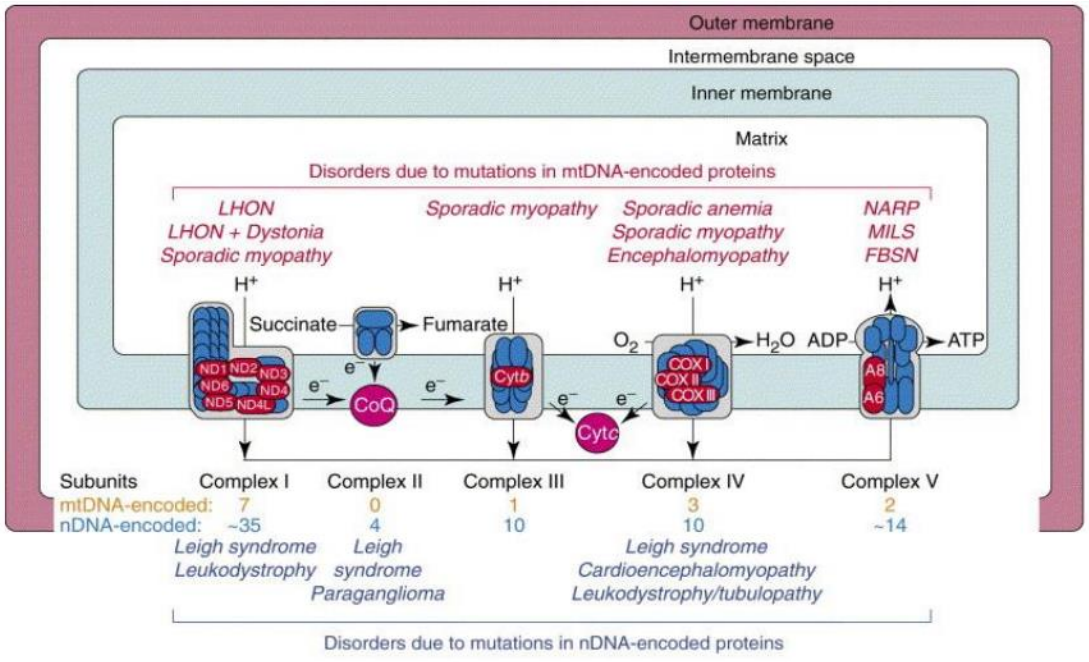
- A. Mitochondrial DNA and OXPHOS Diseases
  - ✓ Small (16,569) base pair, double-stranded, circular DNA
  - ✓ Encodes 13 subunits: 7 (I) , 1 (III), 3 (IV), 2 (Fo)
  - ✓ Also encodes necessary components for translation of its own mRNA: a large and small rRNA and tRNAs
  - ✓ Maternal inheritance, replicative segregation & heteroplasmy
  - ✓ Accumulation of somatic mutations with age
  - ✓ Highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver

OxPhos Diseases (Genetic)

- B. Nuclear Genetic Disorders of Oxidative Phosphorylation
  - ✓ 1,000 proteins
  - ✓ Usually autosomal recessive
  - ✓ Expressed in all tissues
  - ✓ Phenotypic expression with high ATP demand

➢ **LHON disease, is a disorder resulting from a mutation affecting the mitochondrial DNA, which may affect the ETC.**

➢ **Leigh syndrome, disorder resulting from nuclear DNA mutation which affects ETC and OxPhos.**



عن ابن عباس رضي الله عنهما قال: كنت خلف رسول الله صلى الله عليه وسلم يوما فقال: (يا غلام إني أعلمك كلمات: احفظ الله يحفظك احفظ الله تجده تجاهك إذا سألت فاسأل الله وإذا استعنت فاستعن بالله واعلم أن الأمة لو اجتمعت على أن ينفعوك بشيء لم ينفعوك إلا بشيء قد كتبه الله لك ولو اجتمعوا على أن يضروك بشيء لم يضروك بشيء إلا بشيء قد كتبه الله عليك رفعت الأقلام وجفت الصحف).

MITOCHONDRIAL SHUTTLING SYSTEMS "CYTOSOLIC NADH"

NADH is a product of huge number of enzymes, most of them are mitochondrial but some are cytosolic (producing NADH in cytoplasm), Glycolysis is an example, it produces two ATPs and two NADH molecules. Those NADH molecules are loaded with electrons, which could be useful.

NADH does not has carriers, most of its production occurs inside the mitochondria, so no need for the carriers. However, how the NADH can get inside the mitochondria?

Shuttling mechanisms are just like bus, they transport electrons from NADH towards matrix of mitochondria through special carriers.

❖ Malate-Aspartate shuttle

As we all know, through kreb's cycle, Malate is transferred to oxaloacetate, producing NADH through **malate dehydrogenase**. Oxaloacetate can be transaminated into Aspartate, through **aspartate transaminase**. When aspartate gets outside mitochondria, it can be transaminated back to oxaloacetate (In cytosol).

➢ So:

Oxaloacetate >> Aspartate (in Mitochondria).

Aspartate >> Oxaloacetate (in cytosol).

Both reactions are catalyzed by **(Aspartate transaminase).**

There are two copies of **Malate dehydrogenase,** mitochondrial copy **(malate >> oxaloacetate)**, and cytosolic copy **(oxaloacetate >> malate).** In cytosol, malate dehydrogenase can catalyze the reversible reaction, it transfers oxaloacetate to malate (it favors malate over oxaloacetate).

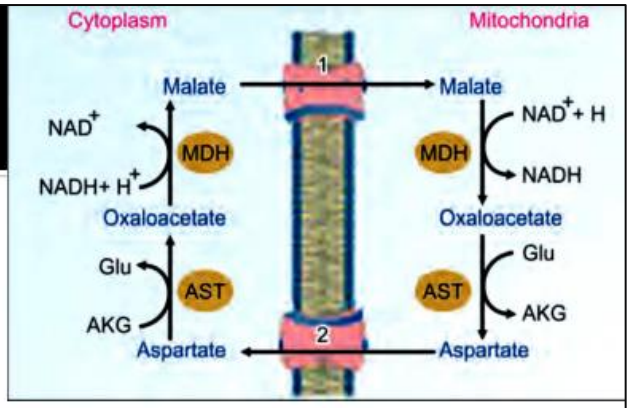
In mitochondria, when **Malate dehydrogenase** transfer malate to oxaloacetate, one NADH molecule is produced.

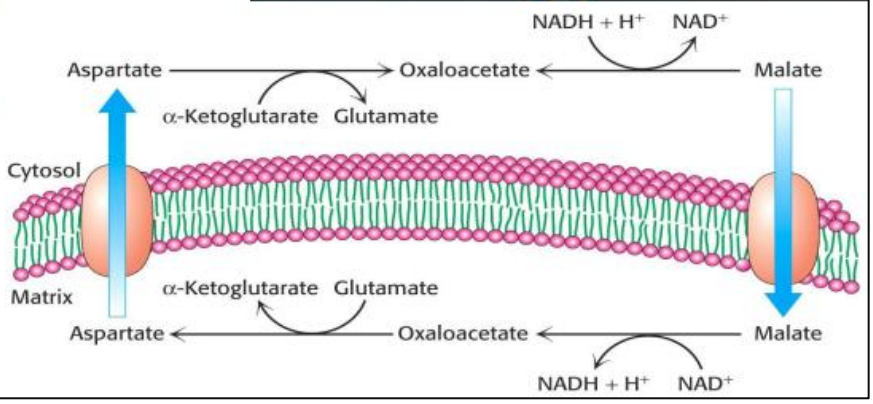
While in cytosol, the reversible reaction uses NADH. NADH is oxidized to NAD+, the two electrons are loaded on malate (alcohol). Malate has a transporter, so it gets inside the mitochondria. Once it's inside the matrix, it gets converted to oxaloacetate by **malate dehydrogenase** enzyme and regenerate the NADH, so it goes to **complex I** to enter TCA cycle.

Remember: NADH produces 10 protons >> 2.5 ATPs.

Mitochondrial shuttling systems - "Cytosolic NADH"

- Malate-Aspartate shuttle
- Operates mainly in liver, kidney and heart
- 2 membrane carriers & 4 enzymes
- Readily reversible (vs. Glycerol 3-phosphate shuttle)
- NADH can be transferred only if the NADH/NAD+ ratio is higher in the cytosol than in the mitochondrial matrix
- Exchange of key intermediates between mitochondria & cytosol





Quick summary to the mechanism:

Oxaloacetate >> malate **"by malate dehydrogenase"** >> uses NADH molecule >> electrons are loaded on malate >> malate enters the mitochondria>> transferred to oxaloacetate >> producing NADH molecule >> NADH goes to Complex I entering TCA cycle.

For more clarification:

https://www.youtube.com/watch?v=nEj8b-sg4ps

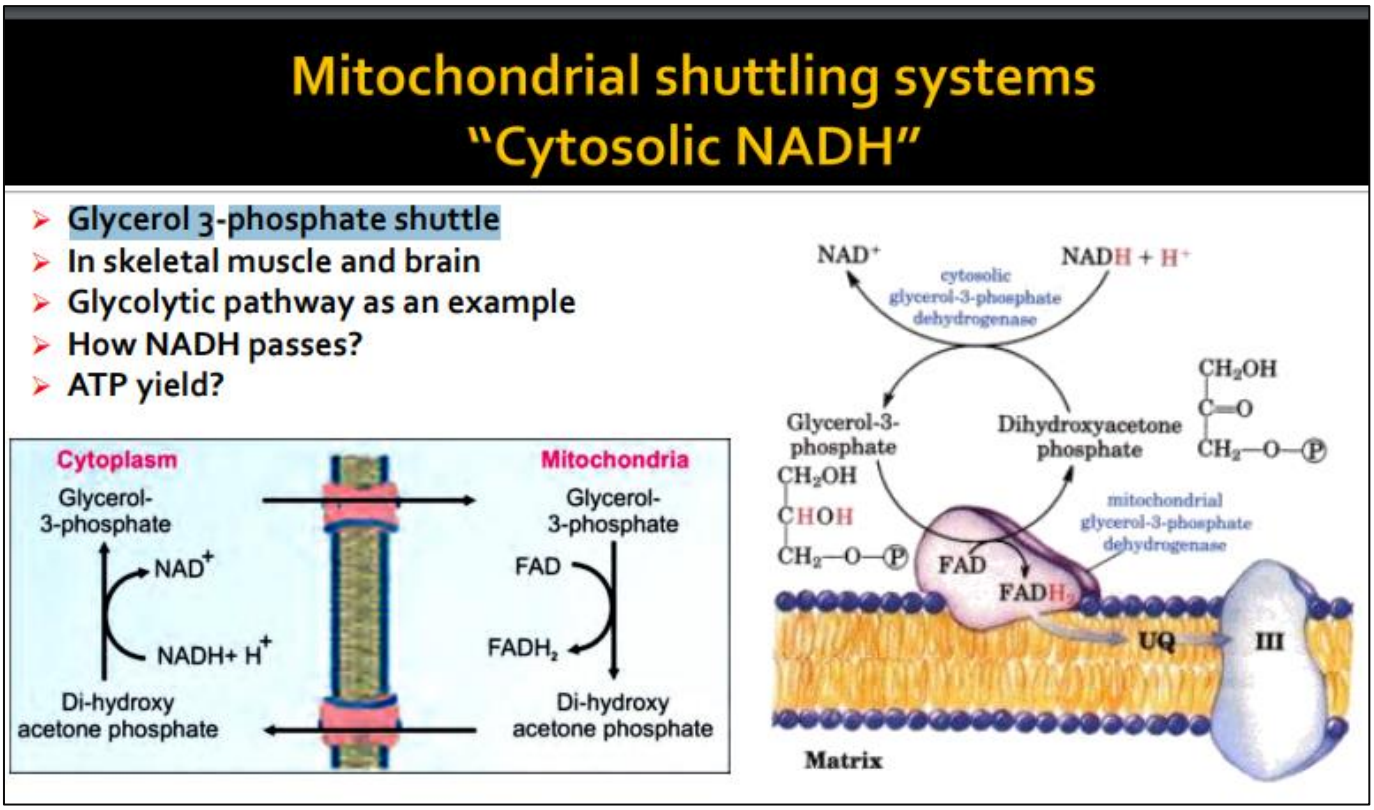
❖ Glycerol 3-phosphate dehydrogenase shuttle

Simplest sugars are aldose (glyceraldehyde) and ketose (dihydroxy acetone). If we add phosphate to ketose, through an enzyme called **glycerol-3-phosphate dehydrogenase**, it becomes **dihydroxyacetone phosphate**.

Glycerol-3-phosphate dehydrogenase has two copies. The cytosolic copy catalyzes a reversible reaction, it transfers **dihydroxyacetone phosphate (DHAP)** to **glycerol-3-phosphate (G3P)** (the keto group is reduced to alcoholic group), using electrons form NADH "which was produced by glycolysis in cytosol".

(G3P) can enter the mitochondria, and there, **glycerol-3-phosphate dehydrogenase** (the mitochondrial copy of the enzyme, which is located on the outer surface of IMM) do the reversible reaction and transfers **glycerol-3-phosphate (G3P)** to **dihydroxyacetone phosphate (DHAP).** It takes electrons from alcohol (G3P) and turns it back to ketone (DHAP). Electrons are loaded on FADH2, Co-Q takes electrons from FADH2 transporting them to complex III producing 4 protons. And then those electrons are transported to complex IV producing 2 protons (6 protons in total).

Remember, source of electrons was NADH, but we got 6 protons instead of 10, because it goes through glycerol-3-phosphate shuttle.



For more clarification:

https://www.youtube.com/watch?v=sglxi7I21-M

❖ ATP-ADP Translocase

Also known as Adenine Nucleotide Translocase (ANT), it's a transporter protein that enables the exchange of cytosolic ADP and mitochondrial ATP across the inner mitochondrial membrane.

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 the energy need.

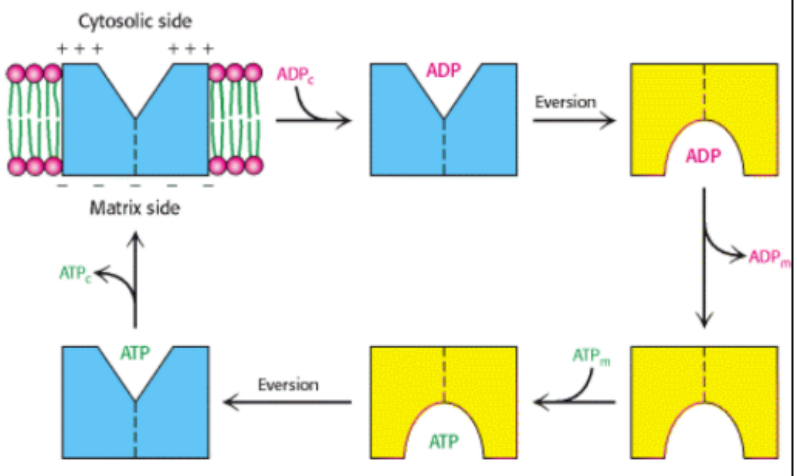
ATP/ADP translocase is embedded inside the IMM, it has like two openings. The inner side binds ATP with high affinity, which lead to conformational changes of the enzyme, it closes inside and opens outside with a low affinity to ATP to release it and higher affinity for ADP. Another conformational change happens to enter ADP to matrix to be converted to ATP through ATP Synthase.

This is an endergonic reaction, 25% of energy yield from ETC is just for this process. 14% of proteins in IMM are ATP/ADP translocase.

For each ATP molecule going outside, there should be one ADP going inside, so the ratio between them should be the same.

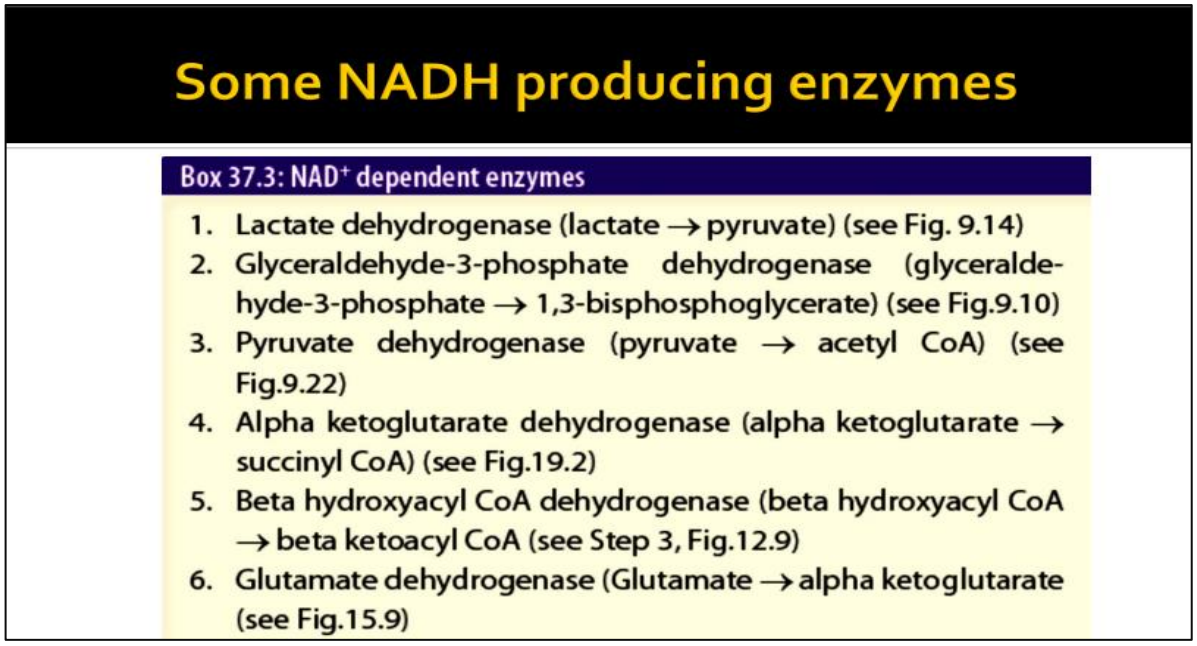
Mitochondrial shuttling systems "ATP/ADP"

- **ATP-ADP Translocase** (also called adenine nucleotide translocase or ANT)
- The flows of ATP and ADP are coupled (ADP enters only if ATP exits, and vice versa)
- Highly abundant (14% of IMM proteins)
- Contains a single nucleotide-binding site (alternates)
- Similar affinity to ATP and ADP
- Endergonic (25% of ETC)
- Inhibition leads to subsequent inhibition of cellular respiration



For more clarification:

https://www.youtube.com/watch?v=k_DQ1FjFuYM



Those are some questions the doctor discussed at the end; they are from previous years exams:

1. **Which is better to the body, high ATP/ADP ratio or low ATP/ADP ratio?**

110/10 or 100/10?

110/10 (higher ratio) is better because ΔG will be greater, remember the equation:

$$\Delta G = \Delta G^{\circ} + RT \ln \left(\frac{[P]}{[R]} \right)$$

ln (100/10) is less than **ln (110/10)**. So, energy wise, a higher ratio is better for the body. And remember that we can change energy through **playing with concentrations**.

In the exam, the question may give you five different concentrations of (ATP, ADP, ip), and you have choose depending on the given concentrations and think how they can increase or decrease energy according to the equation.

2. **Why TCA cycle uses three dehydrogenases with NAD+ as electron carrier, and just one uses FAD as electron carrier?**

Again, it's energy wise. We cannot use NAD+ with succinate dehydrogenase, ΔE when using FAD is positive, so ΔG is negative (favorable). While when using NAD+, ΔE is negative and ΔG is positive (unfavorable), so it will favor succinate formation not fumarate.

3. **If you have a glucose molecule, how many ATP it will give due to full oxidation of glucose?**

In order for glucose to be full oxidized, it must go through 3 processes (glycolysis, TCA and ETC), so we have to calculate how many NADH and FADH2 each process produces, and as we know that each NADH gives (2.5 ATPs) and each FADH2 gives (1.5 ATPs), so finally we can calculate how many ATP we would have.

4. **How many molecular oxygen (O2 molecule) do I need for complete oxidation of glucose?**

Each NADH and FADH2 gives 2 electrons, and each molecular oxygen (O2) needs to receive 4 electrons in order to form water molecule (H2O), and as we know that the complete oxidation of glucose produces 10 NADH molecules and 2 FADH2 molecules, so 6 molecular oxygens (O2) are needed for complete oxidation of glucose.

More questions from the doctor, by Ahmad Allaham.

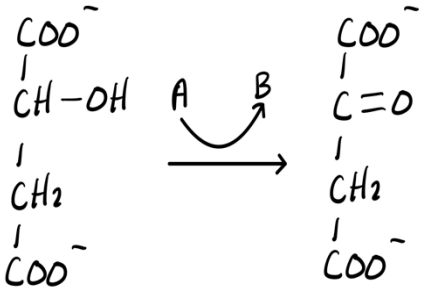
5. **One of the following is an inhibitor for ATP synthase:**
   A) Oligomycin
   B) cyanide
   C) Azide
   D) Antimycin

ANS: A

6. **The advantage of oxidative phosphorylation uncoupling?**
   A) limiting the rate of oxidizing carriers
   B) restriction of the consumption of ATP
   C) generating heat
   D) balancing the pH between inside and outside the mitochondria
   E) regulating mitochondria shuttling systems

ANS: C

7. **What is A/B in this reaction? ***



   A) NAD+/NADH
   B) FAD/FADH2
   C) ATP/ADP

D) NADPH/NADP+

ANS: A

*Malate ————> Oxaloacetate (dehydrogenation reaction NAD+ involved)

8. A characteristic of the glycerol shuttle is:

A) shuttles NADH across the membrane yielding 1.5 ATP per NADH

B) it operates through a cytosolic isozyme and another one located in the MT matrix

C) malate is a key component of the shuttling process

D) it shuttles the electrons from NADH to FADH2 across MT membrane yielding 1.5 ATP per NADH

E) None of the above

ANS: D

9. If MT matrix has a PH of (7.8) whereas the intermembrane space has a PH range between (7-7.4) and an appropriate buffer exists in the matrix, what do you expect to happen if you injected the MT matrix with (0.5) equivalents of NaOH?

A) An increase in the ATP generation efficiency

B) An increase in the level of uncoupling proteins

C) An increase in the level of electron carrying molecules

D) An increase in O2 consumption

E) Nothing would happen

ANS: E

10. ATP synthase can produce ATP using this mechanism as a direct source of energy:

A) The conversion of glucose to pyruvate

B) Oxidation of pyruvate producing CO2 and H2O

C) A proton gradient established in the MT matrix

D) metabolism of amino acids

E) break down of NADH

ANS: C

11. The only TCA cycle enzyme whose name doesn't imply its function is responsible for the production of:

A) five carbon unit molecule

B) NADH

C) CO2

D) Four carbon unit molecule

E) Tricarboxylic Acid

ANS: E

12. TCA cycle is unique because:

A) A cyclic pathway that produce electron carrying molecules
B) It produces ATP molecules through substrate level molecules
C) It has very high efficiency
D) It is an exergonic pathway

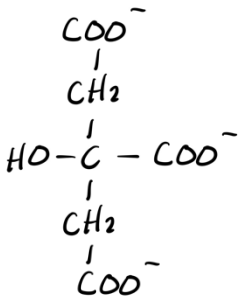
ANS: C

13. The main regulator of the respiratory chain reaction is the level of:

A) O2
B) ATP
C) ADP
D) Calcium ions
E) Electron carriers

ANS: C

14. Choose the correct statement based on the following structures of TCA cycle intermediates:



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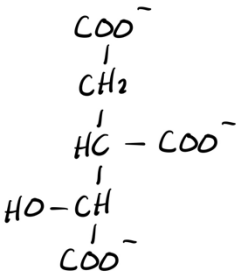

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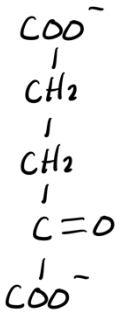

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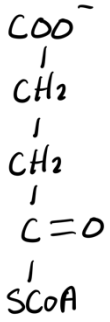

(4)

A) Conversion of (1) to (2) is an oxidative decarboxylation reaction
B) release of COA from (4) accompanies the release of CoA
C) Compound (1) is oxidized but can't be reduced
D) conversion of (3) to (4) is the rate limiting step of the cycle
E) the enzyme that catalyzes the conversion of (2) to (3) is allosterically activated by ADP

ANS: E

فتح الله علينا وعليكم فتوح العارفين