DOCTOR 2020 | JU

METABOLISM

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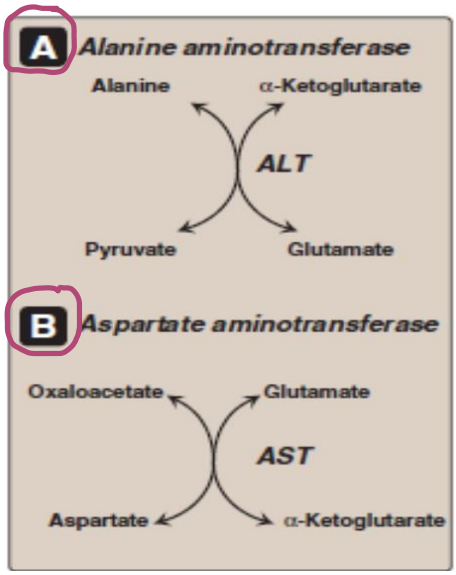
Last lecture we discussed the structure of Amino Acids which has the **alpha carbon** attached to **4 groups** : Amino group (NH3 +),Carboxyl group (COO-),Hydrogen and R group.

Today we will talk about new section of Amino Acid that deals with common pathways and common structures between these A.A and how they get metabolized. Our main concern is the **Nitrogen** of the Amino group because it is a source of Nitrogen, and this nitrogen is going to be released as Ammonia (NH_3) which is a very toxic compound, so we must be careful when we are dealing with these compounds. Also we have to maintain this nitrogen in balance (no increasing or decreasing) because we need it in the synthesis of many other compounds not only A.A .

Transamination:

generally the transamination process is about transferring an amino group(NH_3+) from a nitrogen containing compound to a certain recipient by a certain Aminotransferase (AT). In the case of transamination of AA, we transfer amino group from the AA to **α-ketoglutarate (recipient).**



*When an AA loses its amino group, it still have its carboxyl group, hydrogen and R group which is called it **α-keto acid**.

Simply:

Amino Acid – Amino group = α-keto acid

*So (α-keto acid) is a product from the transamination reaction.

^ each AA has its own α-keto acid due to the differences in the R groups.

^accordingly, there are different α-keto acids derived from different AA.

so, each aminotransferase (AT) is specific for one or few amino group donors.

*The common characteristic between the transamination of all A.A is that they have the same recipient which is **α-ketoglutarate**.

α-ketoglutarate is a TCA's cycle intermediate.

recall that the structure of **α-ketoglutarate** (5 carbons, 1 carbonyl group, 2 carboxylic groups) adding **amino group** to it → transforming it into **GLUTAMATE (AA)**

What is the first step?

-Transfer the amino group from any amino acid to the α-ketoglutarate which becomes Glutamate.

What will happen to the amino acid that lost its amino group?

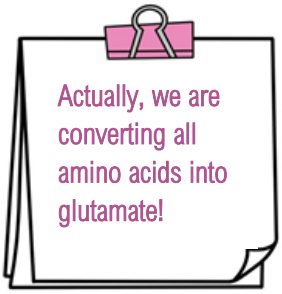
-It will become α-ketoacid.

Alanine Amino Transferase (ALT) another name is Alanine Transaminase.

ALT transfers the amino group from Alanine to α-ketoglutarate which become glutamate and the leftover of Alanine is called pyruvate (α-ketoacid of alanine is pyruvate). pyruvate can be used as a gluconeogenesis intermediate. (See pic A)

Aspartate Amino transferase (AST) transfers the amino group of **Aspartate** to **α-ketoglutarate which becomes Glutamate, and the leftover of Aspartate is called Oxaloacetate** (oxaloacetate is the α-ketoacid of aspartate). oxaloacetate can be used as a gluconeogenesis intermediate. (See pic B).

There are other enzymes for other amino acids that have the same mechanism, but we will focus on these two enzymes (ALT & AST), because they have medical significance.



The reaction of ALT goes in both directions, forward and backward(reversible), but **it favours the forward direction** which is the synthesis of glutamate and conversion of Alanine to pyruvate, so the amino group of the Alanine funnels (تتمركز و تتجمّع)into glutamate.

In this situation we are producing pyruvate by degrading amino acids, and we are degrading them because we're in the **starvation situation** to produce energy and provide gluconeogenesis with its own intermediates (e.g., oxaloacetate and pyruvate).

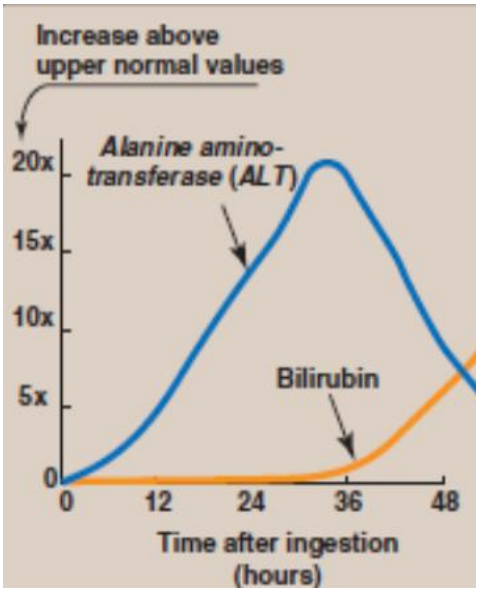
The reaction of AST goes in both directions(reversible), but **generally favors the backward direction**, so during AA catabolism AST transfers amino group from glutamate to oxaloacetate forming aspartate.

In this situation we didn't degrade the Aspartate, we produced it, **why is this happening?**

Because this Aspartate will be used in another pathway related to amino acids metabolism which is the Urea Cycle !
so the amino group of Aspartate doesn't funnel into glutamate.

Clinical Hint : Diagnostic value of plasma aminotransferases

ALT is specific to **L**iver which means it is mostly expressed in hepatocytes .
AST can be expressed by different cell types BUT it is sensitive indicator in every tissue that it is expressed in. for example, if a patient has a problem in the liver (hepatitis, hepatic cancer ..etc) there will be destruction in the cells so they go under apoptosis or necrosis and releases their contents, these contents are taken by the blood stream. So, taking a blood sample from the patient and doing a **diagnostic lab test called "Liver Function Test (LFT)"** which has serve different parameters and variables measured through this test. (ALT&AST) are two of these parameters. If ALT or AST has high levels in the blood sample that indicates a big destruction in the liver because AST and ALT normally found in the blood in low levels.
*High levels of ALT indicate that destruction is happening in liver, so ALT is a **specific indicator.**



*High levels of AST indicate that destruction is happening in many tissues not only the liver (e.g., muscle disorders, myocardial infraction), so AST is a **sensitive indicator**.

→ specificity VS sensitivity:
specificity Deals with the location of disruption, while sensitivity deals with the amount, so small destruction in the organ can reflect a high amount of this enzyme.
→Relation between **specificity & sensitivity:**
sensitivity and specificity are inversely proportional, once specificity is increased, the sensitivity is decreased etc.

after the transamination of all AA To **GLUTAMATE**, the next step is removal of this amino group in the form of ammonia (NH_3) in a step called: OXIDATIVE DEAMINATION.

Oxidative deamination

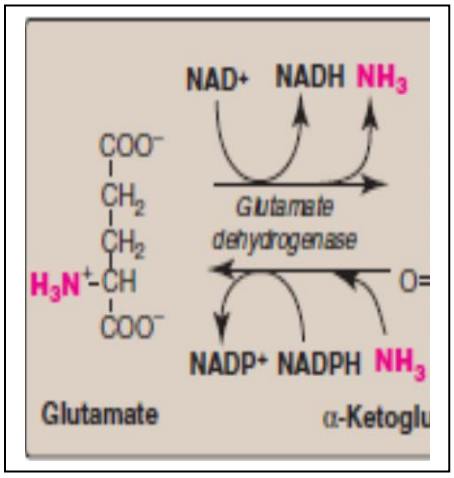
All amino acids are converted into glutamate because Glutamate is the only AA that undergoes rapid oxidative deamination.

Oxidative = loss of hydrogen.

deamination=removal of amino group

*Oxidative deamination by glutamate dehydrogenase result in the liberation of amino group as a free ammonia. So, the products of this reaction can either enter the central pathway of energy metabolism (**α-ketoglutarate**) or enter the urea cycle as a source of Nitrogen(**ammonia**).

*Reactions occur primarily in the liver and the kidney.



→transamination VS oxidative deamination:

transamination: transferring amino group from AA to a recipient (**α-ketoglutarate**).

Oxidative deamination: removing amino group from glutamate and releasing it as a free ammonia (no recipient).

→Glutamate Dehydrogenase:

*Catalyzes the forward and backward reactions but with different co-factors (observe the picture above):

→**In the forward reaction**: (converting Glutamate to α-ketoglutarate) using **NAD+** as a coenzyme which is reduced to **NADH,** and glutamate is oxidized by losing H.

←**In the backward reaction**: (converting α-ketoglutarate to Glutamate) using **NADPH** as a coenzyme which is oxidized to **NADP+,** and α-ketoglutarate is reduced by gaining H.

→Allosteric regulators:

GTP is an inhibitor for glutamate dehydrogenase because GTP is high energy indicator, so there is no need to degrade AA.

ADP is an activator for glutamate dehydrogenase because ADP is low energy indicator, so there is a need to degrade AA.

D-Amino acid oxidase

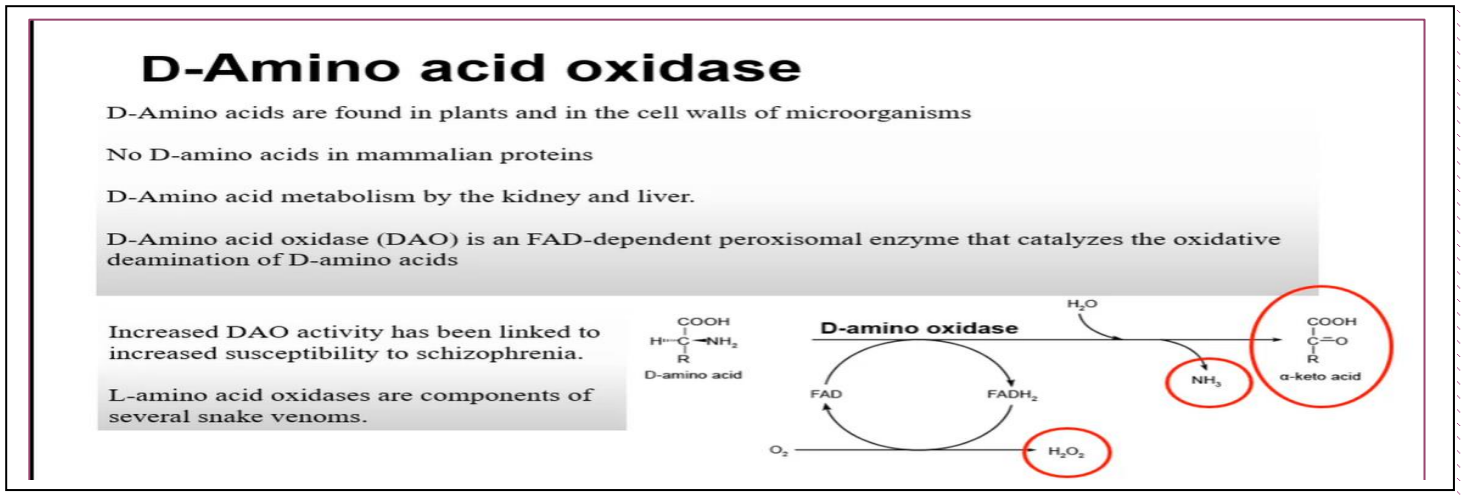
The pathways that discussed before is applied for **L- amino acids** which normally present in our bodies.

D-amino acids can get to our bodies from different sources, and they can be metabolized by the kidney and the liver.

D-amino oxidase (DAO) is a FAD-dependent peroxisomal enzyme that catalyzes the oxidative deamination of D-amino acid. Same mechanism explained above with different co-factor which is **FAD** reduced to **$FADH_2$** and producing H_2O_2 (ROS) from O_2.

Increased DAO activity has been linked to increased susceptibility to schizophrenia (مرض الفُصام). But the mechanism is unknown.

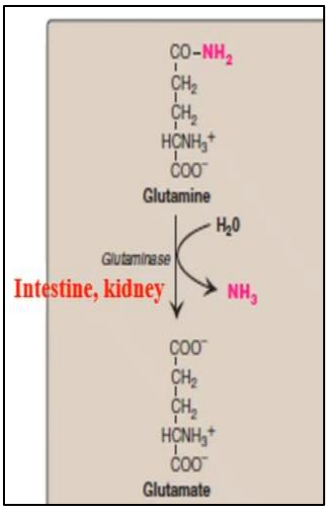
There is L-amino oxidase which is a component of **<u>snake venoms</u>** which will affect the deamination of AA.



Now let's move on and see what will happen to ammonia that was released in oxidation deamination.

Metabolism of Ammonia

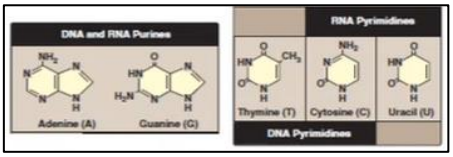
Sources of ammonia:



1) From Glutamine: Most of the ammonia is excreted into urine as NH4+ (acid-base balance).

2) From bacterial action in the intestine (normal flora): ammonia is formed from urea by bacterial urease in the intestinal lumen. This ammonia can be absorbed from the intestine by the portal vein and is converted again to urea by the liver.

3) From amines: amines in diet, and monoamines that acts as hormones and neurotransmitters give rise to ammonia by amine oxidase.



4) From purines and pyrimidines: in the catabolism of purines and pyrimidines, amino group attached to the rings are released as NH_3.

Transport of ammonia to the liver

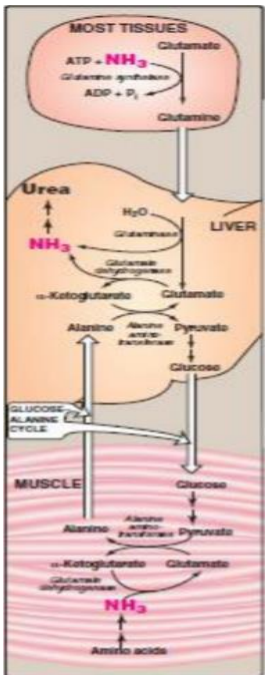
The ammonia that was formed from oxidation deamination in different tissues, is going to be converted to urea to become less toxic. This takes place only in **hepatocytes,** so ammonia must be transported from peripheral tissues to the hepatocytes(liver).

Ammonia is a hydrophilic molecule(polar), so it can move easily but there is a problem in that.

The problem is that Ammonia is a very toxic molecule, so while it is moving in the blood to reach the liver , it causes toxicity to the blood.

So, what is the solution for a such situation?

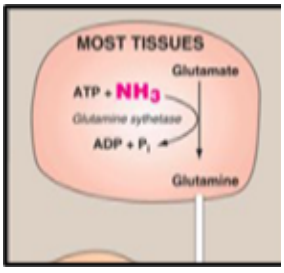
-Transferring ammonia in a hidden form (AA) so it won't cause toxicity to the blood.



Two mechanisms for ammonia transport:

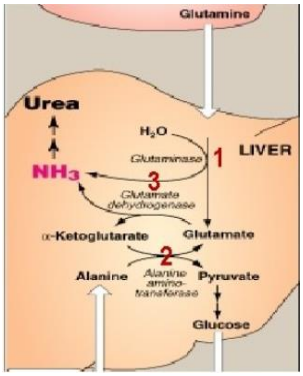
1) By glutamine synthetase.

Glutamine synthetase combines ammonia to glutamate to form glutamine. This reaction is ATP-dependent→ using ATP to avoid toxicity.



NH_3+Glutamate =Glutamine

after we have hidden the ammonia in glutamine. Glutamine is transported from the peripheral tissues by blood and reaches to the liver. Once glutamine reaches the liver, we remove **ammonia,** so it's converted again to glutamate. Then we remove another amino group from glutamate by oxidative deamination which gives **α-ketoglutarate** and **ammonia**, this ammonia can then enter to urea cycle.(see the pic)→

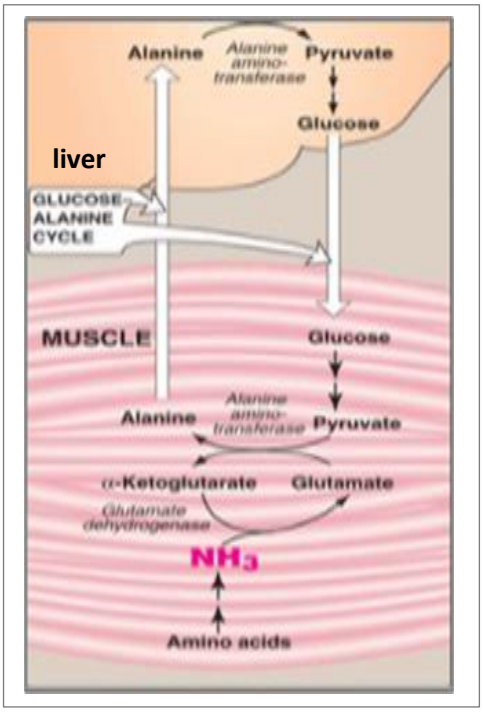


Note: 1 glutamine gives 2 ammonia.

2) By Transamination of pyruvate to form Alanine

Ammonia is transported in the blood from **muscles** to **hepatocytes** in a form of **Alanine.**

In muscles, amination reaction occur to pyruvate converting it to alanine. Alanine gets out of the muscles to the blood stream then to hepatocytes. Once alanine reaches the liver, ALT enzyme works on Alanine removing (ammonia → urea cycle) and the leftover is pyruvate, this pyruvate can be used in gluconeogenesis producing glucose in the hepatocytes. Then glucose leaves the liver and go back to muscles. The whole process is called "**glucose-alanine cycle".** (See the picture)→



Note: 1 Alanine gives 1 ammonia.