Metabolism

فريق طوفان الأقصى

Modified N: 14

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Amino acid metabolism

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Biochemistry for Lippincott. Chapters 19-21

All images were taken from Lippincott's Biochemistry textbook except where noted

Amino acid digestion and metabolism overview

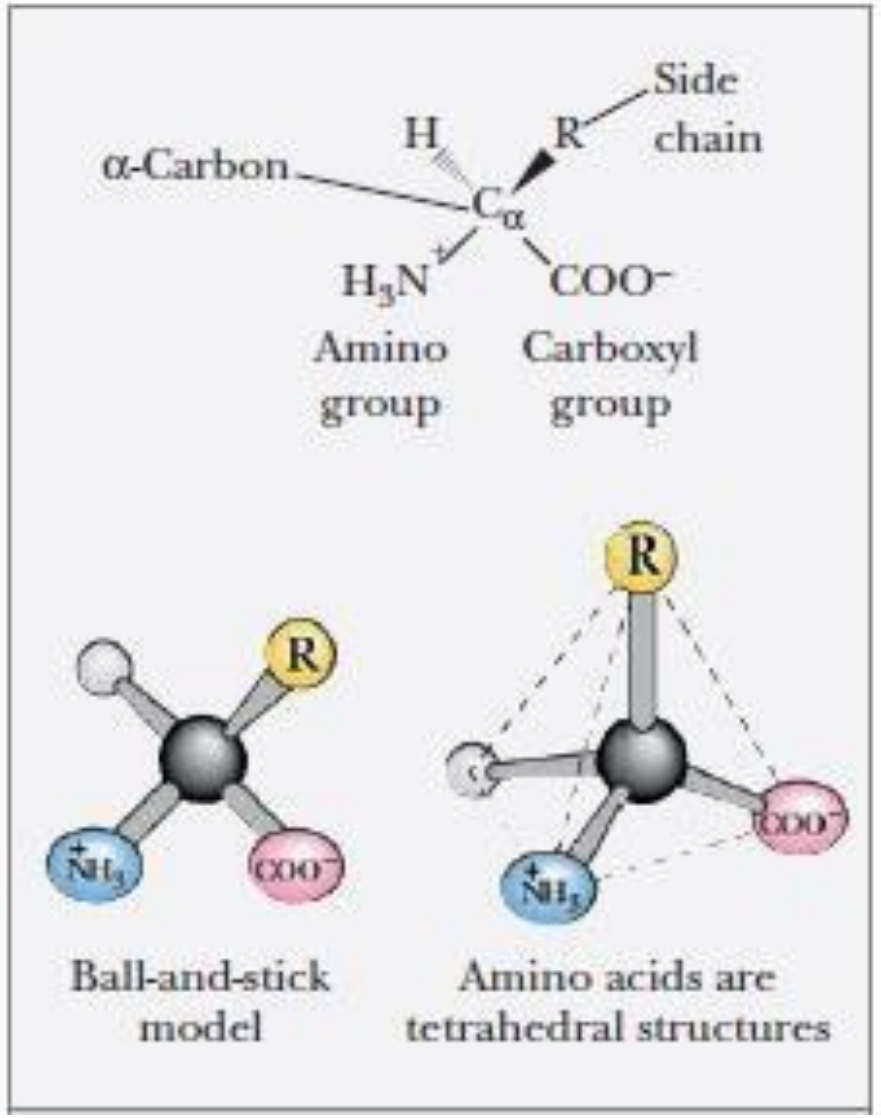
Read all the pictures important!!!!

Amino Acid Structure

- **NOTE:** Amino acids consist of a carbon molecule bound to and amine group (NH3), carboxyl group (COOH), a hydrogen (H), and an R group.

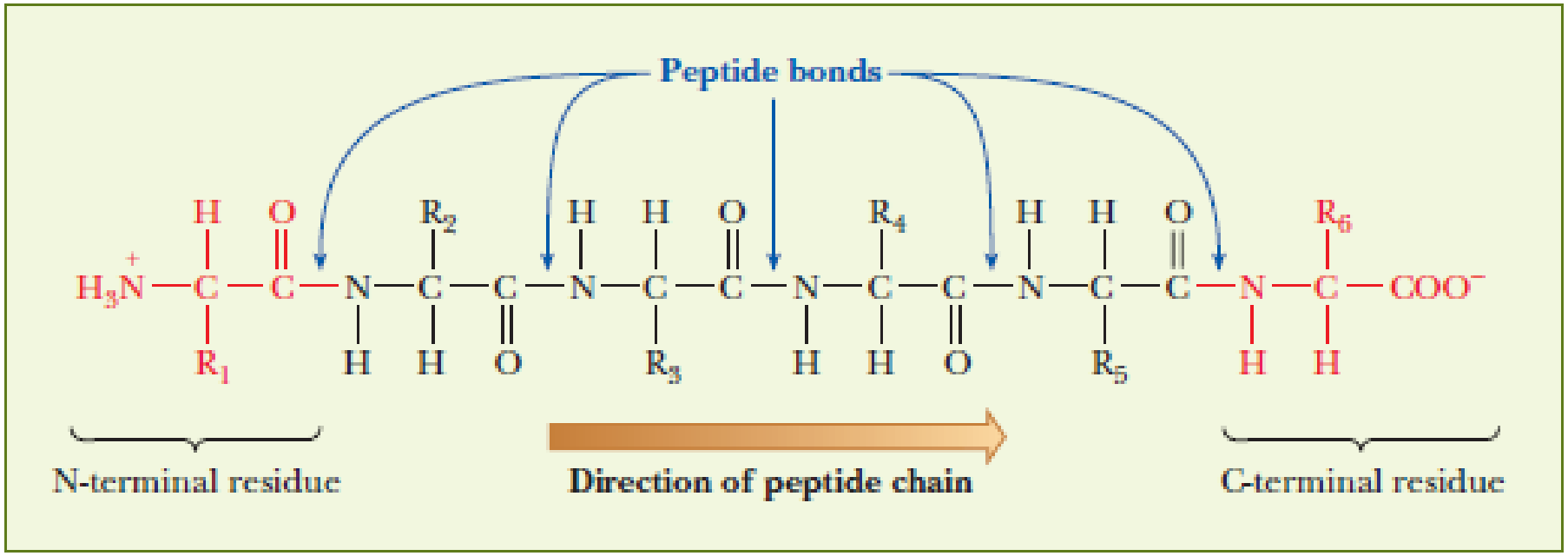
- **NOTE:** Amino acids are chiral except glycine, which has (H) as its R group.

Only 20 are usually found in proteins



- **NOTE:** All amino acids in humans are in L configuration.
- Humans can get amino acids in D configuration from exogenous sources and your cells can deal with them.

Peptide and polypeptide chains



- **NOTE:** **Amino acids are connected together by a covalent peptide bond, NH3 from one amino acid is connected to COOH of the next amino acid.**

Peptides: two to several dozens AA.

Polypeptide chain: many amino acids (usually more than a hundred)

Amino acids (AAs)

NOTE: Peptides and polypeptides start from N terminus and end in C terminus, N terminus is where the amino group is not bound to anything, and C terminus is where the carboxyl group is not bound to anything. In physiological conditions, N terminus and C terminus are charged. (Remember isoelectric point).

- AAs are NOT stored in the body
- AAs sources are diet, de novo synthesis or protein degradation
- AA metabolism overview:

NOTE: since the structure of AAs have common parts of the backbone, we expect to have shared reactions between metabolic pathways and distinctive steps that can deal with R group that differs between amino acids

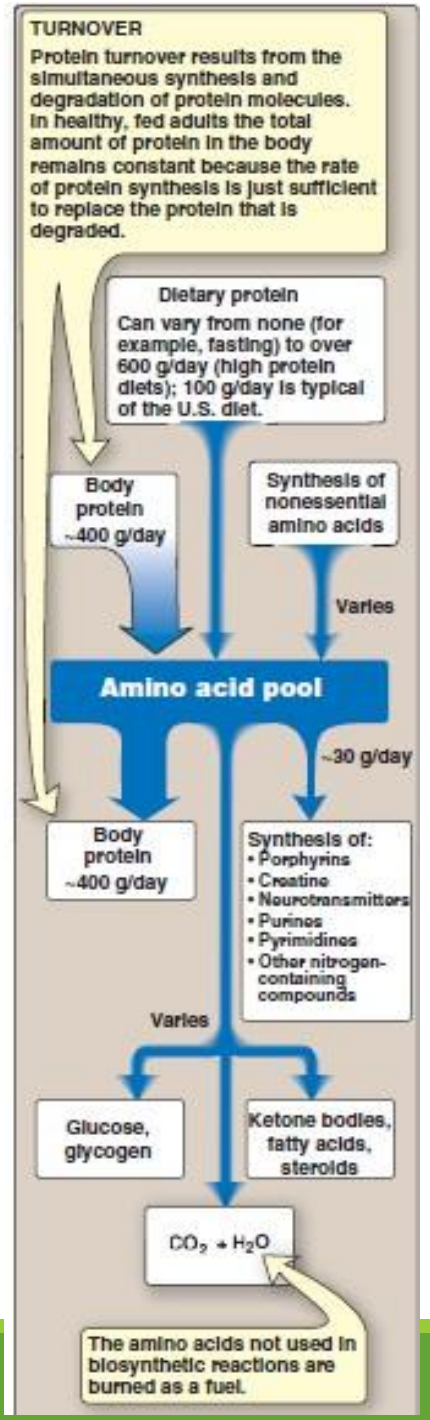
Degradation of AAs whenever I have excess AAs that I can't make use of :

1. α-amino group (amino group of the backbone) removal by transamination(we will talk about it in the next slides) then oxidative deamination(amine group released as free group) (N leaves the body as urea, ammonia or other compounds)
2. The resulting α-keto acids are converted to energy producing intermediates
3. Intermediate metabolism to CO_2, water, glucose, fatty acids, or ketone bodies

The metabolic processes have to keep harmony between amino acid pool and protein turn over

Sources and fates of amino acids

- The AA pool is small ~about 90–100 g of AAs
- The amount of protein in the body is about 12 kg in a 70-kg man.
- Normally, the amount of AAs in the AA pool is balanced by the output (constant amount)
- The amino acid pool is in a steady state, and the individual is in nitrogen balance.



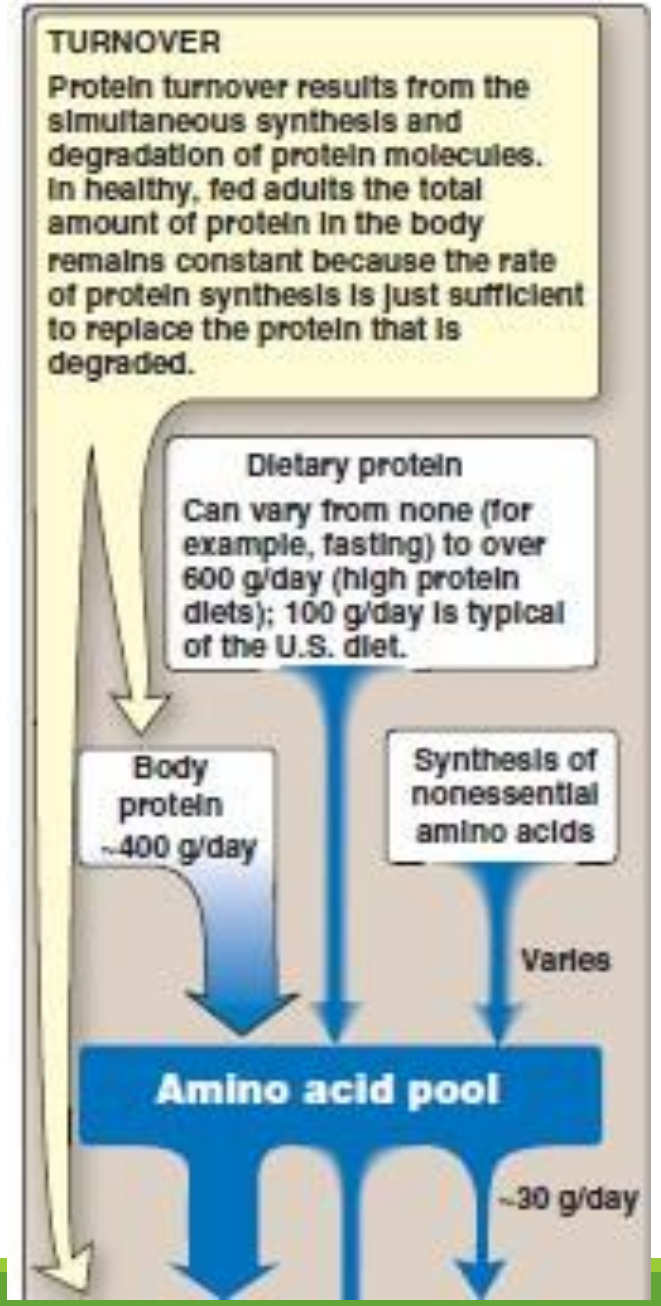
Amino Acid Pool

- AA sources:

1. Endogenous (body) protein degradation

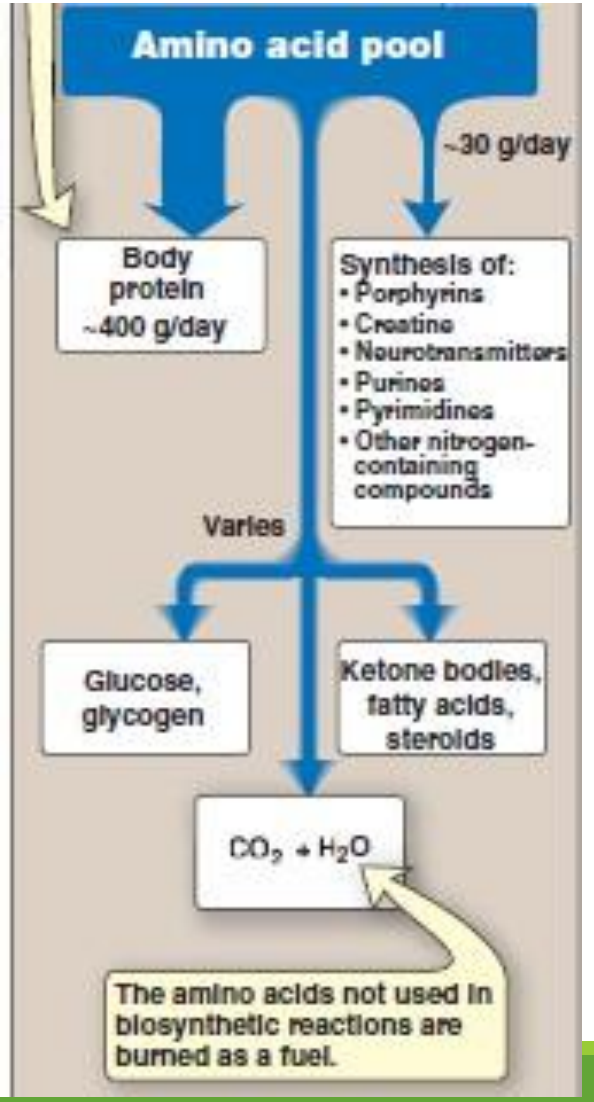
   - An example is misfolded proteins that are directed to degradation with the help of chaperones. Or degradation of unused proteins, unused Enzymes.

2. Exogenous (dietary) protein digestion either free amino acids or proteins.
3. Nonessential amino acids synthesized from metabolic intermediates



Amino Acid Pool Depletion Routes

- AAs are depleted by 3 routes:
- 1) Synthesis of body protein
- 2) AAs consumed as precursors of nitrogen-containing small molecules
  - Like heme group, melanin pigment, epinephrine.
- 3) Conversion of AAs to glucose, glycogen, fatty acids, ketone bodies, or $CO_2 + H_2O$



Protein Turnover

- Protein turnover is the process in which the rate of protein synthesis is sufficient to replace the degraded protein.
- Each day, 300–400 g of body protein is hydrolyzed and resynthesized
- In healthy adults, the total amount of protein in the body remains constant.
- Turnover varies widely for individual proteins.
- Most proteins are long-lived proteins (t1/2 days to weeks)
- Structural proteins, such as collagen, are metabolically stable (t1/2 months or years).

RATE OF PROTEIN TURNOVER

- For many proteins, regulation of synthesis determines the [protein in the cell] and protein degradation is minor
- For other proteins, the rate of synthesis is constitutive, or relatively constant, and [protein in the cells] is controlled by selective degradation.

NOTE:
Protein turnover is the difference between protein synthesis and degradation simply the replacement of old proteins with new ones.

The complement to this slide :

- **Proteins have half life, each protein has a half life depending on its function, so proteins used for a short time have a short half life (like some types of enzymes), and proteins that are used for long time have a long half life (like structural proteins).**
- **In general, structural proteins have longer half life than other proteins.**
- **Protein degradation and synthesis is based on cell demand, for example if I shift from fasting to well fed state, anabolic enzymes will be synthesized, and catabolic enzymes will be degraded.**
- **These demands are based on signals, like hormones that induce transcription factors to synthesize more enzymes for example.**
- **The primary sequence of amino acids in a protein can determine its half life.**

- **Another factor that would affect turnover of these protein is their mode of expression(how your cells synthesis your protein)**
- **If it continuous we call it** constitutively active protein(they are present all the time) like house keeping genes
- Other proteins have inducible expression(we have to have stimulus to activate their expression and this stimulus is connected to hormonal secretion, and these hormones can bind to their receptors activating a cascade of events that result in activation of a transcription factor and expression of type of genes. Like glucagon in fasting state

Protein degradation

Two major enzyme systems are responsible for degrading damaged or unneeded proteins:

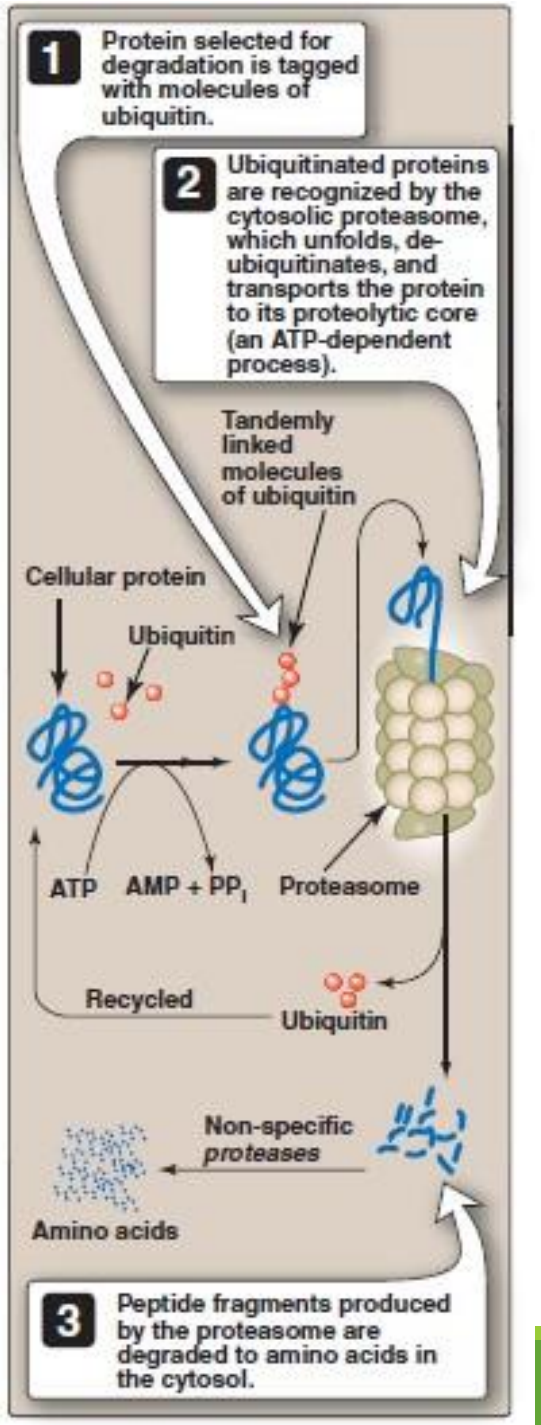
1. The ATP-dependent ubiquitin-proteasome system of the cytosol mainly endogenous proteins (proteins that were synthesized within the cell)

2. The ATP-independent degradative enzyme system of the lysosome

LOOK AT THE PICTURE IN THE NEXT SLIDE WHILE READING!!

- **The complement in this slide:**

- Proteasomes are not organelles but are protein complexes with a core in the middle where the protein to be degraded inters it and get degraded into fragments.
- Ubiquitin is a non-enzymatic small protein.
- Proteins that need to be degraded are tagged with multiple ubiquitin units making a polyubiquitin chain .
- Ubiquitin doesn’t do cleavage it’s just a marker and it doesn’t get degraded so it can used again to mark another protein.
- A proteasome comes to this tagged protein and degrades it to multiple fragments .
- These fragments are further degraded by non-specific proteases.
- This pathway is ATP-dependent.
- This mechanism is responsible for degradation of intracellular proteins.



Ubiquitin-proteasome proteolytic pathway

Ubiquitin (Ub) is a small, globular, non-enzymic protein.

Several Ub units are added by an enzyme-catalyzed, ATP-dependent process to generate a **polyubiquitin chain**.

A proteasome is a large, barrel-shaped, macromolecular, proteolytic complex that recognizes Ub-protein

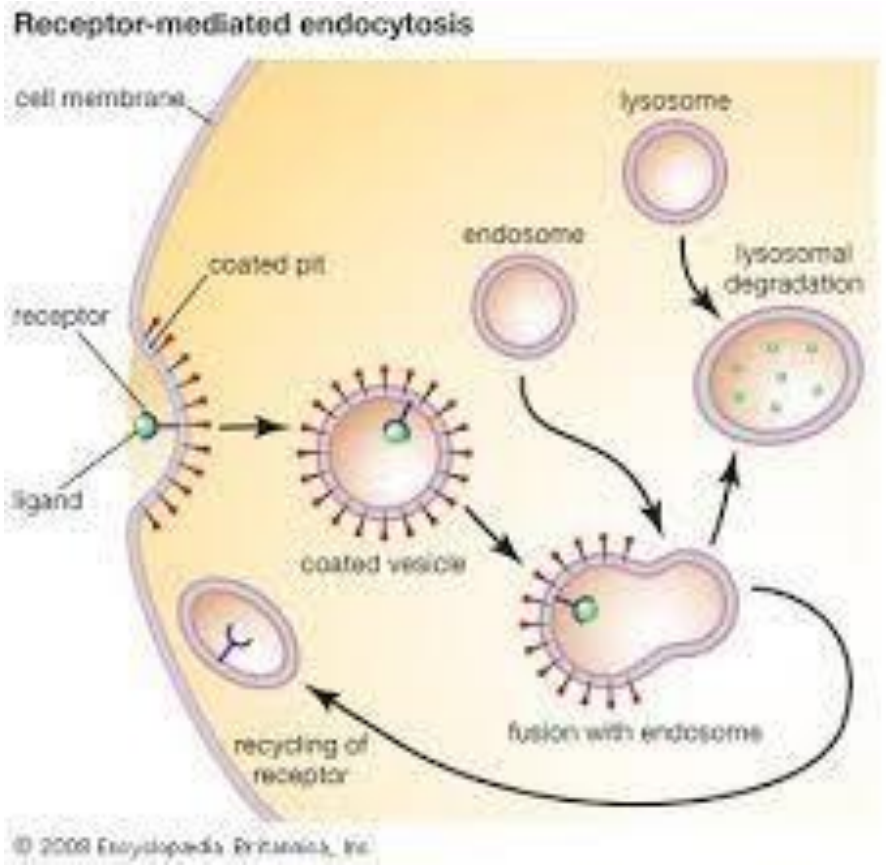
Simple hydrolysis by proteolytic enzymes does not require energy

The ATP-independent degradative enzyme system of the lysosome

Lysosomal enzymes (acid hydrolases) degrade primarily:

A. Extracellular proteins, such as plasma proteins, by endocytosis

A. Cell-surface membrane proteins by receptor-mediated endocytosis.



- **The complement in this slide:**

- This mechanism is ATP-independent.
- It degrades proteins in lysosomes.
- It’s mainly responsible for degradation of extracellular proteins as well as plasma membrane proteins.
- These proteins are endocytoted by receptor mediated endocytosis.
- The vesicle fuse with the lysosome forming endosome, and proteins are degraded by lysozymes in an acidic environment inside the lysosome.

DIGESTION OF DIETARY PROTEINS

70–100 g/day in the American diet

Proteins are too large to be absorbed by the intestine. Protein digestion begins in the stomach

Stomach secretes the gastric juice that contains hydrochloric acid and the proenzyme, pepsinogen.

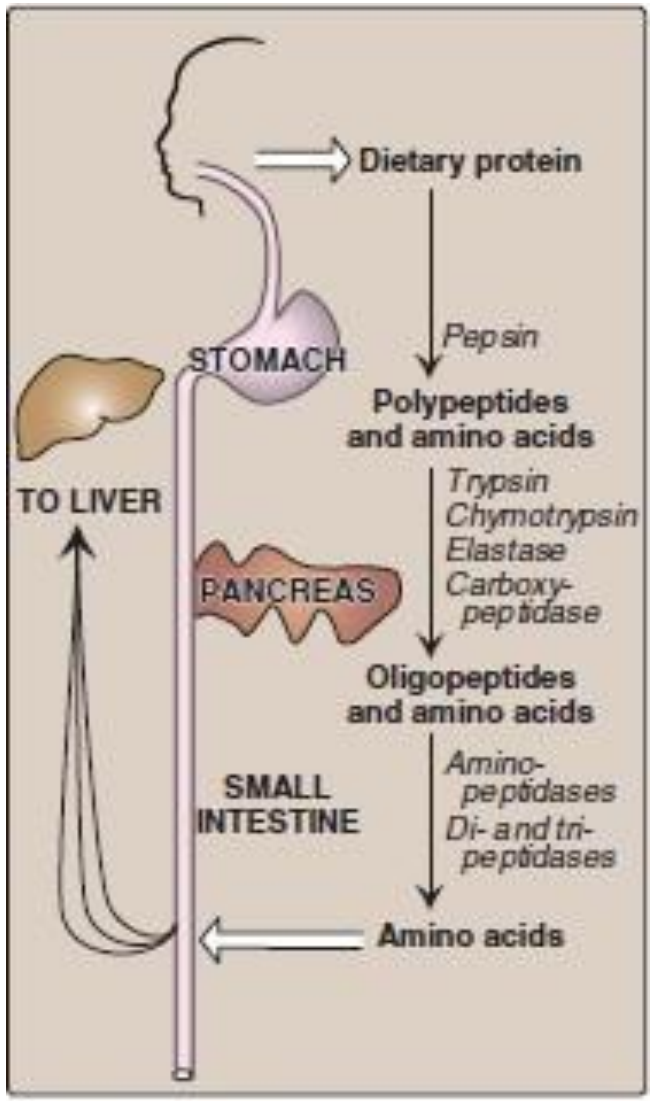
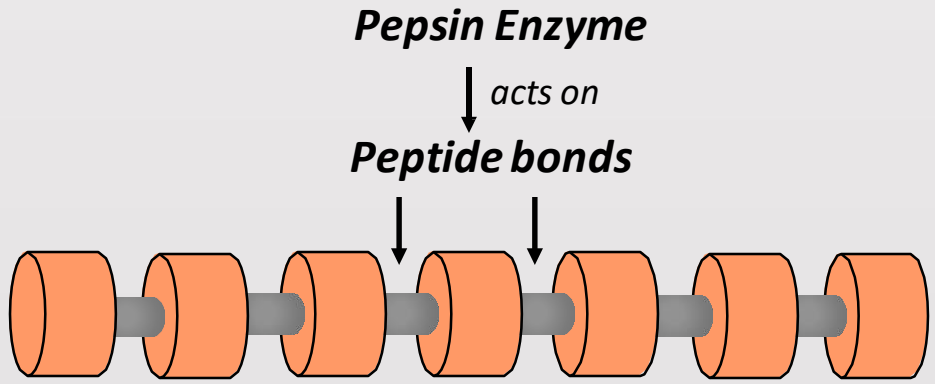


Figure 19.4
Digestion of dietary proteins by the proteolytic enzymes of the gastro-intestinal tract.

- **Note:** **proteins in general speaking are complex molecules so when they enter oral cavity nothing happen to them, so they have to move to the stomach where they encounter digestive enzyme pepsin and acidic environment of the stomach.**

Digestion of proteins by gastric

1. Hydrochloric acid: pH 2–3 to hydrolyze proteins.



HCl is secreted by the parietal cells

HCl functions:

A. kills some bacteria "protection"

B. denatures proteins to make them more susceptible to subsequent hydrolysis by proteases.

C. Activation of pepsin

NOTE: pepsin is inactive in the form of pepsinogen in chief cells so it doesn't digest the cell, and it's only activated when it's secreted to the lumen of the stomach

2. Pepsin: acid-stable endopeptidase

- Is secreted by the chief cells of the stomach as an inactive zymogen (or proenzyme), pepsinogen.

-Pepsinogen is activated to pepsin, either by HCl, or autocatalytically by other activated pepsin molecules.

-Pepsin releases peptides and a few free amino acids from dietary proteins.

- **NOTE:** once this protein is partially digested by pepsin (it's not going to be completely digested (fragments not free AAs)) the Mixture of food and the digestion, pepsin, HCL this Mixture is going to move to the small intestine and once pepsin faces the relatively basic environment of the small intestine, inactivation of it happens.

Digestion of proteins by pancreatic enzymes in small intestine

Release of zymogens: The release and activation of the pancreatic zymogens is mediated by the secretion of cholecystokinin and secretin (two polypeptide hormones of the GIT)

Zymogen activation : Enteropeptidase (enterokinase)— the luminal surface of intestinal mucosal cells converts the pancreatic zymogen trypsinogen to trypsin (removal of a hexapeptide from the N-terminus of trypsinogen)

- **NOTE**: In the small intestine in the duodenum, the secretions of the pancreas are released, and multiple proteases are included in the secretions.
- Pancreas secretes amylase and lipase and also can secrete group of proteases including trypsinogen ,chymotrypsinogen , proelastase ,procarboxy peptidase A and B.
- Again they are synthesized and released as zymogen **to prevent digestion of the pancreas as well as the canal reaching the duct.**

-Trypsin subsequently converts other trypsinogen molecules to trypsin

-Trypsin is the common activator of all pancreatic zymogens

- **The complement in this slide:**

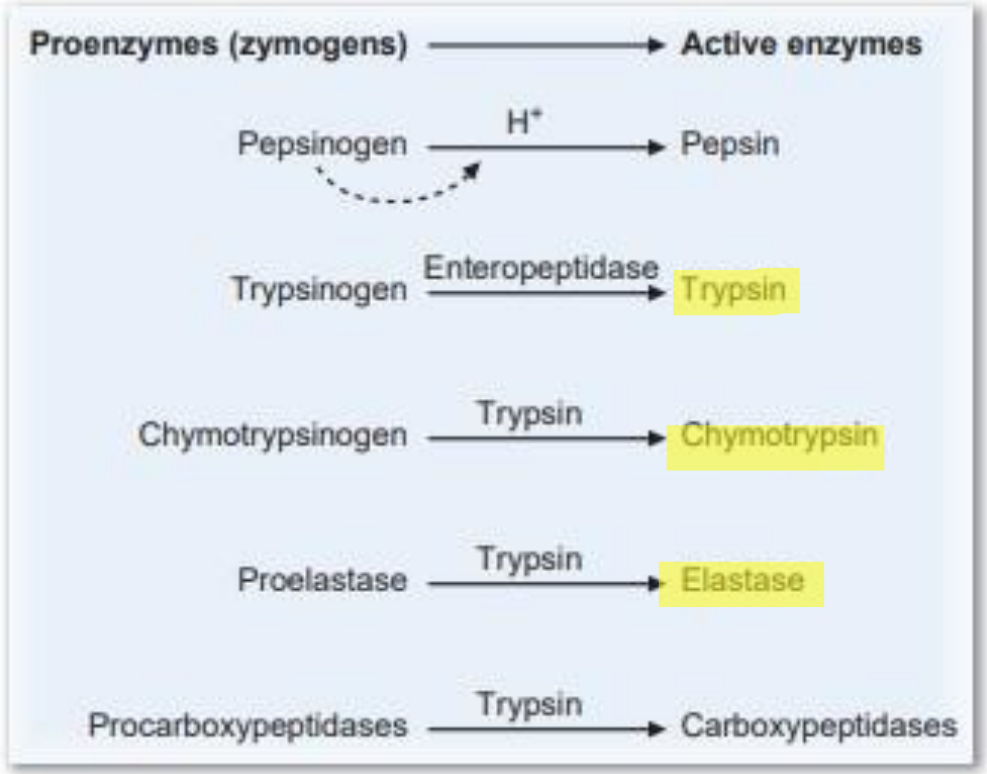
Once they reach the small intestine the first one to be activated is **trypsinogen** and becomes **trypsin** by **Enteropeptidases** and the changes in the environment(the relatively basic environment). Once trypsin is formed it is going to activate all other types(trypsin responsible of cleavage and activation).

The complement in this slide:

We have three enzymes (trypsin,chymotrypsin,elastase) they are endopeptidases act on the middle of structure, and they are serine endopeptidases because they have a serine amino acid in the catalytic site(involved in catalysis).

But carboxypeptidases A and B they are exopeptidases act on the C- terminus.

Each one of them identify certain amino acids around the peptide bonds and cuts before or after them.



NOTE: Trypsin: cleaves after arginine and lysine

NOTE: Chymotrypsin : cleaves after aromatic (tryptophan, tyrosine, phenylalanine) in addition to methionine and leucine

NOTE: Elastase: cleaves after alanine, glycine, serine

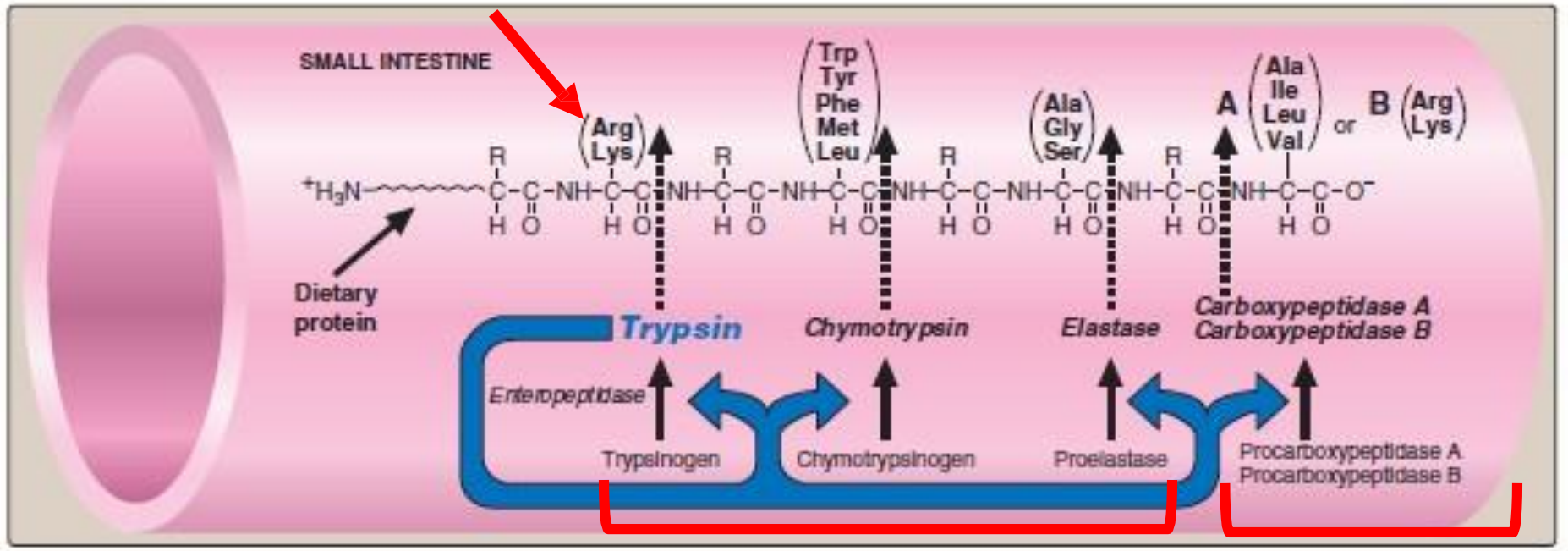
NOTE: Carboxypeptidases:

- A carboxypeptidases: cleaves before alanine, isoleucine, leucine and valine
- B carboxypeptidases: cleaves before arginine or lysine

Digestion of proteins by pancreatic enzymes in small intestine

Large polypeptides produced in the stomach are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases.

Enzyme specificity: Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond



Serine endopeptidases

Exopeptidases

Digestion of oligopeptides by enzymes of the small intestine

Aminopeptidase at the luminal surface of the intestine

Aminopeptidase is an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce smaller peptides and free AAs.

The digestive enzymes digest themselves as well as dietary protein. They also digest the intestinal cells that are regularly sloughed off into the lumen.

- **The complement in this slide:** in this way we covered peptide bonds in the middle of the sequence as well as C-terminus, we still have N- terminus not digested(it's not going to be digested by pancreatic enzymes, but it's going to be digested by intestinal enzymes called **amino peptidase**)
- **Amino peptidase:** is a membrane protein of the small intestinal cells in the the luminal side that protrudes to the lumen of the small intestine.
- This way the polypeptide chain now has free amino acids and di, tripeptides (largest piece) and they can be absorbed now ☺

Absorption of amino acids and small peptides

Free AAs are absorbed into the enterocytes by a Na+-linked secondary transport system at the apical membrane.

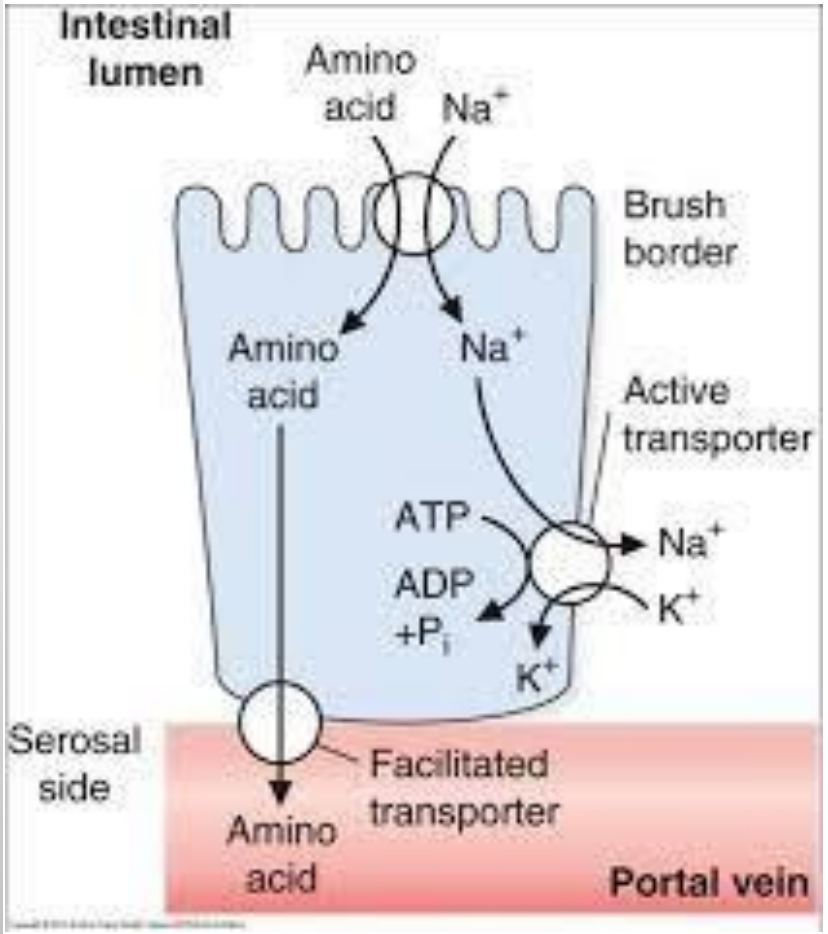
Di- and tri –peptides are absorbed by a H+-linked transport system.

The peptides are hydrolyzed in the cytosol to AAs

AAs are released into the portal system by facilitated diffusion.

AAs are either metabolized by the liver or released into the general circulation.

Branched-chain amino acids are not metabolized by the liver, but are sent from the liver to muscle via the blood



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- **The complement in this slide:**

How we absorb them??(there is difference between absorption of amino acids and absorption of di,tri peptides)

Absorption of free amino acids: amino acids can be uptaken in association with Na+ in cotransport system(secondary transport system with sodium ions), and once these ions are inside, we have to connect them to the sodium potassium pump to maintain the distribution of ions across the membrane. so here we will have consumption of energy in terms of ATP(but here we don't need energy لما ندخل على الخلية AA). **LOOK AT THE PICTURE IN THE PREVIOUS SLIDE TO UNDERSTAND!!**

Absorption of di, tri peptides: enter by a system which depends on protons(proton linked transport system), and once they are inside the intestinal cells their peptide bonds get cleave, and they are converted to free amino acids.

So everything now inside the intestinal cells is free amino acids, their concentration is very high so they move down the concentration gradient by facilitated diffusion through the basolateral surface of these cells to the portal circulation.

From portal vein they go to the liver and then liver distributes them by general circulation to all cells of the body.

■ **The complement in this slide:**

Amino acids are relatively large molecules(polar molecules and even the non polar amino acids have polar backbone groups) so they need transporters to be uptaken by the cells, and there are several types of transporters. some amino acids they have their own transporter like tryptophan. And others share the same transporter like COAL transporter(each letter stands for the one letter abbreviation of amino acid , so four amino acids)

(Cystine , Ornithine, Alanine, Leucine)

Ornithine: it is amino acid but not present in protein structure.

Clinical Hint: Abnormalities in protein digestion and Celiac disease

Pancreatic secretion deficiency due to chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas, results in incomplete fat and protein digestion.

Symptoms: abnormal appearance of lipids (**steatorrhea**), and **undigested protein** in the feces.

- **NOTE:** that's why these diatery components are going to stay in the intestinal lumen and be excreted in the feces(feces in these patients will contain proteins ,sugars , lipids ..)
- Important sign the patient may notice is the presence of lipid molecules in their feces.

- **NOTE:** medical problems related to pancreas, such as acute, chronic pancreatis, pancreatic cancer, cystic fibrosis,..)

All digestive enzymes (lipase, amylase, proteases) are going to be affected (if it was genetic problem, one gene, one protein) so the digestion is going to be affected hence the absorption gets affected.

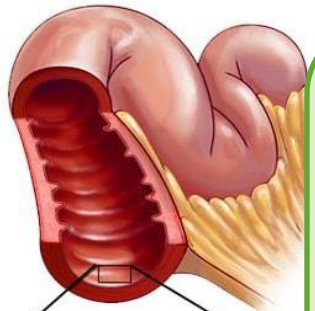
We talked about it in lipid Lec number 1 (:

Celiac disease (celiac sprue) is a disease of **malabsorption** resulting from immune-mediated damage to the small intestine in response to ingestion of **gluten** (or gliadin produced from gluten), a protein found in wheat, barley and rye.

- **NOTE:** the second problem is celiac disease حساسية القمح .
- It's allergy to gluten that is present in wheat as well as other grains, such as barelyالشعير which contain gluten
- Gliadin is part of gluten and the one responsible for producing allergic response in these patients.

- **NOTE:**

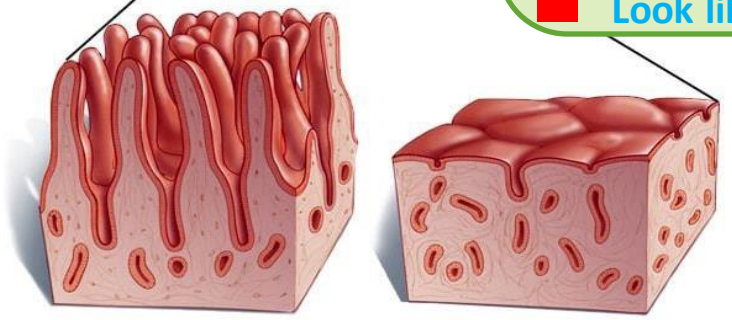
Allergy is going to destruct the intestinal cells that looked normally like this(as shown in the picture) and flattened the surface of the small intestine



- Normal state: villi, microvilli to increase the surface area

- This is going to be missing this way because flatter surface means less surface area, less number of cells,enzymes and transporters. so the patient will have diahrrea in the cause of accumulation of dietry constituents in the intestinal lumen.
- Look like malnutrition.

- Larger surface area means you can fit in more cells, more cells means high concentration of the enzymes and transporters, so more efficient digestion and absorption.



The complement in this slide:

Gluten found in wheat is the protein(wheat protein) and responsible for providing fluffiness of the dough بتعطي شوي مرونة بالعجينة فلما نيجي ناكل الخبز العادي بكون طري وفيه مرونة بالمقارنة مع خبز دقيق الأرز أو دقيق الدرة بكسر لحاله لأنه ما فيه غلوتن بالتالي ما فيه مرونة.ويقال أنه الغلوتن الموجود بالقمح الحالي بالعالم كمياته كثير أكبر من الكمية الحقيقية وهاد بسبب التلاعب فيه جينيًا(بزيدو كمية الغلوتن فيه بمتص مي أكثر فبثقل أكثر وبجيب مصاري أكثر وعشان هيك الخبز بكون كثير دبق)

So it is recommended for celiac disease patients to drink a lot of water to clean the rest of gluten from their bodies.

- **NOTE:** To sum up we talked about sources of amino acids(exogenous /dietary protein degradation, endogenous protein degradation, synthesis of nonessential amino acids(we will talk about it later))

Disposal of Nitrogen

- **NOTE:** now we are going to talk about degradation of amino acids(how we degrade them)
- We will focus now on disposal of nitrogen because we have to maintain nitrogen balance it’s very important!!

Transamination

Substrate specificity of aminotransferases:

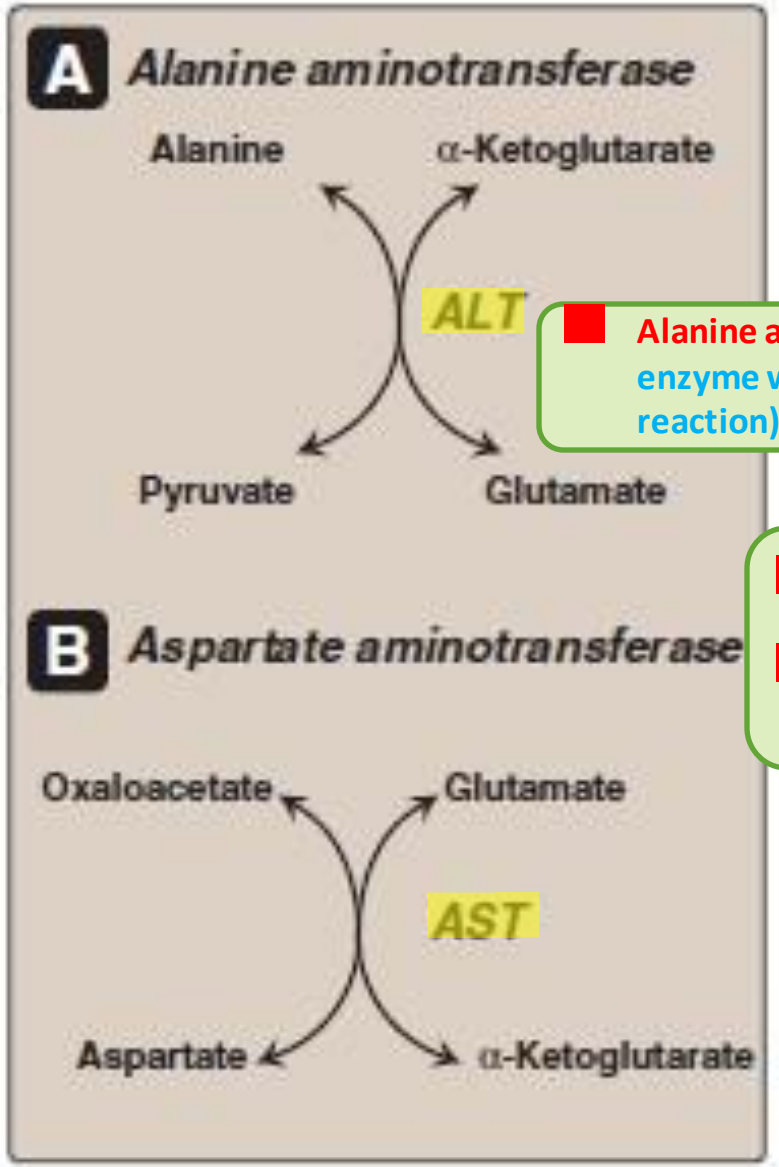
Each aminotransferase (AT) is specific for one or a few amino group donors.

The most important ATs are:

Alanine Aminotransferase (ALT)
Aspartate Aminotransferase (AST)

The equilibrium constant of transamination reactions is near one.

Keq=1 means the reaction functions in both amino acid degradation and biosynthesis according to the cellular needs



- **Alanine aminotransferase** (the enzyme which catalyzes this reaction)

- **NOTE:** it's a reversible rxn (it can catalyze the rxn of both pools)
- Depends whether I need alanine or pyruvate

- A)Alanine aminotransferase: transporting the **amino group of the backbone** from amino acids to a common recipient which is alpha-ketoglutarate.
- Once alpha-ketoglutarate accepts amino groups it becomes glutamate.

Which means alpha-ketoglutarate is the alpha keto acid of glutamate.

Now we have pyruvate from alanine(for example) which is the alpha keto acid of alanine.

- B)Aspartate aminotransferase: transporting the amino group of the aspartate to alpha-ketoglutarate to form glutamate.
- The leftover of aspartate is oxaloacetate(the alpha keto acid of aspartate)
- Again it's a reversible rxn, the enzyme can catalyze the opposite reactions(forward and reverse) using the same enzyme.

Alanine aminotransferase (ALT)

ALT is present in many tissues.

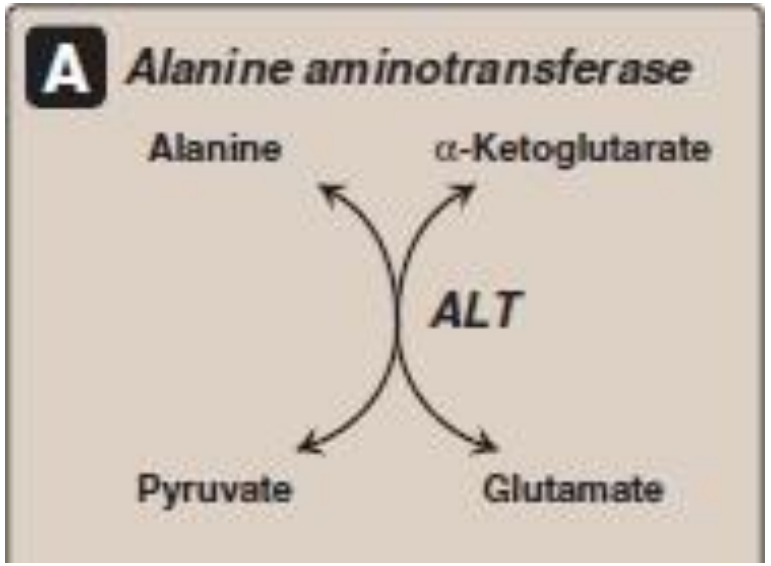
The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate

Reaction products: pyruvate and glutamate

The reaction is reversible.

During amino acid catabolism, ALT functions in the direction of glutamate synthesis.

Glu acts as a “collector” of nitrogen from Ala.



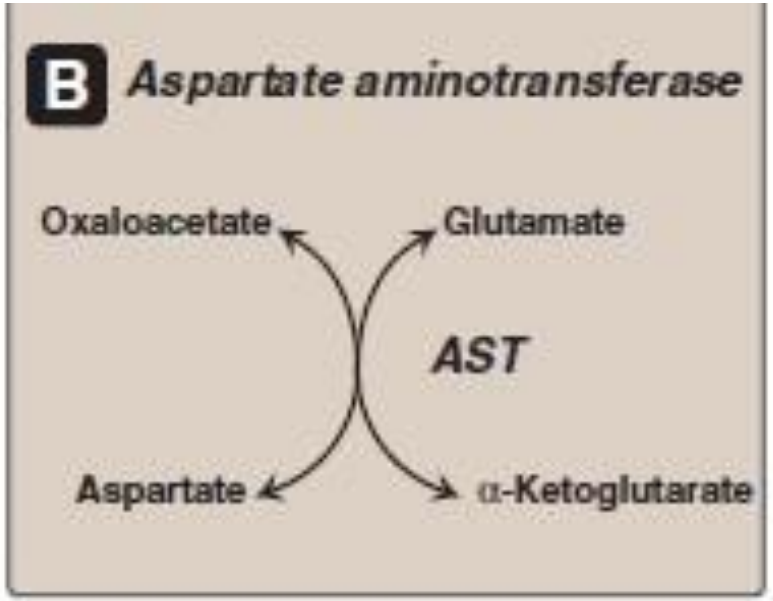
Aspartate aminotransferase (AST)

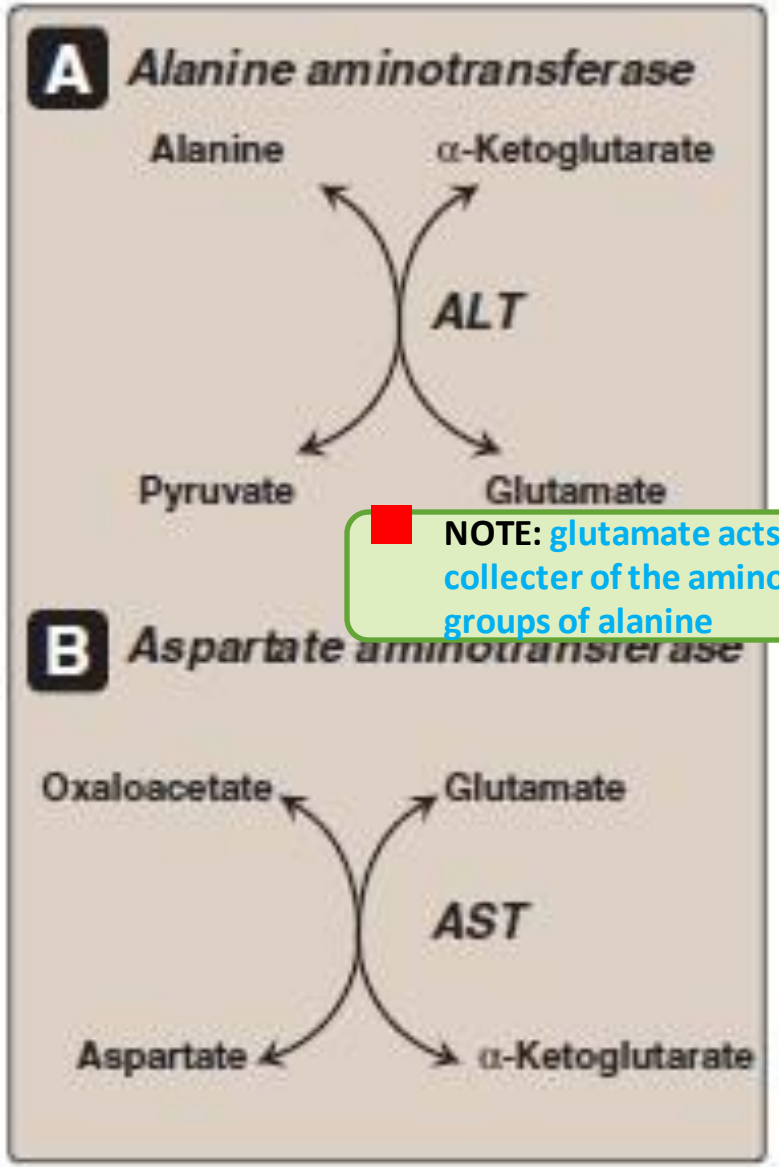
AST does not funnel amino groups to form Glu

During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate, forming aspartate.

Aspartate is used as a source of nitrogen in the urea cycle

The AST reaction is reversible





- **NOTE:** glutamate acts as a collecter of the amino groups of alanine

- Now what is the difference between these two enzymes??(they are not the only enzymes but these two have clinical significance and clinical applications that's why we focus on them)
- The first rxn:ALT transport amino groups of alanine to alpha ketoglutarate to form glutamate, and the pyruvate is the product. this rxn favors the forward direction where pyruvate is produced(why??)

when I start breaking AA this means that I'm in fasting state(we are in glucneogenesis pyruvate is being consumed).

While the second rxn favors the reverse direction(which is formation of aspartate rather than degrading of aspartate to oxalocetate), which means aspartate is being consumed that's why the equilibrium is shifted to the production of aspartate(we consume aspartate in urea cycle 😉).

Clinical hint: Diagnostic value of plasma aminotransferases

ATs are normally intracellular enzymes but low levels in the plasma represent the release of cellular contents during normal cell turnover.

NOTE: ALT is specific to the liver
Changes in levels of ALT mean we have a problem in the liver

AST and ALT have a diagnostic value when found in the plasma.

NOTE: both are part of a test called liver function test(variables in the test are enzymes and molecules such as bilirubin),and it measures the function of the liver واضحة يعني☺

NOTE: AST is expressed in multiple sites(in the liver, heart, skeletal muscles,…)
It's a sensitive marker

a. Liver disease: Plasma AST and ALT are elevated
Examples: severe viral hepatitis, toxic injury, and prolonged circulatory collapse.
ALT is more specific than AST for liver disease
AST is more sensitive because the liver contains larger amounts of AST.

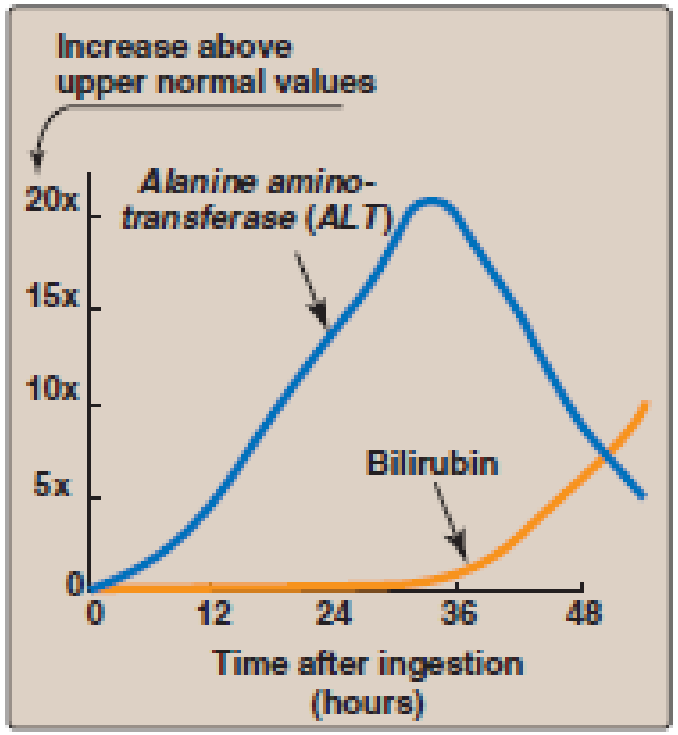
b. Nonhepatic disease: MI and muscle disorders.

- What's the difference between specificity and sensitivity??
- **Sensitive**: becomes affected very quickly, means if there was little destruction(heart, muscle, liver) it's going to get affected, and there is an effects on its levels so I can detect the problem in an early stage.
- **Specificity**: related to certain location
- a single marker cannot be both specific and sensitive.

- **NOTE**: in liver cirrhosis(cellular death due to a pathology, it's going to be necrosis)

In necrosis the cell will release its contents and these contents are uptaken by bloodstream and are shown up as elevation in the levels of these molecules in the blood.

For example in liver function test(it is a blood test) we found AST and ALT are elevated, this indicates a problem in the liver.



- **NOTE:** we need more investigations rather than examining AST and ALT concentrations.

مش أي فحص لهذول كافي انه يأكدلك وجود المرض لازم كمان فحوصات

V2: Slide 11

for example if I shift from fasting to well fed state, anabolic enzymes will be synthesized, and catabolic enzymes will be degraded.

Instead of

for example, in fasting state, anabolic enzymes are synthesized, and catabolic enzymes are degraded.