PROTEINS & AMINO ACIDS METABOLISM

Metabolism of proteins is essentially metabolism of amino acids.

**Recommended Dietary Allowance for protein:** Catabolism of amino acids leads to a net loss of nitrogen from the body, corresponding to approximately 30 to 55 g of protein each/day in healthy adults. This loss must be compensated by the diet in order to maintain a constant amount of body protein. The Recommended Dietary Allowance (RDA) of 56 g of protein per day for a 70 kg man provides a safe margin for replenishing the amino acids lost through anabolic and catabolic pathways.

**Consequences of diets low in protein:** If the diet does not provide adequate amounts of protein, a deficiency of essential amino acids required for the synthesis of body proteins occurs. These results in the net breakdown of tissue protein that can lead to clinical symptoms of protein deficiency, such as those described for kwashiorkor.

**Consequences of diets high in protein:** There is no storage form for amino acids analogous to that for lipid (triacylglycerol) or carbohydrate (glycogen). Therefore, if the diet contains excess protein, providing more amino acids than can be rapidly incorporated into protein or other nitrogen-containing molecules, the excess amino acids are metabolized, with their carbon skeletons being oxidized or converted to glucose or to fat, and their amino groups converted to ammonia.

**DIGESTION OF DIETARY PROTEINS**

Proteins are too large to be absorbed by the intestine and therefore must be hydrolyzed to yield their constituent amino acids, which can be absorbed. Proteolytic enzymes responsible for degrading proteins are produced by three different organs: the stomach, the pancreas, and the small intestine.

**A. Digestion of proteins by gastric secretion**

The digestion of proteins begins in the stomach, which secretes gastric juice, a unique solution containing hydrochloric acid and the proenzyme pepsinogen:

1. **Hydrochloric acid:** Stomach acid is too dilute (pH 2-3) to hydrolyze proteins; however, the acid functions to kill some bacteria and to denature proteins, making them more susceptible to subsequent hydrolysis by proteases.

2. **Pepsin:** This acid-stable endopeptidase is secreted by the serous (chief) cells of the stomach as an inactive zymogen (or proenzyme), pepticinogen. In general, zymogens contain extra amino acids in their sequences, which prevent them from being catalytically active. [Note: Removal of these amino acids permits the proper folding required for an active enzyme.] Pepsinogen is activated to pepsin either by HCl, or autocatalytically by other pepsin molecules that have already been activated. Pepsin releases peptides and a few free amino acids from dietary proteins.
B. Digestion of proteins by pancreatic enzymes

On entering the small intestine, large polypeptides produced in the stomach by the action of pepsin are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases.

1. **Specificity**: Each of these enzymes has a different specificity for the amino acid R groups adjacent to the susceptible peptide bond (next figure).
For example: Trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine. Chemotrypsin cleaves only when the carbonyl group of the peptide bond is contributed by phenylalanine, tyrosine, or tryptophan.

These enzymes, like pepsin described above, are synthesized and secreted as inactive zymogens. They are activated in the lumen of the intestine by trypsin, which cleaves a limited number of specific peptide bonds in the zymogen.

2. 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 digestive tract.

3. Activation of zymogens: Enteropeptidase (formerly called enterokinase), an enzyme synthesized by and present on the luminal surface of intestinal mucosal cells of the brush border membrane, converts the pancreatic zymogen trypsinogen to trypsin by removal of a hexapeptide from the NH₂-terminus of trypsinogen. Trypsin subsequently converts other trypsinogen molecules to trypsin. Enteropeptidase thus unleashes a cascade of proteolytic activity, because trypsin is the common activator of all the pancreatic zymogens (next figure):

4. Abnormalities in protein digestion: In individuals with a deficiency in pancreatic secretion (for example, due to chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas), the digestion and absorption of fat and protein is incomplete. This results in the abnormal appearance of lipids, "steatorrhea", and undigested protein in the feces.

C. Digestion of oligopeptides by enzymes of the small intestine

The luminal surface of the intestine contains aminopeptidase, an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce free amino acids and smaller peptides.

D. Absorption of amino acids and dipeptides

Free amino acids and dipeptides are absorbed by the intestinal epithelial cells in which the dipeptides are hydrolyzed to amino acids in the cytosol before they enter the portal system. Thus, only free amino acids are found in the portal vein after a meal containing protein. These amino acids are either metabolized by the liver or released into the general circulation.

TRANSPORT OF AMINO ACIDS INTO CELLS

The concentration of free amino acids in the extracellular fluids is significantly lower than that within the cells of the body. This concentration gradient is maintained because active transport systems, driven by the hydrolysis of ATP, are required for movement of amino acids from the extracellular space into cells. At least seven different transport systems are known that have overlapping specificity for different amino acids. One transport system is responsible for reabsorption in kidney tubules of the amino acids cysteine, ornithine, arginine, and lysine. In the inherited disorder cystinuria, this carrier system is defective, resulting in the appearance of all four amino acids in the urine.
OVERALL NITROGEN METABOLISM

Amino acid catabolism is part of the larger process of whole body nitrogen metabolism. Nitrogen enters the body in a variety of compounds present in the food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism. The role of body proteins in these transformations raises two important concepts: the **amino acid pool** and **protein turnover**.

**A. Amino acid pool**

Amino acids released by hydrolysis of dietary or tissue protein mix with other free amino acids distributed throughout the body, and collectively constitute the amino acid pool. The amino acid pool, containing about 100 g of amino acids, is small in comparison to the amount of protein in the body (about 12 kg in a 70 kg man). If the only fate of the amino acids contributed to the pool by the degradation of the body’s proteins were to reform those proteins, adults would not have a significant need for additional dietary protein. However, only about 75% of the amino acids obtained through hydrolysis of body protein are recaptured through the biosynthesis of new tissue protein.

Amino acids contain nitrogen in addition to the carbon, hydrogen, and oxygen atoms also found in carbohydrates and fats. This nitrogen cannot be stored, and amino acids in excess of the biosynthetic needs of the cell are immediately degraded. The first phase of catabolism involves the removal of the α-amino groups by transamination and oxidative deamination, forming ammonia and the corresponding α-ketoacids. A portion of the free ammonia is excreted in the urine, but most is used in the synthesis of urea, which is quantitatively the most important route for disposing of nitrogen from the body. In the second phase of amino acid catabolism, the carbon skeletons of the α-ketoacids are converted to common intermediates of energy-producing metabolic pathways. These compounds can be metabolized to CO₂ and water, glucose, fatty acids, or ketone bodies by the central pathways of metabolism.

**B. Protein turnover**

Most proteins in the body are constantly being synthesized and then degraded. In healthy adults, the total amount of protein in the body remains constant, because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and resynthesis of 300 to 400 g of body protein each day.
1. **Rate of turnover**: The rate of protein turnover varies widely for individual proteins. For example, some proteins that function outside cells, such as digestive enzymes and plasma proteins, are rapidly degraded, having half-lives measured in hours or days. However, structural proteins, such as collagen, are metabolically stable and have half-lives measured in months or years.

   N.B. **Half life time** is the time required for ½ material to be broken.

2. **Chemical signals for protein turnover**: Because proteins have different half-lives, it is clear that protein degradation cannot be random, but rather is influenced by some structural aspect of the protein. For example, some proteins that have been chemically altered by oxidation or tagged with ubiquitin (a small heat-stable protein) are preferentially degraded. Further, proteins rich in sequences containing proline, glutamate, serine, and threonine (called PEST sequences after the one-letter designations for these amino acids) are rapidly degraded and therefore exhibit short intracellular half-lives.

C. **Role of dietary protein in overall nitrogen metabolism**

   In contrast to carbohydrates and triacylglycerols whose major function is to provide energy, the primary role of amino acids is to serve as building blocks in biosynthetic reactions, particularly the synthesis of tissue protein. Protein is used secondarily as a fuel, and in a typical diet provides about one fifth of the daily energy requirement.

**Nitrogen balance**

   Nitrogen is the main component of proteins. It represents **about 16%** by weight of any protein. So the amount of protein intake (in diet) or excreted (e.g. as urea in urine) can be expressed by **nitrogen intake and output**:

   1- **Nitrogen balanced**: occurs when nitrogen intake = nitrogen excreted in urine, sweet and feces. Most healthy adults are normally in nitrogen balance.

   2- **Positive nitrogen balance**: occurs when nitrogen intake > nitrogen excretion. It was observed in situations which tissue growth occurs, e.g. in children, pregnancy, or during recovery from an emaciating illness.

   3- **Negative nitrogen balance**: occurs when nitrogen losses > nitrogen intake. It is associated with inadequate dietary protein, lack of essential amino acids, or during physiological stresses such as trauma, burns, illness, or surgery.

**Classification of amino acids:**

   Amino acids can be classified according to:

   1- **The dietary requirement**:

      a) **Non-essential amino acids**: that can be synthesized in the body from the corresponding α-ketoacids which in turn can be synthesized in the body:

         \[ \text{R-CO-COOH} \rightarrow \text{R-CHNH}_2\text{-COOH} \]

      b) **Essential amino acids (EAA)**: that cannot be synthesized in the body as its corresponding ketoacids (carbon skeleton) cannot be synthesized. This include aromatic and branched amino acids as **valine, leucine, isoleucine, lysine, methionine, threonine, phenylalanine, tryptophan and histidine**. **Arginine** is essential only in newborn and infants as in adults it can be synthesized as an intermediate of urea cycle. The animal
proteins contain all the essential amino acids, while plant proteins are deficient in some EAA.

2. The metabolic fate of amino acids:
   a) **Ketogenic amino acids**: which produce ketone bodies on catabolism as leucine and lysine.
   b) **Mixed amino acids**: that produces glucose and ketone bodies e.g. phenylalanine, tyrosine, tryptophan, isoleucine and threonine.
   c) **Glucogenic amino acid**: that produces glucose only and this involves the rest of amino acids.

**REMOVAL OF NITROGEN FROM AMINO ACIDS**

The first step in the catabolism of all amino acids involves the removal of the α-amino group. Once removed, this nitrogen can be incorporated into other compounds or excreted. This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen.

A. **Transamination**: the funneling of amino groups to glutamate:

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate. The products are an α-keto acid (derived from the original amino acid) and glutamate. α-Ketoglutarate plays a unique role in amino acid metabolism by accepting the amino groups from other amino acids, thus becoming glutamate. Glutamate produced by transamination can be oxidatively deaminated, or can be used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of enzymes called aminotransferases (formerly called transaminases). All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism.

1. **Substrate specificity of aminotransferases**: Each aminotransferase is specific for one or at most a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. The two most important aminotransferase reactions are catalyzed by alanine aminotransferase and aspartate aminotransferase:

   a. **Alanine aminotransferase (ALT)**, also called glutamate-pyruvate transaminase (GPT), is present in many tissues. The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily reversible; however, during amino acid catabolism, this enzyme (like most aminotransferases) functions in the direction of glutamate synthesis. Thus, glutamate, in effect, acts as a "collector" of nitrogen from alanine.
b. Aspartate aminotransferase (AST), also called glutamate oxaloacetate transaminase (GOT), is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, aspartate aminotransferase transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is itself used as a source of nitrogen in the urea cycle.

2. Mechanism of action of aminotransferases:
All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B₆). Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid and regenerates the original aldehyde form of the coenzyme.

3. Equilibrium of transamination reactions:
For most transamination reactions, the equilibrium constant is near 1, allowing the reaction to function in both amino acid degradation through removal of α-amino groups (for example, after consumption of a protein-rich meal) and biosynthesis through addition of amino groups to the carbon skeletons of α-keto acids (for example, when the supply from the diet is not adequate to meet the synthetic needs of cells).

4. Diagnostic value of plasma aminotransferases:
Aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferases in the plasma indicates damage to cells rich in these enzymes.
   a. Liver disease: Plasma AST and ALT are elevated in nearly all liver diseases, but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis and prolonged circulatory collapse. Serial enzyme measurements are often useful in determining the course of liver damage.
   b. Nonhepatic disease: Aminotransferases may be elevated in nonhepatic disease, such as myocardial infarction and muscle disorders; however, these disorders can usually be distinguished clinically from liver disease.

B. Oxidative deamination:
In contrast to transamination reactions that transfer amino groups, oxidative deamination results in the liberation of the amino group as free ammonia. These reactions occur primarily in the liver and kidney and provide α-ketoacids (which can enter the central pathway of energy metabolism) and ammonia (which is a source of nitrogen in urea synthesis).
1- **Glutamate dehydrogenase**: As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with \( \alpha \)-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase:

\[
\begin{align*}
\text{Glutamate} & \rightarrow \text{\( \alpha \)-ketoglutarate} \\
\text{NAD}^+ \text{ or } \text{NADP}^+ & \rightarrow \text{NADH}^+ \text{ or } \text{NADPH}^+ \\
\text{H}_2\text{O} & \rightarrow \text{ammonia}
\end{align*}
\]

Therefore, the sequential action of transamination (resulting in the collection of amino groups from other amino acids onto \( \alpha \)-ketoglutarate to produce glutamate) and the subsequent oxidative deamination of that glutamate (regenerating \( \alpha \)-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia.

a. **Coenzymes**: Glutamate dehydrogenase is unusual in that it can use either \( \text{NAD}^+ \) or \( \text{NADP}^+ \) as a coenzyme.

b. **Direction of reactions**: The direction of the reaction depends on the relative concentrations of glutamate, \( \alpha \)-ketoglutarate, and ammonia, and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia. [Note: the reaction can also be used to synthesize amino acids from the corresponding \( \alpha \)-ketoacids]

c. **Allosteric regulators**: ATP and GTP are allosteric inhibitors of glutamate dehydrogenase, whereas GDP and ADP are activators of the enzyme. Thus, when energy levels are low in the cell, amino acid degradation by glutamate dehydrogenase is high, providing \( \alpha \)-ketoglutarate as a substrate for the TCA cycle.
2- **L-amino acid oxidase:**
Most of the ammonia released from \( \alpha \)-amino acids reflects the coupled action of transaminases and L-glutamate dehydrogenase. However, **L-amino acid oxidases** are present in mammalian liver and kidney tissue. They oxidize L-amino acids (using FMN as co-dehydrogenase) to \( \alpha \)-imino acids that add water and decompose to the corresponding \( \alpha \)-ketoacids. The reduced flavin is reoxidized directly by molecular oxygen, forming hydrogen peroxide, which splits to oxygen and water by enzyme **catalase** present in many tissues as liver:

![Diagram of amino acid oxidase](https://via.placeholder.com/150)

3- **D-Amino acid oxidase:**
D-Amino acids are found in plants and in the cell walls of microorganisms but are not used in the synthesis of mammalian proteins. D-Amino acids are, however, present in the diet and are efficiently metabolized by the liver. D-Amino acid oxidase is an **FAD**-dependent enzyme that catalyzes the oxidative deamination of these unnatural isomers of amino acids. The resulting **\( \alpha \)-ketoacids** can enter the general pathways of amino acid metabolism, be reaminated to L-isomers, or catabolized for energy: