Amino Acid and Protein Metabolism

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## Essential Amino Acids

<table>
<thead>
<tr>
<th>Adults and Young</th>
<th>Additional for Young</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Leucine</td>
<td>Glycine (Chickens)</td>
</tr>
<tr>
<td>Lysine</td>
<td>Histidine</td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
</tr>
<tr>
<td>Taurine (Cats)</td>
<td></td>
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<tr>
<td>Threonine</td>
<td></td>
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<tr>
<td>Tryptophan</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
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</tbody>
</table>
Essential Amino Acids

- **BCAA (Branched chain amino acids)**
  - Leucine, Isoleucine and Valine: Oxidized routinely in muscle tissue.

- **Phenylalanine**: It is required for hepatic tyrosine synthesis.

- **Methionine**: Required for cysteine formation.

- **Tryptophan**: Used for serotonin and melatonin synthesis.
Protein Digestion
Protein Digestion

- **Protein digestion** is called hydrolytic degradation of peptide bonds and separation of amino acids.

- Proteins need to be broken down to amino acids in order to be absorbed.
Protein Digestion

- Proteins targeted for digestion in animals originate from three primary sources:
  1. Tissue proteins,
  2. Exogenous dietary proteins, and
  3. Endogenous proteins in the digestive tract from either sloughed cells or those present in exocrine secretions.

- Each day a normal adult mammal degrades about 1-2% of body protein, principally from liver and other visceral organs.
Protein turnover occurs at different rates in different tissue types.

- Immunoglobulins and collagen, for example, normally have half-lives measured in years.
- Liver proteins, plasma proteins and regulatory enzymes have half-lives measured in hours to days.
- In general, the proteins of visceral organs turn over at a faster rate than those of skeletal muscle and connective tissue.
- However, during the latter phases of physiologic starvation or during cachexia, muscle protein is degraded at a faster rate than normal.
Protein Digestion

- Tissue protein degradation, like protein synthesis, is a carefully regulated process. Protein conjugation to the 74-amino acid polypeptide known as **ubiquitin** targets it for degradation.
  - This polypeptide is highly conserved, and has been found in species ranging from bacteria to primates.
  - Animals appear to possess complex mechanisms by which abnormal proteins are recognized and degraded more rapidly than normal proteins.
    - These mechanisms, however, may not operate optimally in some disease states.
About 75% of amino acids generated from tissue protein digestion are used for the resynthesis of new tissue protein.
  • The remainder enter hepatic gluconeogenesis, as well as biosynthetic pathways for a variety of specialized products.
  • Amino acids removed from the tissue pool are replaced through digestion of dietary protein, and from \textit{de novo} biosynthesis.

Amino acids in protein are stored in the body in a functional capacity.
  • For virtually, all proteins are considered to possess anatomic and/or physiologic activity.
Protein Digestion in Stomach

- Protein digestion begins at the **stomach** and is carried out by **pepsin**.
  - It is up to 15%. The rest is digested in the intestine.
  - Proteins are cleaved into polypeptides.
  - **Pepsin** is found in the gastric mucosa, in the form of **pepsinogen**, which is inactive. Once released into the stomach cavity, it becomes an active pepsin by autocatalytic action of HCl.
    - Pepsinogen I: Oxynitic glandular region.
    - Pepsinogen II: Pyloric region.
  - Its secretion is stimulated by «**secretin**».
Protein Digestion in Stomach

- **Pepsin** has an **endopeptidase** effect and primarily cleaves peptide linkages in the protein, especially those that follow the aromatic amino acids, and forms peptide chains.
  - In addition, there are two separate proteolytic enzymes called rennin in calves, lambs and childs, and gastricsin in also newborns child.
  - After digestion done through these proteolytic enzymes, the peptide mixture together with stomach contents are transferred to intestines.
Protein Digestion in Intestines

- Protein digestion (oligopeptides) is maintained through **proteinases** transported to the intestines via the pancreatic secretion.
  - Their release is stimulated by «cholecystokinin».

- The most important of these are **trypsin, chymotrypsin, carboxypeptidase A - B** and **elastase**.

- Proteases produced in the pancreas are synthesized as proenzymes and stored in secretory granules.
  - They are activated by **enterokinases**.
Protein Digestion in Intestines

- **Trypsin** and **chymotrypsin** are **endopeptidases** and they break the peptide bonds on the hydrolytic pathway in the inner part of the peptide chain.
  - Zn containing enzymes.

- While trypsin cleaves peptide bonds that follow the basic amino acids, chymotrypsin exhibits a broad spectrum of action (breaking off peptide bonds that come after more aromatic amino acids).
By the action of these two enzymes, peptide chains with a weight of 600-3000 molecules (come from stomach) are broken down into smaller peptide chains.

These resulting small peptide chains are also cleaved to the mixture of di-, tri-peptides and free amino acids via carboxypeptidases A and B, which are found on pancreatic fluid, and aminopeptidases, which are released from intestinal mucosa cells.
Protein Digestion in Intestines

- **Carboxypeptidases** are exopeptidases and separate the amino acids one by one start from the carboxyl end of the peptide chain.
  - **Carboxypeptidase A** affects the carboxyl end of the peptide chains which are formed by the action of chymotrypsin and carry more aromatic amino acids at the carboxyl ends,
  - **Carboxypeptidase B** affects peptide chains which are formed by the action of trypsin and carry basic amino acids at the C-terminus.
Protein Digestion in Intestines

- The endopeptidases, starting from the amino terminus of these constituent chains, which break the amino acids one by one and are released from the intestinal mucosa cells, are also called aminopeptidases, in which a mixture of free amino acids, di-, tri-, and tetrapeptides are formed in the digestive tract.
Absorption

- **Peptides**
  - Peptide bonds are cleaved by peptidases found in mucosal cells, and the free amino acids formed are released into the portal blood.

- **Amino acids**
  - For amino acid transport there is an active transport system in the mucosal cells that carries a high concentration of energy dependent activity.
  - It is suggested that the transport of amino acids to the mucous cells is dependent on the presence of Na ions and is similar to the transport of monosaccharides.
Dietary Protein Digestion and Absorption

% Radiolabeled Albumin Digested and Absorbed

Stomach  Duodenum  Jejunum  Ileum

About 15-20% Not digested and Absorbed

Source: Engelking, 2014
• Stimulators of pepsinogen release are secretin and acid-stimulation of proton receptors on the gastric mucosal surface.

• Once secreted into the gastric lumen, pepsinogen is further activated to pepsin through the action of HCl.
Proteaz Aktivasyonu

Trypsinogen (Inactive)

Pancreatic Trypsin Inhibitor

(-)

Trypsinogen
Chymotrypsinogen
Procarboxypeptidase
Proelastase

(+)

Trypsin
Chymotrypsin
Carboxypeptidase
Elastase

Active Pancreatic Proteases in the Intestinal Lumen

Duodenal Enterokinase (Enteropeptidase)

Trypsin (Active)

Inactive Cleaved Peptide

Source: Engelking, 2014
Intestinal Mechanism for Protein Digestion and Absorption

• Although some neonates may absorb proteins intact for a short period of time following birth (approx. 24-36 hrs), most neonates absorb only amino acids and small peptides following protein digestion.

C = Protein; B = Peptides; A = Amino Acids
Amino Acid Metabolism
Functions of Amino Acids

1. 20 amino acids function as a building block of protein biosynthesis.

2. Amino acids act as amino groups or nitrogen donors in the synthesis of other nitrogen containing compounds.

3. They play an important role in glucose homeostasis.
Amino Acid Catabolism

- Following protein digestion in the intestinal tract, free amino acids are transported via the portal circulation to the liver, which then plays a pivotal role in determining the type and amount of amino acids released into the systemic circulation for supply to non-hepatic tissues.

- The liver generally prefers to metabolize aromatic amino acids (AAAs; phenylalanine, tryptophan, and tyrosine), while allowing the BCAA to pass on into the systemic circulation.
  - The amino acid concentration leaving the liver in the fed state is usually 2-3 times higher than in starvation, and more than half are BCAAs.
Following intestinal amino acid absorption, AAAs are largely removed by the liver while most BCAAs pass on into the systemic circulation.

Amino acids are utilized by the liver for both energy and biosynthetic purposes, and once deaminated their amine groups normally appear in either urea or glutamine.
Liver

- After ingestion the hydrolyzed amino acids reach the vena porta by active transport, which causes the content of amino acids in the blood of the portal circulation to rise after the protein-rich diet.
  - The absence of this elevation in the systemic circulation indicates that a large part of the absorbed amino acids are taken up by the liver.

- Apart from glutamine (Gln), asparagine (Asn) and BCAAs, many other amino acid catabolisms start in the liver.
Liver

- Amino acids are used
  - For the synthesis of other amino acids or proteins in the liver or
  - Their ammonia is broken off and converted into keto acids.

- Keto acids are either converted to fatty acids or glucose or oxidized to CO$_2$ and water.
Glucogenic and Ketogenic Amino Acids

- **Ketogenic amino acids** enter hepatic mitochondrial metabolic processes at the level of acetyl-CoA or acetoacetyl-CoA.

- **Glucogenic amino acids** enter the gluconeogenic pathway at the level of pyruvate, oxaloacetate, fumarate, propionyl-CoA or α-KG®.

### Glucogenic Amino Acids

<table>
<thead>
<tr>
<th>Glucogenic Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ala</strong></td>
</tr>
<tr>
<td>Arg</td>
</tr>
<tr>
<td>Cys</td>
</tr>
</tbody>
</table>

*: Minor Hepatic Degradation
*: Major Renal Substrate
*: Major Hepatic Substrate

### Ketogenic Amino Acids

<table>
<thead>
<tr>
<th>Ketogenic Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Leu</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Glucogenic and Ketogenic Amino Acids</th>
</tr>
</thead>
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<tr>
<td>*Ile</td>
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</tbody>
</table>

Source: Engelking, 2014
Liver

- Some of the **free ammonia** obtained from amino groups of amino acids can be used as building blocks for **nitrogen-containing compounds**, while the rest are converted into **urea** in the liver, transported through the blood to the kidneys, and excreted in the urine.

- Approximately half of the free amino acids resulting from protein breakdown are used again in protein synthesis.
Hepatic Metabolism of Phenylalanine

- In the fed state, approximately three-quarters of the Phe entering the liver is converted to Tyr, with the other quarter incorporated into proteins.
  - During starvation, Phe can be catabolized in the liver to acetoacetate, a ketone body, and fumarate.

- Conversion of Phe to Tyr requires oxygen and the enzyme phenylalanine hydroxylase. Hereditary deficiencies of phenylalanine hydroxylase leads to accumulation of Phe in blood, tissues, and urine (phenylketonuria).
The BCAAs are primarily metabolized by muscle tissue, where the concentration of BCAA transaminase is high.

This enzyme is only modestly expressed in liver tissue.

In normal animals, the plasma BCAA/AAA ratio is usually about 3:1.

Liver disease can lead to a shift in the ratio from 3:1 to 1.5:1 or less.
Intestinal cells use glutamine (Gln) and asparagine (Asn) as a major energy source.

This use results in several byproducts such as citrulline, ammonium ion, $\text{CO}_2$ and alanine (Ala), which are released into the portal circulation.
Skeletal Muscle

- The catabolism of BCAAs in skeletal muscle for energy purposes.
  - Usually results in the transfer of amino groups to pyruvate (thus forming Ala), and/or glutamate (Glu) (thus forming Gln).
  - More than 50% of amino acid release from skeletal muscle during starvation is accounted for through Ala and Gln.
  - Alanine is the favored gluconeogenic substrate of hepatocytes, while Gln is the favored gluconeogenic substrate of proximal renal tubular epithelial cells.
  - During starvation, Val, which is released in small quantities, can be oxidized by the brain.
When Val is oxidized in brain tissue during starvation, the amino group is normally transferred to Glu, thus forming Gln, which is the preferred manner in which the brain rids itself of ammonia.

Since ammonia is toxic to brain tissue, the ability of supporting glial cells to maintain Gln formation remains integral to neuron survival not only during starvation, but in other physiologic and pathophysiologic states in which hyperammonemia develops.
Kidney

- The kidney is, like the liver, a **gluconeogenic** and **ammoniagenic** organ.
- Most of the ammonium ions produced in the kidney and excreted in the urine originate from **Gln**.
  - It is removed from blood or the glomerular filtrate by proximal tubular epithelial cells of the kidney following release by skeletal muscle, the liver, and brain.
- The renal release of ammonia via **Gln** is formed in two steps and **mitochondrial glutaminase** and **glutamate dehydrogenase (GLDH)** enzymes are involved.
In order for glucose to be synthesized from the metabolic end products of amino acids and to be able to synthesize amino acids again from the intermediates of the TCA cycle, the amino acids and TCA metabolites have to have some common properties.

The most important of these common properties are,

- There is a group such as a $\alpha$-NH$_2$ which reacts with a COOH group at the $\alpha$-carbon atom.
- The most suitable TCA intermediate metabolites with these properties also form pyruvic acid, oxaloacetic acid and $\alpha$-ketoglutaric acid.
Intramolecular Amino Asit Metabolism

- These keto acids are replaced by amino group substitution instead of ketone groups.
  - Alanine from pyruvic acid,
  - Aspartic acid from oxalacetic acid and
  - Glutamic acid from α-ketoglutaric acid are synthesized. In addition, appropriate α-keto acids can also be synthesized by reversible reactions.
Intramolecular Amino Acid Metabolism

- Cells are capable of synthesizing keto acids that can be converted into their appropriate amino acids by transamination.

- Since transamination is a free-reversible reaction, keto acids are formed during amino acid elimination.
Intramolecular Amino Asit Metabolism

- These resulting keto acids can also be converted to the CoA-thioesters of the dehydrogenated decarboxylated 1 C-deficient aliphatic fatty acids.
- For example; acetic acid activated from pyruvic acid (Acetyl CoA), succinic acid activated from α-ketoglutaric acid (succinyl KoA) can be formed.
Transamination Reactions

- **Transamination** is the transport of an amino group of an amino acid to a keto acid and is reversible.

- One of the pairs of substrates entering the reaction in the majority of the transamination reactions is α-ketoglutarate.

- The amino group in the amino acid is removed as a result of the transamination reaction with α-ketoglutarate.
Transamination Reactions

- The amino form of the coenzyme can also form the appropriate shift base with a keto acid, and the amino group gives another keto acid to form a new amino acid.

- In this reaction, defined as transamination, a new amino and keto acid are synthesized from an amino and a keto acid.
There are four coenzymes intimately involved in amino acid catabolism; pyridoxal phosphate (derived from vitamin B$_6$), tetrahydrofolic acid (THFA), biotin (a CO$_2$ shuttler), and vitamin B$_{12}$ (cobalamin).

Most reactions that involve the α-carbon atom of an amino acid require pyridoxal phosphate as a coenzyme (e.g., transaminations, amino acid decarboxylations, and some deaminations).
The α-amino group is transferred to α-carbon of α-ketoglutarate, which is a keto acid, to form glutamic acid. The α-keto acid residue of the amino acid in the α-amino group is left behind.

For example, if aspartic acid loses the α-amino group, then oxaloacetic acid remains.
Transamination Reactions

- Transamination reactions are catalyzed by transaminases (aminotransferases), which pyridoxal phosphate (PLP) is the coenzyme.
Transaminase Reactions

- **Transaminases** are found in the cell's cytosol and mitochondrial compartment, and are found predominantly in all tissues, especially in the liver and muscle.

- **Alanine aminotransferase (ALT, GPT)** and **Aspartate aminotransferase (AST, GOT)** are important transaminases and found mainly in the liver, muscle, brain, kidney and testis.
  - Alanine-Pyruvate: ALT
  - Aspartate-Oxaloacetate: AST
Turnover of the Amino Acid Pool in the Body

De Novo
Non-essential amino acid synthesis

Dietary Protein

Tissue Proteins

Non-Protein Derivatives
Neurotransmitters, Creatine, Carnitine, Heme, Glutathione...

Amino Acid Pool

Ammonium ion

Urea Cycle

Urea

β-Keto Acids

Gluconeogenesis

Acetyl-CoA

Lipogenesis

Ketogenesis

Gluconate

Biochemistry in VET
Deamination

- **Deamination** is the removal of an amino group from a molecule, in other words it is an α-ketoacid conversion event resulting from the removing of an amino group of an amino acid into ammonia.

- **Deamination** events are divided into two groups, oxidative and non-oxidative; mainly by the action of various enzymes and coenzymes found in the liver and kidney.
Oxidative deamination is catalyzed by L-amino acid oxidases and D-amino acid oxidases.

L-amino acid oxidases use FMN as coenzyme; it doesn’t act on glycine and dicarboxylic and hydroxylated amino acids. Oxidative deamination takes place by the addition of one molecule of H$_2$O in the presence of O$_2$. The formation of H$_2$O$_2$ as an intermediate product is important.
Deamination

- D-amino acid oxidases use FAD as coenzyme; expect D-asparagine and D-glutamine, act on D-amino acids, especially glycine.

- The oxidative deamination of glutamate is catalyzed by glutamate dehydrogenase (GLDH). It is mainly found in liver (also in other tissues) and is a mitochondrial enzyme which needs $\text{NAD}^+$ or $\text{NADP}^+$ as a coenzymes.
Deamination

- The non-oxidative deamination of amino acids is carried out by various enzymes.

- Histidine deamination is catalyzed by histidine-ammonia lyase (histidase) and urocanate and ammonium are formed.
Deamination

- **Dehydratases** act on hydroxylated amino acids such as serine and threonine, using pyridoxal phosphate.

  ![Chemical Reaction Diagram](image)

  Serine Dehydratase

- Serine amino acid lose one hydrogen from the $\alpha$-carbon and one hydroxyl group from the $\beta$-carbon and an amino-acrylate molecule. This unstable compound then reacts with one mole of $H_2O$ to form the pyruvate and ammonium ion ($NH_4^+$).
**Deamination**

- **Desulfhydrase** are effective on sulfuric amino acids, they use **pyridoxal phosphate**; together with the amino group, separate the sulfur from the molecule.

\[
\begin{align*}
\text{L-Cysteine} & \quad \text{Desulfhydrase} \\
H_3N-C-CO \text{O}^- + H_2O & \rightarrow O=C-CO\text{O}^- + H_2S + NH_4^+ \\
\text{CH}_2 & \quad \text{2-Ketopropanate} \\
\text{S} & \\
\text{IH} & \\
\end{align*}
\]
Aminotransferases catalyze the reversible transfer of an α-amino group from an α-amino acid to an α-ketoacid.

AST catalyzes reversible transfer of the amino group of Asp to α-KG\(^-\), thus forming Glu and OAA, while ALT catalyzes reversible transfer of the amino group of Ala to α-KG\(^-\), thus forming Glu and pyruvate.

Hepatic oxidative deamination of Glu, catalyzed by GLDH, results in α-KG\(^-\) and NH\(_4\)^+ formation, but in other tissues the equilibrium for this reaction favors Glu formation, thus preventing ammonia toxicity.
Decarboxylation is the separation of the carboxyl group as CO$_2$ from an amino acid structure.

Decarboxylation of amino acids is catalyzed by amino acid *decarboxylases*, which the coenzyme is *pyridoxal phosphate* (PLP), resulting in *biogenic amines* having important effects in cells.
## Decarboxylation

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Biogenic Amine</th>
<th>Önemi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>ß-Alanin</td>
<td>It is found in the structure of the coenzyme A.</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>GABA</td>
<td>It is a mediator in CNS.</td>
</tr>
<tr>
<td>Ornithine</td>
<td>Putrescine</td>
<td>Pre-cursor of polyamines.</td>
</tr>
<tr>
<td>Lysine</td>
<td>Cadaverine</td>
<td>It is the product of microorganisms in the intestine.</td>
</tr>
<tr>
<td>Arginine</td>
<td>Agmatine</td>
<td>It is the product of microorganisms in the intestine.</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cysteamine</td>
<td>It is found in the structure of the coenzyme A.</td>
</tr>
<tr>
<td>Methionine</td>
<td>S-adenosyl methionine</td>
<td>It is necessary for biosynthesis of polyamines.</td>
</tr>
<tr>
<td>Serine</td>
<td>Ethanolamine</td>
<td>It is necessary for biosynthesis of phospholipids.</td>
</tr>
<tr>
<td>Histidine</td>
<td>Histamine</td>
<td>Tissue hormone.</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyramine</td>
<td>It is the product of microorganisms in the intestine.</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Tryptamine</td>
<td>It is the product of microorganisms in the intestine.</td>
</tr>
<tr>
<td>S-hydroxytryptophan</td>
<td>S-hydroxytryptamine</td>
<td>Tissue hormone.</td>
</tr>
<tr>
<td>3-4 dihydroxyphenylalanine</td>
<td>Dopamine</td>
<td>It is a mediator in CNS.</td>
</tr>
</tbody>
</table>
GABA (Gamma aminobutyric acid) is an important inhibitor neurotransmitter. Drugs, such as benzodiazepines, increase the effects of GABA; They are used for epilepsy treatment.
Decarboxylation

- **Histamine** is synthesized and secreted in mast cells.
  - H1 receptors cause vasodilatation and bronchoconstriction allergic reactions.
  - Stimulate gastric acid secretion via H2 receptors.
Tryptophan (Trp) is converted into serotonin (5-HT) through the following steps:

1. Tryptophan is oxidized by tryptophan hydroxylase in the presence of oxygen (O₂) to form 5-hydroxytryptophan.
2. 5-Hydroxytryptophan is decarboxylated to form serotonin (5-HT).

Blood Brain Barrier

L-DOPA

Blocks

Dopamine

Carbidopa

Blood

Brain

L-DOPA → Dopamine

Parkinsonism associated with a reduction in dopamine in brain through loss of neurons in basal ganglia.

Carbidopa + L-DOPA
Decarboxylation

- When the amines have 1 or 2 amino groups, degradation is carried out by monoamine or diamine oxidases. By force of these enzymes, the amines are dehydrated to imines and the imines are liberated from aldehydes and ammonia. Aldehydes that are released also continue to be metabolized to carbonic acids by dehydrogenation and subsequent Beta-oxidation.
Amino acids synthesized by microorganisms and plants which are not synthesized in animal cells are called essential amino acids.
Essential Amino Acids

- Lysine
- Triptofan
- Phenylalanine
- Leucine
- Isolösin
- Treonin
- Methionine
- Valin
- Histidine
- Arginine
- Glycine
- Glutamic acid

HUMAN

DOG
RAT
PIG

CHICKEN
Amino acid biosynthesis in microorganisms and plants

- Which group of amino acids can be synthesized from a common precursor is shown below.
  - **Aspartic acid family:** Amino acids which can be synthesized by transamination of oxalacetic acid which is alpha-keto acid (Lysine, methionine, threonine and isoleucine)
  - **Pyruvic Acid Family:** Amino acids synthesized by transamination of alpha-keto acid pyruvic acid (leucine and valine)
  - **Shikimic Acid Family:** Amino acids consisting of an alpha-ketoacid of 7 C, an intermediate product of glycolysis and pentose phosphate pathway (Phenylalanine, tyrosine and tryptophan).
In this way, the C skeletons are degraded to CO$_2$ and H$_2$O for energy (ATP) production, or they convert to Ketone bodies or glucose.
The amino acids which can be used to synthesize glucose are called **glycogenic amino acids**.

If C skeleton is used in the synthesis of ketone bodies or fatty acids, such amino acids are termed **ketoogenic amino acids**.

The C framework remaining from some amino acids is broken down into smaller subunits and can be used in the synthesis of both glycogen and ketone bodies.
### Catabolism of essential amino acids and metabolic importance

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Yıkılım ürünü</th>
<th>Ketogen</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>2 Acetyl CoA</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>Succinyl CoA</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>Succinyl CoA</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Acetyl CoA and Succinyl CoA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Valine</td>
<td>Succinyl CoA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leucine</td>
<td>Acetyl CoA and Acetoacetate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Fumarate and Acetoacetate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>2 Acetyl CoA and Alanine</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Non-essential amino acids: Biosynthesis, degradation and metabolic importance

- Because the C skeletons of all non-essential amino acids can be converted to oxalacetic acid, all of these amino acids are glycogenic amino acids. In biosynthesis, these amino acids (aspartic acid and glutamine) serve as the amino group donor.

- During the decomposition of non-essential amino acids, alpha-keto acids form oxalacetic acid, alpha-ketoglutaric acid and pyruvic acid.
Oxaloacetic acid formation from Aspartic acid and Asparagine

Asparagine is hydrolytically cleaved to aspartate by removing ammonia, and glutamic acid is synthesized when the amino group is converted to oxalacetic acid by transferring the amino group to alpha-ketoglutaric acid by transamination of the aspartic acid by the AST enzyme.

Metabolic Significance: In many reactions, the amino group acts as a donor.
<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
<th>Metabolic Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transamination</td>
<td>Oxaloacetate</td>
<td>TCA cycle metabolite</td>
</tr>
<tr>
<td>Amide formation</td>
<td>Asparagine</td>
<td>Proteinogenic amino acid</td>
</tr>
<tr>
<td>Condensation of carbamoyl phosphate</td>
<td>Carbamoil aspartate</td>
<td>Reaction that initiates pyrimidine synthesis</td>
</tr>
<tr>
<td>Phosphorylation with ATP</td>
<td>Aspartyl phosphate</td>
<td>The reaction that initiates the synthesis of methionine, threonine, lysine and isoleucine in plants and microorganisms</td>
</tr>
<tr>
<td>Decarboxylation</td>
<td>Beta-alanine</td>
<td>A component of pantothenic acid</td>
</tr>
<tr>
<td>Condensation of citrulline</td>
<td>Argininosuccinate</td>
<td>Amino group carrier in the conversion of Carbamic acid to Urea.</td>
</tr>
<tr>
<td>Condensation of IMP</td>
<td>Adenyl succinate</td>
<td>The amino group carrier in the conversion of IMP to AMP</td>
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Amino acid that form alpha-ketoglutaric acid

- **Glutamic Acid and Glutamine**
  - Act as amino group donor in many biosynthesis.
  - In the kidneys, the ammonia source in control of the acid-base balance.
  - Peripheral organs (brain, muscles) kidneys, liver and intestines form ammonia.
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In the extracellular space, cysteine is present in the form of oxidized form which is cystine. With dehydrogenation of cystine, the disulfide bridge is disrupted and cysteine is released and destroyed by alpha-beta elimination.

The displacement products are pyruvic acid and sulphate.

The resulting sulphate is either used for sulphate transport or detoxification reactions after activation, and is excreted in the urine.

In the cysteine biosynthesis, while the C-skeleton and the amino group originate from the serine, the sulfur atom is removed from methionine.
Ammonia Metabolism
Ammonia Metabolism

- In prokaryotic and eukaryotic cells, ammonia is primarily used for the synthesis of amino acids (especially through glutamine, aspartic acid and lysine), porphyrins, purines, pyrimidines, keratin and other nitrogen containing substances such as amino sugars.
  - Free ammonia, which can also occur in the metabolisms of purines and pyrimidines, as well as originating from amino acid breakdowns in mammals, can be reused in the body during biosynthesis events.
  - The unused portion is converted into 90-95% urea and a small part (5-10%) is excreted as free ammonia in the urine.
Ammonia Toxicity

- Although ammonia is an essential substance, very low levels of free ammonia in humans and animals lead to severe cerebral disorders.

- Some clinical findings of toxicity,
  - Vibrating hands,
  - Weakening of speaking,
  - Visually impaired.
  - In severe cases it results in coma and death.
Blood Ammonia Content

- Except for portal blood with a high ammonia content, the amount of ammonia in the blood is very low.

- The high concentration of nearby ammonia is derived from amino acids taken with food and by the microorganisms that have reached the intestines through gastrointestinal secretions.
Blood Ammonia Content

- Because urea is broken down by urease into ammonia and CO₂, there is no urea in the stool.

- Bacteria containing urease are found in the stomach, intestines and especially in the colon, and disintegrate 20% of the total amount of urea produced in the body.
Absorbed ammonia is rapidly taken up by the liver from the portal blood. There are very low concentrations of ammonia in the liver veins and whole blood. For this reason, the liver protects the organism, especially brain, from ammonia poisoning. In the systemic circulation, most of the ammonia is derived from amino acid metabolism in the brain, muscle, and kidneys.
Ammonia Metabolism

- **Glutamic acid dehydrogenase (GLDH)** and **Glutamine synthetase**
  - In the reaction catalyzed by GLDH, alpha-ketoglutaric acid is first bound to NH$_3$ and $\alpha$-iminoglutaric acid forms. $\alpha$-iminoglutaric acid is also converted to alpha-amino glutaric acid (L-Glutamic acid) by hydrogenation in the presence of NADH+H.
  - Glutamic acid also binds one mole of NH$_3$ under the use of energy by the glutamin synthetase enzyme and glutamine is formed.
Ammonia Metabolism

- Since the glutamic acid dehydrogenase enzyme is present in the liver mitochondria at the highest concentration, the enzyme serum concentrations are used as indicators of degradation in liver cells.

- Glutamine synthetase is found in all tissues.
Glutamic Acid has a key position in amino acid metabolism for the following reasons.

1. Glutamic acid can be formed by linking α-ketoglutaric acid and free ammonia.

2. The amino group of glutamic acid plays a role as an amino group carrier in the plasma, which is formed by transferring pyruvic acid through reversible transamination.

3. The amino group of glutamic acid is reversibly transaminated to oxalacetic acid, which is a keto acid, and aspartic acid is formed. The amino group on aspartic acid can also be used in biosynthesis, mainly urea formation.
Aspartate $\xrightarrow{\text{transaminase}}$ α-ketoglutarate $\xrightarrow{\text{transaminase}}$ Oxaloacetate $\xrightarrow{\text{transaminase}}$ Glutamate

alanine $\xrightarrow{\text{aminotransferase (Transaminase)}}$ α-ketoglutarate $\xrightarrow{\text{aminotransferase (Transaminase)}}$ pyruvate glutamate
Metabolism of Amino Groups

4. Glutamic acid is formed by 1 mole NH$_3$ binding and acts as an amino group donor in biosynthesis in glutamate, as well as mediates transport of NH$_3$, which is activated in peripheral tissues, and influences nitrate uptake.

5. Excess ammonia is broken off from glutamic acid by deamination and used in urea synthesis.
Urea Biosynthesis/Cycle
(Krebs-Henseleit Ornithine Cycle)

\[
\text{H}_2\text{N} \equiv \text{C} \equiv \text{NH}_2
\]
Urea Cycle

- If ammonia, which is removed from amino acids and a toxic matter, is not to be used in the synthesis of compounds containing nitrogen again, it is converted to a non-toxic matter. This conversion requires energy.

- **Urea synthesis is not compulsory** in the majority of aquatic animals, which can leave metabolic debris permanently in the environment they live in. The accumulation of ammonia in their bodies does not occur. **Such animals excrete ammonia directly** and they referred as **ammonoletic living beings.**
Urea Cycle

- There is no chance for ammonia to be continuously released in terrestrial animals (mammals). For this reason they convert the ammonia into urea and form a breakthrough product urea, which is why they are called ureotelic living beings.

- In reptiles and birds with limited water uptake, the end product of amino acid catabolism, ammonia, is excreted in the form of uric acid, and is called such aquatic uricotelic living beings.
Purpose of Urea Biosynthesis

- It is to make the ammonia which is emerged as excess, non-toxic.

- For this purpose in liver cells;
  - 1 mole of free ammonia,
  - 1 mol bicarbonate and
  - The amino group of 1 mole of aspartic acid is combined with a multi-step cycle and the urea is synthesized.
The two primary pathways by which nitrogen is transferred from amino acids to urea involve transamination and deamination reactions.

- Transaminases channel amino groups from several amino acids into glutamate (Glu), which can then be deaminated by GLDH, or the amino group can be transaminated onto oxaloacetate (OAA), thus forming aspartate (Asp) and α-ketoglutarate (α-KG=).
- CO₂ (in the form of HCO₃⁻), the ammonium ion (NH₄⁺) generated from deamination of Gln or Glu, or from ammonia entering directly from portal blood, and Asp now become substrates for hepatic urea synthesis.

\[
\text{NH}_4^+ + \text{HCO}_3^- + \text{Aspartate} + 3\text{ATP} \longrightarrow \text{Urea} + \text{Fumarate} + 2\text{ADP} + \text{AMP} + 4\text{Pi}
\]
The concentration of NH$_3$ in portal blood is usually high following a protein meal, and it may be transiently increased by the release of additional NH$_4^+$ from hepatic glutaminase and GLDH activity.

Ammonia is an allosteric activator of glutaminase. However, by the time hepatic portal blood reaches the systemic circulation, the NH concentration has usually been reduced by about 50-fold.
The importance of the **glutaminase** reaction

- The deficiencies of the urea cycle are corrected by glutamine coordination. This enzyme, which cleaves ammonium ions from glutamine, is localized along with carbamoyl phosphate synthetase-1 in the mitochondria of periportal hepatocytes.
- Glutaminase accelerates the urea cycle by providing more ammonium ions to the carbamoyl phosphate synthase-1, the first enzyme in the urea cycle.
- Since glutamine periportal hepatocytes formed in perivenous liver cells can be diffuse, an intercellular glutamine cycle occurs in which the conversion of ammonium ions coming from the portal blood into the urea is guaranteed.
Transamination and deamination reactions in periportal hepatocytes help to transfer nitrogen (N) from amino acids ultimately to urea.

- CO$_2$, in the form of HCO$_3^-$, NH$_4^+$, and the amine group of Asp are substrates for urea biosynthesis.
Urea Cycle

- **Periportal hepatocytes**
  - They are located near the portal vein, are the first liver cells to receive blood from the intestine.
  - They are rich in **carbonic anhydrase (CA), glutaminase, GLDH, and urea cycle enzyme** activity.

- The five reactions required in **periportal hepatocytes** for urea formation.
  - The first two occur **in mitochondria**, and the last three in the **cytoplasm**.
  - Starvation or an increased protein intake can alter concentrations of individual urea cycle enzymes 10- to 20-fold.
Reactions of Urea Cycle

1. Carbamoyl Phosphate Formation
   - The first and also rate-limiting reaction in the urea cycle which occurs in mitochondria.
   - It is catalyzed by carbamoyl phosphate synthetase-1 (CPS-1).
     - A CPS-2 exists in the cytoplasm, however, it participates in pyrimidine nucleotide rather than urea biosynthesis.
   - **N-Acetylglutamate** is a cofactor required as an allosteric activator of CPS-1.
   - Under the influence of CPS-1, HCO₃⁻, NH₄⁺, 2ATP and H₂O are condensed in the formation of carbamoyl phosphate, 2ADP, and an Pi.
Reactions of Urea Cycle

2. Citrulline Formation

• The second reaction, catalyzed by ornithine transcarbamoylase, involves mitochondrial condensation of carbamoyl phosphate with ornithine, thus forming citrulline and Pi.

• A citrulline-ornithine antiporter, which transports ornithine into mitochondria in exchange for citrulline, is located in the inner mitochondrial membrane.

• Both ornithine and citrulline are basic amino acids, and citrulline can also be formed in mucosal cells of the gut.
Reactions of Urea Cycle

1. Carbonic anhydrase:
   - $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+$
   - $\text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{N}$

2. CPS-1:
   - $\text{H}_2\text{N} = \text{PO}_4^-$
   - $\text{C}=\text{O}$

3. Glutaminase:
   - $\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{N} \rightarrow \alpha\text{-KG} = \text{H}_2\text{O}$

4. GLDH:
   - $\text{H}_2\text{N} \rightarrow \text{H}_3\text{N}^- + \text{CH} + \text{CH}_2\text{CH}_3$

5. Glutamate (Glu):
   - $\text{H}_3\text{N}^- + \text{CH} \rightarrow \text{COO}^-$
   - $\text{H}_3\text{N}^- + \text{CH} \rightarrow \text{CO}_2$

6. Carbamoyl Phosphate:
   - $\text{H}_2\text{N} \rightarrow \text{C}=\text{O}$
   - $\text{PO}_4^-$

7. Ornithine transcarbamoylase:
   - $\text{C}=\text{O}$
   - $\text{NH}_2$

8. Citrulline:
   - $\text{C}=\text{O}$
   - $\text{NH}_2$

9. Mitochondria:
   - Antiport

Source: Engelking, 2014
3. Argininosuccinate Formation

- The third reaction, catalyzed by argininosuccinate synthetase, involves cytoplasmic condensation of citrulline with aspartate, thus forming argininosuccinate.

- Linkage occurs via the α-amino group of aspartate, which ultimately provides the second nitrogen of urea.

- Energy required for this condensation is provided by the hydrolysis of ATP to AMP and 2Pi, and this reaction can also occur in the kidneys.
4. Arginine and Fumarate Formation

- The fourth reaction, catalyzed by *argininosuccinase*, involves cleavage of *argininosuccinate* into *arginine* and *fumarate*.
  - Fumarate forms a link with other pathways, including cytoplasmic reformation of aspartate, or entry into the mitochondrial TCA cycle.
  - **Fumarate** is hydrated to form **malate** in the presence of **fumarase**.
  - The malate so formed may be shuttled into mitochondria to enter the TCA cycle, or it may be converted to **pyruvate** (*malic enzyme*) or **OAA** (*malate dehydrogenase*).
  - The **OAA** may then undergo transamination by accepting an amino group from **Glu** to reform **aspartate**, thus completing an entirely cytoplasmic transamination route for entry of nitrogen into the urea cycle.
5. **Urea Formation**

- The fifth reaction, catalyzed by **cytoplasmic Mn^{++}-containing arginase**, involves removal of the **urea** side chain from **arginine**, thus forming **ornithine**, which is then transported back into mitochondria to undergo another cycle of urea biosynthesis.

- **Urea**, being sufficiently lipophilic and, unlike NH$_3$, a nontoxic end product of mammalian metabolism, diffuses out of periportal liver cells into blood.
Reactions of Urea Cycle

Cytoplasm

Blood

Ornithine

Argininosuccinate Synthetase

3

ATP

AMP + 2Pi

H₃N⁺–CH
(CH₂)₃

+NH₃

COO⁻

H₂O

Arginase

5

Aspartate

AST
(SGOT)

Glu

α-KG==

Argininosuccinate

4

Oxaloacetate

Malate dehydrogenase

NAD⁺

NADH

Fumarase

Malic enzyme

Pyruvate

Mitochondrial TCA Cycle

H₂O

COO⁻

NH

NH

H₂N⁺–CH
(CH₂)₃

NH

H₂N⁺–CH
(CH₂)₃

NH

H₂N⁺–CH
(CH₂)₃

NH

Urea

H₂N⁺–CH
(CH₂)₃

NH
• Acquired or inherited defects in any one of the five urea cycle enzymes may result in protein intolerance, hyperammonemia and CNS depression.

• Since Arg is an important intermediate of the urea cycle, a deficiency of this essential amino acid in cats may also lead to hyperammonemia and CNS depression.
Urea Disposal

Systemic Blood

Urea Cycle

Toxic Metabolites

Decarboxylation

Amino Acids

Bacterial Deamination

NH₃ \xrightarrow{\text{Bacterial Urease}} \text{NH}_4^+

NH₃ \xrightarrow{\text{H}^+} \text{NH}_4^+

Bacterial Protein

75% Urinary Urea

25% Salivary Gland

BUN

Rumen and Colon

Source: Engelking, 2014
An α-keto acid and ammonia formation event as a result of amino group removal from an amino acid is called ......

a. Thiolysis
b. Transamilation
c. Decarboxylation
d. Deamidation
e. Deamination
Your Questions?

Send to serkan.sayiner@neu.edu.tr
References

- Eren Meryem. Prof. Dr. Ders Notları (Teşekkürlerimle)
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