Nitrogen Metabolism

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Nitrogen metabolism

Atmospheric nitrogen N2 is most abundant but is too inert for use in most biochemical processes.

Atmospheric nitrogen is acted upon by bacteria (nitrogen fixation) and plants to nitrogen containing compounds. We assimilate these compounds as proteins (amino acids) in our diets.

Body proteins

Amino acids

α-amino groups

Other nitrogen containing compounds

Lecture III

NH₄⁺

Lecture II

Carbon skeletons

Urea

Lecture I

excreted

Enters various metabolic pathways
Urea Cycle

Disposal of Nitrogen

Nitrogen enters the human body as dietary amino acids (protein) and most of it leaves in the form of urea.
**Essential versus Nonessential Amino Acids**

Cannot be synthesized de novo, hence, must be supplied in the diet.  

<table>
<thead>
<tr>
<th>Essential</th>
<th>Nonessential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Alanine</td>
</tr>
<tr>
<td>Histidine</td>
<td>Aspartate</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Leucine</td>
<td>Glutamate</td>
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<tr>
<td>Lysine</td>
<td>Glycine</td>
</tr>
<tr>
<td>Methionine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Proline</td>
</tr>
<tr>
<td>Phenylalanine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Serine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
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<tr>
<td>Valine</td>
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</tbody>
</table>

<sup>a</sup> Arginine is synthesized by mammalian tissues, but the rate is not sufficient to meet the need during growth.

<sup>b</sup> Methionine is required in large amounts to produce cysteine if the latter is not supplied adequately by the diet.

<sup>c</sup> Phenylalanine is needed in larger amounts to form tyrosine if the latter is not supplied adequately by the diet.

Non-essential amino acids are either synthesized from the products of their catabolism or from essential amino acids.
Amino acids

Amino acid pool (in cells, blood and extracellular fluids) is supplied by three sources:
• Degradation of body proteins
• Derived from dietary proteins
• Synthesis of non-essential amino acids

Amino acids are depleted by three different routes:
* Used to synthesize body proteins.
* Used as a precursors of nitrogen containing small molecules (porphyrins, neurotransmitters, nucleotides etc).
* Converted to glucose, glycogen, fatty acids or CO2.

In normal healthy individuals amino acid pool is constant (stead state).

Amino acids undergo oxidative degradation in three different metabolic circumstances:
* During normal synthesis and degradation of cellular proteins.
* When diet is rich in protein and amino acids are ingested in excess of body needs (amino acids can not be stored).
* During starvation (amino acids are called upon as a fuel).
Protein turnover

There are two major pathways responsible for protein degradation

**Ubiquitin-26S proteasome pathway** (degrades endogenous proteins)

**Lysosomal** (degrades extracellular proteins and organells)
- Microautophagy
- Macroautophagy
- Chaperone-mediated microautophagy
Ubiquitin-26S proteasome Pathway

1. Protein selected for degradation is tagged with molecules of ubiquitin.

2. Ubiquinated proteins are recognized by the cytosolic proteasome, which unfolds and transports the protein to its proteolytic core.

Tandemly linked molecules of ubiquitin

Cellular protein

Ubiquitin

ATP → AMP + PPi Proteasome

Recycled Ubiquitin

Non-specific proteases

Amino acids

3. Peptide fragments produced by the proteasome are degraded to amino acids.

Proteasomes mainly Degrades endogenous Cellular proteins
**Lysosomal Pathway**

Macroautophagy involves the formation of a de-novo-formed membrane sealing on itself to engulf cytosolic components (proteins and/or whole organelles), which are degraded after its fusion with the lysosome.

Microautophagy is the direct invagination of materials into the lysosome.

Chaperone-mediated autophagy involves degradation of specific cytosolic proteins marked with a specific peptide sequence. Chaperone molecules (hsc70) bind to and transport marked proteins to the lysosome via a receptor complex.
Protein degradation is not a random process and is influenced by some structural aspects of the proteins.

- Proteins chemically altered by oxidation
- Tagged by ubiquitin
- Nature of N-terminal residue (serine (long lived, 20 hrs), aspartate (short lived, 3 mins).
- Proteins with PEST (proline, glutamate, serine and threonine) sequences
Fate of Dietary Protein

Dietary proteins are hydrolyzed to amino acids by proteolytic enzymes produced by three different organs: the stomach, the pancreas, and the small intestine.

Enteropeptidase unleashes a cascade of proteolytic activity.

Enzymes have different specificity for the amino acid R-group adjacent to the peptide bond: e.g. trypsin cleaves only when carbonyl group is contributed by arginine and lysine.

Free amino acids are taken into enterocytes by a Na⁺-linked secondary transport system. Di and Tripeptides are taken up by H⁺-linked transport system and hydrolyzed to free aa in cytosol and released into the portal system.
Transport of amino acids into cells

The ATP-dependent active transport systems controls the movement of amino acids from the extracellular space into cells. Hence, the concentration of free amino acids in extracellular fluids is significantly lower than that within cells.

- Seven different transport systems are known so far that have overlapping specificities for different amino acids.

- The small intestine and proximal tubule of the kidney have common transport systems.
Removal of amino group is an obligatory step of amino acid catabolism. The removal of the amino groups of almost all amino acids begins with the transfer of amino groups to just one amino acid - glutamic acid.

-All amino acids, with the exception of lysine and threonine, participate in transamination.

**Transamination:** funnels amino groups to glutamate

1) -α-amino group is transferred to α-ketoglutarate

2) -the reaction is carried out by aminotransferase, also called as transaminases

3) -the products are α-keto acid and glutamate

Oxidatively deaminated or used as a amino group donor in the synthesis of non essential amino acids

\[ \text{NH}_4^+ \]
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.

Pyridoxamine then reacts with an α-keto acid to form an amino acid.
Aminotransferase

-These enzymes are found in the cytosol and mitochondria of cells throughout the body, especially of the liver, kidney, intestine, and muscles.
-There are multiple transaminase enzymes which vary in substrate specificity.
-Two important aminotransferases are Alanine (ALT) and Aspartate (AST) aminotransferases.

ALT
-catalyzes transfer of amino group of alanine to \(\alpha\)-ketoglutarate---- products are pyruvate and glutamate.

AST (AST an exception to aminotransferases that funnels amino groups to glutamate)
-catalyzes transfer of amino group from glutamate to oxaloacetate
-products are aspartate and \(\alpha\)-ketoglutarate

used as a source of nitrogen in the Urea cycle.
Diagnostic value of plasma aminotransferases

Physical trauma or a disease process causing cell lysis, results in release of intracellular enzymes into the blood.

Liver disease: AST and ALT are elevated in all liver diseases e.g. conditions causing cell necrosis, viral hepatitis, toxic injury and prolonged circulatory collapse.

Non hepatic diseases: Aminotransferase are elevated in myocardial infarction and muscle disorders.
In liver, many of the amino groups are removed from α-amino acids by transamination to form glutamate.

Q. How amino groups are removed from glutamate?

Glutamate is transported from cytosol to mitochondria where it undergoes oxidative deamination by Glutamate dehydrogenase.
Glutamate dehydrogenase: the oxidative deamination of amino acids.

- Glutamate is the only amino acid that undergoes rapid oxidative deamination— a reaction catalyzed by glutamate dehydrogenase

- Conoenzymes: Glutamate dehydrogenase is unusual in that it can use both NAD$^+$ and NADP$^+$ as a coenzyme.
- Direction: The direction of the reaction depends on the relative concentrations of glutamate, α-κetoglutarate, and ammonia.
- Allosteric regulators: GTP is an allosteric inhibitor of glutamate dehydrogenase and ADP is an Activator.
Transdeamination of amino acids produce ammonia

Amino groups of amino acids → **Transamination** → α-ketogutarate → α-keto acid (derived from the original amino acid) + Glutamate

**Oxidative deamination**

Glutamate dehydrogenase

α-ketogutarate + NH4

The combined action of aminotransferase and glutamate dehydrogenase is called transdeamination
Two mechanisms that transport ammonia to the liver

I. Glutamine synthetase combine ammonia with glutamate to form glutamine (a nontoxic transport form of ammonia).

   - Glutamine is cleaved by glutaminase to produce glutamate and free ammonia in liver.

   - The second transport mechanism, primarily used by muscles involves two transamination reactions.

II. • Transamination of pyruvate to form alanine in muscles.
• Transamination of alanine to form pyruvate again in liver.
Urea Cycle

The ammonia is converted into urea via Urea cycle in hepatocytes.

The 2 nitrogen atoms of urea enter the Urea Cycle as NH$_3$ (produced mainly via the glutamate dehydrogenase reaction) and as the amino N of aspartate.
The urea cycle consists of five reactions - two mitochondrial and three cytosolic.
Step 1 Formation of carbamoyl phosphate

NH₃ (amino N) and HCO₃⁻ (carbonyl C) that are part of urea are incorporated first into carbamoyl phosphate by the enzyme Carbamoyl phosphate synthetase.

The reaction, which involves cleavage of 2 ~P bonds of ATP, is essentially irreversible.

Carbamoyl Phosphate Synthetase has an absolute requirement for an allosteric activator N-acetylglutamate. This derivative of glutamate is synthesized from acetyl-CoA and glutamate when cellular [glutamate] is high, signaling an excess of free amino acids due to protein breakdown or dietary intake.
Urea Cycle

1) Formation of carbamoyl phosphate

2) Formation of citrulline:
* formation of citrulline from ornithine and carbamoyl phosphate is carried out by **Ornithin transcarbamoylase**.
* release of high energy phosphate of carbamoyl phosphate as inorganic phosphate drives the reaction in the forward direction.
* the reaction product is citrulline which is released into the cytosol.
* Ornithine is regenerated.
* Ornithine and Citrulline are not incorporated into cellular proteins

3) Synthesis of argininosuccinate:
* Citrulline condenses with aspartate to form argininosuccinate.
* reaction is carried out by **Argininosuccinate synthetase**.
* requires one molecule of ATP (third molecule of ATP).

4) Cleavage of argininosuccinate:
* Argininosuccinate is cleaved to yield arginine and fumarate.
* reaction is carried out by **argininosuccinate lyase**

5) Cleavage of Arginine to Ornithine and Urea:
* Arginine is cleaved into Ornithine and Urea.
* reaction is carried out by **Arginase**
* reaction occurs almost exclusively in the liver.
Urea Cycle

1. Carbon dioxide provides the carbon atom of urea.
2. Free ammonia provides one of the nitrogen atoms of urea.
3. The enzyme has an absolute requirement for N-acetylglutamate, which acts as an allosteric activator.
4. Citrulline is transported out of the mitochondrion.
5. The amino group of aspartate provides one of the nitrogen atoms of urea.
6. Ornithine is regenerated and transported into the mitochondrion.
7. Fumarate is hydrated to malate, which is oxidized to oxaloacetate, which is transaminated to aspartate.
8. Tissues in addition to the liver use this pathway to make arginine.
Overall stoichiometry of the urea cycle

Aspartate + NH$_3$ + CO$_2$ + 3 ATP $\rightarrow$ Urea + Fumarate + 2 ADP + AMP + 2 P$_i$ + PP$_i$ + 3 H$_2$O

Four high energy phosphates are consumed in the synthesis of each molecule of urea.

Two ATPs are required to make Carbomoyl phosphate
One ATP is required to make Argininosuccinate

Two ATPs are required to restore 2 ADPs to two ATPs + two to restore AMP to ATP.
Metabolism of Ammonia

Sources of ammonia
1) From amino acids:
2) From glutamine: Glutamine hydrolysis by glutaminase (from kidney and intestine) form ammonia. From kidney, ammonia is excreted into the urine.
3) From bacterial action in the intestine: Ammonia is formed from urea by the bacterial urease. This ammonia is absorbed from the intestine by the way of portal vein and then converted into urea in the liver.
4) From amines: amines from diet, and the monoamines that serve as hormones or neurotransmitters gives rise to ammonia by the action of amine oxidase.
5) From purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as ammonia

Transport of ammonia in the circulation
1) Urea: Formation of Urea in the liver is major route of disposal for ammonia
2) Glutamine: Is a nontoxic storage and transport form of ammonia.

Hyperammonia
Increase levels of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, vomiting, cerebral edema, and blurring vision. At high concs. Ammonia can cause coma and death.

Acquired hyperammonia: Liver diseases—e.g. viral hepatitis, ischemia, or hepatotoxins
Cirrhosis
Hereditary hyperammonemia: Genetic disorders of five enzymes of the urea cycle
Summary

Amino acid pool

- is defined as: All the free amino acids in cells and extracellular fluids
- are produced by degradation of body protein
  - regulated by: Ubiquitin, N-Terminal amino acids, PEST sequences
- are consumed by synthesis of nonessential amino acids
  - requires: α-Keto acids and ammonia
  - involves: Proteolytic enzymes of the GI tract and pancreas

Synthesis of body protein

- is regulated by: Transcription and translation factors
  - biosynthetic pathways
  - intermediary metabolism

Degradation of dietary protein

- involves: Proteasome, Lysosome, Cytosol via nonspecific proteases

Protein turnover

Removal of nitrogen from amino acids

- occurs because amino acids cannot directly participate in energy metabolism
- therefore: Amino groups are removed
  - mediated by: Two sequential reactions
    - first: Transaminases
      - elevated serum levels can detect
      - result in: Liver damage due to
        - Hepatitis
        - Cirrhosis
        - Hepotoxic drugs
        - Defects in enzymes of the urea cycle

Amino groups transferred to α-keto acids forming:

- Aspartate
- Glutamate

followed by:

2. Glutamate dehydrogenase

- results in: Glutamate oxidatively deaminated to α-Ketoglutarate NH₃
  - can be stored and transported as glutamine
  - can release ammonia

Urea cycle

- Nitrogen of aspartate, CO₂, and NH₃ incorporated into urea

Inherited enzyme deficiencies

- characterized by: Hyperammonemia, Mental retardation
- treated by: Drug therapy, Reduction of protein intake