M1 - Renal, Fall 2007

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Nitrogen Metabolism (and Related Topics)

• Amino Acid Metabolism (Nitrogen metabolism)
• Folate Metabolism (“One-Carbon pathways”)
• Nucleotide Metabolism

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There are also PDF’s of class handouts with supplemental information available in the table of contents for this course.

Supplementary study material on the Web:  
http://seqcore.brcf.med.umich.edu/mcb500
Protein Degradation:

• Endogenous proteins degrade continuously
  - Damaged
  - Mis-folded
  - Un-needed
• Dietary protein intake - mostly degraded

Nitrogen Balance - expresses the patient’s current status - are they *gaining* or *losing* net Nitrogen?
Transaminases Collect Amines

General reaction overview:

\[
\text{Amino acid} + \alpha\text{-keto acid (typically alpha-ketoglutarate)} \rightarrow \text{Amino acid} + \alpha\text{-keto acid (typically glutamate)}
\]

Details of reaction mechanism:

1. Amino acid + $\alpha$-keto acid (typically alpha-ketoglutarate)
2. Reaction with pyridoxal phosphate
3. Formation of pyridoxamine phosphate
4. Conversion to pyridoxal phosphate
5. Cycle continues
Transfer the amine back to an acceptor α-keto acid
In peripheral tissues, transaminases *tend* to form Glutamate when they catabolize amino acids.

In other words, alpha-ketoglutarate is the preferred acceptor, and Glutamate is the resulting amino acid:

Some amino acid + \( \alpha \)-ketoglutarate \( \rightarrow \) some alpha keto acid + Glutamate
Glutamate can donate its amines to form other amino acids as needed.

A specific example - production of Aspartate in liver (described a few slides from now):

\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \alpha\text{-ketoglutarate} + \text{aspartate}
\]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{align*}
\text{glutamate} & \rightarrow \text{NAD}(P) \\
& \rightarrow \text{NAD}(P)H \\
& \rightarrow \alpha\text{-keto glutarate} + \text{NH}_3
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \rightarrow \text{ATP} + \text{NH}_3 \\
& \rightarrow \text{ADP} + P_i \\
& \rightarrow \text{glutamine}
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{Glutamine} \rightarrow \text{Glutamate} + \text{NH}_3
\]

Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:}
\]

\[
\begin{align*}
\text{Glutamate} & \rightarrow \text{aspartate} + \text{oxaloacetate} \\
\text{α-keto glutarate} & \rightarrow \\
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate \[ \rightarrow \text{ATP} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate} \]
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[ \text{aspartate} \rightarrow \text{Citrulline} \rightarrow \text{Argininosuccinate} \]

\[ \text{ATP} \rightarrow \text{AMP} + \text{PP}_i \]
Argininosuccinate lyase

Argininosuccinate $\xrightarrow{\text{Argininosuccinate lyase}}$ Arginine

$\text{H} \quad \text{NH}_2$ $\quad \text{H} \quad \text{NH}_2$

$\text{H} \quad \text{NH}_3$ $\quad \text{H} \quad \text{NH}_3$

$\text{(-)}_0 \text{C}-\text{CH}_2\text{CH}_2\text{NH}-\text{C}=\text{NH}_2^{(+)}$ $\quad \text{(-)}_0 \text{C}-\text{CH}_2\text{CH}_2\text{NH}-\text{C}=\text{NH}_2^{(+)}$

$\text{(-)}_0 \text{C}-\text{CH}_2\text{CH}_2\text{NH}-\text{C}=\text{NH}_2^{(+)}$ $\quad \text{(-)}_0 \text{C}-\text{CH}_2\text{CH}_2\text{NH}-\text{C}=\text{NH}_2^{(+)}$

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Urea Cycle Connects to TCA Cycle
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\begin{align*}
\text{glutamate} & \quad \text{NAD}(P) \\
\text{mito} & \rightarrow \quad \text{NAD}(P)H \\
& \quad \alpha\text{-ketoglutarate} + \text{NH}_3
\end{align*}
\]

**Glutamine Synthetase:**

\[
\begin{align*}
\text{glutamate} & \quad \text{ATP} + \text{NH}_3 \\
& \rightarrow \quad \text{ADP} + P_i \\
& \quad \text{glutamine}
\end{align*}
\]
CPS I is Stimulated by NAG

\[
\begin{align*}
\text{glutamate} & \rightarrow \text{N-acetyl glutamate (NAG)} \\
\text{acetyl CoA} & \rightarrow \text{N-acetyl glutamate (NAG)}
\end{align*}
\]

(repeating the figure from page 3 of your handout)
Complicating the picture: Other tissues may be involved
Why is Ammonia Toxic?
Why is Ammonia Toxic?

• Possible neurotoxic effects on glutamate levels (and also GABA) (due to shifting equilibria of reactions involving these compounds)
Why is Ammonia Toxic?

• Possible neurotoxic effects on glutamate levels (and also GABA) (due to shifting equilibria of reactions involving these compounds)

• Possible metabolic/energetics effects:
  - alpha-ketoglutarate levels
  - glutamate levels
  - glutamine
Inherited Defects of Urea Cycle Enzymes: Diagnosis

Defects are diagnosed based on the metabolites seen in the blood and/or urine.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSD</td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td>OTCD</td>
<td>Elevated CP causes synthesis of Orotate</td>
</tr>
<tr>
<td>ASD</td>
<td>Elevated citrulline</td>
</tr>
<tr>
<td>ALD</td>
<td>Elevated argininosuccinate</td>
</tr>
<tr>
<td>AD</td>
<td>Elevated arginine</td>
</tr>
</tbody>
</table>
CPS I is Stimulated by NAG

Glutamate + Acetyl CoA → N-Acetyl Glutamate (NAG)

(repeating the figure from page 3 of your handout)

Bicarbonate → Carboxyl Phosphate → Carbamate → Carbamoyl Phosphate
The diagram illustrates the urea cycle and related metabolic pathways. The cycle begins with the conversion of carbamoyl phosphate into citrulline in the liver mitochondria. Citrulline is then converted into argininosuccinate in the liver cytoplasm. Argininosuccinate is degraded into fumarate and urea. The urea cycle is essential for nitrogen excretion in humans.
Clinical Management of Urea Cycle Defects

- Dialysis to remove ammonia
- Provide the patient with alternative ways to excrete nitrogenous compounds:
  - Intravenous sodium benzoate or phenylacetate
  - Supplemental arginine
- Levulose - acidifies the gut
- Low protein diet
Degrading the Amino Acid Carbon Backbone
Easily-degraded products after transamination:

\[
\begin{align*}
\text{aspartate} & \xrightarrow{\text{transamination}} \text{oxaloacetate} \\
\text{glutamate} & \xrightarrow{\text{transamination}} \alpha\text{-ketoglutarate} \\
\text{alanine} & \xrightarrow{\text{transamination}} \text{pyruvate}
\end{align*}
\]

We also already know how to degrade Glutamine:

\[
\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}
\]

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
Many amino acids are purely glucogenic:
Glutamate, aspartate, alanine, glutamine,
asparagine,…

Some amino acids are *both* gluco- and ketogenic:
Threonine, isoleucine, phenylalanine,
tyrosine, tryptophan

The only PURELY ketogenic Amino Acids:
: leucine, lysine
Amino acids with 5-carbon backbones tend to form α-ketoglutarate.
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

Glycine

Serine Hydroxymethyltransferase:

Serine

Serine Dehydratase:

Serine
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine

\[
\begin{align*}
\text{NH}_3 & \quad (+) \\
\text{O} & \quad (-)
\end{align*}
\]

Enzyme: Phenylalanine hydroxylase

\[
\begin{align*}
\text{Dihydrobiopterin} + \text{H}_2\text{O} & \quad \text{Tetrahydrobiopterin} + \text{O}_2 \\
\text{NH}_3 & \quad (+)
\end{align*}
\]

Tyrosine

\[
\begin{align*}
\text{OH} & \quad (-)
\end{align*}
\]

Homogentisate

\[
\begin{align*}
\text{COO} & \quad (-)
\end{align*}
\]

Phenylpyruvate

Deficiency: Alkaptonuria

“Ochronosis”

Enzyme: homogentisate dioxygenase

(you don’t need to know the rest)
Branched Chain Amino Acids

Isoleucine

\[ \text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^- \]

\[ \text{CH}_3 \text{NH}_3^+ \]

\[ \text{α-KG} \]

\[ \text{Glu} \]

\[ \text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{COO}^- \]

\[ \text{NAD}^+ \text{CoASH} \]

\[ \text{NADH} + \text{CO}_2 \]

Leucine

\[ \text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^- \]

\[ \text{CH}_3 \text{NH}_3^+ \]

\[ \text{α-KG} \]

\[ \text{Glu} \]

\[ \text{CH}_3\text{CHCH}_2 - \text{C} - \text{COO}^- \]

\[ \text{NAD}^+ \text{CoASH} \]

\[ \text{NADH} + \text{CO}_2 \]

Valine

\[ \text{CH}_3\text{CH} - \text{CH} - \text{COO}^- \]

\[ \text{CH}_3 \text{NH}_3^+ \]

\[ \text{α-KG} \]

\[ \text{Glu} \]

\[ \text{CH}_3\text{CH} - \text{C} - \text{COO}^- \]

\[ \text{NAD}^+ \text{CoASH} \]

\[ \text{NADH} + \text{CO}_2 \]

--- Branched-chain α-keto acid dehydrogenase ---

\[ \text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{S-CoA} \]

\[ \text{CH}_3\text{CHCH}_2 - \text{C} - \text{S-CoA} \]

\[ \text{CH}_3\text{CH} - \text{C} - \text{S-CoA} \]

(continues on to degradation path similar to β-oxidation of fatty acids)
Synthesis of Bioactive Amines

Tyrosine → Dihydroxyphenylalanine (L-DOPA) via Tyrosine hydroxylase

Dopamine → Norepinephrine → Epinephrine
Synthesis of Bioactive Amines

Tryptophan $\xrightarrow{\text{Tryptophan hydroxylase}}$ 5-hydroxytryptophan $\xrightarrow{\text{PLP-dependent decarboxylation}}$ Serotonin
Synthesis of Bioactive Amines

\[ \text{Glutamate} \xrightarrow{\text{Glutamate decarboxylase (PLP-dependent)}} \text{\(\gamma\)-aminobutyric acid (GABA)}} \]

\[ \text{Histidine} \xrightarrow{\text{Histidine decarboxylase (PLP-dependent)}} \text{Histamine} \]
NON-Essential Amino Acids:

Glutamate, aspartate, alanine, glutamine, asparagine, (proline), glycine, serine (cysteine, tyrosine)

Essential Amino Acids:

Arginine (!), phenylalanine, methionine, histidine, Isoleucine, leucine, valine, threonine, tryptophan, lysine