M1 - Renal, Fall 2007

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Viewer discretion advised: Material may contain medical images that may be disturbing to some viewers.
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
Amino Acid metabolism

- Amino acids
  - Glu, Gln, Asp, NH₃

Folate metabolism

- Methylene THF
- Met Cycle

Nucleic Acid metabolism

- Purines
  - DNA
  - RNA
  - Pyrimidines
- Uric Acid
  - (energy)
**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:

\[
R_1\text{C} \text{coo}^\text{(-)} + R_2\text{C} \text{coo}^\text{(-)} \xrightarrow{\alpha\text{-keto acid (typically alpha-ketoglutarate)}} R_1\text{C} \text{coo}^\text{(-)} + R_2\text{C} \text{coo}^\text{(-)}
\]

Details of reaction mechanism:

amino acid

\[
\text{R-C-coo}^\text{(-)} + \text{NH}_2 + \text{O} \rightarrow \text{R-C-coo}^\text{(-)} + \text{H}^+ \rightarrow \text{R-C-coo}^\text{(-)} + \text{HCH} \rightarrow \text{R-C-coo}^\text{(-)} + \text{NH}_2 \rightarrow \text{pyridoxal phosphate}
\]

\[
\text{R-C-coo}^\text{(-)} + \text{H}_2\text{O} \rightarrow \text{R-C-coo}^\text{(-)} + \text{H}^+ \rightarrow \text{R-C-coo}^\text{(-)} + \text{HCH} \rightarrow \text{R-C-coo}^\text{(-)} + \text{NH}_2 \rightarrow \text{pyridoxamine phosphate}
\]
Transfer the amine back to an acceptor $\alpha$-keto acid
Some amino acid + α-ketoglutarate → some alpha keto acid + Glutamate

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:
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:**

\[
\text{glutamate} + \text{NAD}^+ \rightarrow \alpha\text{-ketoglutarate} + \text{NH}_3 + \text{NADH} + \text{H}^+ \quad \text{(mito)}
\]

**Glutamine Synthetase:**

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} + \text{ADP} + \text{Pi}
\]

\[
\text{glutamine} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamate} + \text{ADP} + \text{Pi}
\]
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:

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

\[
\text{glutamate} + \text{oxaloacetate} \rightarrow \text{aspartate} + \text{\alpha-keto glutarate}
\]
Carbamoyl phosphate synthetase I
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[ \text{NH}_2\text{C} - \text{OP}_2^{(-)} \]

Ornithine

\[ \text{(-OOC-CH}_2\text{CH}_2\text{CH}_2\text{NH}_2\text{(+)} \]

Citrulline

\[ \text{(-OOC-CH}_2\text{CH}_2\text{CH}_2\text{NH}-\text{C} - \text{NH}_2 \]
Argininosuccinate synthetase

Citrulline → Argininosuccinate

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

Argininosuccinate $\rightarrow$ Arginine

\[ \text{Argininosuccinate} \rightarrow \text{Arginine} \]
Arginase

Arginine $\rightarrow$ Urea $\rightarrow$ Ornithine

$\text{H} - \text{CH}_2\text{CH}_2\text{NH}_2 - \text{C} - \text{NH}_2$

$\text{H}_2\text{O}$
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Argininosuccinate
- Arginine
- Urea
- Aspartate
- Oxaloacetate
- Malate
- Fumarate
- α-Ketoglutarate
- Citrate

The Urea Cycle connects to the TCA Cycle through the intermediates Ornithine, Citrulline, Argininosuccinate, and Arginine. Aspartate, Oxaloacetate, Malate, Fumarate, α-Ketoglutarate, and Citrate are key intermediates in the TCA Cycle, showing their interconnection with the Urea Cycle.
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\text{Glutamate} \quad \xrightarrow{\text{NH}_2} \quad \text{NAD(P)} \quad \xrightarrow{\text{mito}} \quad \text{NAD(P)H} \quad \xrightarrow{\text{O}} \quad \alpha\text{-ketoglutarate} + \text{ammonia}
\]

Glutamine Synthetase:

\[
\text{Glutamate} \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{ADP} + \text{P}_i \quad \xrightarrow{\text{glutamine}}
\]
CPS I is Stimulated by NAG

\[
\text{glutamate} + \text{acetyl CoA} \rightarrow \text{N-acetyl glutamate (NAG)}
\]

(repeating the figure from page 3 of your handout)

\[
\text{bicarbonate} \xrightarrow{\text{ATP}} \text{carbonyl phosphate} \xrightarrow{\text{NH}_3} \text{carbamate} \xrightarrow{\text{ATP}} \text{carbamoyl phosphate}
\]
The diagram illustrates the metabolic pathways involving glucose, pyruvate, glutamate, and alanine, particularly highlighting the processes within muscle and liver tissues. Key pathways include:

- **Muscle**
  - Glucose → Pyruvate → Glutamate → Amino acids (via Amines)
  - Glutamate → α-ketoglutarate → Alanine

- **Liver**
  - Glucose → Pyruvate → Glutamate → Urea
  - Alanine → α-ketoglutarate

These pathways are crucial for energy production and nutrient metabolism.
Complicating the picture: Other tissues may be involved

**Muscle:**
- Amino acids: Transamination, Deamination
- Alanine $\rightarrow$ Glutamate $\rightarrow$ Glutamine $\rightarrow$ NH$_4$$^{(+)}$

**Intestine:**
- Glutamine
- Alanine $\rightarrow$ NH$_4$$^{(+)}$ Citrulline

**Kidney:**
- Glutamine $\rightarrow$ NH$_3$
- NH$_4$$^{(+)}$
- Arginine $\rightarrow$ Citrulline

**Liver:**
- Glutamine $\rightarrow$ Arginine $\rightarrow$ Urea
  - Alanine $\rightarrow$ Glu $\rightarrow$ Aspartate
  - NH$_4$$^{(+)}$
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></th>
<th>Description</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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \text{ATP} \\
\text{carbonyl phosphate} & \quad \text{NH}_3 \\
\text{carbamate} & \quad \text{ATP} \\
\text{carbamoyl phosphate} & \quad \text{ADP}
\end{align*}
\]
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:

- \[ \text{Glutamine} \rightarrow \text{glutamate} + \text{ammonia} \]
- \[ \text{Asparagine} \rightarrow \text{aspartate} + \text{ammonia} \]

We also already know how to degrade Glutamine:

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

...and by analogy, how to degrade Asparagine:

\[ \text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia} \]
Amino Acids are categorized as ‘Glucogenic’ or ‘ketogenic’ or both.

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 $\alpha$-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

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

\[
\begin{align*}
&\text{Tetrahydrobiopterin} + O_2 \\
&\text{Dihydrobiopterin} + H_2O \\
\end{align*}
\]

Enzyme: Phenylalanine hydroxylase

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

Tyrosine

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

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

(You don’t need to know the rest)

Phenylalanine

\[
\begin{align*}
&\text{CH}_2 \\
&\text{CH} \\
&\text{COO} \\
&\text{(-)} \\
\end{align*}
\]

Phenylpyruvate

Phenylketonuria (no phenylalanine hydroxylase)
Branched Chain Amino Acids

Isoleucine

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{CH} \quad \text{COO} \\
\text{CH}_3 & \quad \text{NH}_3
\end{align*}
\]

Leucine

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{CH} \quad \text{COO} \\
\text{CH}_3 & \quad \text{NH}_3
\end{align*}
\]

Valine

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{CH} \quad \text{COO} \\
\text{CH}_3 & \quad \text{NH}_3
\end{align*}
\]

------------------- Transamination -------------------

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

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

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

Tyrosine → Dihydroxyphenylalanine (L-DOPA) via Tyrosine hydroxylase

Tyrosine hydroxylase

Dopamine → Norepinephrine → Epinephrine
Synthesis of Bioactive Amines

Tryptophan $\rightarrow$ NAD$^+$

Tryptophan hydroxylase $\rightarrow$ 5-hydroxytryptophan

PLP-dependent decarboxylation $\rightarrow$ CO$_2$

Serotonin
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent)

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