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
Amino Acid metabolism

Amino acids

Glu, Gln, Asp, NH₃

Urea

Folate metabolism

Methylened THF

Met Cycle

TCA Cycle

oxaloacetate

fumarate

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:

\[
\text{R}_1\text{C}^{\text{coo}(-)} + \text{R}_2\text{C}^{\text{coo}(-)} \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} + \text{R}_2\text{C}^{\text{coo}(-)}
\]

\(\text{R}_1\text{C}^{\text{coo}(-)}\) is the \(\alpha\)-keto acid (typically \(\alpha\)-ketoglutarate).

Details of reaction mechanism:

\[
\begin{align*}
\text{amino acid} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
+ \text{H} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{NH}_2 & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{O} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{H} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{H}_2\text{O} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{N} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{CH}_3 & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{BOCH}_2 & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{OH} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{pH} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\text{pyridoxal phosphate} & \rightarrow \text{R}_1\text{C}^{\text{coo}(-)} \\
\end{align*}
\]

\(\text{R}_2\text{C}^{\text{coo}(-)}\) is the \(\alpha\)-keto acid (typically glutamate).
Transfer the amine back to an acceptor $\alpha$-keto acid
Some amino acid + $\alpha$-ketoglutarate $\rightarrow$ 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:

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

Glutamine Synthetase:

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

Glutamine is hydrolyzed to glutamate and ammonia:

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

Glutamate donates its amino group to form aspartate:
Carbamoyl phosphate synthetase I

bicarbonate → carbonyl phosphate → carbamoyl phosphate → carbamate → carboxamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[
\begin{align*}
\text{Ornithine} & \quad \text{Citrulline} \\
\text{(-)} \text{OOC} - & \quad \text{(-)} \text{OOC} - \\
\text{NH}_2 - C - & \quad \text{C} - C_2 H_5 C_2 H_5 C_2 H_5 \text{NH}_2^{(+)} \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{(+)} & \quad \text{(+)} \\
\end{align*}
\]
Argininosuccinate synthetase

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

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

Argininosuccinate → Arginine

Fumarate
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine → Citrulline → Argininosuccinate → Arginine → Urea

TCA Cycle:
- Oxaloacetate → Malate → Fumarate → α-Ketoglutarate → Citrate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{align*}
\text{glutamate} & \rightarrow (\text{mito}) \rightarrow \text{NAD(P)H} \rightarrow \text{NAD(P)}^+ \\
& \downarrow \quad \quad \quad \quad \text{O} \\
\text{\textit{\alpha}-ketoglutarate} + \text{ammonia} \\
\end{align*}
\]

Glutamine Synthetase:

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


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

(repeating the figure from page 3 of your handout)

bicarbonate  \rightarrow  carbonyl phosphate  \rightarrow  carbamate  \rightarrow  carbamoyl phosphate
Muscle

Glucose → Pyruvate → Glutamate → \( \alpha \)-ketoglutarate → Alanine

(Amines) → Amino acids

Blood transport

Liver

Glucose → Pyruvate → Glutamate

\( \alpha \)-ketoglutarate → Urea

\( \mathbf{N}_2 \)
Complicating the picture: Other tissues may be involved

Muscle:
- Amino acids: Transamination, Deamination
- Glutamine → Glutamate → Alanine
- Purine deamination: NH₄⁺

Intestine:
- Glutamine
- Alanine, NH₄⁺ → Citrulline

Kidney:
- Glutamine → NH₃
- NH₄⁺ → NH₄⁺
- Arginine
- Citrulline

Liver:
- Glutamine
- NH₄⁺ → Arginine, Urea
- Alanine, Glu, Aspartate
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>Acronym</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>
1. Carbamoyl phosphate + ATP + HCO₃⁻ + NH₃ → Cytosol
2. Ornithine + Citrulline + ATP → Liver mitochondrion
3. Arginine + Fumarate + ATP → Liver cytoplasm
4. Urea + Argininosuccinate + ATP → Liver cytoplasm
5. ATP + AMP + P_i → Liver cytoplasm

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)

\[
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\]
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:

\[
\begin{align*}
\text{Glutamine} & \xrightarrow{\text{transamination}} \text{glutamate} + \text{ammonia} \\
\text{asparagine} & \xrightarrow{\text{transamination}} \text{aspartate} + \text{ammonia}
\end{align*}
\]

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 α-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\text{Glycine} \xrightarrow{\text{THF, N}^5\text{-N}^\delta\text{-methylene THF}} \text{CO}_2 + \text{NH}_4^{+}
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine} \xrightarrow{\text{THF, N}^5\text{-N}^\delta\text{-methylene THF}} \text{Glycine}
\]

Serine Dehydratase:

\[
\text{Serine} \xrightarrow{\text{H}_2\text{O}} \text{OOC-CH-NH}_3^{+} \xrightarrow{\text{H}_2\text{O}} \text{OOC-CH-NH}_2 \xrightarrow{\text{NH}_4^{+}} \text{OOC-C-C-O}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine $\rightarrow$ Tetrahydrobiopterin + $O_2$ (via Dihydrobiopterin + $H_2O$)

Enzyme: Phenylalanine hydroxylase

Phenylalanine $\rightarrow$ Tyrosine $\rightarrow$ Homogentisate

Phenylpyruvate

Phenylketonuria (no phenylalanine hydroxylase)

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

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

Isoleucine

\[
\text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NH}_3 \\ 
\text{(+)}
\]

\[\alpha-\text{KG}\]

\[\rightarrow \text{Glu}\]

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

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

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

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

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

Leucine

\[
\text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NH}_3 \\ 
\text{(+)}
\]

\[\alpha-\text{KG}\]

\[\rightarrow \text{Glu}\]

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

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

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

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

Valine

\[
\text{CH}_3\text{CH} - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NH}_3 \\ 
\text{(+)}
\]

\[\alpha-\text{KG}\]

\[\rightarrow \text{Glu}\]

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

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

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

\[
\text{CH}_3\text{CH} - \text{S-CoA} \\
\text{CH}_3 \\
\]
Synthesis of Bioactive Amines

Tyrosine \rightleftharpoons \text{Tyrosine hydroxylase} \rightarrow \text{Dihydroxyphenylalanine (L-DOPA)}

Dopamine \rightarrow \text{Norepinephrine} \rightarrow \text{Epinephrine}
Synthesis of Bioactive Amines

Tryptophan $\xrightarrow{\text{Tryptophan hydroxylase}}$ 5-hydroxytryptophan $\xrightarrow{\text{PLP-dependent decarboxylation}}$ Serotonin

\[ \text{Tryptophan} \rightarrow \text{5-hydroxytryptophan} \rightarrow \text{Serotonin} \]
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

\[ \text{Glutamate} \rightarrow \gamma\text{-aminobutyric acid (GABA)} \]

Histidine decarboxylase (PLP-dependent)

\[ \text{Histidine} \rightarrow \text{Histamine} \]
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