Glutamine and Protein Metabolism in the Newborn
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Protein Metabolism in Vivo

Equilibrium: Input = Output

Proteins

Diet (in)

Amino Acids

- Loss (out)
- Energy

Quantification of Protein Metabolism in Vivo

Proteins

Diet

\[ ^{13}\text{C,2H Tracer AA} \]

Leucine
Phenylalanine
Valine

- Loss [tracer – CO₂]
- Energy

Biological Actions of Glutamine

- Glutamine
- Most abundant dispensable amino acid in blood and tissues.
- Virtually synthesized by every tissue
- Rapid decrease in tissue and plasma levels in acute stress
Congenital Glutamine Deficiency with Glutamine Synthetase Mutations

Fetal-Placental Glutamine Exchange

Glutamine Synthesis from TCA Cycle

Anaplerosis and Cataplerosis

Glutamine and Phenylalanine Kinetics in Term Infants

Leucine and Urea Kinetics in Term Infants

Relationship Between Leucine (C) and Phenylalanine Flux in Neonates
Correlation Between Rate of Appearance of Leucine (N) and Glutamine

\[ r^2 = 0.59 \quad p = 0.001 \]

Correlation Between Ra Glutamine and Urea Synthesis in Newborn Infants

\[ r^2 = 0.39 \quad p = 0.03 \]

**OBJECTIVES**

**Effect of 5 days enteral glutamine (0.6 g·kg\(^{-1}\)·d\(^{-1}\)) on**

- Whole body protein turnover (phenylalanine kinetics)
- Glutamine turnover and de novo synthesis
- Transamination of BCAA
- Urea synthesis

**Enteral Glutamine in LBW Infants**

**Methods**

- **Preterm infants:** Gestational age <32 weeks, Birth Weight <1500 g
- **Control (n=8):** Preterm infant formula (PF24®, Ross Labs), 5d
- **Glutamine (n=9):** PF24®+ glutamine, 0.6g·kg\(^{-1}\)·d\(^{-1}\), 5d
- **Tracers:** \([2H_5]\)phenylalanine, \([1-15N,13C]\)leucine, \([5-15N]\)glutamine, \([15N_2]\)urea

**STUDY DESIGN**

- **Tracer Infusion**
- **Blood Sample**
- **Feeds**
Clinical Characteristics of Study Infants

<table>
<thead>
<tr>
<th>Birth Weight g</th>
<th>Gestation wk</th>
<th>Prewnental Age in Weeks wk</th>
<th>Weight at Birth g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>1161 ± 340</td>
<td>28 ± 3</td>
<td>1827 ± 350</td>
</tr>
<tr>
<td>Glutamine (n=9)</td>
<td>1216 ± 330</td>
<td>28 ± 2</td>
<td>1888 ± 307</td>
</tr>
</tbody>
</table>

Glutamine Kinetics

<table>
<thead>
<tr>
<th>Glutamine Ra</th>
<th>Glutamine De Novo Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>Fed</td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>765 ± 165</td>
</tr>
<tr>
<td>Glutamine (n=9)</td>
<td>690 ± 124</td>
</tr>
</tbody>
</table>

µmoles.kg⁻¹.h⁻¹

Glutamine from Proteolysis

<table>
<thead>
<tr>
<th>Glutamine from Proteolysis</th>
<th>Urea Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>Fed</td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>101 ± 16</td>
</tr>
<tr>
<td>Glutamine (n=9)</td>
<td>102 ± 12</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
</tr>
</tbody>
</table>

µmoles.kg⁻¹.h⁻¹

Phenylalanine and Leucine Kinetics

<table>
<thead>
<tr>
<th>Phenylalanine Ra</th>
<th>Leucine (N) Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>Fed</td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>94 ± 15</td>
</tr>
<tr>
<td>Glutamine (n=9)</td>
<td>90 ± 41</td>
</tr>
<tr>
<td>P</td>
<td>ns</td>
</tr>
</tbody>
</table>

µmoles.kg⁻¹.h⁻¹

SUMMARY

Enteral administration of glutamine in growing LBW infants results in:

- No measurable change in glutamine systemic Ra
- Increase in transamination of leucine
- An equimolar increase in urea Ra
- A positive linear correlation between reamination of BCAA and urea synthesis
- No detectable change in whole body phenylalanine or leucine C kinetics
CONCLUSION

Enterally administered glutamine does not have a measurable impact on whole body protein kinetics. The equimolar increase in urea synthesis and unchanged systemic glutamine Ra suggests a first pass oxidation of glutamine in the gut.

Splanchnic Metabolism of Glutamine

Glutamine Supplementation in LBW Infants

- Clinical advantages
  - Length of hospital stay
  - Ventilatory support
- Lower rate of sepsis
- Lower stimulation of immune system

Glutamine Supplementation - Rationale

In isolated skeletal muscle:
- Positive correlation between (cellular) glutamine levels and protein synthesis
- Glutamine inhibited protein breakdown

Parenteral glutamine

(Neu J. et al; J Pediatr 1997)
(Lacey et al; JPEN 1996)

Parenteral Glutamine Supplement - Rationale

Experimental Depletion of Glutamine:
- No effect on Ra
  - Leucine
  - Phenylalanine
  - Glutamine
- Decreased hepatic
  - (a) release of protein
  - (b) KIC oxidation

(MacLennan et al, 1987)
Effect of parenteral Glutamine on Amino Acid Kinetics In Preterm Infants

Endogenous Ra of Glutamine, Phenylalanine and Urea

SUMMARY

Our data show that in LBW infants, parenteral glutamine supplementation results in:

- a decrease in the rate of appearance (Ra) of glutamine and phenylalanine,
- no change in the rate of urea synthesis, and
- a lower rate of turnover of leucine N.

CONCLUSIONS

Since decrease in whole body proteolysis is associated with protein accretion, parenteral glutamine supplement may benefit LBW infants by enhancing protein synthesis and growth.

Parental Amino Acids and Protein Kinetics

In healthy adults, infusion of amino acids for 3-4 hours
- decreased leucine Ra (proteolysis)
- increased NOD of leucine (synthesis)
- increased incorporation of leucine in skeletal muscle

Phenylalanine tracer – similar data

(Pacey et al 1988; Bennet et al 1989, 1990)
Healthy full term and preterm infants respond to exogenous infusion of amino acids by suppressing protein breakdown (leucine, phenylalanine).

(Denne et al 1996; Clark et al 1997)

Parental Amino Acids and Protein Kinetics

SPECIFIC AIM

Examine the effect of parenteral amino acids on whole body nitrogen kinetics.

HYPOTHESIS

Administration of intravenous amino acids to LBW infants during the immediate neonatal period, when they are acutely stressed, will result in greater amino acid oxidation.

Study Population

Low birth weight infants, <32 weeks gestation
(a) Age 3-5 days
(b) Age <48 h

Isotopic Tracers

ring [1H5]Phenylalanine
[5-15N]Glutamine
[15N2]Urea
[1-13C,15N]leucine

Prime-constant rate infusion

Short Study

Tracers glutamine, phenylalanine, leucine, urea

TPN: 3 g.kg⁻¹.d⁻¹
TPN: 1.5 g.kg⁻¹.d⁻¹

Blood Sample
**Extended Study**

**Blood Sample**

TPN: 1.5 g/kg·d⁻¹

TPN: 3 g/kg·d⁻¹

Blood Sample

**Tracer Infusion**

**Clinical Characteristics**

<table>
<thead>
<tr>
<th>Birth Weight</th>
<th>Gestational Age</th>
<th>Age at Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>wks</td>
<td>d</td>
</tr>
<tr>
<td>Group 1 (12)</td>
<td>1139 ± 276</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Group 2 (5)</td>
<td>1189 ± 324</td>
<td>28 ± 2</td>
</tr>
</tbody>
</table>

Group 1: 1.5 g for 20h, 3 g for 5h. Group 2: 1.5 g for 20h, 3 g for 20h.

Data shown are mean ± SD

**Plasma Concentration of Amino Acid**

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 g</td>
<td>1.5 g</td>
</tr>
<tr>
<td>3.0 g</td>
<td>3.0 g</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leucine</th>
<th>Phenylalanine</th>
<th>Glutamine</th>
<th>Alanine</th>
<th>Serine</th>
</tr>
</thead>
<tbody>
<tr>
<td>97 ± 24</td>
<td>174 ± 34</td>
<td>102 ± 17</td>
<td>198 ± 30</td>
<td></td>
</tr>
<tr>
<td>102 ± 17</td>
<td>198 ± 30</td>
<td>144 ± 40</td>
<td>239 ± 70</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenylalanine Ra During High and Low TPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short (n=12)</td>
</tr>
<tr>
<td>1.5 g</td>
</tr>
<tr>
<td>3.0 g</td>
</tr>
<tr>
<td>1.5 g</td>
</tr>
<tr>
<td>3.0 g</td>
</tr>
</tbody>
</table>

**Glutamine and Urea Kinetics**

**Infants Less Than 48h Age**

<table>
<thead>
<tr>
<th>Birth Weight</th>
<th>Gestational Age</th>
<th>SNAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g)</td>
<td>(wks)</td>
<td>(median)</td>
</tr>
<tr>
<td>Short Study (7)</td>
<td>1234 ± 282</td>
<td>29.4 ± 2.4</td>
</tr>
<tr>
<td>Ext. Study (5)</td>
<td>1047 ± 229</td>
<td>28.6 ± 2.5</td>
</tr>
</tbody>
</table>

Data shown are mean ± SD
Leucine, Glutamine and Urea Kinetics (<48 h age)

Higher amino acid infusion causes a transient effect on whole body rate of proteolysis and protein oxidation (urea synthesis).

SUMMARY (cont’d)

Sustained amino acid load, following the initial response, results in higher rate of glutamine and urea synthesis (cataplerosis and oxidation).

Endogenous Phenylalanine Ra during TPN

Acutely “stressed” LBW infants also respond to acute amino acid load by suppressing whole body proteolysis and protein oxidation.

SUMMARY (cont’d)

Amino Acids

BCAAs (Leucine)

Ketoacid

Glutamate

Glutamine

Urea

Short Study
**Extended Study**

**Amino Acids**

- **BCAA (Leucine)**

**Proteins**

- **Ketoacid**
- **Glutamate**
- **Glutamine**
- **Urea**

**TCA Cycle**

**α-KG**

**SPECULATION**

Adaptation to higher amino acid concentration may have resulted in down-regulation of amino acid transporter or other intracellular signaling systems.

**Effect of Increasing Dose of Amino Acids on Skeletal Muscle Protein Dynamics**

**Skeletal Muscle:**
- Increase synthesis

**Splanchnic Compartment:**
- Increase synthesis
- Inhibit breakdown

**Time Course of Synthesis of Mixed Muscle Protein During Amino Acid Infusion**

**Amino Acids and Protein Dynamics**

**Proteolytic Pathways**

- Autophagic - lysosomal
- Ubiquitin - proteosome
**Autophagic – Lysosomal Pathway**

- Controlled by plasma amino acids
- Activated by:
  - Starvation
  - Amino acid deficiency
  - Hypoxia
  - High temperature

**“Partial autodigestion” to provide nutrients to maintain cell viability.**

- Responsible genes: atg 1……….12
- Regulated by P13 kinase
- IgF_1_ and others activate P13 and inhibit autophagy

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**Synthesis and Breakdown of Proteins by Amino Acids**

**Clinical Trials**

**Parental Glutamine Supplement in LBW Infants**

**Why variable response?**
- Heterogeneity of population
- Difficulties in delivery targeted amino acids and glutamine
- Complex outcome parameters
  - Mortality, sepsis, etc.

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**Lang et al (1996)**  
*Shorter duration of ventilation and TPN; Early full feeds*

**Thompson (2003)**  
*Early full feeds*

**Poindexter (2004) (NICHD)**  
*No effect*
Enteral Glutamine Supplement in LBW Infants

- Lower rate of sepsis
- Less feeding intolerance
  - Neu, J Pediatr 1997
  - van den Berg, AJCN 2005
  - Vaughn P, J Pediatr 2003

My colleagues:

Prabhu Parimi
Chantal Cripe-Mamie
Mark Kadrofske
Colleen Nye
Joyce Nolan

Ed Burkett
Carole Bennett
Clarita Duenas
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