Cysteine Supplementation in Parenteral-fed Critically ill Neonates:

A Randomized Trial Investigating Glutathione Synthesis

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Critically ill neonates (premature and term) are at high risk for developing disease secondary to oxidative tissue injury:
- Retinopathy
- Necrotizing enterocolitis (NEC)
- Bronchopulmonary dysplasia (BPD)

These illnesses contribute significantly to morbidity and mortality of the infant and their healthcare costs:
- Est. 100,000 critically ill neonates per yr in US
- Additional healthcare cost of $280,000 prior to discharge per neonate with NEC alone

Increased oxidative injury can occur from:
- Oxygen therapy
- Inflammation
- Hypoxia-ischemia reperfusion
Oxidative Tissue Injury
Many critically ill neonates receive their nutrition solely from TPN in the first few days to weeks of illness.

Cysteine formulation in TPN is not routine in most centers:
- Cysteine highly unstable unless in acid
- Cystine (cysteine dimer) is insoluble in aqueous solution
- Additional administrative efforts and expense
- Increases risk of metabolic acidosis and need for acetate
- Increases calcium & phosphate solubility in TPN

Cysteine required is the rate limiting substrate of:
- Glutathione (GSH) - primary antioxidant of the body
- Taurine

Neonates do not synthesize cysteine adequately
- Normally cysteine is a non-essential amino acid
Prior Studies

- Infants given cysteine free parenteral nutrition have lower plasma cysteine levels despite adequate methionine intake and plasma levels.

- Infants with lower gestational age have lower plasma cysteine levels, higher plasma cystathionine levels, and lower in vitro RBC glutathione concentrations, despite no difference in methionine intake or plasma levels.

- Cysteine supplementation increases plasma cysteine levels, total sulfur retention, and free urinary cysteine excretion.
Prior Studies

- Higher levels of plasma cyst(e)ine and hepatic glutathione concentrations when cysteine supplemented to TPN of beagle pups

- Higher levels of whole blood, lung, and intestinal glutathione when cysteine supplemented to neonatal cell cultures and animals

- Clinical studies in adults and children demonstrating resolution of illnesses sooner with higher glutathione when administered cysteine as dietary supplement
Intracellular Glutathione Pathways

- Plasma Precursor Pool
- Glutathione (GSH) synthesis:
  - Glu + Cys + Gly → Glutathione Synthetase → GSH
- GSH-conjugates
- GSSG (oxidized glutathione)
- Glutathione Peroxidase
- Glutathione Reductase
- Glutathione Synthetase
- γ-glutamylcysteine Synthetase
Oxidative Stress

GSH

GSSG

Plasma Precursor Pool

Glu Cys Gly

GSH-conjugates

Oxidative Stress (e.g., NEC, BPD)

Cellular protection

Cellular damage

toxin conjugation

GSH-conjugates

Oxidative Stress

GSSG

GSH

Glu Cys Gly
Background Conclusions

- The conditionally essential nature of cysteine for neonates is not conclusive.

- Glutathione concentrations are increased after cysteine supplementation for *in vitro* and animal studies with improvement in clinical outcome for children and adults.

- There has not been an *in vivo* assessment of erythrocyte glutathione synthesis in critically ill neonates given cysteine supplementation.
## Prelim Data: Patient Demographics

<table>
<thead>
<tr>
<th>Cysteine</th>
<th>(-) Cysteine</th>
<th>(+) Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>Birthweight (kg):</td>
<td>1.43 ± 0.45 (SD)</td>
<td>1.43 ±</td>
</tr>
<tr>
<td>0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (wk):</td>
<td>30.0 ± 2.6</td>
<td>29.7 ±</td>
</tr>
<tr>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study weight (kg):</td>
<td>1.38 ± 0.48</td>
<td>1.37 ±</td>
</tr>
<tr>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day-of-life studied:</td>
<td>8.2 ± 2.1</td>
<td>8.3 ±</td>
</tr>
<tr>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days on study TPN:</td>
<td>6.6 ± 1.9</td>
<td>5.7 ±</td>
</tr>
<tr>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP concentrations (mg/L):</td>
<td>7.1 ± 4.1</td>
<td>6.4 ±</td>
</tr>
<tr>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 concentrations (pg/mL):</td>
<td>46 ± 16</td>
<td>49 ± 33</td>
</tr>
<tr>
<td>6.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Prelim Data: TPN Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>(-) Cysteine</th>
<th>(+) Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal/kg·d):</td>
<td>96.3 ± 14.1 (SD)</td>
<td>92.3 ± 10.7</td>
</tr>
<tr>
<td>Protein (g/kg·d):</td>
<td>2.71 ± 0.42</td>
<td>2.93 ± 0.36</td>
</tr>
<tr>
<td>Lipids (g/kg·d):</td>
<td>3.69 ± 0.71</td>
<td>3.48 ± 0.85</td>
</tr>
<tr>
<td>Cysteine (mMol/kg·d):</td>
<td>0</td>
<td>0.77 ± 0.08</td>
</tr>
</tbody>
</table>

n = 10
# Stable Isotopes

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Mass</th>
<th>Natural</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1\text{H})</td>
<td>1.008</td>
<td>99.985</td>
</tr>
<tr>
<td>(^2\text{H})</td>
<td>2.014</td>
<td>0.015</td>
</tr>
<tr>
<td>(^{12}\text{C})</td>
<td>12.000</td>
<td>98.90</td>
</tr>
<tr>
<td>(^{13}\text{C})</td>
<td>13.003</td>
<td>1.108</td>
</tr>
<tr>
<td>(^{14}\text{N})</td>
<td>14.003</td>
<td>99.60</td>
</tr>
<tr>
<td>(^{15}\text{N})</td>
<td>15.000</td>
<td>0.370</td>
</tr>
</tbody>
</table>
Stable Isotopic Dilution Model

1-\(^{13}\text{C}\)-AA Infusion

Plasma AA Pool

Endogenous protein pool

GC / MS
Infusion Protocol

$[^{13}\text{C}_2]\text{glycine}$

$P$ (45 µmol/kg; 45 µmol/kg/hr)

Blood sample

0 1 2 3 4 5 6 7

Hours
Isotopic Labeling: RBC Glutathione

Plasma Precursor Pool

Glu
Cys
Gly

GSH
(-glutamylcysteinylglycine)

U-[¹³C]glycine:glycine

MBB, RBC lysis
HPLC, GSH hydrolysis

4hr
sample

U-[¹³C]glycine:glycine

6hr
sample

MBB, RBC lysis
HPLC, GSH hydrolysis
Fractional Synthetic Rates

Glutathione:

\[
\text{FSR}_{\text{gsh}} \ (\% \ / \ d) = \left( \frac{\text{MPE} \ bgly_2 - \text{MPE} \ bgly_1}{\text{MPE} \ freegly} \right) \times \left( \frac{24 \times 100}{t_2 - t_1} \right)
\]

- **AA enrichments:** HFBA deriv., GC/MS
- **Glutathione:**
  - Conc - MBB, HPLC;
  - FSR / ASR - HPLC, hydrolysis, HFBA deriv., GC/MS
Glutathione Concentration

RBC-glutathione concentration (mM/L)

* * ††

* $P < 0.0001$ vs. Healthy Infants
† $P = NS$, Cysteine: (-) vs. (+)

Infants (n = 11)

Healthy Infants

(-) Cysteine (n = 10)

 (+) Cysteine (n = 10)
Fractional Synthetic Rate

FSR
RBC-glutathione

(% / d)

* P < 0.005 vs. Healthy Infants
† P = 0.005, Cysteine: (-) vs. (+)

(+) Cysteine
(n = 10)

(+) Cysteine
(n = 10)

Infants
(n = 11)

(n = 10)
Absolute Synthetic Rate

ASR
RBC-glutathione (mmol/L/d)

* $P < 0.0001$ vs. Healthy Infants
† $P < 0.005$, Cysteine: (-) vs. (+)

Infants
(n = 11)

(+) Cysteine
(n = 10)

(-) Cysteine
(n = 10)
Current Hypothesis

- Parenteral cysteine-HCl supplementation in TPN-fed critically ill neonates will result in:
  - Higher glutathione concentrations
    - Erythrocyte glutathione
  - Higher rates of \textit{in vivo} glutathione synthesis
    - Fractional synthetic rates of erythrocyte glutathione utilizing IV infusions of $[^{13}\text{C}]$glycine
  - Lower markers of oxidative stress
    - TNF-$\alpha$, IL-6, malondialdehyde concentrations
Critically ill (SNAP > 10) TPN-fed neonates < 1mo:

(Baseline TNF-α, IL-6, CRP, malondialdehyde, plasma AA, RBC-GSH conc)

blind randomization

TPN w/ (-) Cysteine placebo (+) Cysteine (121 mg/kg/d)

amino acid soln (121 mg/kg/d) acetate (2 meq/kg/d)

Outcome Variables (Day 7):

TNF-α RBC - GSH conc.
IL-6 RBC - GSH synthesis
CRP Plasma amino acid
Malondialdehyde

Exclusion criteria: renal or hepatic failure, insulin administration, inherited, metabolic
Enrollment

Over of 5 year period:

- **Group 1:** Neonates weighing less than 1499 grams (total n = 36)
  - Subgroup 1A: Cysteine supplement (n = 18)
  - Subgroup 1B: Non-cysteine supplement (n = 18)

- **Group 2:** Neonates weighing between 1500 to 2499 grams (total n = 36)
  - Subgroup 2A: Cysteine supplement (n = 18)
  - Subgroup 2B: Non-cysteine supplement (n = 18)

- **Group 3:** Neonates weighing greater than 2500 grams (total n = 36)
  - Subgroup 3A: Cysteine supplement (n = 18)
  - Subgroup 3B: Non-cysteine supplement (n = 18)

Enrollment Started Sept 2006
Measured Outcomes

- DSMB oversight with annual closed reviews

- Comparisons between cysteine and placebo groups:
  - erythrocyte glutathione
  - fractional and absolute synthetic rates of erythrocyte glutathione
  - TNF-α, IL-6, malondialdehyde concentrations

- Paired comparisons of each neonate before and after treatment
  - erythrocyte glutathione
  - TNF-α, IL-6, malondialdehyde concentrations

- Secondary outcomes between cysteine and placebo groups:
  - Duration of mechanical ventilation
  - Duration of supplemental oxygen
  - Duration of hospitalization
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