Early Nutrition of LBW Preterm Infants and the Developmental Impact

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Growth
- Prior to birth - intrauterine environment
  Placenta – nutrients, hormones, clear waste products – heat, acids, CO₂, ammonia, bilirubin
  Gravity minimized

Brain Development
- Protein-energy
- Iron
- Zinc
- Copper
- LC-PUFAs
- Choline

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Growth
- Neonate
  High metabolic rate
  Lack of energy reserve – glycogen, fat
  Waste clearance
    immature liver and kidneys
  Organisms – Bacteria, viruses
  Barriers – skin, respiratory tract GI tract

Parenteral Nutrition
Breast Milk – The Gold Standard

Biologic Product
- 20cal/30cc blended mature milk
- Foremilk – high lactose 16 cal/30cc
- Hindmilk – high fat 24 cal/30cc

Breast Milk – The Gold Standard

Biologic Product - Antimicrobials
- Secretory IgA, IgM, IgG
- Lysozyme
- Complement C3
- Bifidus Factor
- Antiviral Mucins
- Oligosaccharides

Breast Milk – The Gold Standard

Biologic Product – Cytokines
- Tumor necrosis factor
- Interleukins
- Interferon
- Prostaglandins
- Platelet activating factor
- Alpha-1 Anti-trypsin

Breast Milk – The Gold Standard

Biologic Product - Hormones
- Insulin
- Prolactin
- Thyroid Hormone
- Corticosteroids
- Oxytocin
- Calcitonin
- Parathyroid hormone
- Erythropoietin

Breast Milk – The Gold Standard

Biologic Product - Growth Factors
- Epidermal Growth Factor
- Nerve Growth Factor
- Insulin-like Growth Factor
- Transforming Growth Factor
- Polyamines

Breast Milk – The Gold Standard

Biologic Product - Digestive Enzymes
- Amylase
- Lipases
- Lipoprotein lipase
- Ribonuclease
**Breast Milk – The Gold Standard**

**Biologic Product - Transporters**
- Lactoferrin
- Folate binder
- Cobalamin binder
- IgF binder
- Thyroxine binder
- Corticosteroid binder

**Breast Milk – The Gold Standard**

**Biologic Product - Stuff**
- DNA, RNA - nucleotides
- Lutein
- Lycopene
- Gamma sleep peptides

**Breast Milk – The Gold Standard**

**Biologic Product - Bad Stuff**
- Environmental contaminants
  - Heavy metals – Pb, Hg
- Antibiotics
- PCB, PBB
- Infectious agents
  - Viruses – HIV, Rubella
- Medications
  - Chemotherapy, Lithium, Antipsychotics

**Essential for Growth**

- **Energy**
- **Protein**
- **Potassium**

**Food and Drug Act – 1938,1941**

**Formula Labeling**
- Moisture
- Calcium
- Protein
- Phosphorus
- Fat
- Iron
- Carbohydrate
- Fiber
- Vitamins A,B,C,D
Energy

- Carbohydrate
- Fats
- Protein

Energy

- Carbohydrate
  - Lactose
  - Maltose
  - Sucrose
  - Glucose Polymers
  - Lactulose
  - Dextrose

Energy

- Carbohydrate
  - Lactose = Glucose + Galactose
  - Maltose = Glucose + Glucose
  - Sucrose = Glucose + Fructose
  - Glucose Polymers = Glu + Glu + ...
  - Lactulose = Galactose + Fructose

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Energy

- Carbohydrate
  - Dextrose
  - Dextrorotary isomer of glucose
  - Polymerizes to glycogen
  - Insulin facilitates cell entry

Energy

- Carbohydrate
- Fats
- Protein
Energy

- Fats
- Triglycerides
- Glycerol + 3 fatty acids
- Essential Fatty Acids
- Linoleic
- Alpha-linolenic
- Cholesterol

Fatty Acid Nomenclature Polyunsaturated Fatty Acids

- PUFA
  - 18:2ω6 or linoleic acid
  - 18:3ω3 or α-linolenic acid

- LCPUFA
  - 20:4ω6 or arachidonic acid (ARA)
  - 20:5ω3 or eicosapentaenoic acid (EPA)
  - 22:6ω3 or docosahexaenoic acid (DHA)

Fatty Acid Profile of Human Milk is Gold Standard

Brain Growth Especially Rapid in the Last Trimester and First 2 Years of Life

DHA Accumulates in the Brain Early and Rapidly

DHA and ARA: Important Building Blocks of the Growing Brain

Membranes, Supports Growth

- Linoleic n-6
- α-Linolenic n-3
- Eicosapentaenoic
- DHA

Critical Biologic Mediators

- Arachidonic (ARA)
- Prostaglandins, etc (+/−)

- Eicosapentaenoic (EPA)
- Prostaglandins, etc (+/−)

- Docosahexaenoic (DHA)
- Docosanoids

Membranes, Especially Retina and Brain (Vision and Cognition)

Note: Linoleic and α-linolenic acids are precursors to ARA and DHA, respectively. All three fatty acids also are present in breast milk.
The Role of Nutrients in Supporting the Immune System

Energy
- Carbohydrate
- Fats
- Protein

Formulas
- Proteins, essential AA
- Minerals - Potassium
- Fats - PUFA, LCFA
- Carbohydrate
- Vitamins

Dietary Proteins
- Milk Caseins and Whey
- Soy Fractions
- Hydrolysates
  - Casein, Beef Heart

Dietary Proteins
- Milk Caseins
- Milk Whey
- Soy Fractions
- Hydrolysates
  - Casein, Beef Heart
**SOY**

Proprietary Soy Pulp Extraction

Heat Stable Proteins

- Beta-conglycin  \( MW \approx 180,000 \)
- Glycinin  \( MW \approx 320,000 \)

Cross reacts with cows milk proteins

React with human IgE

Bind divalent cations


Franck et al, Int Arch Allergy Immunol 128:212, 2002

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**Dietary Proteins**

Milk Caseins

Milk Whey

Soy Fractions

Hydrolysates

Casein, Beef Heart

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**Hydrolysates**

Casein, Beef Heart

Amigen - WWII

IV protein preparation for soldiers with abdominal wounds

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**Dietary Proteins**

Hydrolysates

Casein, Beef Heart

Amigen - WWII

IV protein preparation for soldiers with abdominal wounds

Mead Johnson - Nutramigen

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**Dietary Proteins**

Milk Caseins and Whey

Soy Fractions

Hydrolysates

Casein, Beef Heart
### Milk Protein Homology

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Bovine</th>
<th>Homology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Whey (g/dl)</strong></td>
<td>0.72</td>
<td>0.7</td>
<td>----</td>
</tr>
<tr>
<td><strong>Alpha-lactalbumin (%)</strong></td>
<td>30</td>
<td>19</td>
<td>72</td>
</tr>
<tr>
<td><strong>Beta-lactoglobulin (%)</strong></td>
<td>0</td>
<td>51</td>
<td>----</td>
</tr>
<tr>
<td><strong>Serum Albumin (%)</strong></td>
<td>6.4</td>
<td>6.4</td>
<td>80</td>
</tr>
<tr>
<td><strong>Nonprotein nitrogen (%)</strong></td>
<td>25</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>


### Whey Proteins

#### Bind minerals
- Sodium
- Potassium
- Calcium
- Magnesium
- Zinc
- Iron

### Whey Proteins - Bovine

**All non-casein milk proteins**

- Beta lactoglobulin: 45%
- Alpha lactalbumin: 12%
- Glycomacropeptide: 12%
- Proteose peptones: 12%
- Immunoglobulins: 8%
- Serum Albumin: 5%
- Lactoferrin: 1%
- Lactoperoxidase: 0.5%

### Human Alpha Lactalbumin

**Immunostimulator**

- IL-1 Beta increased from
  - alveolar macrophages
- Lactoimmunopeptides
  - Tyr-Gly, Tyr-Gly-Gly
- proliferation of cultured human blood lymphocytes

### Bovine Whey Proteins – Beta Lactoglobulin

**Milk protein allergy**

- Antigenic and Allergenic
- antigenicity reduced by conjugation with acidic oligosaccharides

Yoshida et al, J Agric Food Chem 53:6851, 2005

### Alpha Lactalbumin

**Inhibits human colon adenocarcinoma (culture)**


**HAMLET** - human alpha-lactalbumin made lethal to tumor cells

- protein lipid complex – lactalbumin and oleic acid
- induces apoptosis of tumor cells
- fully differentiated cells unaffected


**inhibits growth of human skin papillomas (topical)**

Gustafsson et al, NEJM 350:2663, 2004
**Alpha Lactalbumin**

- Rich in tryptophan
- Evening lactalbumin intake
- Following morning
  - Improved morning alertness
  - More attentive to specific tasks

  Markus et al, Am J Clin Nutr 81:1026, 2005

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**Bovine Alpha Lactalbumin**

- Immunostimulating peptide
  - Gly-Leu-Phe
- Binds to neutrophils and monocytes

  Juari et al, Biochim Biophys Acta 1160:251, 1992

- Stimulates neutrophil superoxide anions

  Migliore-Samour et al, Biochem Pharmacol 44:673, 1992

- In mice, protects against Klebsiella pneumoniae infection


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**Low Abundance Proteins**

- 1% or less of the whey protein fraction
- Lactoferrin
- Lactoperoxidase
- Growth factors - TGF beta
- Cytokines
- Nucleosides

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**Lactoferrin**

- Single chain iron-binding glycoprotein
- A natural defense protein –
  - Colostrum, milk, tears, saliva,
  - Bile, pancreatic juice,
  - Mucus, genital secretions
- Neutrophils – bacterial environment

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**Low Abundance Proteins**

**Lactoferrin**

- Stimulates the immune system
- Anti-cancer activity
- Attenuates inflammatory disease
- Antimicrobial activity – macrophage
- Mediated CMV, Enterovirus, Hepatitis C, HIV-1

  Varadhachary et al, Int J Cancer 111:398, 2004
  Krissanen, J Am Col Nutr 26:71S, 2007
Dietary Proteins

Milk Caseins and Whey

Color of Milk

Casein molecule linear bipolar lipophilic and hydrophilic AA sequences in solution, casein associates into complex micelles which bind calcium and phosphate
Lactobezoar – Casein Calcium Phosphate cement

GI Proteins - Medications

Milks tested
woman, mare, goat, cow, ewe, dog, rabbit
Carbon 14 labeled drugs clonazepam, diazepam
Casein fraction of all milks accounted for >90% of drug binding
Stebler, Pharm Res 7:633, 1990

Milk Protein Homology

<table>
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<tr>
<th>Human</th>
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<th>Homology(%)</th>
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<tbody>
<tr>
<td>Total Protein (g/dl)</td>
<td>0.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Total Casein (g/dl)</td>
<td>0.18</td>
<td>2.8</td>
</tr>
<tr>
<td>Alpha (%)</td>
<td>absent</td>
<td>50</td>
</tr>
<tr>
<td>Beta (%)</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>Kappa (%)</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Casein N/Whey N</td>
<td>20/80</td>
<td>80/20</td>
</tr>
</tbody>
</table>


Recommended Protein: Energy Ratios for Premature Infants

Grams protein per 100 kcal

<table>
<thead>
<tr>
<th>LSRO 2002*</th>
<th>Ziegler 2007†</th>
<th>ESPGHAN 2010‡</th>
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<tbody>
<tr>
<td>2.5-3.6</td>
<td>&lt;1000 gm*</td>
<td>1000-1800 gm*</td>
</tr>
<tr>
<td>3.8</td>
<td>3.2-3.6</td>
<td>3.6-4.1</td>
</tr>
</tbody>
</table>

*According to infant's current weight
†LSRO: Life Science Research Office
§ESPGHAN: European Society of Pediatric Gastroenterology, Hepatology and Nutrition
Efficacy of Premature Formula with 3.6 g Protein per 100 kcal

- **Weight Gain, p<0.005**
  - Standard Protein Formula
  - High Protein Formula

- **Protein Accretion, p<0.001**
  - Standard Protein Formula
  - High Protein Formula

Cooke 2006 - Lab Values

- None of the infants developed uremia
- Blood urea was linearly related to nitrogen intake
- None of the infants developed metabolic acidosis
- No differences in total serum protein, albumin, or transferrin concentrations
- Total essential amino acids were ↑ in high protein group
- Non-essential amino acids:
  - 13 were ↑ in high protein group but ↓ cord reference value
  - 6 were ↑ than cord reference value; significance unclear


Pick a Product

**Preterm newborn**

- **CHO** – lactose/glucose polymers
- **Protein** – Cows milk proteins
  - ~50% greater than in term infant formula
- **Fats** – Essential FA, ½ MCT
- **Vitamins** – more Vit D, antioxidants
- **Minerals** – more K, Na, Ca, Mg, Trace

The Future

- Better Proteins
- Better metabolic monitoring
- Epigenetics
- Hormonal augmentation of growth – brain
- Prebiotics
- Probiotics

Probiotics

- **Nonpathogenic, live microorganisms in the food supply that, when consumed in adequate amounts, are capable of conferring a health benefit to the host**
  - Bifidobacteria
  - Lactobacilli

Gut Barrier and Immune Function

- Decreased gut permeability
- Increased mucin production
- Increased IgA secreting cells and secretory IgA
- Increased natural killer cell tumor-killing activity
- Increased production of macrophages and activated phagocytosis
- Immune modulation towards antigen tolerance

Douglas LL, Sanders ME. JADA. 2008;108(3):510-521


Probiotics

Probiotic Characteristics

- Be nonpathogenic in nature
- Be resistant to destruction by technical processing
- Be resistant to destruction by gastric acid and bile
- Adhere to or transiently colonize intestinal epithelial tissue
- Provide a measurable benefit to the host

Probiotics

- Anaerobic, non motile, Gram positive rods
- Tolerate low pH
- Can survive intestinal digestion and appear in stool
- Primary microbiota of breastfed infants
- Non-pathogenic

*Bifidobacterium lactis*

- Isolated from breastmilk
- Reduced infant colic and crying
- Improved GI motility
- Reduced intensity of abdominal pain
- Regulated bowel movements
- Improved feeding tolerance in premature infants
- Reduced diarrhea

Conclusion:

- The results confirm the significant benefits of probiotic supplements in reducing death and disease in preterm neonates.
- The dramatic effect sizes, tight confidence intervals, extremely low P values, and overall evidence indicate that additional placebo controlled trials are unnecessary if a suitable probiotic product is available.

Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates

Giri S, Despande S, Shingide R, Saigal P, and Malhotra A

*Pediatrics* 2010;125;921-930; originally published online Apr 19, 2010.