Micronutrients in Breast milk & Supplements in Infant formulae

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Content and Aim

• Review some of the more established micronutrients in breast milk
• Assess the scientific basis and benefits of supplementing these micronutrients in infant formula
• Aim to provide basic knowledge in counseling on breast feeding vs infant formula
“Breast Best”

• Human milk is much more than a food
• Energy & body building - macronutrients – protein, fat, CHO
• Non energy - micronutrients - Immunological, protective and maturational properties
• > 130 different compounds with biological function
  – Cells, enzyme, hormone, growth factors, cytokines, peptide, amino acid, vitamin, trace element etc
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Class of Ingredient</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>Enzyme</td>
<td>Polysaccharide digestion</td>
<td>Howell et al., 1986</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>Growth factor/hormone</td>
<td>Gastrointestinal growth/differentiation</td>
<td>Donovan and Odle, 1994; Dvorak et al., 2003; Howell et al., 1986</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Growth factor/hormone</td>
<td>Red cell production; possible growth factor for gut and central nervous system</td>
<td>Kling, 2002</td>
</tr>
<tr>
<td>Insulin</td>
<td>Growth factor/hormone</td>
<td>Anablic hormone promotes carbohydrate, protein, and fat accretion</td>
<td>Donovan and Odle, 1994</td>
</tr>
<tr>
<td>Insulin-like growth factor-I</td>
<td>Growth factor/hormone</td>
<td>Primary growth hormone of late fetal/neonatal period</td>
<td>Donovan and Odle, 1994</td>
</tr>
<tr>
<td>Insulin-like growth factor-II</td>
<td>Growth factor/hormone</td>
<td>Unknown function; thought to be weak growth hormone</td>
<td>Donovan and Odle, 1994</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Carrier protein</td>
<td>Increases efficiency of iron delivery</td>
<td>Howell et al., 1986</td>
</tr>
<tr>
<td>Lipase</td>
<td>Enzyme</td>
<td>Triglyceride hydrolysis</td>
<td>Howell et al., 1986</td>
</tr>
<tr>
<td>Nerve growth factor</td>
<td>Growth factor/hormone</td>
<td>Neuronal growth/differentiation</td>
<td>Donovan and Odle, 1994</td>
</tr>
<tr>
<td>Proteases</td>
<td>Enzyme</td>
<td>Unknown if active in protein hydrolysis</td>
<td>Howell et al., 1986</td>
</tr>
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<td>Relaxin</td>
<td>Growth factor/hormone</td>
<td>Regulates morphological development of the nipple</td>
<td>Donovan and Odle, 1994</td>
</tr>
<tr>
<td>Transforming growth factor-alpha</td>
<td>Growth factor/hormone</td>
<td>Gastrointestinal growth</td>
<td>Donovan and Odle, 1994; Dvorak et al., 2003</td>
</tr>
<tr>
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<td>Function</td>
<td>Reference</td>
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<tr>
<td>Antiproteases (e.g., secretory immunoglobulin A and trypsin inhibitor)</td>
<td>Enzyme</td>
<td>Inhibits breakdown of anti-infective immunoglobulins and enzymes</td>
<td>Howell et al., 1986; IOM, 1991</td>
</tr>
<tr>
<td>Arylsulfatase Catalase</td>
<td>Enzyme, Enzyme</td>
<td>Degrades leukotrienes</td>
<td>Hanson et al., 1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degrades hydrogen peroxide; protects against bacterial breeches of intestinal barrier</td>
<td>Lindmark-Mansson and Akesson, 2000</td>
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<tr>
<td>Fibronectin</td>
<td>Opsonin</td>
<td>May present debris to macrophages</td>
<td>IOM, 1991; Mestecky et al., 1990</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>Lipids</td>
<td>Antiviral (coronavirus); antiparasitic (Giardia, Entamoeba)</td>
<td>Mestecky et al., 1990</td>
</tr>
<tr>
<td>Granulocyte-colony stimulating factor</td>
<td>Cytokine</td>
<td>Causes endothelial cell migration and proliferation</td>
<td>Wallace et al., 1997</td>
</tr>
<tr>
<td>Hemagglutinin inhibitor</td>
<td>Opsonin</td>
<td>Prevents bacterial adherence</td>
<td>Neeser et al., 1988</td>
</tr>
<tr>
<td>Ingredient</td>
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<td>Function</td>
<td>Reference</td>
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<tr>
<td>Histaminase</td>
<td>Enzyme</td>
<td>Degradates histamine</td>
<td>Hanson et al., 1988</td>
</tr>
<tr>
<td>Immunoglobulin G</td>
<td>Immunoglobulin</td>
<td>Immune protection</td>
<td>Howell et al., 1986; IOM, 1991</td>
</tr>
<tr>
<td>Interleukin-1-beta</td>
<td>Cytokine</td>
<td>Activates T-cells</td>
<td>Mestecky et al., 1990</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Cytokine</td>
<td>Enhances immunoglobulin A and C-reactive protein production</td>
<td>Mestecky et al., 1990</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>Cytokine</td>
<td>Chemotaxis</td>
<td>Maheshwari et al., 2002</td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>Cytokine</td>
<td>Decreases inflammatory cytokine synthesis</td>
<td>Goldman et al., 1996</td>
</tr>
<tr>
<td>Lactadherin</td>
<td>Protein</td>
<td>Prevents rotavirus binding</td>
<td>Peterson et al., 2001</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Carrier</td>
<td>Anti-infective; may prevent iron from being bioavailable to microbes</td>
<td>Howell et al., 1986; IOM, 1991</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>Live cell</td>
<td>Cytokine production by T-cells; direct in vivo roles of B-cells, macrophages, and neutrophils</td>
<td>IOM, 1991; Mestecky et al., 1990</td>
</tr>
<tr>
<td>Lipases</td>
<td>Enzyme</td>
<td>Releases bacteriostatic and bacteriocidal free fatty acids</td>
<td>Howell et al., 1986; IOM, 1991</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Enzyme</td>
<td>Bactericidal</td>
<td>Howell et al., 1986; IOM, 1991</td>
</tr>
<tr>
<td>Macrophage colony stimulating factor</td>
<td>Protein</td>
<td>Macrophage proliferation</td>
<td>Goldman et al., 1986</td>
</tr>
<tr>
<td>Mucin</td>
<td>Inhibits E. coli binding to gut epithelium</td>
<td>Peterson et al., 2001</td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides, polysaccharides, gangliosides</td>
<td>Carbohydrates, glycoconjugates</td>
<td>Receptor analogs block binding of enteric bacteria; growth promoters for Lactobacillus</td>
<td>Coppa et al., 1999; IOM, 1991; Rivero-Urgell and Santamaria-Orleans, 2001</td>
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<tr>
<td>Peroxidases</td>
<td>Enzyme</td>
<td>Bactericidal</td>
<td>Furukawa et al., 1993</td>
</tr>
<tr>
<td>Platelet activating acetyl hydrolase factor</td>
<td>Enzyme</td>
<td>Catabolizes platelet activator factor</td>
<td>Howell et al., 1986</td>
</tr>
<tr>
<td>Prostaglandin E2, F2-alpha</td>
<td>Prostaglandin</td>
<td>Intestinal cytoprotection</td>
<td>Howell et al., 1986</td>
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<tr>
<td>Ribonuclease</td>
<td>Enzyme</td>
<td>Prevents viral replication</td>
<td>Nevinsky and Bunev, 2002</td>
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<tr>
<td>Secretory immunoglobulin A</td>
<td>Immunoglobulin</td>
<td>Immune protection (broad spectrum antiviral, antibacterial, antiparasitic)</td>
<td>Howell et al., 1986; IOM, 1991</td>
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<tr>
<td>Soluble intracellular adhesion molecule-1</td>
<td>Cytokine</td>
<td>Alters adhesion of viral or other molecules to intestinal epithelium</td>
<td>Xyni et al., 2000</td>
</tr>
<tr>
<td>Transforming growth factor-beta</td>
<td>Cytokine</td>
<td>Produces immunoglobulin A and activates B-cells</td>
<td>Borchert et al., 2000</td>
</tr>
<tr>
<td>Tumor necrosis factor-alpha</td>
<td>Cytokine</td>
<td>Mobilizes amino acids</td>
<td>Mestecky et al., 1990</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Small molecular-weight nitrogenous compound</td>
<td>Antioxidant</td>
<td>Van Zoreen-Grobben et al., 1994</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Class of Ingredient</td>
<td>Function</td>
<td>Reference</td>
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<td>Choline</td>
<td>Amino acid</td>
<td>Neurotransmitter synthesis</td>
<td>Zeisel et al., 1986</td>
</tr>
<tr>
<td>Insulin-like growth factor-1</td>
<td>Growth factor/hormone</td>
<td>Neuronal growth/differentiation</td>
<td>Cheng et al., 2003; Donovan and Odle, 1994</td>
</tr>
<tr>
<td>Long-chain polyunsaturated fatty acids</td>
<td>Essential/semiessential fat</td>
<td>Visual acuity</td>
<td>Uauy-Dagach and Mena, 1995</td>
</tr>
<tr>
<td>Nerve growth factor</td>
<td>Growth factor/hormone</td>
<td>Neuronal growth/differentiation</td>
<td>Donovan and Odle, 1994</td>
</tr>
<tr>
<td>Oligosaccharides (fucose, mannose, <em>n</em>-acetylglucosamine, sialic acid)</td>
<td>Carbohydrates</td>
<td>Neuronal cell-cell communication</td>
<td>Hynes et al., 1989</td>
</tr>
</tbody>
</table>
Composition of infant formula

• Standards set by Authorities
  – Codex Alimentarius of UN/WHO, FDA, Scientific committee on Food of the European Commission
  – Mainly cover the macronutrients, vitamins and minerals

• Increasing knowledge on the micronutrients and advancement in technology
  – Attempts to supplement formulae with various micronutrients to mimic human milk
  – For better health of infants ± better marketing
Oligosaccharides (低聚糖) / Prebiotics (益生素) / Soluble fibre

• Oligosaccharides are sugars consisting of approximately 2-20 saccharide units
• resistant to enzymatic digestion in the upper gastrointestinal tract – remain intact in small intestine
• Similar molecular structure with the binding sites in respiratory, gastrointestinal and urinary tracts
  – act as competitive receptors
  – bind to pathogens and prevent their adhesion to the gastrointestinal epithelium
• as substrates for bacterial fermentation in the colon (Prebiotics effect)
  – Favour the growth of bifidobacteria and lactobacilli
  ⇒ ↓ potentially pathogenic bacteria of the colonic flora
Effects of Oligosaccharides

- bind to specific receptors on mucosal cell surfaces and to act as receptor analogues thereby preventing adhesion of a number of bacterial and viral pathogens.
- undigested oligosaccharides may serve as substrates for colonic fermentation
  - Enhance growth of Bifidobacteria and lactobacilli

*Pediatr Res* 61: 2–8, 2007
Oligosaccharides in Human milk

• > 130 different oligosaccharides in human milk
• concentration
  – 15–23 g/L in colostrum
  – 8–12 g/L in transitional and mature milk
  – > amount of total protein in human milk
  – only trace amount in cow milk

• Because of the variety, variability, complexity and polymorphism of their structure, it is currently not feasible to replicate the oligosaccharide component of human milk in infant and follow-on formulae
Oligosaccharide supplement in infant formula

- Aim at prebiotic effect / as dietary fibre
- 2 non-digestible oligosaccharides currently used in infants in USA and Europe
  - fructo-oligosaccharides FOS
  - galacto-oligosaccharides GOS
- In Japan, also include
  - isomalto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides and xylo-oligosaccharides.
Oligosaccharide / prebiotics supplement in infant formula

- **ESPGHAN** committee on nutrition position paper 2011
- Prebiotics supplement in AF vs No supplement
  - **Growth**
    - Limited data, effect on improved growth is modest at best
  - **Stool pH**
    - Potential to reduce faecal pH - clinical benefit not established
  - **Stool frequency**
    - Limited data, potential to increase stool frequency – clinical significance unclear
  - **Stool consistency**
    - Limited data, potential to soften stools. – clinical significance unclear
  - **Gut flora**
    - Higher stool colony counts of bifidobacteria and lactobacilli.
    - Limited reduction of pathogenic bacteria

JPGN 2011;52: 238–250
ESPGHAN position paper

• For healthy infants - no safety concerns with regard to growth and adverse effects
• The clinical effects and safety of a prebiotic product should not be extrapolated to other prebiotics.
• Administration of formula supplemented with some prebiotics is associated with some clinical effects, such as increased stool frequency and stool softening, the clinical relevance of which remains questionable.
• Lack of data on the long-term effects
• **DOES NOT** recommend the routine use of formula supplemented with prebiotics in infants
Sialic acids 唾液酸
( N-acetyllneuraminic acid NANA)

• 9-carbon sugar
• Present in essentially all body tissue as component of **oligosaccharide** chains, glycoprotein and glycolipid
• Highest concentrate in CNS
• Structural and functional component of brain gangliosides
  – Neural cell membranes contain 20 x sialic acid than other types of membranes
• **Conditionally essential**
  – infant liver has limited capacity for synthesizing sialic acid during early postnatal life
Functions of Sialic Acid

• Play a role in neuronal growth & development, synapse formation & neural transmission

• involvement in memory formation

• Sialic acid containing oligosaccharides – prevent infection
Sialic acid in Human milk and cow milk

- Human milk is rich in SA containing oligosaccharides
- SA vary with the infant’s gestation, the duration of lactation, diurnally (highest in the evening)
- 3 fold-variation among mothers at the same stage of lactation – one of the most variable fractions of human milk
- Highest concentration in colostrum decrease over time, 20% of the initial content by 3 month
- Small amount in cow milk or formula (< ¼ of level in mature human milk)
- The structure and composition of sialic acids / oligosaccharide different greatly between cow milk and human milk
<table>
<thead>
<tr>
<th></th>
<th>Colostrum</th>
<th>Mature milk</th>
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</thead>
<tbody>
<tr>
<td><strong>Human milk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides (g/l)</td>
<td>24</td>
<td>12–14</td>
</tr>
<tr>
<td>Number of structures identified</td>
<td>88</td>
<td>88</td>
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<tr>
<td>Number of sialylated types (as %)</td>
<td>38 (43%)</td>
<td>38 (43%)</td>
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<tr>
<td>Total sialic acid content (mmol/l)</td>
<td>$3.72 \pm 0.15$</td>
<td>$1.48 \pm 0.07$</td>
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<tr>
<td>Structure of sialic acid</td>
<td>100% N-acetylneuraminic acid (Neu5Ac)</td>
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<td>Linkage of Neu5Ac to the galactose</td>
<td>2 → 6 bond (28 of 38) or 2 → 3 (19 of 38)</td>
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<tr>
<td><strong>Cow’s milk</strong></td>
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<tr>
<td>Free oligosaccharides (g/l)</td>
<td>0.025</td>
<td>0.01</td>
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<tr>
<td>Number of structures identified</td>
<td>20</td>
<td>4</td>
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<tr>
<td>Number of sialylated types (as %)</td>
<td>11 (55%)</td>
<td>0</td>
</tr>
<tr>
<td>Total sialic acid content (mmol/l)</td>
<td>0.48 (cow’s milk based infant formulas)</td>
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<tr>
<td>Structure of sialic acid</td>
<td>73% Neu5Ac</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>27% Neu5Gc</td>
<td>0</td>
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<tr>
<td>Linkage of Neu5Ac or Neu5Gc to the galactose</td>
<td>2 → 3 bond (5 of 11), 2 → 6 bond (6 of 11)</td>
<td></td>
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</tbody>
</table>
Effect of Sialic acids administration

- **Animal (Rat pups)**
  - ↑cerebral & cerebellar ganglioside & glycoprotein SA concentration, & ↑ scores in learning / performance tests,
  - Administrated SA incorporated into the brains, in the synaptosomal fraction

- **Human**
  - ↑sialic acid (22-32% higher) in the brain of breast fed infant vs formula fed
  - Breast-fed preterm infants in the 1st 3 mth of life had level of SA in saliva 2x that of cow’s milk fed
  - No clinical trial of Sialic acid supplement in infant formula !!
Recommendation

  – In the absence of adequate scientific data NO recommendations can be made on sialic acid

http://ec.europa.eu/food/fs/sc/scf/out199_en.pdf
Docosahexaenoic acid (DHA)
Arachidonic acid (AA)

- Fatty acids contain a carbon backbone of variable length
- 2 specific long chain (18 carbon) polyunsaturated fatty acids (PUFA) cannot be synthesized by mammals and must be obtained in diet - “essential fatty acids”
  - lack the requisite enzymes to insert a double bond at the n-3 (Ω-3) or n-6 (Ω-6) position of the fatty acid chain
  - α-linolenic acid (ALA) - precursor of the n-3 PUFA
  - Linoleic acid (LA) - precursor of the n-6 PUFA
- DHA - omega-3 fatty acid with 22 carbon atoms
  - Synthesize from ALA
- Arachidonic acid (AA) - omega-6 fatty acid with 20 carbon atoms
  - Synthesize from LA
recent studies show that conversion of ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is very limited in humans.

consider EPA and DHA to be conditionally essential especially in newborn, liver disease
DHA / AA

• DHA and AA are found in high proportions in the structural lipids of cell membranes, particular in retina and central nervous system.

• derivatives of both n-3 and n-6 pathways (LCPUFA)
  – critical components of cell membranes and is important for brain growth and myelination
  – precursors of prostaglandins
DHA / AA

- Breast milk is rich in LCPUFA.
- DHA and AA are the major LC-PUFA in human milk.
- Standard infant formulae only contain essential fatty acid (EFA) - ALA & LA. Formula fed infants have to synthesis their own DHA and AA from the EFA precursors taken.
- Formula fed infants have lower levels of DHA and AA in RBC and the cerebral cortex.
- ↓ IQ scores and sight skills observed in formula fed babies compared to the breastfed babies might be attributed to the ↓ DHA found in these formula-fed babies.
- DHA and AA are added to infant formulae with the anticipated benefits on visual and intellectual development for both the term and preterm infants.
Cochrane Review 2008

- **Term infants** on formulae with or without LCPUFA supplementation
  - 17 RCT included (n=1719), methodology varies considerably between studies
  - feeding term infants with formulae supplemented with LCPUFA had **no proven long term benefit** on vision, cognition and physical growth

Cochrane Review 2011

- **Preterm infants** on formulae with or without LCPUFA supplementation
  - 14 RCT included, methodology varies considerably between studies
  - **No proven long-term benefit** on visual development, neurodevelopment or growth
established a **cause and effect relationship**

- between the *intake of DHA supplemented* formula (at levels around 0.3% of total fatty acids) from birth up to 12 months and visual function at 12 months for term infants
- also true for breastfed term infants taking the DHA supplemented formula after weaning up to 12 months

- DHA supplement in infant formula does not show any benefit over BF
- Approve the health claim application from a milk company “DHA contributes to the visual development of infants”.

Recommendation on DHA

Codex Alimentarius, 1981 & ESPGHAN Coordinated International Expert Group, 2005

- AA and DHA could be added as optional ingredients in infant formulae.
- The level of DHA should not exceed 0.5% of totally fat content, and AA contents should be at least the same concentration as DHA

• Summary
  - The anticipated benefits on intellectual development in term and preterm infants have not been substantiated based on current evidence from meta-analysis of randomized controlled trials with evaluation of long term clinical outcomes.
  - DHA may have positive effect on visual development in term infant

JPGN 41:584–599, 2005
www.codexalimentarius.net/download/standards/288/CXS_072e.pdf
α-Lactalbumin & Tryptophan 色氨酸

• α-lactalbumin
  – dominant whey protein in human milk (2-3g/L, 41% of whey protein, 28% of total protein)
  – appropriate balance of essential amino acid in human milk with high proportion of tryptophan, cysteine and lysine
  – facilitate absorption of Zinc and Calcium, enhanced immune function and prebiotic function

• β- lactoglobulin
  – dominant whey protein in formula milk
  – different in essential amino acid composition (cf human milk)
  – low concentrate of tryptophan and cysteine

• Concentrations of protein in formula must be > human milk (≥15 g/L vs 9-11g / L) in order to meet all essential amino acid requirement
  ⇒Protein content > infant requirement
  ⇒Higher renal solute load
Free Tryptophan supplement

- Tryptophan - one of the essential amino acids
  - Precursor of serotonin, a neurotransmitter and the neurosecretory hormone melatonin

- Problems in infant formula
  - Plasma tryptophan concentrations of formula-fed infants still < breastfed infants despite higher protein content
  - Protein content in formulae more than required

- Studies evaluate lower protein formula with addition of free tryptophan
  - Can achieve plasma tryptophan concentrate similar to breastfed
  - Ratio of tryptophan to other large neutral amino acids (e.g., leucine, isoleucine) still lower than BF
    - Affect the uptake of tryptophan into the brain
**α- lactalbumin**

- Advances in dairy milk technology leads to better extraction of α- lactalbumin from cow milk.
- Attempt to mimic human milk by adding higher α-lactalbumin content.

**FIGURE 3.** Concentrations of α-lactalbumin in bovine milk, infant formula, and human milk (human milk data from reference 20).
α- lactalbumin

• Recent studies on α- lactalbumin enriched formula
  – support normal growth
  – plasma essential AA levels (including tryptophan) ~ BF
  – GI tolerance ~ BF
  – Better iron status (? Better absorption)

• Current no recommendation from scientific bodies
Taurine (牛磺酸)

- the major intracellular free amino acid in human
- the most abundant free amino acid (not protein bound) in breast milk.
- **conditionally essential** for newborns, esp preterm
- important for visual and auditory development; fat absorption, liver functions and antioxidant effect
- low plasma taurine levels in early infancy correlated with poor developmental outcome in preterm
- Low in cow milk or infant formula
- added to nearly all infant formulae including preterm formulae and most of the follow-on formulae since the early 1980s
- However anticipated benefits on visual, auditory and intestinal development of the recipient infants have not been substantiated based on the current evidence from meta-analysis of randomized controlled trials with evaluation of long term clinical outcomes.
Taurine

Recommendations from scientific / regulatory bodies

- *Codex Alimentarius, 1981 & ESPGHAN Coordinated International Expert Group, 2005*
- Taurine could be an optional ingredient added to infant formula in amounts up to 12 mg/100 kcal. There is no need for mandatory supplementation
- Consensus statements by experts in neonatal nutrition (1993) recommended that formula milk fed preterm infants receive about 4.5 to 9.0 mg/kg of Taurine per day

JPGN 41:584–599, 2005
www.codexalimentarius.net/download/standards/288/CXS_072e.pdf
Nutritional Needs of the Preterm Infant: Scientific Basis and Practical Guidelines (Williams & Wilkins) (1993) by Reginald C Tsang, MD
Choline 膽鹼

- Physiological function

Fig 1: Diagram showing the major functions of choline in the body
Choline 膽鹼

- **Deficiency**
  - Liver derangement in adults eg prolonged TPN
  - Low choline intake in pregnant women associated with 4x risk of neural tube defect in fetus

- **Animal studies**
  - Choline deficiency during pregnancy cause abnormal brain function esp memory
  - Availability of choline postnatally cause significant, life long alterations in the spatial memory capacity
Choline

- Different forms of Choline
  - free choline
  - phospholipid bound choline
  - Phosphatidylcholine
  - Sphingomyelin
  - phosphochochine
  - glycerophosphochochine

- Choline contents and compositions of infant formulae and mature human milk are different
  - choline content of human milk doubles during the first week after birth
  - Mature human milk contains total choline content ~12.6 mg/100 kcal

- The choline content of most formulae was comparable with that in colostrum, but below that of mature milk
### Table 3
The concentrations of free choline and choline compounds in human breast milk

<table>
<thead>
<tr>
<th>Samples</th>
<th>n</th>
<th>FCh (μmol/L)</th>
<th>PC (μmol/L)</th>
<th>SM (μmol/L)</th>
<th>GPCh (μmol/L)</th>
<th>PCh (μmol/L)</th>
<th>Total Ch (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrums</td>
<td>21</td>
<td>132±21</td>
<td>146±18</td>
<td>129±13</td>
<td>176±13</td>
<td>93±26</td>
<td>676±35</td>
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<tr>
<td>Mature breast milks</td>
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<td></td>
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</tr>
<tr>
<td>12–180 days</td>
<td>95</td>
<td>228±10*</td>
<td>104±11</td>
<td>94±9</td>
<td>499±16*</td>
<td>551±33*</td>
<td>1476±48*</td>
</tr>
<tr>
<td>12–28 days</td>
<td>14</td>
<td>299±36*</td>
<td>103±9</td>
<td>91±14</td>
<td>596±83*</td>
<td>506±42*</td>
<td>1595±82*</td>
</tr>
<tr>
<td>75–90 days</td>
<td>12</td>
<td>286±21*</td>
<td>155±21</td>
<td>97±26</td>
<td>465±40*</td>
<td>438±69*</td>
<td>1441±84*</td>
</tr>
<tr>
<td>165–180 days</td>
<td>11</td>
<td>132±15**</td>
<td>97±23</td>
<td>84±18</td>
<td>629±135*</td>
<td>407±48*</td>
<td>1349±105*</td>
</tr>
<tr>
<td>Infant formulas</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4</td>
<td>201±15</td>
<td>89±25</td>
<td>21±7**</td>
<td>469±44</td>
<td>401±26</td>
<td>1173±103</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>43±2**</td>
<td>49±1**</td>
<td>12±1**</td>
<td>131±28</td>
<td>77±13**</td>
<td>311±18**</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>723±12**</td>
<td>50±4**</td>
<td>5±1**</td>
<td>1429±176**</td>
<td>36±14**</td>
<td>2241±169**</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>122±14**</td>
<td>89±7</td>
<td>23±6**</td>
<td>1355±140**</td>
<td>773±85</td>
<td>2270±174**</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>56±6**</td>
<td>78±17</td>
<td>8±1**</td>
<td>712±83</td>
<td>368±33</td>
<td>1328±113</td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>172±8**</td>
<td>128±8</td>
<td>20±3**</td>
<td>914±44**</td>
<td>836±11**</td>
<td>2114±132</td>
</tr>
<tr>
<td>G</td>
<td>4</td>
<td>521±34**</td>
<td>50±7**</td>
<td>&lt;5</td>
<td>405±55</td>
<td>&lt;10</td>
<td>983±138</td>
</tr>
</tbody>
</table>

Colostrum (expressed at 0–2 days after birth) and mature breast milk (expressed at 12–180 days after birth) and infant formulas were assayed for the contents of free choline (FCh), phosphatidylcholine (PC), sphingomyelin (SM), glycerophosphocholine (GPCh) and phosphocholine (PCh). Commercial powdered infant formulas were: A, Aptamil-2 (Milupa-Numil Gida Urnleri); B, Aptamil-3 (Milupa-Numil Gida Urnleri); C, Ulker Hero Baby-2 (Ulker-Avda de Murcia, Hero Gida Urnleri); D, SMA plus (Wyeth Ilaclari); E, SMA gold (Wyeth Ilaclari); F, SMA-2 (Wyeth Ilaclari); G, Nutricia Nutrilon Soya (Nutricia-Numil Gida Urnleri). Data are expressed as mean±S.E.M. and were analyzed by using one-way ANOVA followed by Tukey’s multiple comparison test. $n$=number of measurements.

* $P<.001$ compared with the values from the colostrum.

** $P<.05–.001$ compared with the values from the mature breast milk at 12–180 days after birth.
Choline

- Serum free choline concentration in BF infants
  - significantly > formula fed.
  - positively correlated with free choline, phosphocholine, glycerophosphocholine and total choline contents in their mothers’ breast milk
- the amount of free choline extracted by brain $\propto$ serum level

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>FCh (µmol/L)</th>
<th>PLB-Ch (µmol/L)</th>
<th>PC (µmol/L)</th>
<th>SM (µmol/L)</th>
<th>GPCh (µmol/L)</th>
<th>PCh (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula-fed infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>30–150 days</td>
<td>12</td>
<td>10.8±0.7</td>
<td>2100±63</td>
<td>1003±85</td>
<td>1032±116</td>
<td>34±5</td>
<td>2.3±1.0</td>
</tr>
<tr>
<td>Breast-fed infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12–28 days</td>
<td>14</td>
<td>27.1±1.8**</td>
<td>1998±93</td>
<td>1083±105</td>
<td>1032±106</td>
<td>40±4</td>
<td>2.4±0.6</td>
</tr>
<tr>
<td>75–90 Days</td>
<td>12</td>
<td>21.8±2.2**</td>
<td>2059±126</td>
<td>1071±83</td>
<td>1042±74</td>
<td>36±3</td>
<td>2.5±1.2</td>
</tr>
<tr>
<td>165–180 days</td>
<td>11</td>
<td>17.3±0.3****</td>
<td>2245±222</td>
<td>1121±108</td>
<td>1124±116</td>
<td>29±5***</td>
<td>1.9±0.9</td>
</tr>
</tbody>
</table>

Free choline (FCh), phospholipid-bound choline (PLB-Ch), phosphatidylcholine (PC), sphingomyelin (SM), glycerophosphocholine (GPCh) and phosphocholine (PCh) concentrations were measured in blood samples obtained from the formula-fed and breast-fed infants after 12–180 days after birth. Data are expressed as mean±S.E.M. and were analyzed by using one-way ANOVA followed by Tukey’s multiple comparison test. n = number of observations.

* $P<.05$ compared with the values for formula-fed infants.

** $P<.001$ compared with the values for formula-fed infants.

*** $P<.05$ compared with the value for the 12–28 days of breast-fed infants.
Choline

• The international expert group of ESPGHAN (2005)
  • **recommend** Choline supplementation in infant formulae
    – minimum of 7 mg/100 kcal
    – maximum of 50 mg/100 kcal
  
  • No clinical trial in human infant on the benefit of choline supplement in formula milk
β-Carotene 胡蘿蔔素

- Convert into retinol Vitamin A - Provitamin A
- Antioxidant effect
- Lowered DNA damage with in vitro study
- Regulator of immune response
- modulates the growth and differentiation of epithelial and bone cells
- essential for retinal photoreceptors
- required for reproduction, testosterone synthesis
- mammals are not able to synthesize carotenoids
**β-Carotene**

- four major carotenoids (α-carotene, β-carotene, lycopene, and cryptoxanthine)
- Breast milk contains the whole spectrum of carotenoids present in the human diet and serum
  - Colostrum 5x more carotenoids than mature breast milk
- formulas contain low concentrations of carotenoids or a limited variety of carotenoids
Fig. 2 Carotenoid contents in different brands of formula preparations in comparison to mature breast milk. Concentrations of formula preparations are given in µg/l as means of three measurements. Values of breast milk are given in µg/l as interquartile ranges. Crypt: cryptoxanthine, Lyc: lycopene, α-caro: α-carotene, β-caro: β-carotene.
Plasma carotenoid profile

- Lycopene and α-carotene not detectable in formula fed
  - lycopene - one of the most potent antioxidant

- Significantly ↓ β-carotene levels in formula fed
β-Carotene

• β-carotene levels of some formula fed newborns up to 4x higher than those in BF infants,
  – possibly due to feeding with preparations containing very high β-carotene concentrations

• may compensate for the decreased antioxidant capacity caused by the absence of other carotenoids.

• However, β-carotene cannot be expected to supply all the individual functions of the depleted carotenoids.
β-Carotene

• The rate of absorption of β-carotene and its bioequivalence to retinol in infants is unknown
  ⇒ equivalence factor for the vitamin A activity of β-carotene cannot be derived for infants
• Requirement for β-carotene cannot be derived from the well defined requirement of Vit A
• Higher β-carotene content in formulae may increase plasma β-carotene level but not produce similar carotenoid profile as BF
Nucleotide 核酸

- non-protein nitrogen compounds present in human milk
- precursor units of RNA and DNA
- Essential compounds in the energy transfer systems (i.e. in ATP and GTP)
- important role in carbohydrate, lipid, protein and nucleic acid metabolism
- Important for growth and maturation of GI tract
- Development of neonatal immune function
Nucleotide

• Concentration of nucleotide in breast milk was initially quoted from 12 to 33 mg/l (before 1995)

• **Total Potentially Available Nucleotides (TPAN)** - newer method of analysis
  – much higher human milk content ~ 72 +/- 24 mg/l discovered
Nucleotide

Table 3  Clinical studies: nucleotide supplementation of infant formula

Effect on intestinal flora: two studies with conflicting results.\textsuperscript{49,50} Sepsis and diarrhoea: no effect on various types of infection, but one study showed reduced first episode of diarrhoea in a periurban slum population.\textsuperscript{51,52} Immune function: enhanced mononuclear function, increased antibody response, and increased plasma levels of IgM and IgA.\textsuperscript{53,54} Stimulated production of long-chain polyunsaturated fatty acids and increased serum lipoprotein concentration.\textsuperscript{55,56,58} No effect on growth in appropriately grown for gestation infants, and increased growth rate in term small for gestational age infants.\textsuperscript{59,60}

Nucleotide supplement

• Meta-analysis - nucleotide-fortified formulae compared to breast milk or control formulae
  – better antibody response to immunisation with Haemopillus, diphtheria toxin and oral polio vaccine (cf both BF and control formulae)
  – fewer episodes of diarrhoea (with control formulae only)
  – No differences in the risk of upper respiratory infections
  – Sufficient evidence to support the addition of nucleotides to infant formulae based on health benefit related findings

• International Expert Group (2005) of ESPGHAN still considered as optional ingredients

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JPGN 41:584–599, 2005
Summary

• Supplement Recommended
  – Taurine – for preterm and optional for term
  – Choline
  – Nucleotide – optional
  – DHA / AA - optional

• No recommendation / insufficient data
  – Sialic acid
  – α-lactalbumin
  – Free tryptophan
  – β-carotene

• Supplement Not recommend
  – Oligosaccharide / prebiotics / soluble fibre
Conclusions

• Many micronutrients are added to infant formulae trying to mimic breast milk or to improve outcome
• Not all of these supplements are based on sufficient evident from RCTs
• Infant formulae still not yet close to human milk