Importance of Adequate DHA/ARA in Preterm Infants

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## Faculty Disclosures

### Michael Caplan, MD

<table>
<thead>
<tr>
<th>Position</th>
<th>Company</th>
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<tbody>
<tr>
<td>Research Support</td>
<td>Mead Johnson Nutrition</td>
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<tr>
<td>Consultant</td>
<td>Sigma Tau Pharmaceuticals</td>
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<td>Speakers Bureau</td>
<td>Mead Johnson Nutrition</td>
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<tr>
<td>Research Support</td>
<td>Abbott Nutrition, Alcresta Therapeutics</td>
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<td>Fresenius Kabi, Mead Johnson Nutrition</td>
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<tr>
<td>Scientific Advisory Board</td>
<td>Laurent Pharmaceuticals, Prolacta Bioscience</td>
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Learning Objectives

1. Describe the role DHA/ARA plays in developing infants
2. Recognize the importance of adequate DHA/ARA balance in preterm infants
3. Associate current NICU practices with DHA/ARA accretion rates in preterm infants
THE ROLE OF DHA/ARA IN INFANT GROWTH
Overview of Docosahexaenoic Acid (DHA)

- Long-chain, highly unsaturated omega-3 fatty acid (22:6n-3)
- Metabolized from α-linolenic acid
- Found in seafood, especially fatty fish, and products derived from seafood

Overview of Arachidonic Acid (ARA)

- Long-chain, highly unsaturated omega-6 fatty acid (20:4n-6)¹
- Abundant in brain, muscles, and liver¹
- Immediate precursor for adrenic acid²
  - Pathway for ARA utilization in infants to meet increase needed for neural tissue development

Functions of DHA/ARA

- Structure and function of tissues
- Retinal development
- Brain development
- Immune function
DHA/ARA: Important Building Blocks of the Growing Brain

- DHA and ARA play an important role in neuronal cell division and signaling

- Mammalian brains are 60% fat
  - About **25% of the total fatty acid content of the brain** is made up of DHA or ARA

- DHA and ARA are primarily in the form of phospholipids
  - Found in **neural cell membranes**, providing structural support

DHA Signaling: NPD1 and BDNF

BDNF, brain-derived neurotrophic factor; NPD1, neuroprotectin D1.

BDNF Levels Are Lower in Preterm Infants

BDNF, brain-derived neurotropic factor. *P < .05; **P < .001.
Prospective study in 30 healthy term and 15 healthy preterm neonates. BDNF measured by enzyme immunoassay.

DHA/ARA in Infant Retinal Development

- DHA/ARA play a role in the maturation and survival of photoreceptor cells\(^1\)\(^-\)\(^3\)

- Incorporation of DHA into phospholipids is important for retinal function\(^4\)

- Animals raised on fatty acid–free diets develop abnormal electroretinograms\(^5\)

Electroretinography (ERG) is an eye test that detects function of the retina by measuring electrical response of the light-sensitive cells in eyes.

DHA and ARA Metabolites Attenuate Lung Injury†

†C57/BL6 pups were randomized at birth and treated IP on days 0, 3, 6, and 9. On day 10, mice were sacrificed and lungs were collected for morphometric analyses. Hyperoxia, >90% oxygen.

Mechanisms of Maternal-Fetal Fatty Acid Delivery

- Mechanisms involved in fatty acid transfer across placenta are poorly defined.
- DHA may selectively accumulate in fetal tissue through higher placental uptake.
- Fatty acid transfer can occur through passive diffusion or facilitated transfer.
DHA Accumulates in the Placenta at a Higher Rate Than Other Fatty Acids\textsuperscript{1,2}

- In a study of 11 pregnant women given radio-labeled fatty acids, the maternal-fetal distribution was evaluated.

- Twelve hours after oral intake of fatty acids, an elective cesarean section was performed.

- Mean ratios between cord and maternal plasma area under the curve (AUC) concentration of $^{13}$C-fatty acids (expressed as percentages) are shown in the figure.

Accumulation of DHA/ARA in Fetal Tissue at End of Pregnancy\textsuperscript{1-5}

- During the final weeks of pregnancy, the DHA and ARA content in fetal plasma is almost twice as high as in the mother’s blood\textsuperscript{1-4}

- Towards the end of the pregnancy, levels are several times higher in fetal adipose tissue than in the maternal adipose tissue: \textsuperscript{1-4}
  - DHA: 16 times higher
  - ARA: 90 times higher

FABPpm Transports DHA Selectively Across the Placenta\textsuperscript{1-4}

- Membrane associated fatty acid binding protein (FABPpm) is a peripheral membrane protein and fatty acid transporter
- FABPpm selectively binds LC-PUFA on the maternal side to transfer fatty acids to the placenta\textsuperscript{1,2}
- FABPpm binds only 10% of total fatty acids, with a high affinity for DHA/ARA:
  - ARA: 98%
  - DHA: 87%
  - Smaller quantities of LA and OA (oleic acid)\textsuperscript{3}

Enteral Sources of DHA/ARA for Term Infants

**Human milk:** DHA/ARA is always present\(^1\)
- Concentration of DHA/ARA is related to maternal diet
- Breastfeeding is ideal, but the majority of infants receive some formula during the first year of life\(^2\)

**Formula:** DHA/ARA is added in the US since 2001 based on worldwide averages in human milk\(^3\)
- 0.2% to 0.4% DHA
- 0.35% to 0.7% ARA

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IMPORTANCE OF ADEQUATE DHA/ARA BALANCE IN PRETERM INFANTS
DHA/ARA Are Stored in the Brain and Adipose Tissue

• Since maternal diets may not always meet DHA/ARA needs, these fatty acids are stored in the adipose tissue for later use

• Storage in adipose tissue increases substantially in the final weeks of pregnancy

• Preterm infants may not have the same levels of DHA/ARA accretion as term infants

DHA Levels Rapidly Decline in the First Postnatal Week

Median DHA levels at birth in **TERM** infants (n = 10), only one data point collected

Median DHA levels at birth in **PRETERM** infants (n = 88)

Retrospective cohort study of 88 infants born at <30 weeks' gestation. Fatty acid profiles and infant outcomes were assessed during the first postnatal month.

Plasma Levels of DHA Approximate Brain Levels†

†Study in 22 pregnant baboons/neonates randomized to term breast-fed, term formula-fed, preterm formula-fed, or preterm DHA/ARA-supplemented formula-fed

ARA Levels Are Rapidly Altered in the First Postnatal Week

Retrospective cohort study of 88 infants born at <30 weeks’ gestation. Fatty acid profiles and infant outcomes were assessed during the first postnatal month.

Linoleic Acid (LA)
- Median LA levels at birth in TERM infants (n = 10)
- LA levels observed in TERM infants (n = 10)
- Median LA levels at birth in PRETERM infants (n = 88)
- LA levels observed in PRETERM infants (n = 88)

Arachidonic Acid (ARA)
- Median ARA levels at birth in TERM infants (n = 10)
- Median ARA levels at birth in PRETERM infants (n = 10)
- Median ARA levels at birth are comparable across gestational ages
- Median LA levels at birth are comparable across gestational ages

DHA/ARA Imbalance Associated With Disease in Preterm Infants

- Suboptimal cognitive development
- Retinopathy of prematurity
- Chronic lung disease
- Sepsis and infection

DHA/ARA Levels Are Linked With Improved Neurodevelopment

- In a study of 60 preterm infants, red blood cell fatty acid composition was evaluated

- A 1% increase in DHA levels was associated with 4.3-fold reduced risk of intraventricular hemorrhage

- Higher DHA and lower LA levels early after delivery were associated with better developmental scores at a mean follow-up of 33 months

Omega-3 and Retinopathy of Prematurity†

• Mice receiving ω-6-PUFAs had a significantly greater vaso-obliterated/total retinal area of 21.5% vs 13.7% in those receiving ω-3-PUFAs

• Mice receiving ω-3-PUFAs were significantly protected from pathologic neovascularization (5.7% vs 9.0%)

†Mouse pups exposed to 75% O₂ from P7-P12. Scale bar, 1 mm. P17 retinal vasculature stained with isolectin B4-FITC. Omega-6, n = 14; omega-3, n = 27.

Lipid supplement with 2:1 ARA:DHA ratio reduced severe ROP


Low DHA Levels Are Linked to the Development of Chronic Lung Disease

- In a retrospective cohort study of 88 preterm infants, fatty acid profiles were measured for the first 4 weeks.

- Decreased levels of DHA were associated with a **2.5-fold increased risk of chronic lung disease (CLD)**.

- Imbalanced DHA/LA levels were associated with an **8.6-fold increased risk of CLD**.

Association With Increased Risk of Chronic Lung Disease and Late-onset Sepsis

- In the same study, fatty acid levels and imbalance were associated with late-onset sepsis.
- Imbalanced LA:DHA was associated with a 4.6-fold increased risk of late-onset sepsis.

<table>
<thead>
<tr>
<th>Chronic lung disease</th>
<th>OR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>0.9 (0.7, 1.1)</td>
<td>.4</td>
</tr>
<tr>
<td>ARA</td>
<td>0.9 (0.6, 1.3)</td>
<td>.6</td>
</tr>
<tr>
<td>DHA</td>
<td>2.5 (1.3, 5.0)</td>
<td>.001</td>
</tr>
<tr>
<td>LA: DHA</td>
<td>8.6 (1.4, 53.1)</td>
<td>.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Late-onset sepsis</th>
<th>Hazard ratio (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>0.8 (0.7, 0.96)</td>
<td>.02</td>
</tr>
<tr>
<td>ARA</td>
<td>1.4 (1.1, 1.7)</td>
<td>.02</td>
</tr>
<tr>
<td>DHA</td>
<td>1.4 (1.0, 2.0)</td>
<td>.08</td>
</tr>
<tr>
<td>LA: DHA</td>
<td>4.6 (1.5, 14.1)</td>
<td>.007</td>
</tr>
</tbody>
</table>

Models adjusted for gestational age, gender, growth restriction, severity of illness, total Intralipid intake.

DHA/ARA SUPPLEMENTATION IN PRETERM INFANTS
Current Nutritional Practices Are Inadequate to Maintain Optimal Levels of Fatty Acids in Preterm Infants

In a study of 40 preterm infants (≤28 weeks gestational age), nutritional data were collected for the first 4 weeks of life.

- DHA deficit was greater for low-birth-weight infants.
- In preterm infants, DHA accumulation is half that of term infants at 1 month of age.

†Nutritional data were collected for the first 28 days of life in 40 preterm infants born with a gestational age ≤28 weeks at a single center.

Challenges in Achieving Adequate DHA/ARA Intake in Preterm Infants

• Delivery of DHA/ARA\(^1\)
  
  • Breast milk and formulas provide DHA/ARA
  
  • Intravenous lipid emulsions available for routine use do not include DHA/ARA
  
  • Extremely premature infants may rely on intravenous lipid emulsion for the first weeks of life,\(^2\) contributing to DHA/ARA deficiency

Challenges in Achieving Adequate DHA/ARA Intake in Preterm Infants

• Providing additional DHA is dependent on the infant’s ability to tolerate full-volume enteral feedings
  • Variable among NICUs
  • Dependent on size, clinical status, and gestational age

DHA Supplementation Alleviates DHA Deficiency in Preterm Infants†

<table>
<thead>
<tr>
<th></th>
<th>Baseline DHA, mol%</th>
<th>Full-Feedings, DHA mol%</th>
<th>Discharge, DHA mol%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo preterm (n = 29)</td>
<td>2.91 (0.45)</td>
<td>2.83 (0.50)</td>
<td>2.87 (0.50)*</td>
</tr>
<tr>
<td>DHA preterm (n = 31)</td>
<td>2.88 (0.68)</td>
<td>3.03 (0.54)</td>
<td>3.55 (0.44)**</td>
</tr>
<tr>
<td>Term (n = 30)</td>
<td>4.31 (0.95)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†Double-blind, randomized, controlled trial evaluating DHA supplementation (50 mg/day) for preterm infants (24-34 weeks gestational age) beginning in the first week of life.

Results expressed as mean (SD) mol%.

*Groups compared with term reference peers via ANOVA; \( P < .001 \).

**Placebo vs DHA comparison via linear mixed models; \( P < .001 \).
Weight of Preterm Infants Fed DHA/ARA Formula Is Closer to Term Breast-fed Infants

- Double-blinded study of 361 preterm infants fed until 92 weeks postmenstrual age, with follow-up to 118 weeks postmenstrual age.
- Breast-fed term infants > all preterm groups (P < .05).
- Infant formulas not different from term breast milk, both > control and fish/fungal formulas.
- Infant formulas > control formulas.

**Enhanced Immune Function With DHA/ARA Supplementation†**

- Adding DHA/ARA to preterm formula resulted in immune function more consistent with breast milk-fed infants¹
- In another study, infants supplemented with DHA had reduced levels of inflammatory cytokines believed to play a role in type 1 diabetes development²
- DHA/ARA supplementation may affect the ability of an infant to respond to immune challenges¹,²

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†Study of 44 preterm infants with gestational ages between 27 and 36 weeks receiving 100% enteral nutrition by day 14 of life.

*P < .05, compared with Day 14. #P < .05, compared with breast milk, Day 42.

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Effect of Emulsified DHA/ARA Enteral Supplementation on Serum DHA Levels in Preterm Infants

Randomized, double-blind, placebo-controlled trial of 30 very low-birth-weight infants given low dose or high dose PUFA via nasogastric tube. Dosing started within first 72 h of life and was given for 8 weeks or until discharge, whichever came first.

Effect of Emulsified DHA/ARA Enteral Supplementation on Serum ARA Levels in Preterm Infants†

†Randomized, double-blind, placebo-controlled trial of 30 very low-birth-weight infants given low dose or high dose PUFA via nasogastric tube. Dosing started within first 72 h of life and was given for 8 weeks or until discharge, whichever came first.

DHA/ARA Supplementation and Neurodevelopment in Preterm Infant Neurodevelopment at 6 Months and 1 Year†

- In a double-blind study, preterm infants between gestational age of 30 and 37 weeks were randomly assigned to receive formula with or without DHA/ARA supplementation.

- DHA/ARA supplementation for 6 months led to significantly increased measures of neurodevelopment in preterm infants.

<table>
<thead>
<tr>
<th>Age</th>
<th>6 mo</th>
<th>1 y</th>
<th>6 mo</th>
<th>1 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>+DHA/ARA (n = 16)</td>
<td>96.1±8.6</td>
<td>98.7±8.0</td>
<td>102.2±10.5</td>
<td>98.0±5.8</td>
</tr>
<tr>
<td>-DHA/ARA (n = 11)</td>
<td>91.7±10.4</td>
<td>90.5±6.9</td>
<td>95.4±13.2</td>
<td>86.7±11.1</td>
</tr>
</tbody>
</table>

†Double-blind, randomized study of preterm infants >2000 g body weight and >32 weeks of gestation in full feeding status who received formula with or without DHA/ARA for 6 months.

MDI, Mental Development Index; PDI, Psychomotor Development Index.

DHA/ARA Supplementation Improves Cognitive Development at 6 Months†

<table>
<thead>
<tr>
<th>Score, Mean (SD)</th>
<th>Control (n = 55)</th>
<th>DHA/ARA supplementation (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total score</td>
<td>215 (39)</td>
<td>221 (32)</td>
</tr>
<tr>
<td>Communication</td>
<td>46.6 (9.1)</td>
<td>45.4 (7.9)</td>
</tr>
<tr>
<td>Gross motor</td>
<td>30.9 (11.1)</td>
<td>33.3 (11.5)</td>
</tr>
<tr>
<td>Fine motor</td>
<td>45.8 (14.3)</td>
<td>45.2 (10.7)</td>
</tr>
<tr>
<td>Problem-solving</td>
<td>49.5 (9.5)</td>
<td>53.4 (7.0)*</td>
</tr>
<tr>
<td>Personal-social</td>
<td>42.2 (12.3)</td>
<td>43.2 (12.8)</td>
</tr>
</tbody>
</table>

†Randomized, double-blind, placebo-controlled study of 141 infants with birth weights <1500 g. Intervention = 32 mg DHA + 31 mg ARA per 100 mL human milk started 1 week after birth and continued through hospital discharge (mean, 9 weeks). Cognitive development assessed at 6 months of age using Ages and Stages Questionnaire.

DHA/ARA Supplementation Improves Bayley Scores at 118 Weeks Postmenstrual Age in a Double-Blind Study


MDI, Mental Development Index; PDI, Psychomotor Development Index. *Breast-fed term > control, algal-DHA, fish-DHA (P < .05); †Algal-DHA > control (P = .056); ‡ Fish-DHA > control (P < .05); #Algal-DHA, fish-DHA > control (P < .05). Parentheses indicate number of infants per group.

Double-blinded study of 361 preterm infants. MDI and PDI assessed at 118 weeks postmenstrual age.

MDI, Mental Development Index; PDI, Psychomotor Development Index. *Breast-fed term > control, algal-DHA, fish-DHA (P < .05); †Algal-DHA > control (P = .056); ‡ Fish-DHA > control (P < .05); #Algal-DHA, fish-DHA > control (P < .05). Parentheses indicate number of infants per group. Double-blinded study of 361 preterm infants. MDI and PDI assessed at 118 weeks postmenstrual age.

Conflicting Results Regarding Benefit of DHA/ARA Supplementation in Preterm Infant Neurodevelopment: No Significant Difference†

Bayley MDI Scores at 18 Months’ Corrected Age

![Graph showing mean scores for different birth weights and genders](image)

†Randomized, double-blind controlled trial of 657 infants born at less than 33 weeks' gestation at 5 Australian tertiary hospitals with follow-up to 18 months. Standard-/high-DHA enteral feeds were given from day 2 to 4 of life until term corrected age.

Long-Chain PUFA Supplementation in Formula

- Systematic review and meta-analysis of 17 randomized trials (13 classified as high quality) of formula supplemented with LCPUFA to assess safety and benefit to preterm infants
- Infants enrolled in the trials were relatively mature and healthy preterm infants
- Assessment schedule and methodology, dose and source of supplementation and fatty acid composition of the control formula varied between trials
- On pooling of results, no clear long-term benefits or harms were demonstrated for preterm infants receiving LCPUFA-supplemented formula

Enteral Strategies for Increasing DHA/ARA Intake in Preterm Infants

- Maternal supplementation
- Breast milk (mother’s own)
- Structured lipids
- Pre-emulsified lipids
- Enzyme technologies

Update (Nov-2021):
**Sole DHA-infant or maternal supplementation is potentially harmful (reinforcing needing to provide both DHA/ARA)**


Donor Milk Levels of DHA Are Lower Than Mean Reported Levels\textsuperscript{1†}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Comparison of DHA and ARA levels in different breast milk samples.}
\end{figure}

\textsuperscript{†}Descriptive meta-analysis of 65 studies of human breast milk, including 2474 women.

Fatty Acid Replacement in Formula Is Challenging and Requires Standardization

- Defining target levels
- Determining dietary balance of n-3:n-6 fatty acids
- Ensuring optimal sn-position for absorption and incorporation into cellular phospholipids
- Optimizing digestion and absorption
- Achieving adequate levels at the tissue, cellular, and molecular levels
CURRENT NICU PRACTICES INVOLVING DHA/ARA IN PRETERM INFANTS
Current Practices

• Provide long chain PUFA in enteral feedings

• DHA/ARA are available in breast milk and commercially available infant formula

• Very premature infants do not reach full enteral feedings for several weeks

  • Standard parenteral lipid emulsions do not provide DHA/ARA

  • After reaching full enteral feedings, standard dietary DHA provision is not adequate to alleviate deficiency of prematurity

# Current Parenteral Nutrition Practices†

## Lipid emulsion ≤28 days (mean [SD])

<table>
<thead>
<tr>
<th></th>
<th>Birth (n = 17)</th>
<th>2 weeks (n = 14)</th>
<th>4 weeks (n = 17)</th>
<th>8 weeks (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>3.6 (1.6)</td>
<td>8.7 (2.1)</td>
<td>8.8 (2.1)</td>
<td>7.9 (1.9)</td>
</tr>
<tr>
<td>ALA</td>
<td>0.03 (0.02)</td>
<td>0.08 (0.03)</td>
<td>0.07 (0.03)</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td>DHA</td>
<td>5.3 (1.7)</td>
<td>3.6 (1.0)</td>
<td>3.7 (1.1)</td>
<td>4.2 (1.9)</td>
</tr>
<tr>
<td>ARA</td>
<td>14.8 (2.2)</td>
<td>12.0 (2.0)</td>
<td>12.7 (1.8)</td>
<td>11.5 (2.5)</td>
</tr>
</tbody>
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## Lipid emulsion >28 days (mean [SD])

<table>
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<th></th>
<th>Birth (n = 17)</th>
<th>2 weeks (n = 14)</th>
<th>4 weeks (n = 17)</th>
<th>8 weeks (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>3.6 (1.0)</td>
<td>10.1 (2.2)</td>
<td>11.3 (2.4)</td>
<td>10.1 (3.1)</td>
</tr>
<tr>
<td>ALA</td>
<td>0.03 (0.01)</td>
<td>0.09 (0.04)</td>
<td>0.12 (0.04)</td>
<td>0.12 (0.06)</td>
</tr>
<tr>
<td>DHA</td>
<td>5.5 (1.4)</td>
<td>3.0 (1.0)</td>
<td>3.1 (0.4)</td>
<td>2.7 (0.6)</td>
</tr>
<tr>
<td>ARA</td>
<td>15.7 (3.8)</td>
<td>11.7 (2.3)</td>
<td>11.4 (1.9)</td>
<td>9.4 (1.6)</td>
</tr>
</tbody>
</table>

- Infants showed a decline in DHA/ARA over time
- Longer exposure to intravenous lipid emulsion had a significantly greater decline in RBC DHA levels
- Infants who received intravenous lipid emulsion >28 days had longer time to start enteral feeds and reached full enteral nutrition 33 days later

†Prospective cohort study of 26 extremely low-birth-weight infants given intravenous lipid emulsion for ≤28 days or >28 days to assess change in LCPUFA Levels reported as weight % (g/100 g).

Transition to Enteral Nutrition

Even after transitioning to full enteral feedings, premature infants cannot overcome the decline in whole blood DHA/ARA levels apparent after birth\(^1\)

Suggests limited activity of desaturase enzymes\(^2\) or increased utilization of DHA/ARA due to disease or disease severity

The Role of Clinicians

• Help families navigate supplementation fads by sharing evidence from high-quality studies
  • Discuss problems with observational and cross-sectional findings used by companies to promote products that are not regulated directly to parents
• Consider better ways to supplement DHA/ARA in the highest risk patients

The Role of Nurses and Dietitians

• Provide diet-related information to pregnant and lactating women to increase omega-3 fatty acid intake

• Collaborate with colleagues to determine what supplements/formulas would be appropriate to help ensure adequate DHA intake

• Continue to monitor supplement/formula developments

The Role of Hospital Pharmacists

• Provide advice
  • Appropriate choice and correct use of formula
  • Appropriate choice of foods and drinks in weaned infants
  • Vitamin and fatty acid supplementation

• Collaborate with colleagues to ensure adequate information is provided throughout pregnancy to make informed choices about feeding

• Evaluate for and counsel on potential medication and supplement interactions

Future Considerations

• Define goals for fatty acid levels at specific, clinically relevant times

• Ongoing monitoring
  • Feeding advancement
  • Adverse events
  • Effects on other essential fatty acid levels

• DHA/ARA supplementation
  • Provide additional daily enteral DHA (start before full enteral feedings are reached and at a dose approximating in utero accretion rates)

ANY Questions?