



Importance of Adequate DHA/ARA in Preterm Infants

Overview

Michael Caplan, MD, and **Camilia Martin, MD, MS**, discuss the important functions of adequate docosahexaenoic acid (DHA) and arachidonic acid (ARA) balance in the preterm infant, with a focus on the role of DHA/ARA in neurodevelopment, growth, and retinal development. During the first postnatal week, in preterm infants, DHA levels rapidly decline, and ARA levels are also altered. Faculty highlight the challenges of achieving adequate DHA/ARA intake in preterm infants, the benefits of DHA/ARA supplementation, as well as the current NICU practices of DHA/ARA supplementation, and how those practices might evolve in the future.

Target Audience

This activity was developed for neonatologists, nurses, nurse practitioners, dietitians, hospital pharmacists, and other health care providers who have an interest in newborns, infants and toddlers.

Learning Objectives

At the conclusion of this activity, participants should be better able to:

- Describe the role DHA/ARA plays in developing infants
- Recognize the importance of adequate DHA/ARA balance in preterm infants
- Associate current NICU practices with DHA/ARA accretion rates in preterm infants.

Faculty

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Camilia R. Martin, MD, MS

Research Support Abbott Nutrition, Alcresta Therapeutics – clinical area: infant nutrition

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This activity was released on December 13, 2019 and is eligible for credit through December 13, 2021.

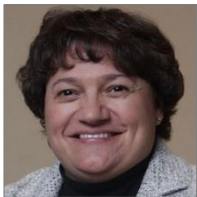


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THE ROLE OF DHA/ARA IN INFANT GROWTH



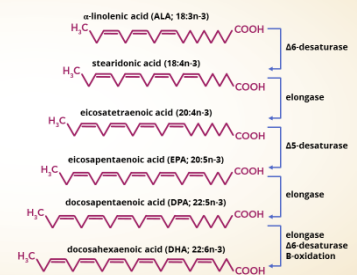
Camilia Martin, MD, MS: Let's proceed with the first part of this webinar and discuss the role of DHA and ARA in infant growth. I think it's important to emphasize here that we're not speaking of

growth just in terms of accrual of weight and height or head circumference, but also in terms of organ development. The latter component is mainly what will contribute to overall health and risk of disease in infants.

First, let's step back and go over the molecules that we're going to be talking about. First, DHA is a long-chain, highly unsaturated fatty acid. Unsaturated means that this molecule has double bonds between the carbons. It's an omega-3 fatty acid because that first double bond is at the third carbon from the terminal end. DHA is metabolized from α -linolenic acid, or ALA, which is considered an essential fatty acid. ALA must be acquired through our diet. Omega-3 fatty acids are found in seafood, especially fatty fish.

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



Calder PC. Ann Nutr Metab. 2016;69 Suppl 1:7-21.

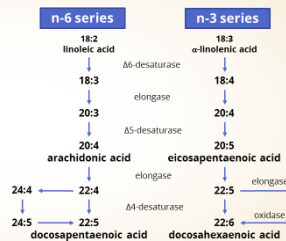
Slide 1 - Overview of Docosahexaenoic Acid (DHA)

ARA is also a long-chain unsaturated fatty acid, but ARA comes from the n-6 series because of the position of the first double bond. It's derived from linoleic acid, which is the essential fatty acid for the n-6 series. It's very abundant in brain, muscles, and liver, and ARA is also a precursor for adrenic fatty acid. We want to talk about DHA and ARA because they are important during the fetal period for organogenesis and during the postnatal period. These fatty acids are important for the development of the brain, and, in fact, DHA and ARA account for many of the fatty acids that are in the brain.

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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

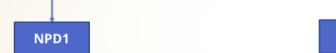


1. Hadley KB, et al. *Nutrients*. 2016;8(4):216. 2. Wijendran V, et al. *Lipid Res*. 2002;43(5):762-767.

DHA Signaling: NPD1 and BDNF



15-LOX-1 MAPK



- Protective roles**
- Inhibits retinal ganglion cell death
 - Protects against oxidative stress
 - Downregulates pro-inflammatory signaling
 - Upregulates antiapoptotic proteins
 - Downregulates proapoptotic proteins

- Protective roles**
- Promotes neuronal survival
 - Increases synaptic plasticity
 - Plays a role in neurogenesis

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

1. Niemöller TD, Bazan NG. *Prostaglandins Other Lipid Mediat*. 2010;91(3-4):85-89.

Slide 2 - Overview of Arachidonic Acid (ARA)

When we discuss DHA and ARA in infants, we have to go beyond what we've learned from the adult literature where much is known about their inflammatory properties. In infancy, DHA and ARA have many more functions that are critical for infant development, such as developing and maintaining the structure and function of tissues—particularly for brain development, retinal development, and also immune function.

Both DHA and ARA are important for neuronal tissues. The mammalian brains are 60% fat, and of that, 25% of the total fatty acid content of the brain is made up of both DHA and ARA.¹

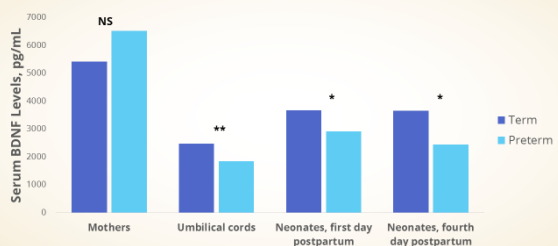
When these fatty acids exist in tissues, they are in the form of phospholipids, which are found on the cell membranes, providing structural support. But these fatty acids are also important in mediating self-signaling as well as regulating downstream pathways that play a role in organogenesis. An example would be the role of DHA signaling in influencing NPD1 and BDNF. Through the 15-LOX-1 pathway, DHA is converted to NPD1, which inhibits cell death, protects against oxidative stress, and downregulates pro-inflammatory signaling. Through the MAPK pathway, DHA mediates BDNF, which has protective roles, including promoting neuronal survival, increasing the brain's plasticity, and playing a role in neurogenesis.

Slide 3 - DHA Signaling: NPD1 and BDNF

In our preterm infants, they're very vulnerable to DHA deficits early in the postnatal period, which we're going to discuss further, but they're also vulnerable to having low fatty acid precursors and fatty acid metabolites, such as BDNF.

This is a study in which investigators evaluated BDNF levels in the mother, the umbilical cord, and the neonates over the first few days of life [Slide 4].² The preterm infants are in the light blue and the term infants are in the dark blue. You can see in the maternal circulation, there's no difference in serum BDNF levels between preterm and term infants. But even with maternal transfer, there's already a reduction in BDNF levels in the preterm infants, and this deficit of BDNF persists out to the first 4 days of life. Without adequate precursor DHA, the infants are at risk of having continued deficits in BDNF levels that may alter brain development.

BDNF Levels Are Lower in Preterm Infants



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

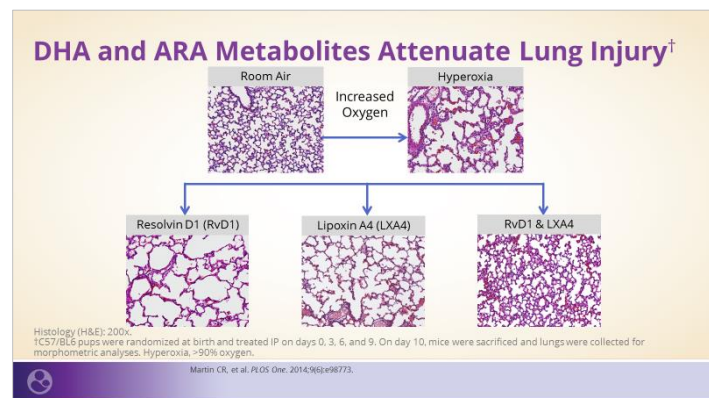
1. Malamitsi-Puchner A, et al. *Early Hum Dev*. 2004;76(1):17-22.

Slide 4 - BDNF Levels Are Lower in Preterm Infants

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In addition to brain development, DHA and ARA are important in neural tissues, such as the retina. These fatty acids play an important role in with the maturation and survival of photoreceptor cells, in which DHA is incorporated into the phospholipids for retinal function. Animal studies support the roles of DHA and ARA in retinal development. If animals are raised on fatty acid-free diets, they develop abnormal electroretinograms.³

When our group performed this study and saw the results [Slide 5],⁴ my enthusiasm for long-chain fatty acids really accelerated. Because again, it proves the point that fatty acids are not just important for regulating inflammation; for our infants, it's also about organogenesis at the very earliest stages of their life. In this study, we investigated the role of fatty acids in a mirroring model of hyperoxia-induced lung injury. On the top 2 panels, you see that the mice raised on in-room air have a typical lung architecture, multiple numerous small alveoli and very thin septal wall. When the mice are exposed to oxygen, there's alveolar simplification, a reduction in alveolar number, and an increase in septal wall thickness.

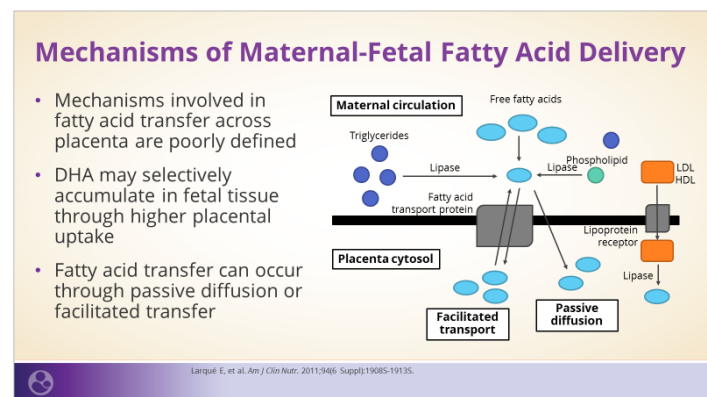


Slide 5 - DHA and ARA Metabolites Attenuate Lung Injury

We performed this study in several stages. In the panel on the bottom left, a molecule called resolvin D1 (RvD1)—whose parent is DHA—was administered. You can see that the alveolar simplification persisted, but septal wall thickness

was reduced. Those walls are very thin. When given lipoxin A4 (LXA4), whose parent is ARA, we saw an improvement in both the septal wall thickness and the number of alveoli. When we provided both of those terminal mediators together (bottom right panel), the lung architecture was very much similar to what was in room air. The combination of DHA and ARA appeared to completely attenuate the lung injury. Probing that even further, we saw that these mechanisms were from regulating inflammation, as somewhat anticipated, but also through growth factors regulating alveologenesis, which is something our babies need.⁴ We have to continue to think about these results in the early postnatal period when the accrual of these fatty acids is not as high as we're hoping for.

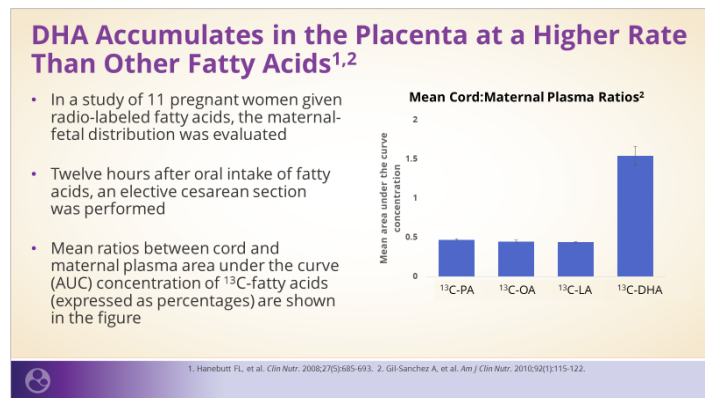
If we look at what we know about maternal-fetal transfer of fatty acid delivery, it is not surprising that fatty acids play an important role in fetal development and early postnatal development. We know that there are mechanisms to specifically transfer fatty acids across the placenta to the baby. Although we say here that they're poorly defined [Slide 6], several mechanisms have been identified, and they continue to be discovered. It's a hot topic of research. There are specific fatty acid-binding proteins that mediate transfer, and transfer can also occur through passive and facilitated diffusion.



Slide 6 - Mechanisms of Maternal-Fetal Fatty Acid Delivery

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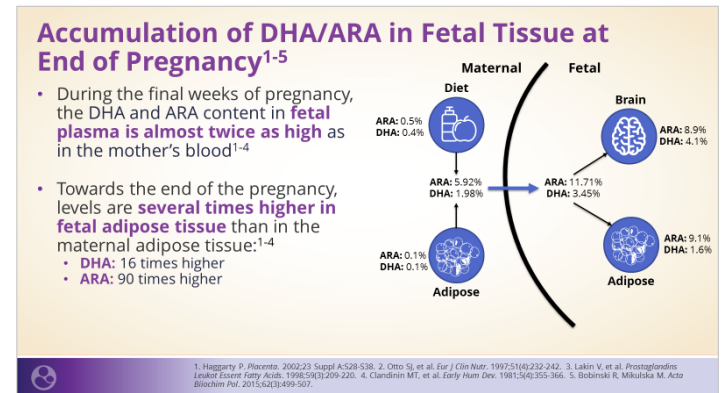
What's most important is the selectivity of the fatty acid transfer. Not all fatty acids are transferred; there are mechanisms of transfer that biomagnify the levels of DHA and ARA in the fetus. Here's an example for DHA. In this study [Slide 7], mothers received a diet with radiolabeled fatty acids right before an elective cesarean section.⁵ Twelve hours after that oral intake, the cesarean section was performed, allowing the investigators to evaluate cord levels of fatty acids relative to maternal levels. What you see in the graph is that, for many of the fatty acids, the cord levels were less than maternal plasma. However, for DHA (far right), the ratio was about 1.5-fold greater in the cord relative to the maternal plasma. That's where this concept of biomagnification came from.



Slide 7 - DHA Accumulates in the Placenta at a Higher Rate Than Other Fatty Acids

But it's not just DHA, it's also ARA that gets selectively transferred. We have to remember that they coexist in a balance. If you look at this figure [Slide 8], you can see that the relative plasma mol% of ARA and DHA in the fetal compartment is twice that of the maternal compartment.⁶ Towards the end of the pregnancy, adipose tissue acts as a reservoir for these fatty acids, allowing continued delivery of fatty acids to the neonate even after delivery. Here you see that DHA and ARA concentrations in fetal adipose tissue are extraordinarily higher than the maternal adipose tissue, with DHA levels about 16 times higher and

ARA levels about 90 times higher. I don't think this was by accident. I think this is important in fetal development and early postnatal development of our babies.



Slide 8 - Accumulation of DHA/ARA in Fetal Tissue at End of Pregnancy

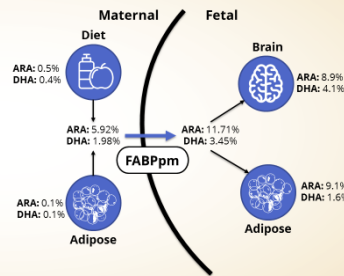
The final slide in this concept is Slide 9. There is a plasma membrane fatty acid binding protein (FABPpm) that has been shown to be important for selectivity of fatty acid transfer. Even though FABPpm is a fatty acid binding protein, it actually only binds 10% of the total fatty acids. But of those fatty acids, DHA and ARA are the primary component—90% or higher—due to high binding affinity.

As you can see, there appear to be specific mechanisms to ensure that the fetus receives concentrated levels of DHA and ARA relative to the mother to support fetal development in important tissues.

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FABPpm Transports DHA Selectively Across the Placenta¹⁻⁴

- **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^{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)³



1. Kaufmann P, Scheffen I. In Fetal and Neonatal Physiology, 1998, pp 59-70. 2. Campbell FM, et al. Life Sci. 1998;63(4):235-240. 3. Schmitz G, et al. Prog Lipid Res. 2008;47(2):147-155. 4. Bobinski R, Mikulska M. Acta Biochim Pol. 2015;62(3):499-507.

Enteral Sources of DHA/ARA for Term Infants

- **Human milk:** DHA/ARA is always present¹
 - 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 life²
- **Formula:** DHA/ARA is added in the US since 2001 based on worldwide averages in human milk³
 - 0.2% to 0.4% DHA
 - 0.35% to 0.7% ARA

1. Hadley KB, et al. Nutrients. 2016;8(4):216. 2. <http://www.cdc.gov/breastfeeding/data/reportcard.htm>. Accessed October 28, 2019. 3. Brenna JT, et al. Am J Clin Nutr. 2007;85(5):1457-1464.

Slide 9 - FABPpm Transports DHA Selectively Across the Placenta

Slide 10 - Enteral Sources of DHA/ARA for Term Infants

What happens after delivery? In preterm infants, the adipose tissue may not have sufficient levels of DHA and ARA to supply the infant—that reservoir of fatty acids is missing. The low levels of fatty acids can't carry them over during that early postnatal period.

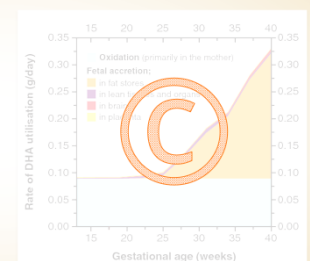
That's when the dietary source of fatty acids becomes important. Preterm infants are very dependent on the external diet and nutritional practices. We know that human milk has DHA and ARA, but the concentration can vary among mothers, across populations, and by source of milk (mother's own or donor milk).⁷ We have to be cognizant of that. DHA and ARA have been present in formulas since 2001, with the concentration based on human milk, but we also know that the actual bioavailability of what's in formulas can vary. Therefore, there are practices we can use to optimize DHA and ARA levels and to make sure that any changes in postnatal fatty acids are minimized to help maintain proper development of the organs.

IMPORTANCE OF ADEQUATE DHA/ARA BALANCE IN PRETERM INFANTS

We've already talked about DHA and ARA accrual in the brain, but again, I want to emphasize that there is accrual in the adipose tissue, and this is a good example on Slide 11. The graph shows the fetal accretion in each compartment.⁸ You can see that the brain has a very small line (red, at the top), but the majority is in fat stores, shown in orange. That's where these fatty acids are primarily going. However, our preterm babies do not have those fatty acid fat stores. They are very vulnerable to postnatal nutritional strategies.

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



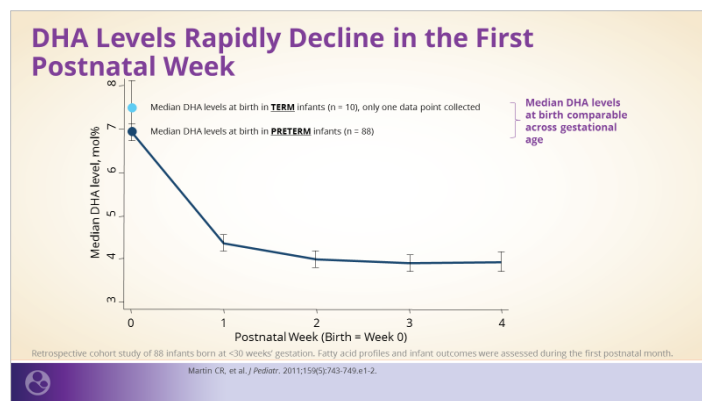
Haggarty P. Annu Rev Nutr. 2010;30:237-255.

Slide 11 - DHA/ARA Are Stored in the Brain and Adipose Tissue

Our group looked at the postnatal period in preterm infants to understand more about what happens to fatty acids after birth. This is a graph of median DHA levels across time, with week 0 being time of birth

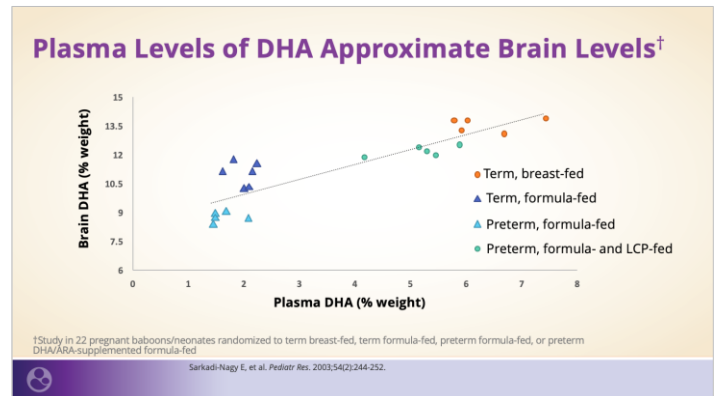
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[Slide 12].⁹ We then evaluated the levels at 1, 2, 3, and 4 weeks. We compared these values to those from a group of 10 term infants, shown in the light blue dot. As you can see, at birth, the levels of DHA in term and preterm infants were relatively close to each other even though the preterm population was less than 30 weeks of gestation; however, pretty quickly within that first week of life, a deficit began to occur where levels were almost half of where they started just a week prior. That deficit was maintained throughout the course of the NICU hospitalization and was never regained or even lessened.



Slide 12 - DHA Levels Rapidly Decline in the First Postnatal Week

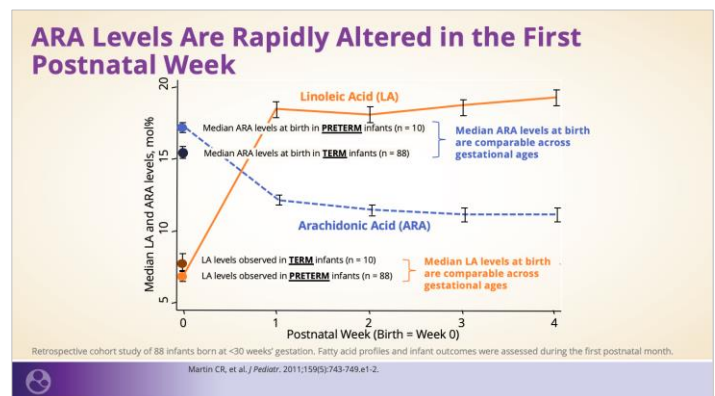
Our prior discussion about transfer, cord levels, and peripheral fatty acid levels in infants is important because that's our window—our biomarker—of what's happening at the tissue level, which we can't easily investigate. But in this study [Slide 13],¹⁰ researchers showed that fatty acid levels in the plasma are likely reflective of levels in the tissues. Investigators compared plasma DHA levels with brain DHA content in neonatal baboons, and they were strongly and linearly correlated. This means that DHA levels that we measure in infant plasma is likely similar to the levels in other tissues.



Slide 13 - Plasma Levels of DHA Approximate Brain Levels

These deficits that accrue in preterm infants should be alarming. ARA is in blue in Slide 14, and the levels are very similar to DHA.⁹ You can see that it's close to the levels of term infants at birth, but within that first week, ARA levels drop to almost half of where they started.

The orange line is the linoleic acid and increases to 2 to 3 times higher. This is probably iatrogenic from the types of diets that we currently provide. Within a week, we reverse ARA and LA ratios relative to each other as well as absolute levels of these fatty acids. We know already that if the babies had remained in utero, they would be at the level we see on day 0, but they get farther from that as the NICU hospital stay continues.⁹



Slide 14 - ARA Levels Are Rapidly Altered in the First Postnatal Week

We have to pay attention to DHA and ARA balance when we consider supplementation strategies. For

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cognitive development, we've seen that a 2:1 ARA:DHA ratio gives the most sustainable effects on neurocognition.¹ Furthermore, we've already discussed how derivatives of both the n-3 and n-6 pathways were critical in alveologenesi and chronic lung disease. Later in the talk, we'll also show you how these fatty acids are important for sepsis and infection.

The studies on the role of fatty acids in neurodevelopment have been mixed. This is an example of a study that was positive; increasing DHA levels were correlated with reduced risk of neural injury and improved neurodevelopment [Slide 15].¹⁰ However, the literature has been mixed, and I think a lot of that just reflects the challenges of developing a strategy that allows for adequate and sustainable levels for both short- and long-term effects. I think part of it is because we have to think about the context and how we're delivering these fatty acids. Dr. Caplan is going to talk more about that in his section.

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**

Tam EW, et al. *Pediatr Res*. 2016;79(5):723-30.

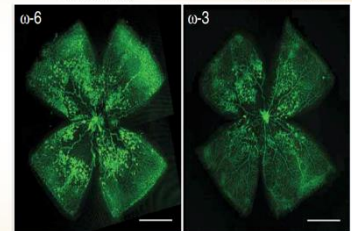
Slide 15 - DHA/ARA Levels Are Linked With Improved Neurodevelopment

This is a lovely study from across the street at [Boston] Children's Hospital [Slide 16].¹¹ In an animal model of retinopathy of prematurity, [Dr. Connor] was able to show that providing the n-3 pathway in excess of the n-6 pathway was associated with an increase in the typical markers for retinopathy of prematurity. But we also know that n-3-dominant strategies may not be optimal

for all organs or developmental systems that we look at.

Omega-3 and Retinopathy of Prematurity[†]

- Mice receiving ω -6-PUFAs had a significantly greater vaso-obiterated/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.

Connor KM, et al. *Nat Med*. 2007;13(7):868-873. Reprinted by permission from Springer Nature.

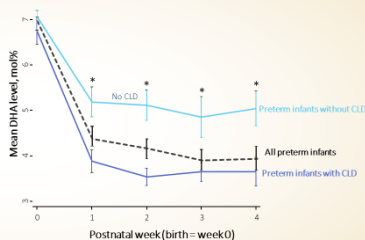
Slide 16 - Omega-3 and Retinopathy of Prematurity

Furthermore, going back to the study where we investigated longitudinal DHA levels in preterm infants, our population showed a decrease in DHA levels as a whole.⁹ But also, the relative DHA deficits during this postnatal period actually did make a difference of whether or not the babies went on to develop chronic lung disease. For example, those infants that did not develop chronic lung disease are shown in the light blue line and had higher average levels of DHA [Slide 17]. The dark blue line shows infants that did develop chronic lung disease, and the black line was the entire population. Even though both sets of infants had a decline in DHA levels, a smaller decrease in DHA levels was associated with protection from chronic lung disease. Every 1 mol% drop in DHA levels increased the risk for chronic lung disease by 2.5-fold.

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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**



Martin CR, et al. *J Pediatr*. 2011;159(5):743-749.e1-2.

Slide 17 - Low DHA Levels Are Linked to the Development of Chronic Lung Disease

But again, we must pay attention to the selectivity of the role of each fatty acid. On the bottom half of this table, showing late-onset sepsis [Slide 18], we can see that every mol% drop in ARA led to a 40% increase in the infants getting those illnesses.⁹

I liked this study because we didn't just say that a decrease in fatty acid causes problems in everything. Instead, it highlighted the great selectivity of what these fatty acids do and how they may protect against multiple conditions that concern us in the NICU.

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

Chronic lung disease		
Fatty acid	OR (95% CI)	P
LA	0.9 (0.7, 1.1)	.4
ARA	0.9 (0.6, 1.3)	.6
DHA	2.5 (1.3, 5.0)	.001
LA: DHA	8.6 (1.4, 53.1)	.02
Late-onset sepsis		
Fatty acid	Hazard ratio (95% CI)	P
LA	0.8 (0.7, 0.96)	.02
ARA	1.4 (1.1, 1.7)	.02
DHA	1.4 (1.0, 2.0)	.08
LA: DHA	4.6 (1.5, 14.1)	.007

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

Martin CR, et al. *J Pediatr*. 2011;159(5):743-749.e1-2.

Slide 18 - Association With Increased Risk of Chronic Lung Disease and Late-Onset Sepsis

Now I'd like to turn over the rest of the talk to my colleague, Dr. Caplan.

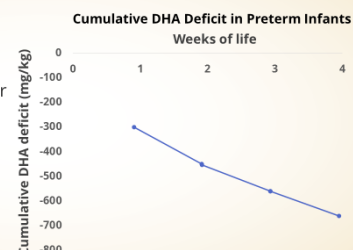
DHA/ARA SUPPLEMENTATION IN PRETERM INFANTS



Michael Caplan, MD: Here is the cumulative DHA deficit in preterm infants that occurs over the first 4 weeks of life [Slide 19].¹² There was a dramatic linear decrease in DHA levels in these 40 very preterm infants (less than 28 weeks gestation). What was striking is that this deficit was greater for low-birthweight infants compared with [higher-birthweight infants]. By 1 month of age, the DHA accumulation in preterm infants was half that of term infants, which is why we think that DHA supplementation might be most critical in preterm infants—but it's also important in term 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-birthweight 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.

Lapillonne A, et al. *Neonatology*. 2010;98(4):397-403.

Slide 19 - Current Nutritional Practices Are Inadequate to Maintain Optimal Levels of Fatty Acids in Preterm Infants

There are many challenges in achieving adequate DHA supplementation and intake in preterm infants. We know that the delivery of DHA and ARA is dependent on the breast milk and formula concentrations that are provided. We also know, and this is critically important, that intravenous lipid emulsions routinely used in the NICU are devoid of mature DHA and ARA. And while these lipid emulsions do have DHA and ARA precursors, these precursors have variable amounts of conversion into mature DHA and ARA. Therefore, it's unpredictable what those levels will be in preterm infants.

Importance of Adequate DHA/ARA in Preterm Infants

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

Back ML, et al. *J.ped.* 2016;51(4):423-433.

DHA Supplementation Alleviates DHA Deficiency in Preterm Infants[†]

	Baseline DHA, mol%	Full-Feedings, DHA mol%	Discharge, DHA mol%
Placebo preterm (n = 29)	2.91 (0.45)	2.83 (0.50)	2.87 (0.50)*
DHA preterm (n = 31)	2.88 (0.68)	3.03 (0.54)	3.55 (0.44)**
Term (n = 30)	4.31 (0.95)		

[†]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.

Back ML, et al. *J.ped.* 2016;51(4):423-433.

Slide 20 - Challenges in Achieving Adequate DHA/ARA Intake in Preterm Infants

Another challenge for achieving adequate DHA and ARA intake in preterm infants is related to the ability of the preterm infant to tolerate full-volume enteral feedings. NICUs vary in when they start feedings and how quickly they advance feedings, and the amount of DHA in breast milk or formula can markedly impact the amount of DHA that gets into the plasma—or even into the brain, as Dr. Martin described. All these factors seem to play a critical role in the amount of DHA and ARA in the developing preterm infant.

In this study, DHA supplementation alleviated DHA deficiency in preterm infants [Slide 21].¹³ The investigators provided 50 mg per day of DHA to [31 preterm infants]. You can see that the baseline levels in these preterm infants was quite low, under 3 mol% compared with the term babies who were at 4.3 mol%.

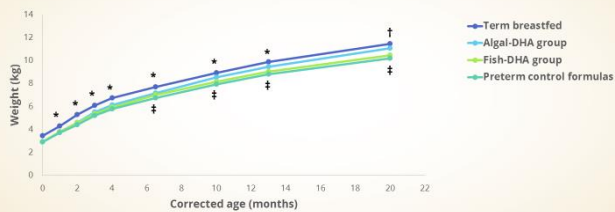
Slide 21 - DHA Supplementation Alleviates DHA Deficiency in Preterm Infants

When the infants began full feedings, DHA-supplemented babies had an average of 3.03 mol%, which was slightly higher than the baseline level. In contrast, the level in placebo-treated infants was lower than baseline. Strikingly, when you look at the discharge DHA level in the supplemented group, it increased to 3.5 mol%, while the group that only received placebo remained stable at the low level of 2.87 mol%.¹³ In this study, providing additional DHA allowed endogenous levels to increase.

In other studies, it's been shown that DHA/ARA supplementation may affect growth in terms of weight in preterm infants.¹⁴ In Slide 22, you can see the top, dark purple line that represents the weights for term breastfed infants. On the bottom, the dark green line shows the weights for preterm controls that didn't receive DHA supplementation. In the middle 2 lines (light blue and light green), weights for preterm infants who received algal and fish DHA-supplemented formula are shown. Over the first couple of years of life for preterm infants who received algal DHA supplementation, you can see that their weight approximates weight for the term breastfed group. This was statistically significant. These results suggest that DHA supplementation may not only just affect organ development but also growth in preterm infants.

Importance of Adequate DHA/ARA in Preterm Infants

Weight of Preterm Infants Fed DHA/ARA Formula Is Closer to Term Breast-fed Infants[§]

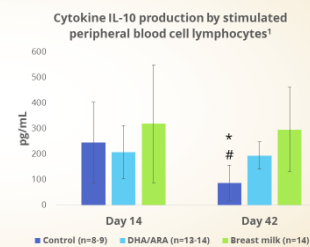


[§]Double-blinded study of 361 preterm infants fed until 92 weeks postmenstrual age, with follow-up to 118 weeks postmenstrual age
^{*}Breast-fed term infants > all preterm groups ($P < .05$).
[†]Infant formulas not different from term breast milk, both > control and fish/fungal formulas.
[‡]Infant formulas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

Candrian MT, et al. *J Pediatr*. 2005;146(4):461-468.

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 challenges^{1,2}



¹Study 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.

1. Field CJ, et al. *J Pediatr Gastroenterol Nutr*. 2000;31(3):291-299. 2. Chase P, et al. American Diabetes Association Scientific Sessions 2010. Orlando, Florida; June 25-29. Abstract 1918-P.

Slide 22 - Weight of Preterm Infants Fed DHA/ARA Formula Is Closer to Term Breast-fed Infants

We also know that DHA and ARA supplementation can enhance immune function. In adult studies, there have been many reports that demonstrate a variety of beneficial effects of DHA and ARA on immune responses. In this particular study [Slide 23], investigators demonstrated that IL-10, an anti-inflammatory cytokine, can be stimulated in peripheral blood lymphocytes by giving DHA and ARA to preterm infants.¹⁵ In the graph, IL-10 levels in peripheral blood cell lymphocytes for the control group (dark blue) were similar to the DHA-supplemented (light blue) and the breast milk-fed (green) groups at baseline, which was day 14 of life. But at day 42, at 6 weeks of age, the control group had decreasing levels of IL-10, while the DHA-supplemented group was similar to a breast milk-fed group. These results suggest that anti-inflammatory or immune host defense properties may be made whole by providing a DHA/ARA blend for preterm infants.

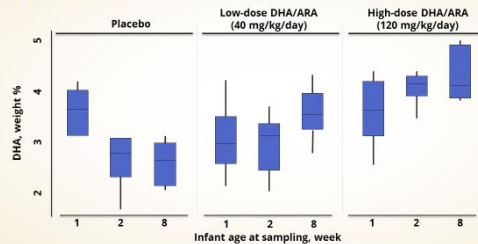
Slide 23 - Enhanced Immune Function With DHA/ARA Supplementation

In this study, we looked at the effect of emulsified DHA/ARA as another way to supplement feedings in preterm infants to provide beneficial endogenous DHA levels [Slide 23].¹⁶ In the placebo-treated groups (left), baseline DHA level in the first week of life was low, slightly under 4 weight%, and by 2 weeks and 8 weeks, that level decreased much more significantly. For DHA supplementation, various doses can be considered. In this study, we chose 40 mg/kg/d as a low dose and 120 mg/kg/d as a high dose. The DHA levels at 2 and 8 weeks of age in the low-dose group (middle) showed that the decrease in DHA after 1 week was prevented simply by providing this low-dose supplementation.

With high-dose supplementation, the reduction in DHA levels over the first 8 weeks is prevented. And in this study, high-dose supplementation demonstrated significantly increased levels that more approximated those levels in full-term infants.¹⁶

Importance of Adequate DHA/ARA in Preterm Infants

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



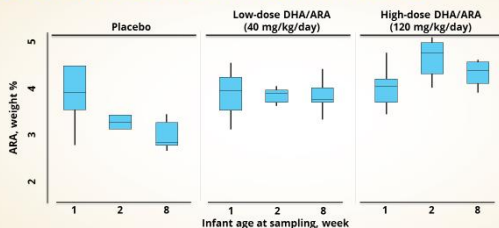
Randomized, double-blind, placebo-controlled trial of 30 very low-birth-weight infants given low dose or high dose PUFA via nasogastric tube. Dosing started within first 72 h of life and was given for 8 weeks or until discharge, whichever came first.

Frost B, et al. Pediatric Academic Societies Meeting, April 24 - May 1, 2019, Baltimore, MD.

Slide 24 - Effect of Emulsified DHA/ARA Enteral Supplementation on Serum DHA Levels in Preterm Infants

In the same study, when we looked at the ARA levels, the results were very similar to those for DHA levels [Slide 24]. In the placebo-treated group, after the first week without supplementation, the ARA levels decreased over time. However, that reduction in ARA levels was prevented by the low-dose DHA/ARA blend that we provided. Furthermore, the high-dose DHA/ARA blend seemed to not only prevent the reduction but allowed for an increase, approximating levels closer to those for full-term infants.¹⁶

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.

Frost B, et al. Pediatric Academic Societies Meeting, April 24 - May 1, 2019, Baltimore, MD.

Slide 25 - Effect of Emulsified DHA/ARA Enteral Supplementation on Serum ARA Levels in Preterm Infants

Additional studies have demonstrated that DHA and ARA supplementation can affect neurodevelopment at 6 months and 1 year of life. As Dr. Martin suggested, results are somewhat variable depending on the type of study, the age of

the infant, and the type of preparation provided. In this particular study [Slide 26], DHA and ARA were provided to babies between 30 and 37 weeks gestation.¹⁷ They were heavier than 2000 g, and they were followed and evaluated with the Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI). While the numbers of babies in these groups were somewhat low—we only had 16 in the treated group and 11 in the control group—you can see that the MDI scores at 6 months and 1 year were improved by DHA and ARA supplementation. The PDI scores were also improved by the DHA and ARA supplementation, suggesting that this might play an important role in infant neurodevelopment.

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

Age	Mean ± SD MDI score		Mean ± SD PDI score	
	6 mo	1 y	6 mo	1 y
+DHA/ARA (n = 16)	96.1±8.6	98.7±8.0	102.2±10.5	98.0±5.8
-DHA/ARA (n = 11)	91.7±10.4	90.5±6.9	95.4±13.2	86.7±11.1

[†]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 Developmental Index; PDI, Psychomotor Developmental Index.

Fang PC, et al. *Chang Gung Med J*. 2005;28(10):708-715.

Slide 26 - DHA/ARA Supplementation and Neurodevelopment in Preterm Infant Neurodevelopment at 6 Months and 1 Year

This is another study that looked at 141 infants [Slide 27].¹⁸ These were smaller babies, under 1500 g, and the babies were provided either a placebo control or 32 mg of DHA and ARA per 100 mL of milk. They started supplementation at 1 week of life and were treated for 9 weeks, but they were assessed at 6 months using the Ages & Stages Questionnaire. While many of these outcome measures assessing cognitive development were not improved with DHA/ARA supplementation, the mean problem-solving score of the Ages & Stages Questionnaire was significantly improved with DHA and ARA supplementation from 49.5 to 53.4.

Importance of Adequate DHA/ARA in Preterm Infants

DHA/ARA Supplementation Improves Cognitive Development at 6 Months[†]

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

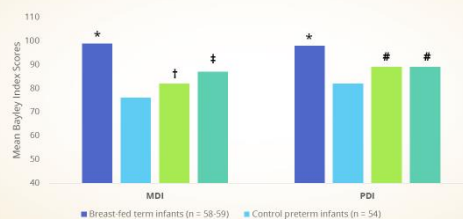
[†]Randomized, double-blind, placebo-controlled study of 141 infants with birth weights <1500 g. Intervention = 32 mg DHA + 31 mg ARA per 100 mL human milk started 1 week after birth and continued through hospital discharge (mean, 9 weeks). Cognitive development assessed at 6 months of age using Ages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

Hemiksen C, et al. *Pediatrics*. 2008;121(6):1137-1145.

Slide 27 - DHA/ARA Supplementation Improves Cognitive Development at 6 Months

This is a study by Dr. Clandinin that was published 15 years ago [Slide 28], which measured the effect of algal or fish DHA supplementation on neurodevelopment in a number of preterm infants.¹⁹ In this particular study, we're again looking at a Bayley scales of MDI and PDI. At 2 years of life, the MDI scores for the algal DHA-treated (light green) and fish DHA-treated infants (teal green) were significantly improved compared with control preterm infants (blue) and were closer to approximating a breastfed term infant (dark blue). Now, please note the scale on the y-axis—the scoring there is pretty dramatic. These effects include maybe 8 or 9 points for the MDI. In the right graph, similar effects occurred in the PDI score of about 8 or 9 points compared with the control patients. That's a huge difference for these particular patients.

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 > control ($P < .05$). †Algal DHA, fish-DHA > control ($P < .05$). #Algal DHA > control ($P < .05$); † 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.

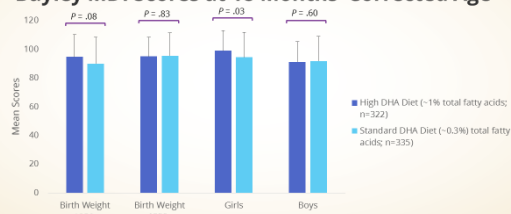
Clandinin MT, et al. *J Pediatr*. 2005;144(4):461-468.

Slide 28 - DHA/ARA Supplementation Improves Bayley Scores at 118 Weeks Postmenstrual Age in a Double-Blind Study

There are conflicting results, as we've mentioned, on the effect of DHA and ARA supplementation looking at neurodevelopment. This was a very large study that was well designed by Dr. Makrides and colleagues [Slide 29].²⁰ While the initial results seem to suggest that there might not be a significant impact, they used a high-DHA diet in this study (dark blue), which was 1% total fatty acids. This was compared with a standard DHA diet (light blue). If you look at the birthweight of the babies with weights less than 1250 g, the P value was 0.08, which is not statistically significant but certainly trended towards having an effect for the high DHA diet. For the bigger, less ill babies, greater than 1250 g, there seemed to be no difference between the groups.

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

Bayley MDI Scores at 18 Months' Corrected Age



[†]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.

Makrides M, et al. *JAMA*. 2009;301(2):175-182.

Slide 29 - Conflicting Results Regarding Benefit of DHA/ARA Supplementation in Preterm Infant Neurodevelopment: No Significant Difference

Importance of Adequate DHA/ARA in Preterm Infants

It's important to realize, though, that the control babies still got a significant amount of DHA. In this study, we're evaluating whether a diet with maybe 3-fold higher levels of DHA, compared with the standard DHA diet, will have an effect. If you look at the difference between girls and boys, there were some significant gender differences in the effect of DHA. Girls receiving the high-DHA diet had a significantly improved mean Bayley MDI score at 18 months compared with those that received the standard diet. For some reason—it might have to do with genetic background—boys didn't have that same effect.²⁰

Long-chain polyunsaturated fatty acid formula supplementation has been evaluated in many different studies. A meta-analysis was performed with 17 randomized trials evaluating the safety and efficacy of supplementation in formula [Slide 30].²¹ There was quite a bit of variability in the trials with regards to how preterm the patients were (ie, weeks of gestation), and there was some difference in the methodology, dose, and source of supplementation across these trials. But when investigators pulled all of these results together, there were no clear long-term benefits or harms of long-chain polyunsaturated fatty acid supplementation in these various supplementation trials.

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

Schulzke SM, et al. *Cochrane Database Syst Rev*. 2011;22:CD006375.

Slide 30 - Long-Chain PUFA Supplementation in Formula

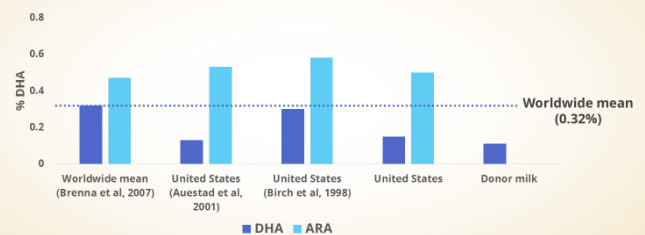
Nonetheless, there is still interest as to whether enteral strategies for increasing DHA and ARA

intake in preterm infants might make some sense. Lots of different approaches could be evaluated. We could give mothers supplementation during pregnancy, which would allow for transfer across the placenta prenatally. We could also give moms supplementation during lactation so that breast milk levels would be higher. We know that DHA would transfer to the baby from the breast milk and allow for higher levels in the plasma and in the brain. There are other approaches to provide structured lipids. We could provide supplemental pre-emulsified lipids to the developing infants, and we could use enzyme technologies to convert more of the precursor into these mature, functional products.

Looking at DHA levels across different populations, what sticks out is the variability throughout different studies. There are particularly low levels measured in donor milk that's banked across United States milk banks.²²

We know that diet in the mother is so important for DHA levels. That's demonstrated here [Slide 31] in the differences in DHA levels in the Auestad et al findings in 2001 and the Birch et al findings in 1998.²²⁻²⁴ We know that donor DHA levels are even lower,²² so babies who are getting donor milk might need even more supplementation to develop reasonable standard levels.

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



[†]Descriptive meta-analysis of 65 studies of human breast milk, including 2,474 women.

1. Valentine C, et al. *J Pediatr*. 2010;157(6):906-910. 2. Auestad N, et al. *Pediatrics*. 2001;108(2):372-381. 3. Birch EE, et al. *Pediatr Res*. 1998;44(2):201-209. 4. Brenna J, et al. *Am J Clin Nutr*. 2007;85(6):1457-1464.

Slide 31 - Donor Milk Levels of DHA Are Lower Than Mean Reported Levels

Importance of Adequate DHA/ARA in Preterm Infants

Fatty acid replacement in formula is challenging. It requires standardization, and for many reasons, requires further evaluation. We need to define what target levels are important. We need to determine what's the important balance between the n-3 and n-6 fatty acids. Dr. Martin provided some evidence to suggest that that balance is important in the biology and the pathophysiology of developing preterm infants. We need to know what the optimal sn-positions are for absorption and incorporation into cellular phospholipids. We need to optimize digestion and absorption. Ultimately, we want to achieve appropriate levels at the tissue and cellular levels, which are difficult to measure, and we use the plasma to approximate that.

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

Slide 32 - Fatty Acid Replacement in Formula Is Challenging and Requires Standardization

CURRENT NICU PRACTICES INVOLVING DHA/ARA IN PRETERM INFANTS

Finally, let's talk a little bit about current NICU practices involving DHA and ARA for the preterm infant.

We know that DHA and ARA are available in breast milk and commercial infant formula, so we're providing these in enteral feedings. But we have already suggested that very preterm infants don't get to full enteral feeds, sometimes for up to several weeks. As we already suggested, standard intravenous lipid emulsions don't provide adequate DHA and ARA—except probably for Omegaven®

and Smoflipid®, which aren't generally used as standard of care. Therefore, preterm infants are very much at risk for deficiency.

In this study several years back [Slide 33], investigators evaluated DHA and ARA levels in babies who were on lipids for less than 28 days, meaning they were started on enteral feeds adequately.²⁵ In the bottom table, the infants were on lipids for longer than 28 days of life, meaning they didn't get up to full feeds as quickly and efficiently.

Current Parenteral Nutrition Practices[†]

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

Lipid emulsion >28 days (mean [SD])				
	Birth (n = 17)	2 weeks (n = 14)	4 weeks (n = 17)	8 weeks (n = 17)
LA	3.6 (1.0)	10.1 (2.2)	11.3 (2.4)	10.1 (3.1)
ALA	0.03 (0.01)	0.09 (0.04)	0.12 (0.04)	0.12 (0.06)
DHA	5.5 (1.4)	3.0 (1.0)	3.1 (0.4)	2.7 (0.6)
ARA	15.7 (3.8)	11.7 (2.3)	11.4 (1.9)	9.4 (1.6)

- 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).

Robinson DT, et al. J Pediatr. 2013;162(1):56-61.

Slide 33 - Current Parenteral Nutrition Practices

If we look specifically at the DHA levels, you can see a significant decline over the first 8 weeks of life in these babies who were getting lipids in only the first 4 weeks, but then these levels start to plateau. By 8 weeks, babies receiving enteral feeds started to get protection from that DHA deficiency. However, on the bottom table, for infants that remained on lipids for longer than 28 days, the DHA levels went down, and at 8 weeks of life, they went down to 2.7 g per 100 g.²⁵

We believe that these very significant low nadirs of DHA might put a developing preterm infant at risk for a variety of neurodevelopmental outcomes as well as additional organ function risks, such as liver malfunction.

As we transition to enteral feedings, it's important that we provide the appropriate amount to prevent that decline in whole DHA and ARA levels. As we've

Importance of Adequate DHA/ARA in Preterm Infants

suggested, it's really not clear whether there's limited activity of these desaturase enzymes, which would convert the precursor linoleic acid or α -linolenic acid into DHA and ARA, or whether there's increased utilization of these fatty acids due to disease or disease severity.

Nonetheless, it's the role of clinicians to help families navigate this supplementation issue and to prevent these significant and clinically important low nadirs of DHA and ARA over the first many weeks of life.

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

Cowden JD. *NEJM Journal Watch*. November 7, 2018. www.nejm.org/doi/full/10.1056/NEJMp1811107;can-docosahexaenoic-acid-supplementation-help-toddlers

Slide 34 - The Role of Clinicians

Furthermore, it's really important for nurses and dietitians to provide diet-related information to pregnant and lactating women to increase their omega-3 fatty acid intake. I can tell you that most neonatologists, when they're taking care of all the critical issues, such as intraventricular hemorrhage and respiratory distress syndrome, we forget sometimes to remind the mothers to use adequate DHA supplementation. I think the nurses and dietitians can further collaborate with their colleagues, determine what those supplements should be and what those concentrations should be for those moms during this difficult time.

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

Brooks SL, et al. *MCN Am J Matern Child Nurs*. 2000;25(2):71-75.

Slide 35 - The Role of Nurses and Dietitians

Finally, the hospital pharmacist can play an important role in DHA and ARA supplementation because they can provide advice about the right choice and correct use of formula in collaboration with the neonatal clinicians as well. They can help talk about vitamin and fatty acid supplementation and they can collaborate with their colleagues to provide adequate information for the mothers and the clinicians.

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

Mason P. *Pharmacist J*. 2000;26(4/708):471-475.

Slide 36 - The Role of Hospital Pharmacists

When we consider the future in this field, we need to define the goals for DHA and ARA levels at specific clinically relevant times and what those levels should be for preterm infants. We need to consider ongoing monitoring with regards to how quickly we advance feeds, what those adverse events might be, and the effects of those changes on other essential fatty acids. I think, more importantly, we need to provide additional daily

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enteral DHA, particularly before full enteral feedings are provided or when we're using exclusive intravenous lipid emulsions that don't have DHA mature products provided in them. We want to approximate in utero accretion rates.

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)

Black M, et al. Lipids. 2016;51(4):423-433.

Slide 37 - Future Considerations

In summary, what we've suggested today is that DHA and ARA are critically important molecules for the developing preterm infant. The levels are low in preterm infants and we need to find better ways to supplement these infants so that they can grow and develop as well as possible over the first several years of life.

QUESTION & ANSWER

Editor's Note: This is a transcript of audience questions together with Dr. Martin's and Dr. Caplan's responses from the November 25, 2019, audio webcast.

In your clinical NICU practice, how frequently do you monitor fatty acid levels in preterm infants?

Dr. Martin: We don't, outside of any research studies that we may have going on. And I would say the only other exception, which is outside of the scope of this talk, is that with fish oil-based lipid emulsions, we have to be careful that we might drive down some essential fatty acids and develop an essential fatty acid deficiency. I am aware that most hospital policies do have some element of checking periodic fatty acid levels, but it's specifically for essential fatty acid delivery.

Why that hasn't been implemented in practice so far is just because, as Dr. Caplan mentioned, we're not sure about the target levels. What we do know is they go into deficit with current strategies. We know that supplementing them and increasing them from their nadir seem to have beneficial health outcomes. But what that absolute target level is still unclear. I feel very comfortable that we have to supplement but not so strong that we have to monitor fatty acid levels.

In the low- and high-dose DHA/ARA early enteral supplementation study,¹⁶ what were the implications from the study on the NICU practices at your institution?

Dr. Caplan: Well, we were struck by the levels that we were able to attain using the low-dose and high-dose supplementation with DHA and ARA. Based on our previous studies where we had such low levels without supplementation, we were excited to consider the opportunity to supplement our babies.

Now, we're a NICU that gets onto feeds fairly rapidly. We start all of our babies in the first day of life on some trophic feedings. But as we all know, when those babies are sick on vasopressors and have a PDA and have lines in, we don't advance them very quickly, and it might still take a few weeks before we get them on full feedings. So we have continued to look through the literature and do studies to try to find ways to provide supplementation so that we don't get these low level nadirs in these at-risk preterm infants.

Can you elaborate on the protocol and outcomes for this study?

Dr. Caplan: Well, we did a randomized blinded study where we provided placebo, 40 mg/kg/d of DHA, or 120 mg/kg/d of DHA. The nurses and doctors were both blinded to what these babies received. We measured the serum DHA and ARA levels at 1, 2, and 8 weeks of life. We really didn't do anything else different for the babies at all. The

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babies were fed according to regular NICU practice. We didn't interfere in any of the feedings. We have a ton of data from this study that we analyzed with respect to differences in feeding approach, amount of breastmilk, and breastmilk DHA levels. As it all turned out, the only thing that really made a significant difference in those DHA levels was the supplementation that we provided. The groups were matched fairly closely for DHA delivered through the normal diet that they were getting. What that suggests and implies to us is that babies who are getting mother's milk or formula still might not be getting quite enough of the DHA and ARA supplementation.

What is a minimal volume of feeds at which enteral DHA supplementation can be started?

Dr. Martin: I think the information we have there is 2-fold from which you can extrapolate. Dr. Caplan could talk a little bit about when they started supplementation in their study,¹⁶ and I'm not sure what Dr. Baack did in her study.¹³ We do know just from maternal diets and animal studies that DHA supplementation is relatively safe. As long as they can provide it in safe and small volumes that mimic our clinical practice in the way that we deliberately advance each day, I think it could be started as soon as you're administering any enteral feedings.

I think it's our challenge to actually figure that out and do it early because in all of the studies that we showed—Dr. Robinson's and mine—the drop in the DHA occurs within the first week of life.^{9,25} If you were to dive deep into a daily level, it's probably happening by day 3 or 4. You don't want to wait very long until you feel comfortable that the baby will tolerate it. It's really our challenge to be able to deliver it in small volumes. It's otherwise a fatty acid that's typically well tolerated.

Dr. Caplan: Yeah, I would agree with you, Dr. Martin. That's exactly what we found in our studies as well. We were able to start these babies early, even before they were getting on any advanced

feedings, and they tolerated the supplement just fine. It's usually 1 mL or at most 2 mL, and there didn't seem to be any adverse events associated with the delivery.

What standard practices or supplementation do each of your institutions utilize in your respective units?

Dr. Caplan: I would say that the emulsified DHA/ARA supplement is now available if people wanted to buy it and use it in their NICUs. I'll tell you that our neonatal team is just reviewing this. They started their discussion last week to try to identify who they wanted to use it in, how they wanted to use it, and in what doses they wanted to use it. I'll be quite transparent and say that they haven't made their final decision on exactly how that's going to go. But the tone of their discussion I'm told was that, without a doubt, they want to provide it to the most at-risk babies. Those would be the smallest and most premature. Those babies that are most likely not to get to full feedings in a reasonable time frame and would have the most potential benefit from getting supplementation. I don't know if you've looked at it, Dr. Martin, in your NICUs.

Dr. Martin: Right. Pretty much at the stage that you just discussed. We're going to have to talk about it as a group and decide which babies and when with regard to supplementation. I appreciate and am happy there are options. It's not necessarily standardized yet across all the units. I know from talking to some and from my travels that, because clinicians understand the importance of DHA and ARA, they would previously devise other ways to deliver it—even drawing up the liquid in adult capsules, which scares me. I really like the idea to get something out there specific for our babies so that at least we have options, and we'll take it back to the nursery and discuss who, when, and how.

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What are the differences between algal and fish oil DHA and formulas?

Dr. Caplan: I would like to hear what Dr. Martin has to say, but what I can suggest is that I think some people were excited about an algal preparation compared with the fish preparations simply because of families that were vegan and didn't want to be exposed to fish. What we've seen, or at least what I've been able to see from the studies so far, is that both the algal and the fish oil seem to provide reasonable supplementation with respect to levels. In studies that have looked at both, you are able to accomplish reasonable outcomes of both preparations. Dr. Martin, do you have any other insight into the differences or the potential benefits of each?

Dr. Martin: I agree with you, especially about the latter half that no, I don't know too much about the differences except that, at least from the biological point of view, they both seem to accomplish increased levels of the fatty acid that you're providing. I think there might be something on the manufacturing end with the algae-based fatty acids, which allows for a little bit more concentration and flexibility in which one you're drawing out for your fatty acid preparation. But outside of that, biologically, I haven't seen much literature, or I'm unaware of it, about the distinction between the 2.

What do you think should be the ratio between ARA and DHA for supplementation of the breast milk?

Dr. Martin: I think on the maternal end, it really is something that is okay where a sole DHA supplementation happens, because the way an adult processes additional ARA is very distinct from how a baby processes it through their diet. In our adult human diet, we're n-6 rich. Our ARA levels are pretty high, probably higher than they should be for our overall health. So detection of ARA in milk is never that much of an issue.

I would be comfortable that for the mother, as you see with prenatal vitamins, that's a DHA story and a DHA supplementation strategy. And the infant will get ARA through there. I think separately, it's important for the infant to get ARA through the diet in which they receive the parental fatty acids and metabolize from there. Because as I've showed, there are metabolic products of ARA that go to very specific purposes that won't necessarily be met just by elevating arachidonic acid.

Dr. Caplan: I would agree with you, but I would also suggest that we don't really know what the right ratio is. I don't think we've studied that nor do we have evidence-based medicine to tell us exactly what ratios. I know that many studies have given just DHA, and some studies have given DHA and ARA. There may be some risks with giving just DHA alone. But I don't think we really know the answer, and it's incredibly difficult to get that answer. I'm afraid we're going to be stuck with what we have, and we just have to suggest that probably a balance is important. It's unlikely that we're going to create any toxicity with the amount of fatty acids that we're going to provide to these preterm infants.

Can DHA be given while a neonate is receiving parenteral nutrition?

Dr. Martin: Yes. I say that because, if you look back at the studies that look at fatty acid levels when being provided with parenteral nutrition, whether it's Intralipid®, Smoflipid®, or even Omegaven®—but Omegaven® should not be used as a maintenance lipid strategy—we can see that even with the delivery of parenteral nutrition and lipids, there's still a decline in DHA. It is slightly higher than what we see in the Intralipid® group, but there is still a deficit that accrues. So as Dr. Caplan suggested, I don't think we're going to run into a lot of problems by starting DHA early and continuing it even through the parenteral nutrition phase as they transition to enteral feeding. My gut sense is that it would be okay to begin that early.

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Do either of you have any plans or know of any plans for longer follow-up studies to test the differences in higher level or executive functioning?

Dr. Caplan: I think it would be very worthwhile to do a phase 3 trial looking at supplementation at high-dose levels and following kids to measure neurodevelopment at 2 years and even getting into older age executive functioning. I don't know about Dr. Martin, but I'll probably be retired before we get those results. But I think it would be very important to do and I think it's smart for clinicians to be asking for those kinds of studies. In lieu of those studies though, I think it makes sense to me just theoretically that we should be using these supplements. But I really do advocate for additional clinical trials so that we can more clearly demonstrate the unique effects of these molecules on these important outcomes.

Dr. Martin: I agree, Dr. Caplan, and you'll be around! I think it is important, and there's so much

to fine tune here. The relevance I think is clearly stated. The premise in the data we have thus far is clearly promising. But we need to continue to dive deep into all the things we don't understand yet. What are those target levels? Even though they're relatively safe, I think we should always be concerned about safety in our babies. So monitoring safety and looking at the interaction between DHA and ARA in context-specific areas. There's still so much more to learn and I think there's a lot of biological promise in fatty acids that can do so much good. But there are still a lot of questions.

Abbreviations

ALA	α-linolenic acid	MAPK	mitogen-activated protein kinase
ARA	arachidonic acid	MDI	Mental Development Index
BDNF	brain-derived neurotrophic factor	NICU	neonatal intensive care unit
DHA	docosahexaenoic acid	NPD1	neuroprotectin D1
FABPpm	membrane-associated fatty acid binding protein	PDA	patent ductus arteriosus
IL	interleukin	PDI	Psychomotor Development Index
LXA4	lipoxin A4	RvD1	resolvin D1

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