Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS

Innovations That Are Closing the Gap Between Breast Milk and Infant Formula

Presented by

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CE/CME Information

Target Audience

This activity was developed for physicians, pediatric nurse practitioners, nurses, registered dietitians, and other health care professionals who have an interest in newborns, infants, and toddlers.

Learning Objectives

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

- Summarize what is known about the bioactive components found in human milk
- Recognize how cognitive development is impacted by DHA (docosahexaenoic acid) and MFGM (milk fat globule membrane)
- Examine how bioactive components are closing the nutritional gap between infant formula and human milk

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Research Support	DSM
Consultant	Mead John Nutrition

Clinical area: infant nutrition for all companies listed above.

Olle Hernell, MD, PhD

Research Support	Arla, Dairy Goat Company, Hero, Mead Johnson Nutrition
Consultant	Arla, Dairy Goat Company, Hero, Mead Johnson Nutrition
Speakers Bureau	Hero, HiPP, Mead Johnson Nutrition, Nestlé, Semper

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CE/CME Information, *cont.*

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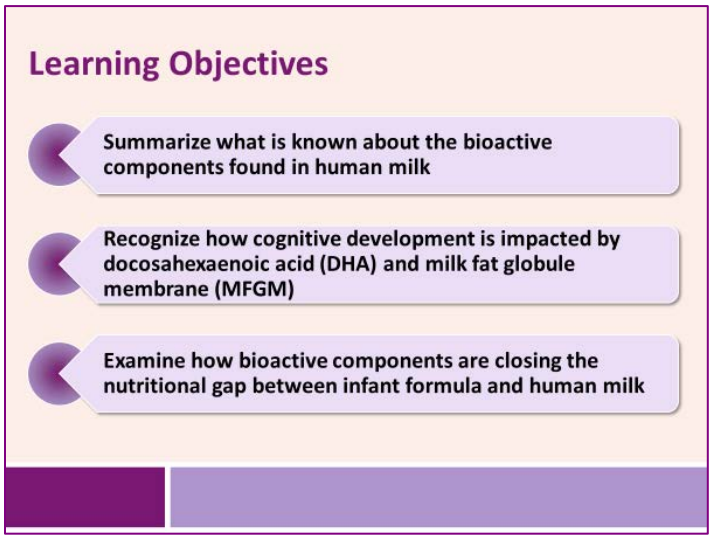
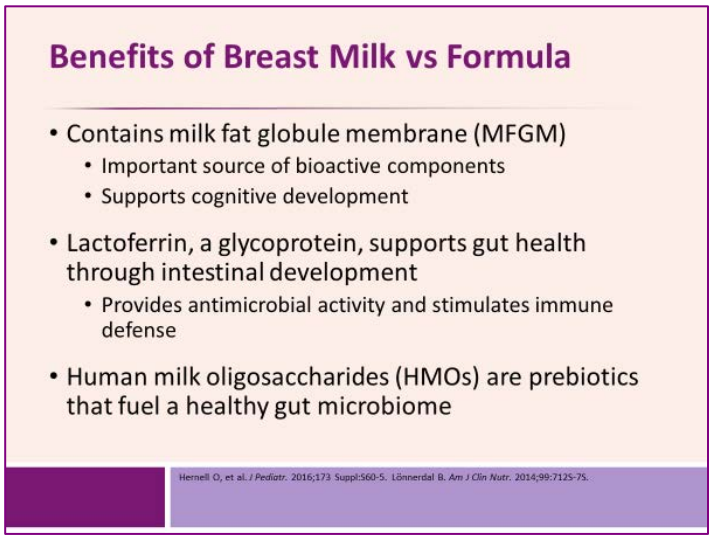
Innovations That Are Closing the Gap Between Breast Milk and Infant Formula

Dr. Susan Carlson: Today we're going to discuss bioactive components found in human milk and the emerging evidence that they influence cognitive development as well as other aspects of physiology, such as the immune system, favorably. Their addition to infant formula is closing the nutritional gap between human milk and infant formulas.



All health professionals recognize that human milk is the gold standard for infant feeding. Breast feeding provides optimal support for physiological growth and development of term infants including the development of the cognitive system, brain development, immune development, and for optimal growth. But, we also know that some women need to feed an infant formula and that many women do mix milk and formula feeding during infancy.

Some of the compounds in human milk not traditionally included in infant formula include the milk fat globule membrane (MFGM), which is an important source of bioactive components, as we will discuss, and supports cognitive development. Also, lactoferrin, which is a glycoprotein that supports gut health through intestinal development and provides antimicrobial activity and stimulates immune defenses. As well, the human milk oligosaccharides, which act as prebiotics or fuel for the bacteria in the gut to fuel a healthy gut microbiome.



We do know that breastfed vs formula-fed infants have different growth patterns. They have fewer acute otitis media and gastrointestinal, and possibly other infections; reduced risk of obesity, and reduced

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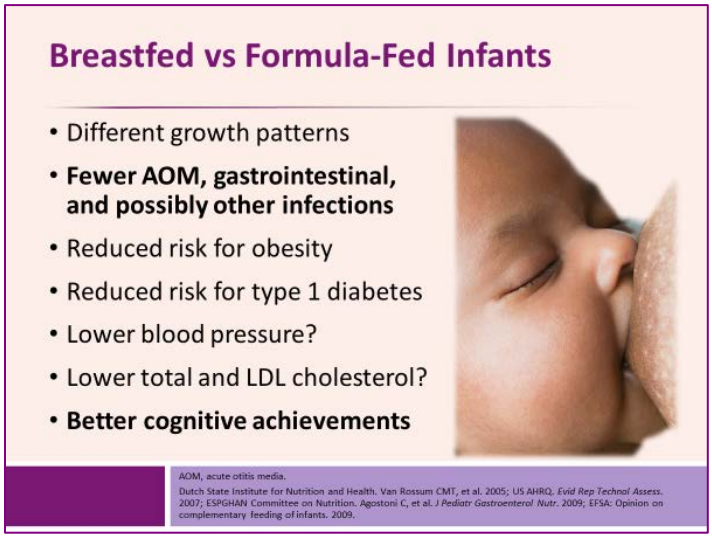
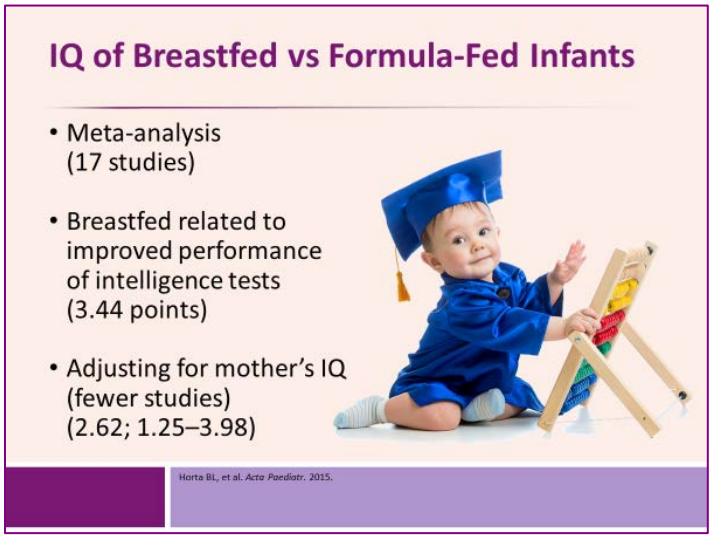
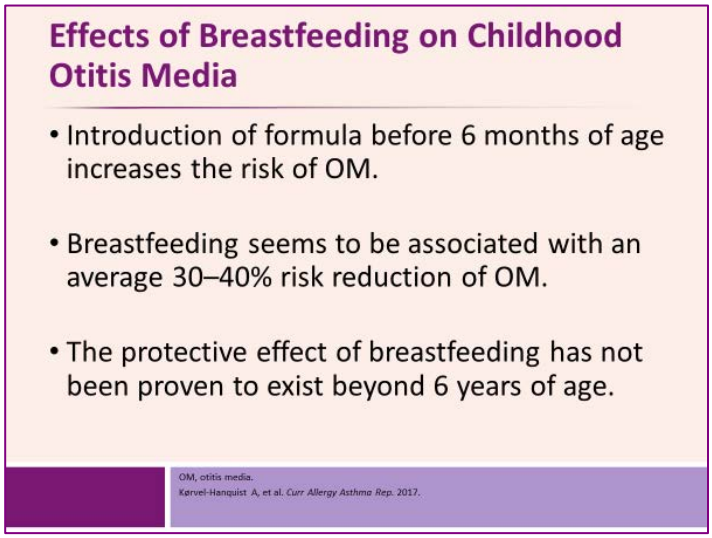
risk of type 1 diabetes, as well as better cognitive achievement. There may be at least some evidence on the fact that breastfed babies have lower blood pressure and better lipid profiles later, well after they complete breastfeeding, so, later in childhood.



We also have evidence from a meta-analysis that includes 17 studies that the IQ of breastfed infants is higher than in formula-fed infants. The amount of increase in IQ is about 3.5 points. But even adjusting for mother's IQ, which we know is a factor of course in the IQ of the offspring, even there, there's about a 2.6-point increase in IQ in breastfed compared to formula-fed infants.



Dr. Olle Hernell: In another recent meta-analysis, it was concluded that introduction of infant formula before 6 months of age increases the risk of otitis media. That means that breastfeeding seems to be associated with a risk reduction that has been estimated to around 30%-40%. But, this effect has not been shown to exist beyond 6 years of age.



So, the question is, if these differences between breastfed and formula-fed infants could actually be—at least partly explained by—the milk fat. Ninety-five or maybe 98% of the milk fat is triglycerides. Now these triglycerides, they form the core of the milk fat globule synthesized within the epithelial cell. When the milk fat globule is extruded through the epithelial cell, the apical part of the epithelial cell, it becomes enveloped by the cell membrane. Which means that the core of the globule containing all the triglycerides, virtually, and other hydrophobic lipids/cholesterol esters are enveloped by a more hydrophilic membrane (or it's actually a triple membrane of phospholipids). Now these phospholipids are glycerophospholipds; there are sphingolipids, there are cholesterol, but also [glycosphingolipids]. And, the membrane also contains numerous proteins, some of which will be discussed further.

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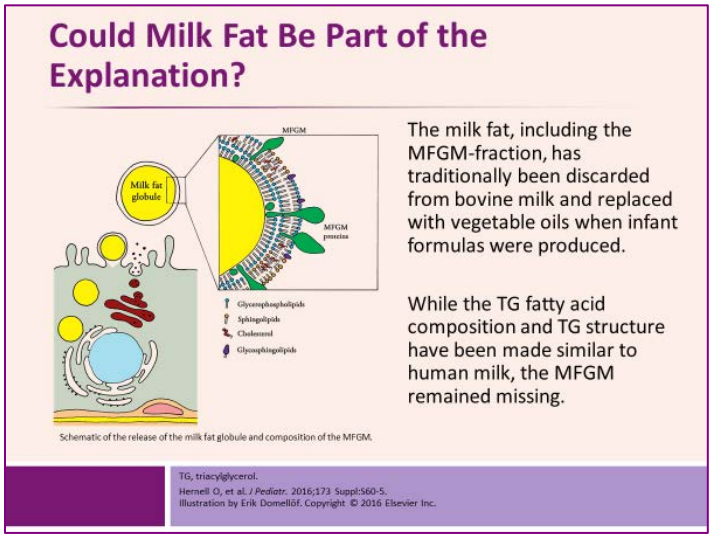
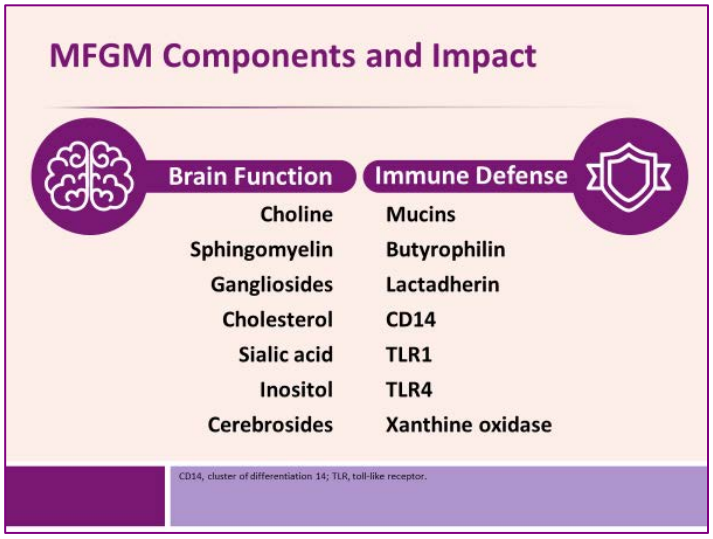
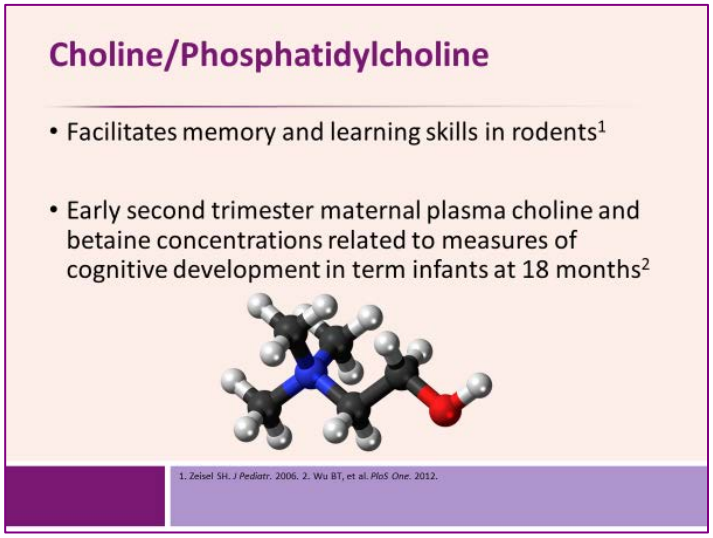




The interesting thing here is that the milk fat, including also milk fat globule membrane fraction, has traditionally been discarded from bovine milk and replaced by a blend of vegetable oils when infant formulas have been produced. It has been possible to, more or less, mimic the composition of the fatty acid composition using these blends of vegetable oils and also the triglyceride structure, particularly using a structured triglyceride with palmitic acid in the 2-position of the triglyceride. But, the MFGM components have remained missing.

Now if we look a little closer on the MFGM components and what impact they might have. They have those components that might affect brain function, or at least we know that they are needed for a normal development of the infant brain. These are choline, as a component of phosphatidylcholine, we have sphingomyelin, we have gangliosides, cholesterol, sialic acid, inositol, and cerebrosides. All these are components of the milk fat globule membrane and are associated to brain development. And then we have the proteins, many of which are actually having a biological effect. We have mucins, butyrophilin, lactadherin, CD14, toll-like receptors (TLR), and xanthine oxidase. And, these proteins, some of them, do have a direct antimicrobial effect and others have an impact on the immune defense development.

Then we have choline as a component of phosphatidylcholine. It has been shown in rodents, again, that it facilitates memory and learning skills. In the human, it has been shown that plasma choline and betaine concentrations during early, second trimester are related to measures of cognitive development in term infants at 18 months.



If we then look at sphingomyelin, another lipid component of the MFGM, it has been shown again in rodents that supplementation to rats accelerates the myelination of cortex. In humans it has been shown that when supplemented to breast milk—so that one group got regular milk and the other a milk fortified with sphingomyelin—there was an association between supplementation with sphingomyelin and neuro-behavioral development at 18 months in low-birthweight infants.

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Sphingomyelin

- Supplementation to rats accelerated myelination of cortex[1]
- When supplemented to breast milk (milk with or without added SM), neuro-behavioral development at 18 months improved in very low-birthweight infants (n=24)[2]

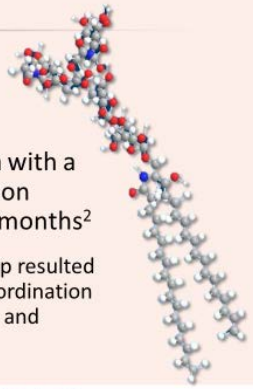
Sphingophospholipides (sphingomyelin)—
Space-filling model of molecule

SM, sphingomyelin.
1. Oshida K et al. *Pediatr Res.* 2003; 2. Tanaka K, et al. *Brain Dev.* 2013.

If you go to sialic acid as a component of the gangliosides, supplementation with sialic acid improves learning and memory in piglets. And, again in humans, supplementation of infant formula with a ganglioside-enriched milk fat fraction improved neurodevelopment at 6 months. That was shown using the Griffith Scales. The treatment group that was supplemented with the MFGM fraction resulted in increased scores for hand-and-eye coordination, performance, and general IQ.

Sialic Acid/Gangliosides

- Sialic acid supplementation improves learning and memory in piglets[1]
- Supplementation of infant formula with a ganglioside-enriched milk fat fraction improved neurodevelopment at 6 months[2]
- Using the Griffith Scales, treatment group resulted in increased scores for hand-and-eye coordination IQ (P<0.006), performance IQ (P<0.001), and general IQ (P=0.041)[2]



1. Wang B, et al. *Am J Clin Nutr.* 2007; 2. Gurnida DA, et al. *Early Hum Dev.* 2012.

Why should infections be affected by MFGM? We know that there are many factors in human milk that provide expected or proven antimicrobial effects. Some are components of the MFGM: and I already mentioned butyrophilin, mucin, lactadherin, toll-like receptors, xanthine oxidase, but also the gangliosides and oligosaccharides. And again, we can give a few examples: When MFGM was fed in formula to rat pups, that promoted development of the intestinal epithelium; it increased gut integrity, impacted the microbiota development, and was—interestingly—protective against *Clostridium difficile* toxin-induced inflammation. So, it had an anti-inflammatory effect. In a double-blind, placebo-controlled trial in healthy adults, a concentrate rich in natural bioactive phospholipids and sphingolipids reduced symptoms when challenged with a diarrheagenic *E. coli*.

Why Infections Are Affected

Many factors in human milk provide expected or proven antimicrobial effects

Some are components of the MFGM (e.g., butyrophilin, MUC1, lactadherin, CD14, toll-like receptor 1 and 4, xanthine oxidase, gangliosides, oligosaccharides)

Examples:

- MFGM in formula fed to rat pups promoted development of the intestinal epithelium and gut integrity, impacted the microbiota development, and was protective against *C Difficile* toxin-induced inflammation[1]
- In a DBPCRT in healthy adults, a concentrate rich in natural bioactive phospholipids and sphingolipids reduced symptoms when challenged with a diarrheagenic E. coli[2]

DBPCRT, double-blind, placebo-controlled randomized trials.
1. Bhinder G, et al. *Sci Rep.* 2017; 2. Ten Bruggencate SJ, et al. *J Nutr.* 2016.

So, then one might ask how human milk bioactive proteins work. Well, they can do that in different ways. We have proteins that are present in high concentrations in human milk, for instance caseins and alpha-lactalbumin. These are, of course, a major source of amino acids, but they can also exert biological functions in recipient infant. Other proteins are present in low concentrations, and that is typical for those in the milk fat globule membrane. They are not so important with respect to amino acids, as a source amino acids or protein synthesis, but still they can exert biological functions in the infant. And, that means that functions can be exerted by the intact protein, and these proteins are often resistant to proteolysis. But, it can also be that peptides formed during the digestion of these proteins exert a biological function. Then we have complex molecules, such as glycolipids and glycoproteins, where the carbohydrate moiety of these lipids and proteins might be the most important for their function.

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How Human Milk Bioactive Proteins Work

- Proteins present in high concentration
  - Source of amino acids
  - Exert biological function(s) in recipient infant
- Proteins present in low concentration
  - Unimportant as amino acid source
  - Exert biological function(s) in recipient infant
- Function(s) can be exerted by:
  - Intact protein (resistant to proteolysis)
  - Peptides formed during digestion
  - Complex molecules, such as glycolipids and glycoproteins

Functional/Bioactive Compounds in Human Milk

Lipids

- Gangliosides
- Sphingomeylin
- Oligosaccharides

Proteins

- Growth factors
- Enzymes
- Enzyme inhibitors
- Hormones
- Cytokines, chemokines
- Binding proteins
- Immunoglobulins

So, these bioactive components may contribute to optimal neurodevelopment, including cognitive development. They can improve nutrient absorption. They can protect against pathogens, and they can enhance immune system development.

Bioactive Components

- Contribute to optimal neurodevelopment, including cognitive development
- Improve nutrient absorption
- Protect against pathogens
- Enhance immune system development

Demmelmair H, et al. *Nutrients*. 2017;9. pii: E817.

Just to summarize: we have functional/bioactive compounds in human milk. Some of them are present in the milk fat globule membrane. These are lipids like the gangliosides, sphingomyelin. We have carbohydrates, like oligosaccharides. We have proteins, and these are growth factors; we have enzymes (for instance the bile salt-stimulated lipase that improves fat absorption). We have enzyme inhibitors, particularly, inhibitors of proteases. We have hormones, cytokines, chemokines, binding proteins, immunoglobulins, and most importantly immunoglobulin A.

We should not forget that there are also, in the MFGM, the long-chain polyunsaturated fatty acids [LCPUFA], particularly the inner MFGM layer contains phospholipids rich in long-chain polyunsaturated fatty acids, docosahexaenoic acid (DHA), or arachidonic acid (ARA). These are linked to myelination and synaptic transmission. They are present in high concentration in the brain. They are linked to brain development. And DHA and arachidonic acid were added to infant formulas some time ago, or rather quite a few years ago, and were shown to have positive effects.

LCPUFAs Are Also Bioactives

- Inner MFGM layer contains phospholipids rich in
  - Long-chain polyunsaturated fatty acids (LCPUFAs)
    - Docosahexaenoic acid (DHA)
    - Arachidonic acid (ARA)
- Linked to myelination and synaptic transmission
- Present in high concentration in the brain
- Linked to brain development
- DHA and ARA were added to infant formulas some time ago, with positive effects

Colombo J, et al. *Am J Clin Nutr*. 2013; 98: 403–412. Demmelmair H, et al. *Nutrients*. 2017;9. pii: E817.

Then I leave the work to you, Dr. Carlson.

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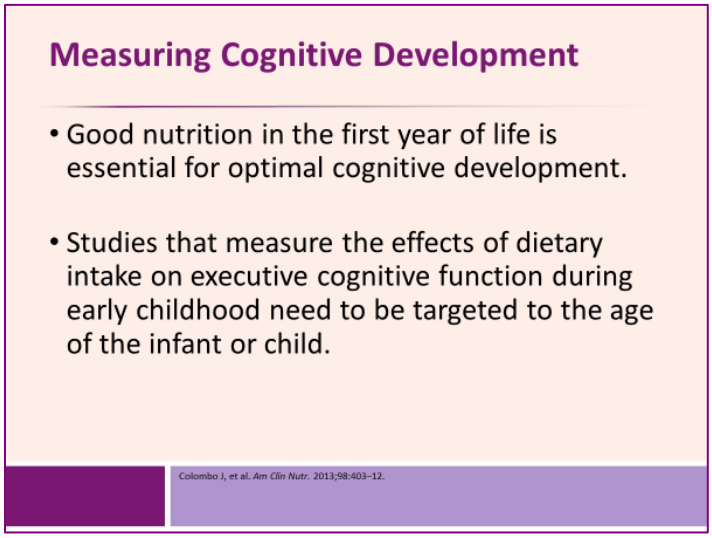
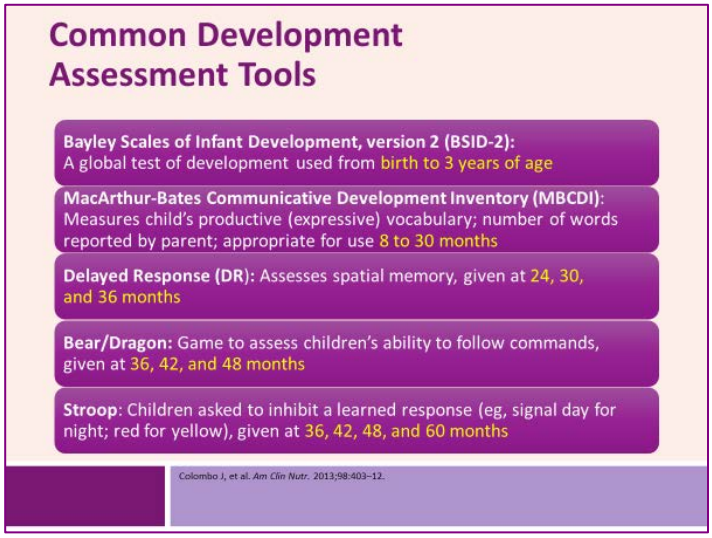
Dr. Carlson: Coming back to cognitive development and, of course, we all have a general understanding that good nutrition in the first year of life is essential for optimal cognitive development. But, in clinical trials or studies where we're trying to determine whether there's a benefit for cognition, we have to follow certain rules, and we have to try to use the best tests for measuring cognition. And there's a very important point that I want to emphasize here—and I'll talk in a moment about some of the tools that we have used in our studies in Kansas City—but it's very important to recognize that measures, when you try to measure the effect of dietary intake on executive or cognitive function during early childhood, you need to use tests that are targeted to the appropriate age for cognitive development.



Some of the tools that we have used in our studies, and others as well, and the ages that we used, are highlighted here in yellow. The Bayley Scales of Infant Development: this is a commonly used test. Version 2, there's now a version 3, but both versions are really global tests of development, and they are typically used in the first several years of life. By global, though, they aren't necessarily targeted to any specific cognitive domain. The reason for using more targeted tests, for example, the MacArthur-Bates, which is targeted towards language, and tests of spatial memory, tests of ability to follow commands, ability to inhibit responses, and so on. The reason for using these is because not all cognitive domains may be affected by a particular nutrient, even if you choose the appropriate age to look at those. But I think it's important to emphasize this, and I'll go to the next slide because these are some of the other tests we've used.



As children get a little bit older, Dimensional Change Card Sort, which measures whether children can basically discriminate after they've learned a rule, if they can reverse that and listen and change. Do they persevere with the old rule or can they learn the new rule? This is a test of mental flexibility—if you will—being able to put together puzzles. As children get older, we can start to use more tests that are more standardized, like the Peabody Picture Vocabulary Test, which we, in our studies, use up to age 5 years, but you can use even in adults, up to any age really, to determine vocabulary. It's kind of a quick measure of verbal IQ. Then, for children, again, the WPPSI, which is a widely used test of intelligence.

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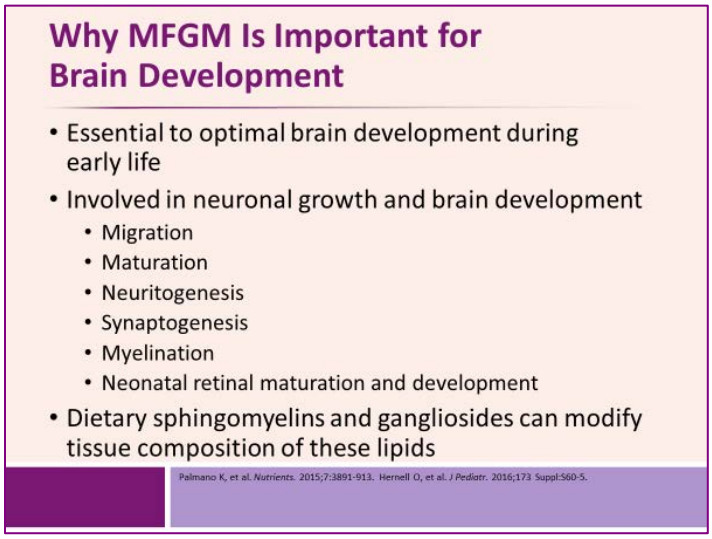
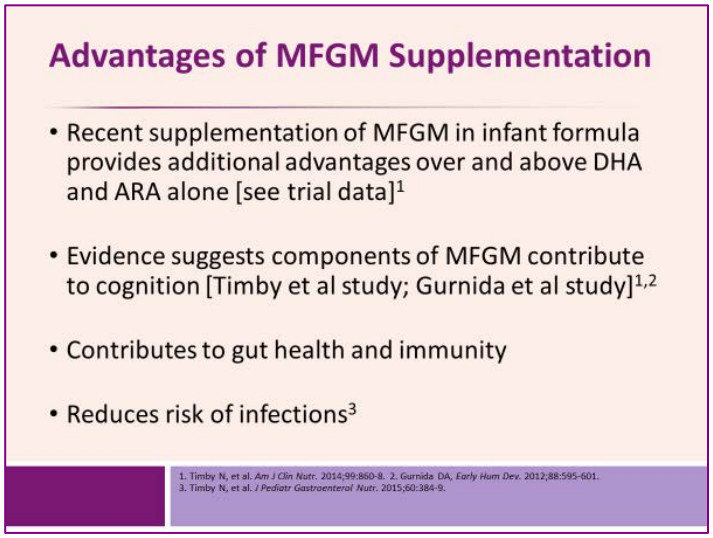
All of these are measures—and I think it's important to mention them—because if you look at the literature, you will often find a variety of different tests that are used, and sometimes people don't understand that those have been targeted to a certain age of the child. But it also makes it more confusing because some people don't recognize what is a cognitive outcome, and this has been actually plagued the meta-analysis in this area, I would say.

With that preamble I will turn it over to Dr. Hernell to talk about MFGM and brain development.

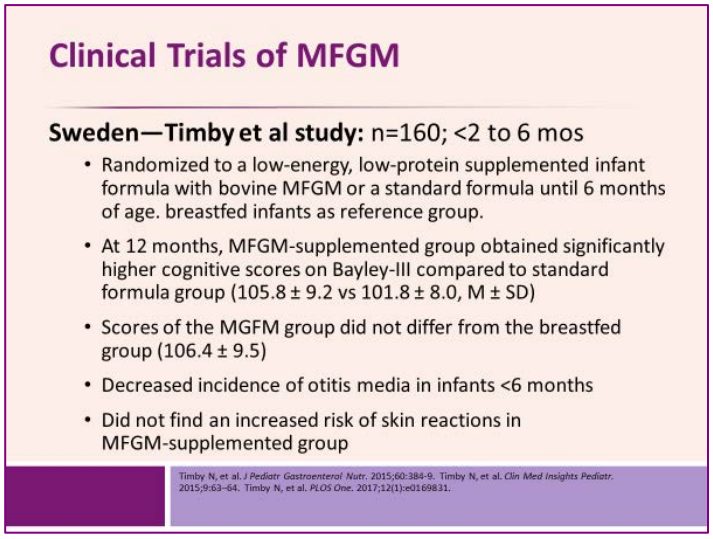
Dr. Hernell: So, why MFGM is important for brain development. They are essential to optimal brain development during early life. They are involved in neuronal growth and brain development: migration, maturation, neuritogenesis, synaptogenesis, myelination, and neonatal retinal maturation development. We know that dietary sphingomyelins and gangliosides can modify tissue composition of these lipids.



So, we have seen that in a recent study that supplementation of MFGM in infant formula provides additional advantages over and above what DHA and arachidonic alone have been shown to do. And, evidence suggests that components of MFGM contribute to cognitive development (and we come back to those studies). They have also shown that these components contribute to gut health and immunity and reduce risk of infections.



Now we will go through the studies that have been done so far showing the effects of MFGM in infants and young children. The first one here is a study that we did in Umeå, Sweden, where we compared a standard infant formula that was reduced in energy and protein, and then supplemented with an MFGM fraction, so that MFGM proteins constituted 4% of the total protein. The intervention was from 2 weeks up to 6 months—actually, it was from 2 months up to 6 months—[I'm sorry] of age. Breastfed infants were the reference group.



At 12 months, it was shown that the MFGM group had a little better cognitive development, and I can come back to that. They also had decreased incidences of otitis media during the first 6 months.

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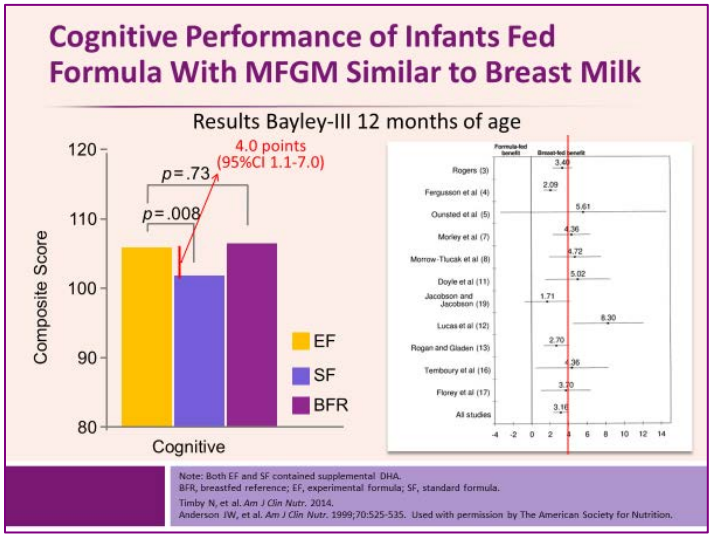
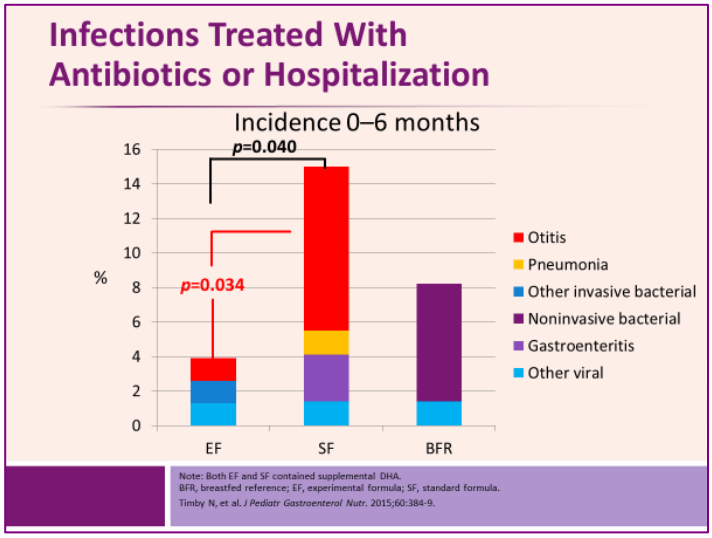
This shows, in more detail, the results. Here you have in the diagram to the left, in yellow, you have the experimental formula. In the middle is the standard formula, and to the right, the breastfed reference group. And, this shows a result to the Bayley-III test at 12 months of age. What you can see here is that the cognitive development is significantly higher in the experimental formula group that got the supplemented MFGM, compared to the standard formula group. The difference is 4 points, which corresponds quite well to what has been found as a difference between formula-fed and breastfed infants.



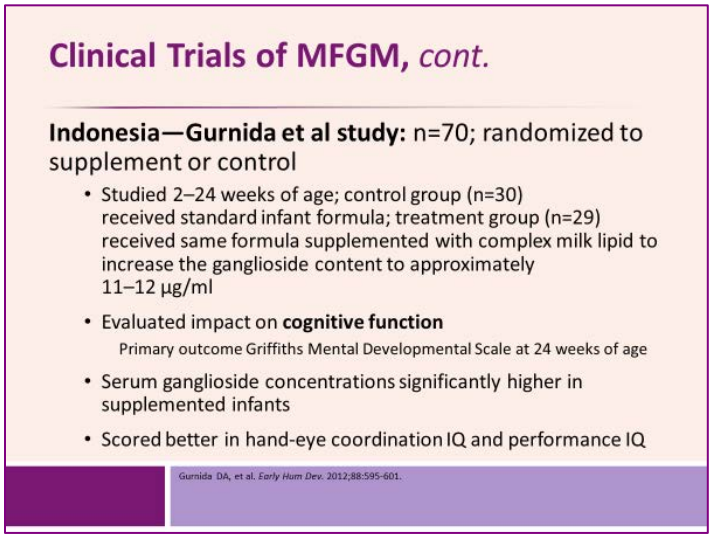
This shows a meta-analysis, and you can see that the average difference is 3 points higher for the breastfed compared to the formula-fed group, which is pretty much similar to what we found in the study. Interestingly enough, we didn't see a difference between the experimental group and the breastfed reference group.

We can now look at infections. You can see that these are the incidence of infections during the first 6 months, and infections was defined as infections treated with antibiotics, or if the infant was hospitalized due to infection. There is a significant difference between the experimental formula and the standard formula, with lower incidence of infections in the group that got the MFGM supplement. If you break it down to different infections, as you can see to the right here, that was particularly a difference with a lower incidence of otitis media. This group—the experimental formula group—also had a lower use of antipyretics.

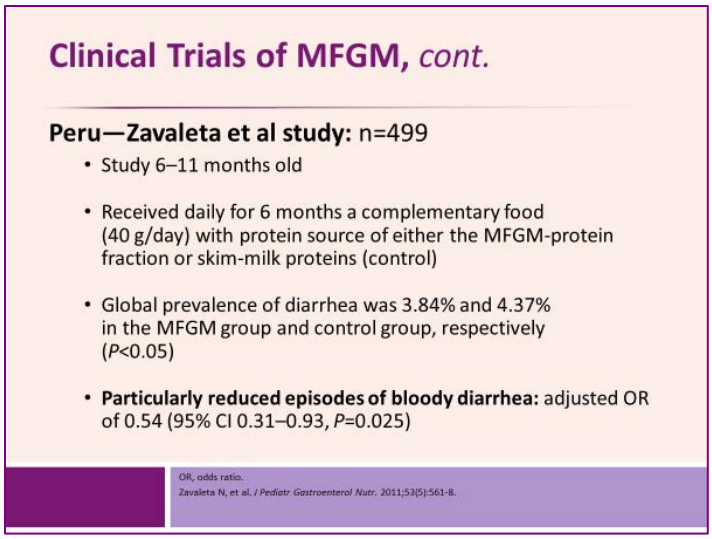


Dr. Carlson: I want to talk about a couple of other clinicals trials that have been done with complex milk lipids. One relatively small study done in Indonesia provided a formula with complex milk lipids that increased the ganglioside contents, specifically, of the formula. In that study the formula was fed till the infants were six months of age. They were evaluated using the Griffiths Mental Developmental Scale at that age, and they scored significantly higher in hand-eye coordination and another measure of performance IQ. Of course, this is a relatively small study. It only had 70 subjects, and it also has the limitation that it's rather difficult to look at cognitive function early on in the first six months of life. Nevertheless, the children did show higher level of performance.

Innovations That Are Closing the Gap Between Breast Milk and Infant Formula

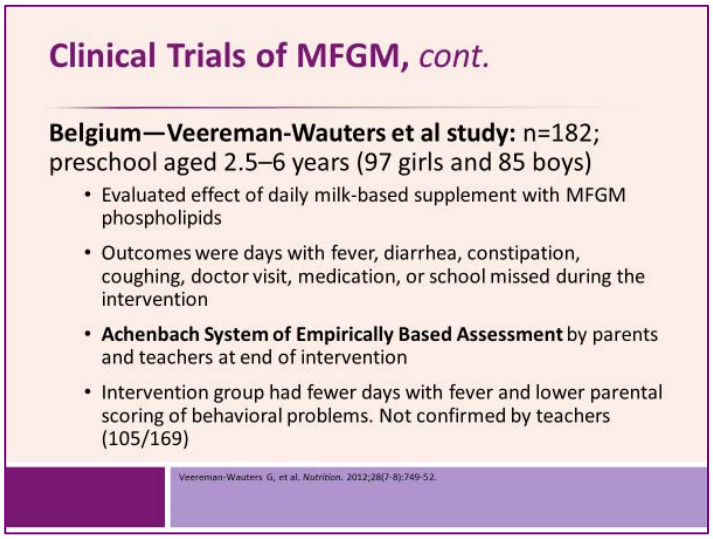


The much larger study done in Peru, by Zavaleta, included almost 500 children who were 6 to 11 months of age during the study. They received a complementary food that included MFGM proteins. What they found was significant reduction in global prevalence of diarrhea in the children who received the MFGM. This is a very nice outcome in a developing country where there might be some issues with GI infections.



I'll turn it over to Dr. Hernell to talk about some additional trials with MFGM.

Dr. Hernell: In this study from Belgium, that was a study that was carried out in older children, 2.5 to 6 years. The major outcome here was days with fever, but there were secondary outcomes, as you can see. What they studied was the effect of a daily milk-based supplement with a milk fat globule membrane fraction that was particularly enriched in phospholipids. At the end of the intervention, the parents and the teachers answered the questionnaire that evaluated behavior problems. What they found here was that the intervention group that was given the MFGM fraction had fewer days with fever and lower parental scoring of behavioral problems. This difference in behavioral problems, or improvement in behavior problems, that was not confirmed by the teachers when they answered the same questionnaire.



In France and Italy, there was another study, a non-inferior study carried out, and in this study they compared a standard formula with the same formula enriched with a MFGM fraction, enriched in lipids, and another one enriched with a protein-rich MGFM fraction. So, they compared 2 different MFGM fractions with a standard formula. The primary outcome here was weight gain and safety of these formulas.

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Clinical Trials With MFGM Components

France & Italy—Billeaud et al study: n=119

- Infants age ≤14 days were randomized to standard infant formula (control), standard formula enriched with a lipid-rich MFGM fraction (MFGM-L), or standard formula enriched with a protein-rich MFGM fraction (MFGM-P)
- **Primary outcome:** Mean weight gain and safety of infant formulas enriched with bovine milk MFGM fractions, protein-enriched or phospholipid-enriched
- Higher incidence of eczema (not clear how diagnosed or at what age) in group receiving high-protein MFGM preparation; however, in Timby et al, same protein-enriched MFGM preparation did not observe sign of increased incidence of skin reactions

Billeaud C, et al. *Clin Med Insights Pediatr.* 2014;8:51-60. Timby N, et al. *Clin Med Insights Pediatr.* 2015.

What they found was, actually, that there was no difference between the MFGM formulas—the 2 different MFGM formulas—or between these formulas and the control formula. Surprisingly enough, they found that the infants fed the MFGM protein-enriched formula had higher incidence of eczema. It was not really clear from the study how eczema was diagnosed or at what age it was diagnosed, and there were actually—in two of the groups—there was a quite low incidence of eczema, unexpectedly low. When we saw these results back to our studies that we did in Sweden, and the parents had actually noted every day if the infant had a rash or not. When we look through these data, we couldn't see that there was any difference between the groups with respect to rash. So, I think it's a little bit of a question mark here.

The last study is a study conducted in India. Here they used the milk powder supplemented with 2 g of a spray-dried ganglioside concentrate—so an MFGM fraction enriched in ganglioside this time. They assessed diarrheal morbidity, particularly rotavirus. Unfortunately, they didn't find any difference in primary outcome of rotavirus diarrhea or in secondary outcomes, including all-cause diarrhea. The reason was that they had an unexpectedly low incidence of diarrhea during the intervention, which means that, given this lower incidence, the study was underpowered to find a difference. But, they found that supplementation with this ganglioside preparation was acceptable and no adverse effects noted, and concluded that we need more randomized controlled trials.

Clinical Trials of MFGM, *cont.*

India—Poppitt et al study: n=450; infants 8–24 months

- Daily dose of milk powder supplemented with 2 g of a spray-dried ganglioside concentrate for 12 weeks
- Assessed diarrheal morbidity
- No difference in primary outcome of rotavirus diarrhea, or in secondary outcomes, including all-cause diarrhea
- High-ganglioside CML was acceptable for long-term consumption in infants ages 8 to 24 months
- More RCTs needed

CML, complex milk lipid; RCT, randomized controlled trials.
Poppitt SD, et al. *J Pediatr Gastroenterol Nutr.* 2014;59:167–171.

This just shows a table with the 6 studies that had been conducted so far.

MFGM Clinical Trials Summary

Study	Age	Supplementation	Results from MFGM Group
Timby et al 2015	<2 to 6 mo	MFGM	Higher cognitive score. Lower incidence of otitis media.
Gurnida et al 2012	2–8 to 24 wks	Complex milk lipids	Higher hand and eye coordination IQ, performance IQ and general IQ.
Zavaleta et al 2011	6 to 11 mos	MFGM	Lower longitudinal prevalence of diarrhea and incidence of bloody diarrhea.
Veereman-Wauters et al 2012	2.5 to 6 yrs, during 4 mos	MFGM	Fewer fever days; lower parental score of internal, external and total behavioral problems.
Billeaud et al 2014	14 days to 4 mo	Compared lipid-rich and protein-rich MFGM fractions	Noninferior weight gain or adverse effects. Higher rate of eczema in protein-rich MFGM group.
Poppitt et al 2014	8–24 mo, during 12 wks	Complex milk lipids	Diarrhea (rotavirus) prevention. No difference between groups.

What you can see here, if we go to the next slide, is that all these use different milk fat globule membrane fractions in these studies. They are carried out in different age groups. They looked for different outcomes. All that means is that these studies are not really comparable. You can't use them, for instance, to do a correct meta-analysis. Which means that we actually, before we can draw firm conclusions, we need more studies and preferentially with the same MFGM fractions. And, it would be great to have a dose-response study (where you have different doses to see whether you have an impact on the outcomes). Longer follow-up times would be good, as well, and also larger cohorts.

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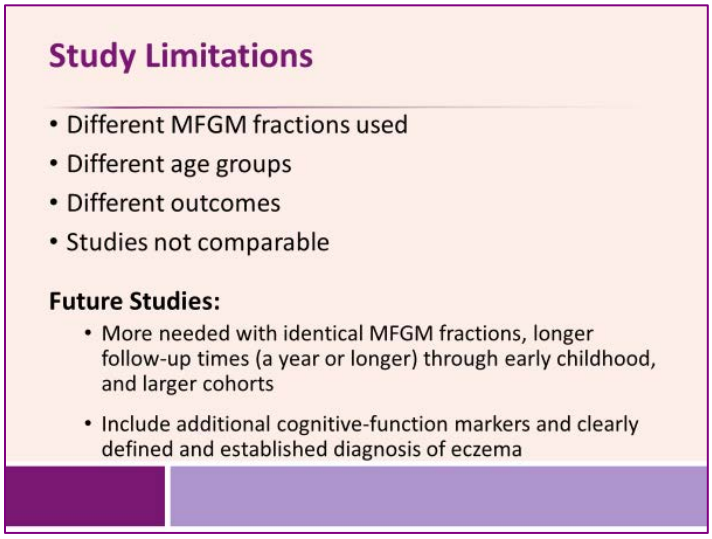
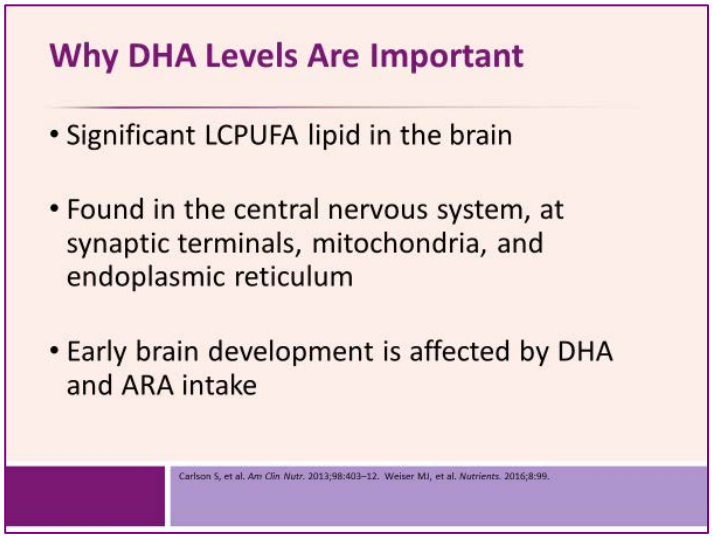




In such studies, of course, we would like to answer if there is an effect of eczema using better defined diagnostic criteria for that, and, also more cognitive function markers in such studies. So, these previous studies we discussed now, they will probably be a good base to design better studies or more studies with better power to draw firm conclusions.

And then I leave the work to you again, Dr. Carlson.

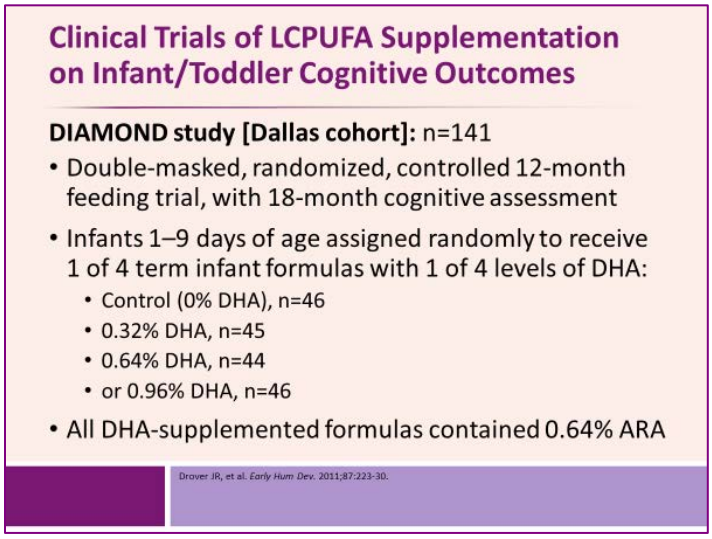
Dr. Carlson: I want to talk a bit about DHA because there is a long history of study of DHA in infant formula, and it provides kind of a model for maybe going forward with MFGM, in terms of what Dr. Hernell has just indicated that would be nice to have longer studies with longer outcomes. DHA, as many of you probably know, has been added worldwide to infant formulas for at least the last 15 years. It was initially studied because there are significant long-chain polyunsaturated fatty acids in the brain, particularly DHA and arachidonic acid. These are particularly located around synaptic terminals. That really promoted the idea that there could be some need for this nutrient, since it wasn't included in infant formula prior to those years.

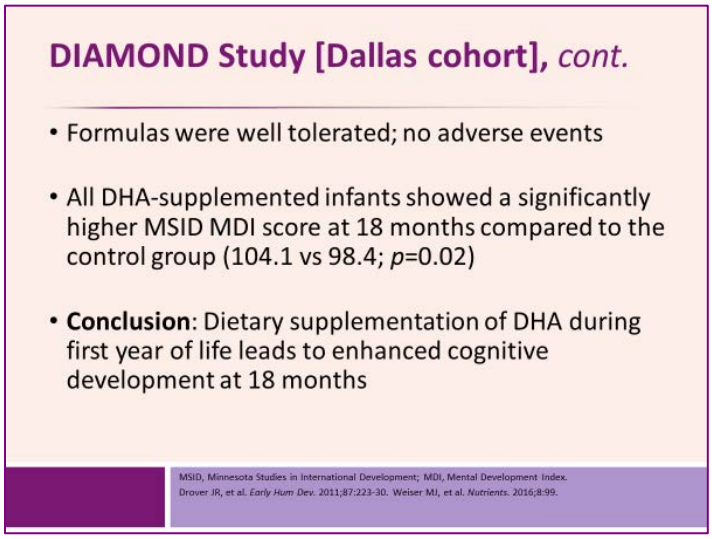
I want to talk about the DIAMOND trial, which was conducted in Dallas, Texas and in Kansas City. I think it has the advantage of being the longest studied trial of DHA and arachidonic acid supplementation that is in the literature. It began in 2002, and it concluded in 2004, as far as the intervention, which was supplementation in the first year of life with either a standard infant formula—at that time without LCPUFA—and then one of 3 different levels of DHA in the infant formula. The primary outcome of that trial was to look at the effect of DHA and arachidonic acid on visual acuity, and both sites combined their data for that. They showed a significant improvement in visual acuity, which is, of course, an important outcome. In addition, both sites asked for permission to follow their cohorts independently looking at cognitive development.

The slide that I'm going to show you here is from the Dallas cohort. They managed to follow 141 children from that cohort through 18 months. At 18 months, they looked at the Bayley Scale, I mentioned the Bayley Scales of Infant Development before as a more general global test of cognition. All the formulas included arachidonic acid, and that's important to say, because this is another important fatty acid in the brain. We didn't vary the arachidonic acid content. In the end, what they showed was that the formulas were very well tolerated at both sites. But, the DHA-supplemented infants, if they combined the groups, the 3 groups that got DHA had a significantly higher score on the Mental Developmental Index at 18 months compared to the control group. It was a rather large difference of almost 6 points on that scale. They

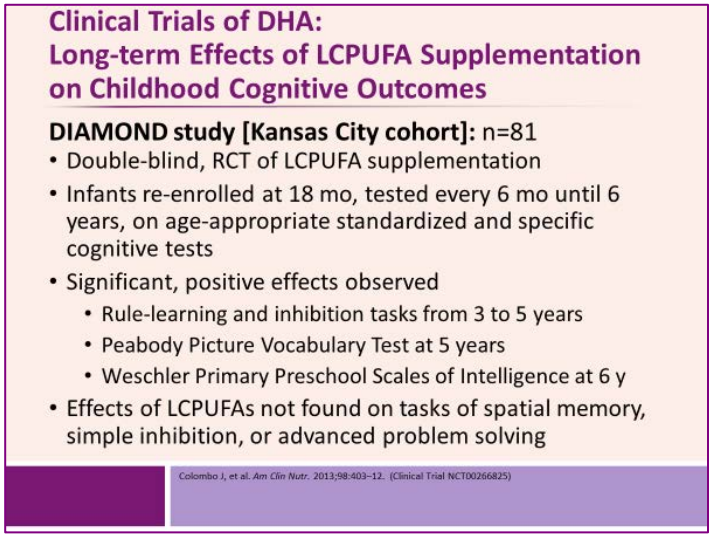
Innovations That Are Closing the Gap Between Breast Milk and Infant Formula

concluded that dietary supplementation of DHA during the first year of life leads to enhanced cognitive development.

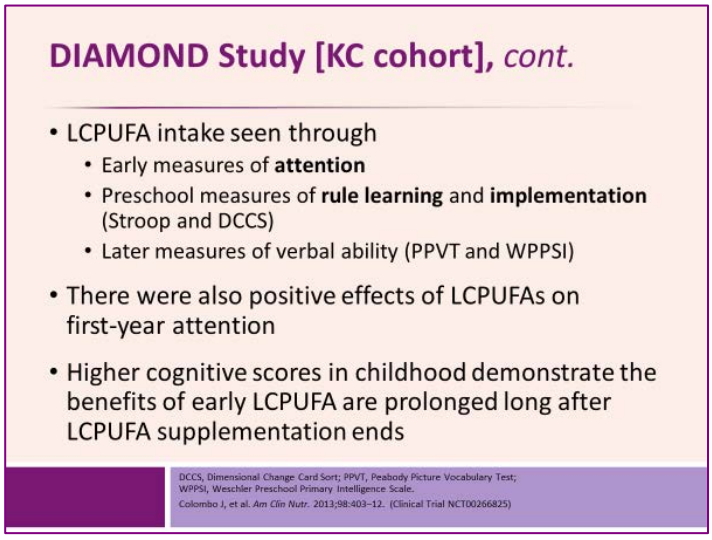




We also followed those children. We had 81 children from that, about evenly divided among the 4 groups. There were 3 groups that got DHA, as I said. We looked at the Bayley Scales, but we didn't have enough power to find a difference on the Bayley. However, we tested the children every 6 months until they were 6 years of age using these targeted tests that I mentioned earlier. What we found is not all domains were affected. For example, we didn't find effects on spatial memory or simple inhibition or advanced problem solving, but we did find some very significant effects on rule learning and inhibition, on processing speeds. We found higher verbal IQ using the Peabody and using the Weschler when these children were 5 and 6 years of age.



The LCPUFA intake really did affect... Also, and I didn't mention this, we published earlier that when the children were in infancy, they also showed improved attention, as well as what I just talked about: better rule learning and implementation during preschool and later verbal IQ. These were all positive findings that were all considered cognitive findings in infancy.



The significance of DHA in infant formula has been proven. The evidence shows that supplementation is important in the diet of infants for the first 24 months. The role we believe it's playing is its role in nerve cells and myelination and neurotransmission. In addition, these studies have all shown that DHA and arachidonic acid, which is typically added with DHA,

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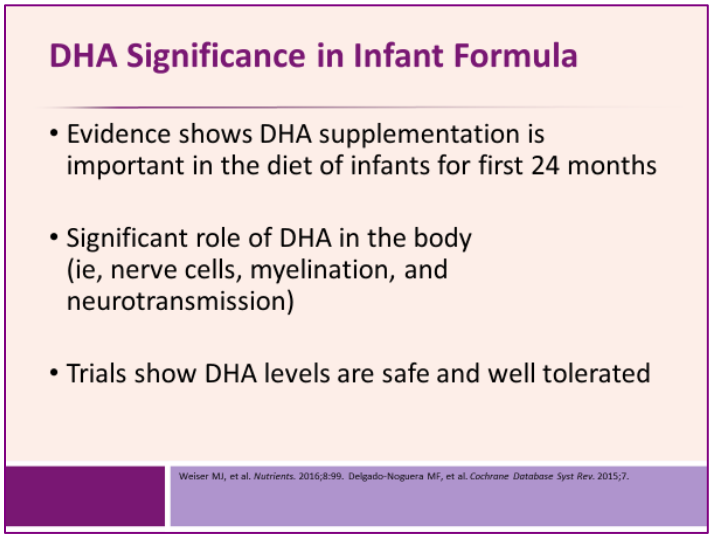
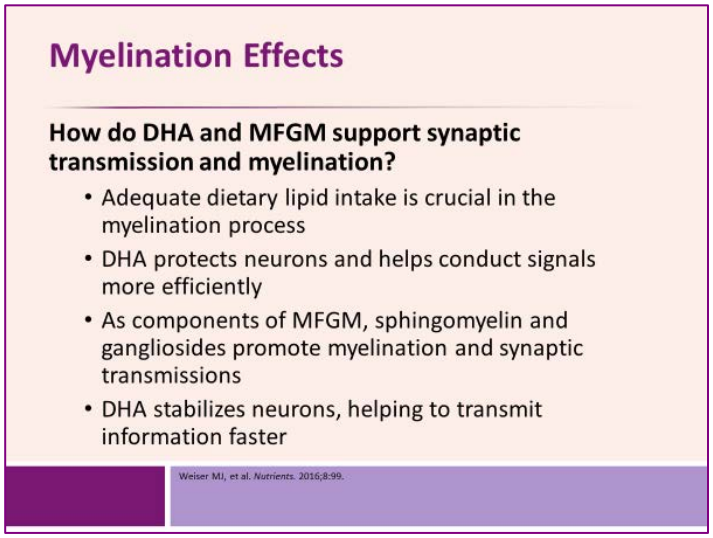
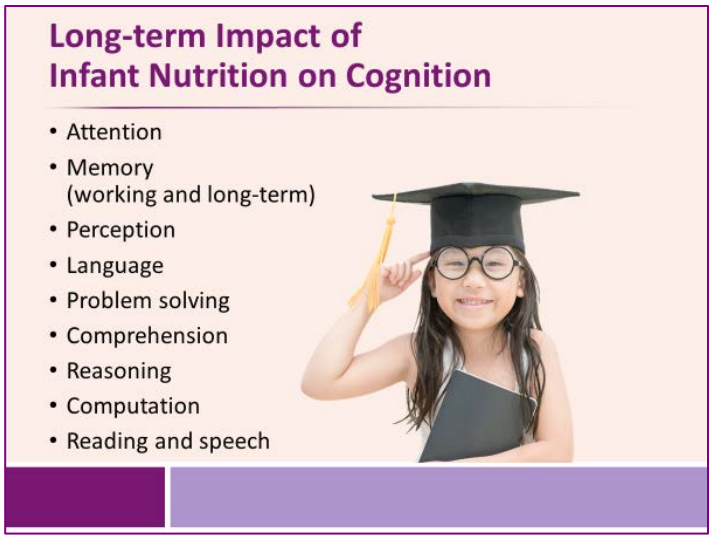
are the formulas are safe and very well tolerated. They have now been fed to millions of infants around the world.



I wanted just to say something here about myelination, because both DHA and the milk fat globule membrane support myelination. There's very old data from Manuela Martinez in Barcelona who studied children with Pseudo Zellweger. Those children cannot synthesize DHA because of their peroxisomal disorder. She showed, many years ago, that those children did not myelinate their brain until they were given a source of DHA from the diet. Even though they can't synthesize it, if you feed it to them they can then put DHA into their brain, and they can begin to myelinate. We've known for a long time that DHA is important for myelination, but, in addition, we know that the MFGM contains the very lipids that are found in myelin and can promote myelination and synaptic transmission because that's... that's actually one of the reasons we believe processing speeds could be faster in children who get DHA because, in fact, they are perhaps doing a better job of myelinating their brain, but that is really not a proven finding at this time.



Long-term impact of infant nutrition on cognition includes many kinds of outcomes that are considered cognitive, like attention and speed of processing, memory, perception, language, problem solving, comprehension, reasoning, computation, and speech and verbal abilities.



MFGM and DHA supplementation, as well, provide additional benefits for immune function. As well as they play a vital role in cognition and visual development, allergic, and respiratory diseases.

Innovations That Are Closing the Gap Between Breast Milk and Infant Formula

Behavioral Regulation Benefits in Young Children

- MFGM and DHA supplementation provide additional benefits for immune function
- Continued studies are needed to examine evidence for safety and tolerance
- LCPUFA plays a vital role in health, cognition, and visual development, allergic and respiratory diseases

I'll turn it over to Dr. Hernell to summarize some of this that we've been talking about.

Dr. Hernell: I think we can conclude now that the dairy industry has developed technology that now allows the addition of bovine MFGM to infant formula. The double-blind RCTs, so far, provide some evidence that inclusion of MFGM and DHA in formula is safe. No serious adverse effects have been shown. The inclusion of MFGM and DHA enhances neurodevelopment and improves defense against infections.

Technological Advances in Infant Formula

- Technology now allows the addition of bovine MFGM to infant formula
- Double-blind RCTs provide evidence:
  - Inclusion of MFGM & DHA in formula is safe
  - Inclusion of MFGM & DHA enhances neurodevelopment and improves defense against infections

Hernell O, et al. *J Pediatr.* 2016;173 Suppl:S60-5.

MFGM and DHA enrichments in the infant formula have shown cognitive outcomes, which are pretty similar to breastfed infants. Although, as mentioned, we need more studies. Studies so far show that MFGM and DHA supplements can provide important nutrients to diets of infants. Again, although we need more studies, those studies that we have show promising results regarding both neurodevelopment and defense against infections.

How Bioactives Are Reducing the Nutritional Gap

- MFGM and DHA enrichments in infant formula have shown cognitive outcomes similar to breast milk [see DIAMOND trial & Timby et al 2015 study].
- Although more are needed, studies show MFGM and DHA supplements can provide essential nutrients to diets of infants.
- Although more are needed, studies show promising results regarding both neurodevelopment and defense against infections.

Hernell O, et al. *J Pediatr.* 2016;173 Suppl:S60-5.

To summarize, then, DHA and MFGM are found in breast milk. Advances in dairy technology allow MFGM and DHA to be supplemented at levels similar to breast milk. Bovine MFGM [is] similar to human MFGM; they are not identical, but they are pretty similar. DHA plus MFGM supplemented at clinically effective levels have demonstrated improved cognition similar to breastfed infants.

DHA and MFGM Combined Benefits

- DHA and MFGM are found in breast milk
- Advances in dairy technology allow MFGM and DHA to be supplemented at levels similar to breast milk
- Bovine MFGM similar to human MFGM[1,2]
- DHA plus MFGM supplemented at clinically effective levels have demonstrated improved cognition similar to breast milk

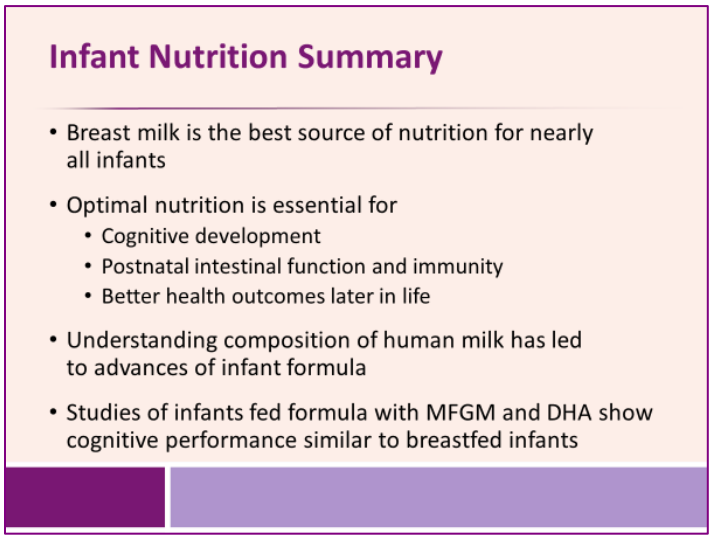
1. Lönnerdal B. *Am J Clin Nutr.* 2014;99:712S-7S. 2. Timby N, et al. *J Pediatr Gastroenterol Nutr.* 2015;60:384-9.

And then I leave the work to you again, Dr. Carlson.

Innovations That Are Closing the Gap Between Breast Milk and Infant Formula

Dr. Carlson: In summary, as we've tried to point out in our presentation today, breast milk is the best source of nutrition for nearly all infants. Optimal nutrition is essential for cognitive development, postnatal intestinal function, and immunity and better health outcomes later in life. Understanding the composition of human milk has led to advances in infant formula. Studies of infants fed formula with MFGM and DHA show cognitive performance similar to breastfed infants, but there may be other health benefits, as well of these compounds. The story on immunity is certainly emerging. These questions can be explored in new and larger studies, particularly with MFGM.

With that, I'll turn it over to our moderator and let her entertain questions for us.



Question & Answer

Is MFGM-supplemented formula available in the US?

Dr. Hernell: To the best of my knowledge, yes, it is.

Are there studies currently underway which address some of the MFGM study limitations you noted?

Dr. Carlson: I happen to know that there is discussion by a number of different people of doing larger studies, but whether those are…, there some ongoing? I couldn't speak to that.

Dr. Hernell: I know this about one study that has been conducted in China. It has not been published, yet, but it is ongoing. I would guess that there are also studies, but I don't know any details about that.

If an infant formula contains DHA and ARA, is MFGM supplementation necessary?

Dr. Carlson: I would say that the MFGM, from the Timby study, which is a large study in a healthy population, is still showing an additional benefit of the MFGM for cognitive function, so I think that's quite interesting. Of course, we would like to repeat that study. We always like to have more than one study that shows that, but I'm impressed by that data that the MFGM may be adding some additional benefit. Quite honestly, it would be my thought that it would because I have been interested for some time in the interesting lipids that are in human milk that are not in infant formula, like the gangliosides, like sialic acid. It doesn't surprise me that there would be additional benefit.

You indicated that DHA levels were safe and well tolerated. How safe and well tolerated were the MFGM-supplemented formulas?

Dr. Hernell: All studies so far have shown that it is safe, and you wouldn't expect anything else because MFGM is nothing new. I mean, everyone who consumes milk (bovine milk) with milk fat will consume MFGM. It's not anything new; it's just an enriched fraction from bovine milk.

Dr. Carlson: I would just add to that, that it would be very surprising to me to have significant problems with these because these are primarily lipid fractions. There's very little protein in there, and the infant formulas that they're being compared to already have cow-milk proteins. It's probably not making any real impact on the composition other than mainly the lipids, and they are very, very specific lipids found in infant formula.

Dr. Hernell: There are proteins in the MFGM fraction, and I don't think you can exclude... For instance, if you are allergic to milk, I don't think you can recommend a fraction of MFGM. Most of the fractions that have

been used in the studies that we have discussed, they contain bovine made proteins.

Are there specific subgroups of infants who might benefit more from MFGM-supplemented formula than others? For example, preterm infants.

Dr. Carlson: Well, we know that the brain of the preterm infant is less mature, and it needs to accumulate these compounds at a higher rate than the term baby. It wouldn't be surprising, but of course it's speculative, because I'm not aware of any studies in preterm infants that have used MFGM. Certainly, this is an area to explore.

Should formulas with DHA and ARA supplementation be recommended for normal-term infants that are fed by formula as a public health message? Do you think there is adequate data to support this?

Dr. Carlson: I will take that answer in 2 parts. The first answer is yes. The second answer is yes. I do want to qualify by saying that it would be very hard to find an infant formula without DHA and arachidonic acid because this has become the standard, at least in the United States.

Evidence to support: I want to say that this has been very confused by the meta-analysis people who are often math people and not biologists. There's actually an ongoing debate going on in the country right now among people who are actually scientists and people who are writing things like the ARC analyses who don't necessarily understand what is a cognitive function, but then opine about cognitive function. They have actually based most of their conclusions on the Bayley, because they don't understand some of these cognitive outcomes that I explained in the talk. They don't understand their cognitive outcomes, so they don't quite know how to put them into the analysis. Based on our own results, I am very convinced that it's important to DHA and arachidonic acid in infant formula.

Why is MFGM removed and then added back? Why don't formulations just leave MFGM in? Is MFGM in formula available in Canada?

Dr. Hernell: The last part of the question, I don't know. I don't know whether it is available in Canada.

The reason why milk fat has not been used in infant formula, it goes back a long time when we had the idea that fat from dairy products [is] saturated and increases cholesterol levels, and it should be avoided. Because we are very cautious about feeding newborn infants things that we think [are] not healthy. That is the reason why the milk fat was discarded and replaced by vegetable oils. Now, several companies have started to add milk fat and MFGM to the formulas. So, it's coming back. So, the question is a good question, I think.

Dr. Carlson: I think people aren't aware that infant formulas have been made traditionally with vegetable oil, which is probably a throwback to the '50s when people, as Dr. Hernell says, became very concerned about saturated fat. We started feeding babies like 50-year-old men with cardiovascular issues.

In the studies where powdered formula, a ready-to-feed formula was used, was the water used for mixing formula sterilized?

Dr. Hernell: It should be, of course. The reason being that we should always be very careful to have clean water when you prepare an infant formula. You should also heat the product to at least 67° or even more, 70°, which will have a pasteurizing effect, so you get rid of any bacteria.

Does MFGM affect the oral microbiome?

Dr. Hernell: Yes, it does. It affects the oral microbiome. One thing that we have found—in a paper that we have published—was that compared to infants fed a standard formula, infants fed the same formula but with the MFGM fraction, had a lower frequency of *Moraxella catarrhalis*. *Moraxella catarrhalis* is actually associated with otitis media. We think that might be at least one of the reasons why infants fed the MFGM formula had lower incidence of otitis media.