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TRANSCRIPT

Supporting Neurodevelopment With Brain-Building Nutrition

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The First 1,000 Days: A Critical Period of Growth and Development

Magnus Domellöf, MD, PhD: I will talk a little about *The First 1,000 Days: A Critical Period of Growth and Development*. So, we humans, we have big brains, and we are very proud of our big brains. We have the largest brains of all species on the planet except for the large mammals, such as elephants, but we have many more neurons than they have. Also, the increase in brain size was very instrumental in our evolution and led to evolutionary advantages. So, we're really happy with the big brains.

During the fetal development and during the early child development, the brain has a growth spurt. The brain is actually the fastest growing organ in infants and toddlers and, at birth, the average weight of the brain is 400 g, and, at 3 years of age, the brain has tripled its size to 1200 g, which means that it's almost at the adult size. So, really fast growth of the brain, but it also has very rapid development during this same period. So, we can see here, during the fetal development, we start with the neural proliferation, where the neural stem cells are differentiating into the glial progenitor cells and the neuroblasts and developing all these neurons. And then, at the same time or parallel to that, we have the migration process where the neurons migrate from the ventricular area in the central brain and out and forming the cerebral cortex. And then the next stage, which occurs a little later during pregnancy, which stretches all through infancy and toddlerhood, is the arborization of the neurons where they form dendrites and later on, synapses and connections. And last, we have the process of myelination, which you can see on top here, which also goes on from before birth and beyond 2 years of age.

We can see that the first 1,000 days are extremely important for brain development as well as brain growth.

Assessing and Measuring Developmental Outcomes

John Colombo, PhD: The development of the brain presents some interesting challenges for the assessment and measurement of developmental outcomes, in particular in clinical trials. We conceptualize this—I'm a developmental psychologist and a neuroscientist—and the way we, in our field, conceptualize these cognitive functions is in terms of a 2-tiered system. There are simple lower-order components that are mediated or controlled by parts of the brain that develop very early, and these include things that basically get information into the brain and then moderate the processes that lead to the expression of an action. These include things like attention: a stimulus will occur, then you can, of course, turn that into neural energy. You attend to that event or stimulus; you may store it for a brief period (and there are 2 types of storage), and then you basically choose—or it leads to, initially, an action.

What's missing from this particular diagram is the development of higher-order components that actually control all of these processes. And these aspects are mediated by parts of the brain that develop relatively late—that is, relatively in the second, third and fourth year, and then continue on through adolescence and into early adulthood—which are the frontal lobes. These higher-order components control all of these lower-order components, and we consider these to be what we call executive functions: the decision to inhibit responses, the organization of behavior around attaining goals, the organization around behaviors that are dictated by rules, mental flexibility. All of these—and its highest 1, problem-solving—are, in fact, mediated by these executive functions that interface with all the lower functions and which develop a little bit later.

As I said, these developmental functions develop—have their own courses. If you're going to measure something in a clinical trial that happens very early, you might choose a basic vital function. If you are measuring in the first to second year, you might measure these lower-order components. But what's

really of interest is what happens—if you're interested in the long-term effects of a nutrient or any kind of intervention happening early, the way you would do that is you would measure something in the executive function range, which is anywhere from about 2½ to 5 or 6 years of age. And I'll be talking a little bit about how we do that in the next few slides.

And there's ways to do this in different ways. There's ways to do this using different kinds of measures. You can use screening assessments. You can use parent-report measures. You can use standardized global developmental measures. You're probably familiar with screening assessments that are meant just to detect whether a child is grossly out of range in terms of attaining developmental milestones. Parent-report measures are not always the best source, but they can be useful at times because you're getting this through a parental filter. Standardized global measurements are good for, again, attaining whether or not a child is delayed, but sometimes they're not particularly sensitive to variations within the normal range or within the typically developing ranges.

I'm a fan of using specific cognitive skills tests—especially with respect to measuring individual differences, and we'll talk about some of these now. An example of how we measure executive function at 3 years and above is something called the Stroop task, which is basically the idea that you ask a child to give a response to a stimulus that is not what they would normally do. So, in order to do this, what they have to do is they have to inhibit the normal response—the typical response. They have to remember that rule for inhibiting, and then they have to hold that information in working memory to express the correct answer. And here are 2 examples. We will often present these on a computer, either a red screen or a yellow screen, or if the child doesn't know their colors, we associate these with a fruit, for example. And if we present the red screen, we ask the child to give us the wrong answer. We ask them, say, "When you see a red screen, I want you to tell me that it's yellow" or "When I show you a yellow screen, I want you to tell me that it's red." Again, the idea here is that the child has to inhibit the normal response, the typical response, the prepotent response, and give you the other response. The other variant of this task is the day/night where I show a picture of a sun, the child has to say night. Or I will show them a picture of a moon and stars, and they have to say day. So, you basically get the idea there.

There's another task that measures executive function. This is called the Dimensional Change Card Sort. And here we have stimuli that can be sorted on more than 1 dimension. That is, they are comprised of either geometric shapes or items that have particular shapes and then also that are of a different color. And here, you have to actually have the child learn the rule for how to sort these, and then you go through a series of items to demonstrate mastery of that before telling them that you now have changed the rule. And then they have to adapt to the new rule. So, you're measuring cognitive flexibility.

Here are examples of how we go about doing this. The simple rule is you sort by color, that would be an example. So, you put blue things with blue things irrespective of whatever shape they might be or red things with red things. Once they've mastered that, you then say, "Well, we're now going to sort by the other dimension, by shape." Putting circles with circles, for example here, irrespective of their color, and squares with squares. There is a final one, a final phase, if children actually pass through all of these, where we then move into an area where the child has to behave conditionally, and where you have the cards without borders, which should be sorted by 1 dimension, for example by color, but if there's a border there, then you sort by shape. And this, again, is a measure of conditional thinking, which is pretty advanced and sometimes doesn't happen until about 5, 5½ years of age.

One last task, which we use with brain measures, is the Go/No-Go. And basically, this seems like a very easy task, but it's often found that children cannot inhibit or cannot do this task very well until often about 5, 4½ or 5 years of age. Here what you do is, you present a series of stimuli that are similar and for which the child is asked, for example, "Hit the space bar every time you see these." They're presented 1 at a time. And these are called the Go stimuli, basically you "go," you hit the bar when you see these. But your child is asked also to—when they see a stimulus that is similar, but which is different, then that's your distractor—you don't. You withhold the button press, so they withhold pressing the space bar. And it's very interesting to watch real young kids do this because they often, they cannot inhibit the bar press early on. At later ages, they still can't inhibit

it, but they at least show remorse when they do. And then, finally, they begin to follow the directions.

Human Milk: The Model for Optimal Early Nutrition

Domellöf: The American Academy of Pediatrics advocates for improving nutrition during the first 1,000 days to support optimal development. And understanding nutrition requires an understanding of the "complex interplay" of the various nutrients that can contribute to brain development. And this is a very simplified list of nutrients and what, as you can see, many of these nutrients (protein, energy, fatty acids, iron, iodine, zinc, choline, B vitamins) are important for different developmental processes that were mentioned previously. So, certainly some of these nutrients are very important for brain development and we have shown, for example, the importance of iron in some studies.

But I think not only the nutrients are important, but also the matrix, the whole food is important, and we know that breastfeeding is the gold standard for infant nutrition, and health benefits of breastfeeding include reduced risk of infections and improved brain development, which we will talk about more today. We know that if you compare individuals who were breastfed when they were young and those that were formula-fed, we know that, at school age and also in adulthood, they have higher intelligence quotient (IQ) scores and the difference is usually about 3 to 5 points. Of course, these are observational studies, and it's difficult to demonstrate causality, but there have been efforts done. This is a very nice meta-analysis where they included 17 studies, and they adjusted for multiple confounders, including socioeconomic status and maternal education. Most of these studies are from high-income countries, and they still observed these differences. Also, we can see that, in this study, the breastfed subjects did achieve higher IQ with a mean difference of 3.4 points and, interestingly, there were similar results in the smaller studies and the larger studies, which suggest that this is not publication bias, and there were some studies that actually controlled for maternal IQ. They actually measured the IQ in the mother and then controlled for it, and still there was a significant difference of about 2.6 points. So, certainly this is not a huge difference on an individual level, but it is on a societal level. It's quite a large difference.

What are the things in breast milk that can have such an effect on brain development? Well, breast milk is a highly complex biological tissue. It's not just any food. This is, to the right here, we can see an electron microscope picture of what breast milk looks like in the electron microscope. It contains oligosaccharides, nonprotein nitrogen, nucleotides, complex lipids, growth factors, hormones, cytokines, bioactive peptides, enzymes, immunoglobulins, leukocytes, live bacteria, exosomes, stem cells, etc. So, it's a really, really complex substance. And we have focused a lot on different bioactive components of breast milk, and those are defined as components having a health effect beyond their purely nutritional contribution, for example energy and macronutrient intakes. So, and the bioactive components may improve immune function, promote neurodevelopment and/or prevent morbidities.

The component that we'll talk a little more about today and that we have studied is called the milk fat globule membrane (MFGM). And you know that milk is white because it contains a lot of small fat droplets, and those fat droplets are produced in the mammary gland and when they are excreted, they will be covered by a triple-layer phospholipid membrane, as you see here, and it contains a lot of phospholipids and complex lipids and also proteins and glycoproteins, etc. And some of these components have been shown in animal experiments to be important for brain development, such as choline, sphingomyelin, gangliosides, etc. And some have been shown to be important for immune defense, such as mucins, butyrophilin, lactadherin, etc. And actually, these membranes are present in breast milk, but they're also present in cow's milk, but when we talk about infant formula, when you produce it from cow's milk, actually, in the regular formulas, you would normally discard the dairy fat and you would include, instead, vegetable fat. So, you would throw away these membranes. So, we think that these can be very important bioactive components.

MFGM: Structure and Functions

Colombo: As you can probably guess from Magnus' description, the MFGM has potential benefit to a lot of different

aspects of biologic activity, including neural activity. As you indicated, the MFGM is a 3-layer membrane. It consists of fats or lipids, glycolipids and protein and this membrane actually encapsulates the fat or triacylglycerol-rich milk fat globules. This membrane is actually—as the fats are excreted from the mammary gland, they are encased in this through exocytosis, and so it's just a natural process and a natural aspect of human milk to have these. As you might expect, these components will have potential wide-ranging effects. For example, brain—and Magnus has already talked about immune and gut and microbiome functions—in particular, we are interested, and I am as a developmental neuroscientist, in the potential neural effects here, in particular sphingomyelin and glycosphingolipids, lipids or gangliosides, are really highly concentrated in the brain and if MFGM is extracted from formula, you're missing these. Over the last few years, there's been mounting evidence that these aspects are directly related to synaptogenesis and myelination, both fundamental aspects of brain development. Synaptogenesis, the connection of neurons with one another, and myelination (the generation of myelin), which improves neural transmission and increases the speed of signaling inside the brain.

We would expect that you would see improved cognitive scores, improved developmental function, developmental status, potentially, if these things are affecting executive function, you might expect to see improved social and emotional management, behavioral management in children, improved memory and you might actually expect to see fewer clinical problems as a result of adding these back in. Our team has done quite a bit of research on MFGM and its components over the last 5 or 10 years and I will be able to talk a little bit about those benefits in a minute.

Potential Benefits of MFGM Supplementation in Infant Formula

Human milk and standard infant formula vary in whether they have these membranes. In human milk fat droplets, there is high sphingomyelin content. It varies across different stages of lactation. The fat globules are very large and there is this phospholipid bilayer membrane. But in standard infant formula, we don't have that. While there is a high phospholipid content and a high phosphatidylcholine content, the globules, due to homogenization, are really small and there's no phospholipid bilayer membrane. And that's 1 of the things that we're interested in putting back in formula.

Our own research, which was recently published in 2 papers in *The Journal of Pediatrics*, looked at the effects of adding the MFGM back into infant formula, and this was the Lighthouse MFGM Clinical Trial, which we ran in Shanghai. We enrolled 450 infants at birth and randomly assigned them to 2 different formulae for feeding for the first 12 months of life. One was a standard cow's milk-based formula, and the other one was that same formula, but with added bovine MFGM and also with lactoferrin, which is part of a standard formula, formulation. Our initial thoughts were to examine the differences in these 2 groups on the Bayley Scales at 12 months, which is a standard global sort of development status assessment, and then to also look at tolerability, safety, growth and other measures of development.

I'll say up front that both formulas here were tolerated quite well, but that we actually had fewer adverse events with the MFGM and lactoferrin formula. There were no differences in growth, so it didn't affect growth, and we did take other measures of development along the way. In particular, we did take—with a sample this large, 1 of the ways you have to operate if you want to get data on everyone on a sort of continuing basis, is to measure, is to ask parents how these children are doing in terms of their development. And although I'm not a huge fan of parental report on these kinds of measures, this was a randomized, controlled, double-blind study, so no one knew what formula they were getting. And so I was encouraged to see, in fact, that if you actually measured, asked parents how their children were doing at 3 different points across the first year, on all of the domains (communication, gross motor, fine motor, problem solving and personal social skills), we saw big differences—statistically significant differences on all those domains in favor of the MFGM formula. And when we measured the Bayley at 12 months, we also saw significantly improved scores on cognitive domain, on the language domain, and the motor domain. And that's represented by the red bars on the MFGM part of the graph here. No differences in social-emotional. No difference on general adaptive function, but we did see these. Now, when

we followed these kids back up at 18 months, we didn't see these differences, but on the language measure, we did see significant differences that made us think that, in fact, and these were complex, language complexity, language sentence complexity, the addition of grammatical markers, that made us think that it was worth going back out and actually measuring these kids again later on.

So, we followed them up again in a follow-up design where we took the children who were enrolled for the first 12 months and measured them at 5½ years. And I want to remind everybody that we stopped feeding them at 12 months. We stopped feeding them these formulas at 12 months. And we were able to bring back quite a few participants in this follow-up study where we actually measured IQ at 5½ years of age as well as a couple of other executive function measures. And we'll talk about this here in the next few slides.

Visual spatial was marginally significant and although infants who were fed the MFGM were higher on both fluid reasoning, working memory, those particular domains did not attain significance there. Of most interest to us here was the fact that infants fed MFGM were significantly better on processing speed, and if you remember that sphingomyelin in MFGM is supposed to be contributing to the development of myelination, this makes perfect sense. The full-scale IQ, which is a composite of all of these domains, actually also attained statistical significance, and I would point out that, as someone who's worked in the area of human intelligence for the last 30 years of my career, improving IQ is not an easy thing to do. And I think this is a particularly interesting and important finding, especially given that we fed these children for the first 12 months and that we continue to see differences here at 5½ years of age.

As I've mentioned before, we did follow up these children with executive function scores on the Stroop tasks and we found that, in fact, on both the day/night and the red/yellow tasks, children fed MFGM and lactoferrin did better on those outcomes. And then we also found that, on the Dimensional Change Card Sort, children fed MFGM and lactoferrin were significantly more likely to pass the most difficult aspect of the Dimensional Change Card Sort task, which is the conditional phase, where if the stimulus is surrounded by a border, you use 1 rule and if the stimulus is not surrounded by a border, you use a different rule. This particular phase, I would remind everybody, is really pretty challenging and even for adults who I've tested with over the years.

Other things that are potential benefits include improvements in adaptive behavior, reduction in infection rates which, and it's important to remember that if a child is healthy, that will indirectly affect cognition. Maintenance of intestinal barrier integrity, again improving health, and lastly modulation of the gut microbiome which actually can have potential behavioral effects as well.

MFGM: Additional Data, Clinical Applications, and Ongoing Questions

Domellöf: We actually did also a randomized, controlled trial on MFGM, and it was published 10 years ago, so it was a long time ago. And we were randomizing 160 healthy formula-fed infants to receive either a standard formula or a standard formula supplemented with MFGM up to 6 months of age. And we also had a breastfed reference group, and as you can see here, when we tested these children using the Bayley Scale of Infant Development, we did not find any difference in verbal or motor score, but we did find actually a significant difference in cognitive score. You can see the red bar here represents the MFGM group, and the second bar is the standard formula, and the blue bar is the breastfed. You can see that the MFGM group actually had 4 points higher on this Bayley cognitive test at 12 months of age. So that's very interesting results and aligns with what John presented.

We also found—in addition to these improved cognitive scores at 12 months—we also observed a reduced infection rate from during the first 6 months of life when the babies consumed this formula, especially the effect was strong on acute otitis media. When we followed up—we also did a follow-up study of these children—however, we did not find any remaining effect on neurodevelopment or anthropometric or metabolic effects.

Those were our results, but there has been a lot of interest in this area and since our study, there have been a number of studies. I think these are supposed to be a comprehensive summary of all of them since 2014, and you can see some of them have used just MFGM as the intervention, and the rest of

them have been MFGM in combination with other components. Three of them have actually had safety and tolerance and growth as the outcome and they have shown—so I think we can conclude that, for this intervention, at least that it's safe. And then we have 3 studies, including the 1 that John talked about, that did study neurodevelopment, and with these 3 published studies, all of them show some positive effects, even though they have tested different things and they had different interventions.

Where I would say that MFGM is—as a supplement to infant formula—is still very promising. It seems, it might have a positive effect on neurodevelopment. Actually, 4 out of 4 RCTs have shown some effect, 1 out of 3 have shown a remaining effect at 5 to 6 years of age. It also might have an effect of prevention of infections: 5 out of 7 RCTs have shown some effect. However, there have been different interventions and outcomes and time periods. There are some remaining challenges because all of these studies have used different MFGM products with different lipid and protein composition, so we really don't know which is the optimal composition—which is the optimal version of MFGM. And so, we need more high-quality, randomized, controlled trials with well-defined MFGM fractions and a long-term follow-up.

In the future perspectives, if we think that MFGM is beneficial in formula-fed infants, would some at-risk group of breastfed infants benefit from additional MFGM, for example, preterm infants? We know that these are at increased risk of cognitive impairment and infections, so if we could prevent that, that will be excellent. Also, maybe infants with immune deficiency or infants with acquired brain lesions. So, these studies remain to be done.

And, in conclusions for clinical practice, I would say that breastfeeding should be supported. It ensures MFGM intake and also the intake of all other bioactive components and it ensures the best health outcomes. But, for those who cannot breastfeed, MFGM-supplemented infant formulas are available. They have been shown to be safe. They have possible health benefits, but I would say, as a scientist, that more studies are needed to prove the clinical effects of this intervention.

Key Takeaways

Colombo: There are a number of things that we would like you to come away from this presentation thinking about. The first 1,000 days is a critical period for brain development. The brain develops quite rapidly in those first 1,000 days, and it's a time during which the environmental conditions and the events that are encountered by the organism can directly affect structure and function. And so, if, during the time when the brain is growing fast, developing most rapidly, is the time when you can affect it most efficiently and most optimally for, and get it to an optimal state of development. And, in fact, understanding nutrition really is part of a larger picture of understanding how the brain develops, and it's important to think about the multifaceted aspects of nutritional components in understanding that interplay and how these various nutrients contribute to brain development. For example, the story on long-chain polyunsaturated fatty acids is actually a complicated story that has to do with balance, and looking at all of these components of MFGM is a great goal to aspire to.

At the same time, breastfeeding should be supported as the gold standard for infant nutrition. There are numerous studies out there over decades of work showing that it's been associated with improved neurodevelopment and that should be emphasized as the continuing gold standard for infant nutrition.

Back to MFGM though, the bioactive components in breast milk may improve a number of domains of behavioral and biologic function. It's been shown to have effects on immune function, to promote neurodevelopment and to improve health. In particular, the MFGM, which Magnus and I have both done research on, appears to be an important component of this. This, again, MFGM—a 3-layer membrane of lipids, glycolipids and proteins, that surround the triglyceride-rich fat globules in mammalian milk. And compared to infant formula, these milk droplets have higher sphingomyelin content in human milk than in typical infant formula.

And if you add MFGM back in, in a number of randomized trials, the evidence suggests that this MFGM has improved cognitive outcomes, and I would point out that these are lasting and meaningful outcomes that are, in fact, quite remarkable. Supplementation has also been shown to reduce the risk of

infection—and remember, improved health is directly related to improved cognitive outcome—and may maintain intestinal barrier integrity and also may modulate gut microbiome effects that are relevant to behavioral outcomes.

For those who cannot, and it's important even though we are emphasizing the importance of breastfeeding, for those many, many moms and families who cannot breastfeed, MFGM-supplemented formulas are safe and they may have health benefits that may approach the level of benefits that we see with breast milk, although research is ongoing and obviously we need to know more about the clinical effects of MFGM supplementation.

Audience Questions

✧ In the Swedish MFGM study, can you speculate on why the benefits of MFGM supplementation did not persist at 6 years?

Domellöf: Yes, it's really hard to tell. We know that some interventions—especially nutritional interventions—can have effects in the short-time perspective and then, when you test the children later on, there are so many other factors, from life and from what happens socially and the parents, the school, preschool, etc. So, I would say that the effect gets diluted with time, but even if it's a transient effect on neurodevelopment, it might have positive long-term effects that we didn't really measure, or the alternative can be that there is no real long-term effect. We don't really know, and we don't—we must do further studies really with long-term follow-up to really know this for sure.

✧ For women following a vegan diet, would they have less MFGM in their breast milk?

Domellöf: I haven't seen any studies of it, but I'm pretty sure—I would be very surprised if they didn't have just normal MFGM. Actually, the MFGM, the production of MFGM, in the breast gland, the mammary gland, is really preserved through mammalian evolution, so it's really not depending on the mother's diet. It's produced from the mammary gland, so I think, I'm quite sure that it doesn't affect, is not affected by the diet.

✧ If MFGM is present in cow's milk fat, is there any reason to not use the bovine fat instead of the vegetable oils currently used in most infant formulas? Will the processing of the cow's milk to manufacture formulas destroy the MFGM?

Domellöf: I think this is a question you have to put to the infant formula manufacturers. I don't think we are experts on this processing bit. But I think it's kind of interesting that, in the 1970s, it was decided that vegetable fat was probably healthier, so they substituted it in the infant formulas without really any good evidence for that. So, I think maybe the trend is going back now to using more dairy fat. I wouldn't be surprised.

✧ Do you think MFGM is beneficial for older children as well?

Colombo: Sure, that—you know, we're scientists—and so that remains to be seen. I'm not aware of any studies with older children where MFGM has been reintroduced. I suspect older children are getting a fair amount of MFGM if they're drinking milk anyway or milk-based products, but I'm not aware of any clinical trials that have assessed that. Perhaps Magnus does.

Domellöf: I think there are some studies showing maybe some positive effect on infection prevention, but certainly nothing on neurodevelopment that I have seen. And, as John says, if the children are consuming milk, I guess it would have less of an effect.

✧ What future research projects would you like completed to see more complete effects of MFGM?

Domellöf: Yeah, first of all, I think we would need—there have been some really nice studies performed—but I think we really need some additional studies maybe using the same interventions as in previous studies, the same products and with the long-term follow-up and looking at both infections and neurodevelopment. I think that would be interesting. Then, of course, as I mentioned, it would be nice to check also in different patient groups like at-risk groups, preterm infants and so on, but for the term infants, I would say just similar studies that have been performed, but we just need more of them because I would say they are not completely conclusive yet.

✧ **Can you talk more about the process of executive function testing, and can any of the tests that measure cognitive development in the research setting be adapted for use in pediatricians' offices?**

Colombo: Executive function measures are typically taken 1-on-1 and a lot of these tests can be pretty easily administered. There are developments afoot to computerize these assessments and so there are, for example, in the US, the National Institutes are developing a baby toolbox that will make available computerized tasks to measure early executive function that would theoretically be available to the public. They are a few years off. There are commercial assessments. You can look at the Cambridge Computerized Tasks; it's called the CANTAB. I recommend that you go to their website. They have examples of measurements of executive function there. This is a developing question; it's a great question, and this is a development that's happening over the next decade. I think we'll see this become more commonplace.

Abbreviations

IQ	intelligence quotient
MFGM	milk fat globule membrane

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