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TRANSCRIPT

Human Milk and Preterm Infant Brain Development

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We're going to switch gears a little bit, still on the theme of growth and nutrition, but now focusing in on human milk and the benefits for preterm infant brain development, what we can take from the science.

Three main points that I want to touch on. One, again, just emphasizing that the NICU hospitalization coincides with an important critical period. Here, we're going to be focused on brain development specifically for preterm infants. Then this idea that a fortified maternal milk-based diet promotes better neurodevelopment compared with formula feeding. This is particularly true among those most vulnerable to impairment. We'll get into that data. Then finally talk about brain MRI which is emerging as a very useful tool to assess the brain structure and even function in utero to establish timing and also to try to understand potential mechanisms underlying the beneficial effects of maternal milk on preterm infant brain development. This is where we're headed.

Again, a critical period is an important concept. This is a specific window of time during development when an organism is particularly sensitive to environmental stimuli or experiences and we all know that there are many stimuli and experiences that occur in the NICU, both positive and negative. It is important to recognize that, despite our best efforts, hospitalized very preterm infants do experience under-nutrition during a critical period in their brain development. This is a really classic paper in the field published by Richard Ehrenkranz when I was still a med student. These are fetal growth curves and you can see 3 red dots here. These are 3 different groups of infants plotted on their curve at birth and you can see that they're all sitting at the 50th percentile for a fetus born at that gestational age, so 24, 26 and 28 weeks. But they very quickly— you can see by the dotted line—fall of that fetal growth curve. Evidence of postnatal growth faltering during this critical third trimester.

I think it's also fair to say that preterm infants represent a nutritionally vulnerable population within pediatrics, and why is that? Well, first, they miss in utero nutrient transfer during the third trimester, so a lot of the nutrients that are supposed to transfer from mother to infant never make it because the baby is born too soon, and then they also may be born with already accumulated deficits. For example, if there was placental insufficiency and IUGR, so not only did the baby miss sort of future nutrient transfer, but they also may have been short on nutrients transferred even before they were born preterm. Then after birth, many challenges including immature feeding, motility, digestion, absorption, metabolism, so those are on the kind of GI side. But then, even outside of the GI tract immaturity, we know that critical illness and inflammation impair the accretion of nutrients, so maybe nutrients are being provided but the baby just can't use those to build new tissue. A lot of vulnerabilities here. Of course, we all know that this population is equally vulnerable to neurodevelopmental impairments.

This is a recent publication in *JAMA* from the NRN data and you can see just in summary here that only half of extremely preterm infants have either no or mild neurodevelopmental impairment at 22 to 26 months, meaning the other half have either moderate or severe impairments.

Let's bring these 2 things together, nutrition and neurodevelopment. We do know that nutrients are required for brain development during this NICU critical period, and this is just a graphic that shows all the different stages of brain development that are happening across the life course, but honing in, here, on the time from conception to birth. By the time that these babies are being born, we're at the late stages of neurogenesis and neuronal migration and really getting into apoptosis, synoptogenesis, gliogenesis, and then the early stages of myelination. And this is really smack dab in the middle of when our babies are experiencing the NICU environment and then, very specifically, all of these steps require specific nutrients to support their progression.

To put a visual to this, these are serial MRI images that I think just really drive home the tremendous amount of brain growth and development that's happening. You can see that the brain of this 1 infant, imaged at 28, 31, 34 and 38 weeks, is doubling in size and then also goes from being essentially a smooth structure to having like almost an adult-looking formation with gyri and sulci. Just really a tremendous amount of changes that are occurring.

I think everyone knows that you need good nutrition for your brain to grow, but this is also a population of infants who experience varying degrees of brain injury around the time of birth, and I think we don't measure that very well so we're not necessarily aware of what's happening. But we know that the pre-oligodendrocyte cells are highly susceptible to inflammation, hypoxia, ischemic and injury to those cells leads to a dysmaturation and hypomyelination that can be seen again on brain imaging, also can result in impaired brain growth.

There is also the potential here for a neurorestorative function of nutrition after early brain injury. Both necessary for normal growth and development, but also may be helpful in rehabilitation after an injury around the time of birth.

We talked about this a little bit in my last talk, but I think it's important to note that this is not just a theoretical situation, but rather that there are several very classic randomized, controlled trials that have established a causal role of the NICU diet on brain development. Although this is the talk about human milk, this is just, I think, a very important trial to understand why we practice the way we practice. This is a multisite trial of preterm vs term formula in very low-birth-weight, preterm infants done back in the 80s. And they randomized infants to these 2 groups and then they had subgroups of babies who were receiving exclusively vs partially formula. Not only do they find improved weight gain and head growth during the period of the intervention in the hospital, but they also found that babies who received this nutrient-enriched formula had higher Bayley scores at 18 months and then they actually went on to follow this cohort into school age and adolescence and saw evidence of continued benefit. Emphasizing this critical period concept and showing causality through clinical trial.

Let's now talk about human milk. Conceptually, I think it's useful to think about the mammary gland as an organ that assumes many of the functions of the placenta after the baby's born. There are actually many similarities between the composition of amniotic fluid and maternal milk and that makes a lot of sense. For a preterm infant, there is an abrupt interruption of gestation that happens early so the baby stops swallowing lots and lots of amniotic fluid and switches to maternal milk and I think there is a possibility that breast milk can compensate for that early separation from the placenta in some ways.

What do I mean by that? You can think about human milk as having 3 main functions for the infant. One is obviously nourishment, but then there are also ways in which the mother communicates with the infant through the milk, through different signals, and then there's also a protective function outside of nourishment, for example, protection from infection.

I think another sort of theoretical way to think about this is that there's this coadapting triad, so there's the mother, there's the baby and then there's actually the milk. And all of this has sort of evolved as a system that has these 3 different components but they're really all coevolved together and I think a great example of this is how we see altered milk composition after preterm vs a term birth. Preterm milk tends to have higher protein, just for example, and that is an adaptation within the system to account for the higher nutrient requirements of a preterm infant.

Why does that all matter? Well, I think we're all here to think about clinical translation and so really trying to understand the unique health benefits of human milk, not just for infants in general, but for this vulnerable population of very preterm infants.

This is some work that we did in collaboration with a group in Adelaide. We did a secondary analysis of data from a large randomized trial, really trying to understand the extent to which feeding maternal milk as compared with formula during the NICU hospitalization is associated with long-term neurodevelopmental outcomes and this dataset has really advantages of high, high follow-up, all the way from NICU discharge to 7 years of age in this cohort with detailed neurodevelopmental testing, and then they also collected very detailed information about the NICU diet, so we took advantage of that.

I'll walk you through the results and so this is how the graphs are organized. There are different domains, IQ, academic achievement, behavior and executive functioning. These are specific scales and then the x-axis is the difference in these outcomes per 25 mL/kg/day of human milk or its actually maternal milk, there is no donor milk, as compared with formula in the NICU. You can see that for each additional 25 mL/kg/day of human milk, there was higher IQ, higher math and reading scores and then fewer symptoms of ADHD. All positive outcomes.

I think 1 question that people have is whether nutritionally maternal milk, even when fortified, is adequate for those teeny, teeny, tiny, lowest gestational age babies and so, because of the size of this dataset, we were actually able to look at that. This is a stratified analysis looking at the association between maternal milk intake in the NICU and IQ, just overall IQ score, stratified by gestational age. And I think what jumps out pretty easily is that all of this overall association is actually driven by the babies born at less than 30 weeks. The beneficial effect of maternal milk seems to be strongest among those babies born at the lowest gestational ages.

We had the opportunity to replicate the study in another cohort, this one with collaborators in Melbourne. This is the VIBeS cohort, also a cohort of very preterm infants born around the same time, also with very similar outcomes assessed at school age, so 7 years, and you can see actually many of the same findings, higher IQ, math, working memory and motor scores associated with more maternal milk, as compared with formula, in the NICU.

I do want to just give 1 caveat. In both of these studies—and also in other contemporary studies that have looked at the

relationship between maternal milk feeding or diet type and neurodevelopmental outcome— those studies reflected routine use of human milk fortifier to ensure that these babies are receiving adequate nutrient delivery and to support growth. I do want to share a paper that we just published this year with collaborators from Brazil in which we actually used data collected through the Vermont Oxford Network in 12 Brazilian NICUs, so over 4,000 very preterm or very low-birth-weight infants. We looked at the association between the infant's diet at discharge and these different indicators of growth. We're not talking about neurodevelopment; we're talking growth. You can see pretty clearly, and I will just explain that the way the data are collected is exclusive human milk means no fortifier, no formula, just human milk. A mixed diet is human milk with formula and/or fortifier. And then there's exclusive formula. Just like a little nuanced in terms of the definitions, but I think you can see pretty clearly that those babies who were fed an exclusive human milk diet, they may have received some fortifier early in the hospitalization, but they lost 2 *z* scores of weight and 1.3 *z* scores of head circumference which was more extreme than the babies who received a mixed diet and exclusive formula. I understand that the practices around fortification are more variable in Brazil specifically, so I think this is evidence that although their use of human milk is amazing and really something for other countries to strive for, that effective and successful promotion of lactation in this very difficult population, it does seem like those babies are getting lower nutrient delivery. I just wanted to just make that caveat that the work that I'm presenting is mainly about fortified maternal milk and that's important.

I think people also want to know about donor milk, so I wanted to share a trial that was published earlier this year looking specifically at neurodevelopmental benefits with donor milk and here we were able to do a trial, right? You can't randomly assign a baby to receive their own mother's breast milk or not, but you can, in a situation where the own mother's breast milk is not available, randomly assign a baby to receive donor milk or formula. This was an NRN [National Research Network] study, the NICHD [National Insitute of Child Health and Human Development] Milk Study. They enrolled babies under 29 weeks or under 1 kg who had little or no access to their own mother's breast milk. Those babies were randomized to receive fortified donor milk or preterm infant formula until discharge or death or 120 days, whichever came first. This study was designed to look at neurodevelopment and you can see here that there was really no difference. The babies who received donor milk had an average Bayley cognitive score of 81 and the babies who received preterm formula had an average Bayley score of 81. Really no differences. There were some differences seen in the expected direction in terms of reduced risk of NEC in the donor milk group, but this was really a study that was aiming to look at neurodevelopment and they did not find any differences.

Why is that? I think one possibility is that slower growth in the donor milk group may have offset potential benefits of donor milk. That's really a speculation, but I think it's just interesting to note that the babies in the donor milk group had slower weight gain, so 22 vs almost 25 g/kg/day and then less decline in their weight *z* score in the formula group, so -.09 *z* scores vs almost a half standard deviation drop in the weight. Impossible to know for sure, but I think that's just an important caveat to this study. They did provide fortification with the donor milk, but whether the nutrient delivery was the same, probably not.

I think another question that people have when we're looking at especially observational data linking maternal milk with neurodevelopmental outcomes months or even years later is whether that's just all confounding. At least in the United States, there's very strong patterning of breast-feeding and even human milk use in the NICU with social class and, of course, that is also predictive of neurodevelopment. One thing that's been really interesting is to start looking at MRI data at term-equivalent age to really see some of these benefits that are detectable even before NICU discharge. Even before some of these potentially confounding influences of the home environment. This is another part of the analysis that I showed earlier using data from the VIBeS [Victorian Institute Brain] study. We were really interested to find that not only was more maternal milk in the NICU associated with better long-term outcomes at school age, but we were also able to see a larger volume of the deep nuclear gray matter, so thalamus, basal ganglia, and then also the hippocampus which, of course, is very important for memory and learning. That correlated with our finding of better working memory associated with more maternal milk. Really helpful in understanding that these differences are actually observable, even before the baby goes home from the NICU.

There's some other studies that have found similar results. This is a very similar study published by a group in DC in which they found again that a human milk diet was associated with larger total and regional brain volumes and actually the same structures that we found in our study, the deep nuclear gray matter and the hippocampus, were the regional volumes that they observed as well as just a larger total brain overall. Then looking at other aspects of brain development, so there's a technique called diffusion tensor imaging that can be used to understand the progression of white matter maturation, so looking at the white matter of early myelinating white matter tracts. They found that breast milk diet vs the formula diet was associated with more mature microstructure in the corpus collosum and the posterior limb of the internal capsule, which again are these early myelinating white matter tracts in the infant brain.

Another aspect of brain development is cortical development, and this is using MRI data to evaluate different aspects of cortical development and the conclusion here is that a breast milk diet was associated with a more full-term-like cortex than a formula diet.

What's going on here? What is it about maternal milk that is promoting better neurodevelopment? There is, I'm sure, some confounding influence of the environment, differences in the environment that the babies go home to, but that's not possibly explaining these MRI findings that are happening even before the baby goes home. Let's look to the composition of the milk and see that there are both nutrients and then also nonnutrient bioactive factors that may be at play.

This is like a proof-of-concept study that I just want to share. This is a trial of formula in full-term infants. So, not anything that is available for our babies, but I just think it's an interesting concept. This is called the COGNIS Study and this study evaluated an experimental formula that contained all of these different bioactive factors. A milk fat globule membrane, symbiotics, some probiotics, LCPUFAs, gangliosides, nucleotides, sialic acid, they added like a whole soup of nutrients and nonnutrient bioactives that are found in human milk and weren't previously found in formula and they compared infants randomized to the experimental formula to those who received a standard formula. They found, with the experimental formula, fewer behavioral problems at 18 months and 2½ years and then higher language scores at 4 years. I will also point out that they included a breast-fed reference group, and that group performed better in all tasks at all ages. This is really specific to formula feeding, but I think it just highlights the idea that there are components of breast milk that may be responsible for the benefits on brain development and then later neurodevelopmental outcomes.

They also used MRI to study longer-term outcomes in this cohort and found that, by 6 years of age, they were able to detect differences in brain volume and cortical thickness in the babies who had received that experimental formula back when they were young infants.

Let's now move back to the preterm infant and think about nutrients that are in maternal milk that may contribute to the neurodevelopmental benefits that we're seeing. And I think a classic example is DHA, so N3 fatty acids, which I do not believe are added to preterm infant formula, but are present in varying amounts in maternal breast milk and in donor milk as well. This is the N3RO trial which is a trial of enteral DHA supplementation for hospitalized infants born below 29 weeks and the dose of the supplement was 60 mg/kg/day compared with a placebo who didn't receive any supplement, but everybody was receiving their own maternal milk or formula with some DHA at baseline. This actually was a trial designed to look at BPD as a primary outcome, but they did go on to follow this cohort to 5 years of age, and despite many, many challenges during the pandemic, followed a very high percent at age 5 and published the results just a couple of years ago.

What they found was that at age 5, those babies who had received the DHA supplement in the NICU had about 3.5 points higher on an IQ scale which was consistent with their hypothesis and, although that's not a large difference, it is pretty impressive that a diet intervention in the NICU could have a detectable effect all the way at 5 years. So, that's nutrients. There are, of course, other nutrients but I think that's just a good example to think about.

Then there are also nonnutrient bioactives that may contribute to neurodevelopmental benefits and I think this is really an area of active investigation. There is still a lot to learn, but the idea is that if we could identify specific components of breast milk that are promoting better brain development, we could make sure that all babies are able to have access to those components, either because there is variation in maternal milk, we could supplement or potentially add to formula or fortifier. Understanding this will drive better diet-based strategies.

One component of breast milk that is of interest to us has been lactoferrin. This is an antimicrobial, anti-inflammatory immunomodulatory protein that's found abundantly in milk and I think most people are familiar with lactoferrin as a protein that protects against infection. But we're really interested in understanding the extent to which this might be promoting neurodevelopment in preterm infants, and this is a very talented master's student who worked in the lab a couple of years ago who's now a medical student at Brown. We had a cohort of infants and were able to analyze breast milk that these infants received for lactoferrin and you can see, just looking across, so the x-axis is just individual participant and the y-axis is the lactoferrin concentration. You can see that there's actually tremendous variation in the lactoferrin content of milk. Babies are getting different amounts in their diet, depending on how much is in their own mother's milk, just like other nutrients. We grouped this cohort into low, medium and high lactoferrin exposure, based on the lactoferrin that was present in the mother's milk, and then we used that categorization to try to understand how lactoferrin intake was associated with later brain growth-related outcomes.

To orient you, we looked at the lowest tertile of lactoferrin, so that low group as the reference group, and then we were mainly interested in how this high group compared to the low group in terms of total brain volume and then some of these regional volumes, cortical gray matter, deep gray matter, white

matter and hippocampus, cerebellum, same areas of the brain that we've looked at before. You can see here that we did see that babies in that high lactoferrin group had bigger brains and some larger regional structures than babies in the low group. This is even after adjustment for factors, such as gestational age, birth weight *z* score, age at scan, and sex. Concluding that lactoferrin may be partly contributing to increased brain growth during this NICU critical period.

I think just trying to tie everything together, there's a lot of old and then also new evidence coming out that is connecting maternal milk generally, but then also specific components of maternal milk, with improved brain development during this NICU critical period. And all of this affirms what the AAP has already recommended for a human milk diet that mother's own milk, appropriately fortified, is the optimal nutrition source for very low-birth-weight infants.

Leaving you with some take-home points, again the NICU hospitalization is this critical period in brain development and NICU-based diet interventions, we already know, can improve long-term outcomes and they're also highly feasible to implement. Next, a fortified maternal milk-based diet promotes neurodevelopment, particularly among those most vulnerable to impairments. And taking that to the next step, we know that maternal milk has a myriad of effects on brain development in the NICU which may be mediated by specific nutrients and bioactives and this underlies the long-term benefits that we're seeing in developmental testing. Then finally, that brain MRI is emerging as a useful tool to establish timing and also to investigate the potential mechanisms underlying effects of maternal milk on preterm infant brain development and specifically understanding the role of nutrients, such as DHA, and bioactives, like lactoferrin, may be very important in advancing our care practices.

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