Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS

COURSE TRANSCRIPT

Physiology and Targeted Nutrition in Infants

Overview

Participants will learn how targeted nutrition impacts physiological development in infants during neonatal intensive care, and the long-term development of pre-term and term infants. Michael K. Georgieff, MD, and Camilia R. Martin, MD, discuss specific micro- and macro-nutritional components and their impact on developing physiology.

Dr. Martin provides the specifics of nutrient-directed metabolic programming in the early postnatal period and the risk of neonatal morbidities. Dr. Georgieff discusses how nutrition in the neonatal and postnatal period relates to brain development.

Target Audience

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

Section

Learning Objectives

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

- Evaluate how targeted nutrition impacts physiological development
- Apply evidence-based nutrient research to disease risk and long-term development in preterm and term infants
- Recognize the role of nutrition in early brain development to improve postnatal outcomes.

Faculty

Michael K. Georgieff, MD
Professor of Pediatrics and Child Development
University of Minnesota School of Medicine
Director, Center for Neurobehavioral Development
The University of Minnesota Masonic Children's Hospital
Minneapolis, Minnesota

Camilia R. Martin, MD, MS
Associate Professor of Pediatrics
Harvard Medical School
Associate Director, NICU
Department of Neonatology
Director for Cross-Disciplinary Research Partnerships
Division of Translational Research
Beth Israel Deaconess Medical Center
Boston, Massachusetts

Accreditation and Certification

The Annenberg Center for Health Sciences at Eisenhower is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The Annenberg Center for Health Sciences at Eisenhower designates this enduring material for a maximum of 1.5 *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Physiology and Targeted Nutrition in Infants

Annenberg Center for Health Sciences at Eisenhower is accredited by the American Association of Nurse Practitioners as an approved provider of nurse practitioner continuing education. Provider number: 040207.

This program is accredited for 1.5 contact hours.
Program ID #5720-EM

Annenberg Center for Health Sciences is accredited as a provider of continuing nursing education by the American Nurses Credentialing Center's Commission on Accreditation.

A maximum of 1.5 contact hours may be earned for successful completion of this activity.

Provider is approved by the California Board of Registered Nursing, Provider #13664, for 1.5 contact hours. To receive credit for education contact hours outside of the state of California, please check with your state board of registered nursing for reciprocity.

Annenberg Center for Health Sciences at Eisenhower is a Continuing Professional Education (CPE) Accredited Provider with the Commission on Dietetic Registration (CDR). Registered dietitians (RDs) and dietetic technicians, registered (DTRs) will receive 1.5 continuing professional education units (CPEU) for completion of this program/material.
Provider number: AC857
Activity number: 148483

Disclosure Statement

It is the policy of the Annenberg Center for Health Sciences to ensure fair balance, independence, objectivity, and scientific rigor in all programming. All faculty and planners participating in sponsored programs are expected to identify and reference off-label product use and disclose any relationship with those supporting the activity or any others with products or services available within the scope of the topic being discussed in the educational presentation.

The Annenberg Center for Health Sciences assesses conflict of interest with its instructors, planners, managers, and other individuals who are in a position to control the content of CE/CME activities. All relevant conflicts of interest that are identified are thoroughly vetted by the Annenberg Center for fair balance, scientific objectivity of studies utilized in this activity, and patient care recommendations. The Annenberg Center is committed to providing its learners with high-quality CE/CME activities and related materials that promote improvements or quality in health care and not a specific proprietary business interest of a commercial interest.

In accordance with the Accreditation Council for Continuing Medical Education Standards, parallel documents from other accrediting bodies, and Annenberg Center for Health Sciences policy, the following disclosures have been made:

Faculty

Camilia R. Martin, MD, MS

Research Support	Abbott Nutrition: Clinical Area– Infant Nutrition Alcresta Therapeutics: Clinical Area– Infant Nutrition
Consultant	Fresenius Kabi: Clinical Area– Infant Nutrition
Scientific Advisory Board	Laurent: Clinical Area– Cystic Fibrosis Prolacta: Clinical Area– Infant Nutrition

The following faculty has no relevant financial relationships to disclose:

Michael K. Georgieff, MD

The faculty for this activity has disclosed that there will be discussion about the use of products for non-FDA approved indications.

Additional content planners

Anna Nowak-Węgrzyn, MD, PhD (peer reviewer)

Research Support	ITN NIAID: Clinical Area– Immunotherapy for Food Allergy FARE: Clinical Area- Immunotherapy for Food Allergy DBV Technologies: Clinical Area– Immunotherapy for Food Allergy Astellas Pharma: Clinical Area- Immunotherapy for Food Allergy Nestlé: Clinical Area– Hypoallergenic Infant Formulas Nutricia: Clinical area– Hypoallergenic Infant Formulas

Physiology and Targeted Nutrition in Infants

	Thermofisher Scientific: Clinical Area– Immunotherapy for Food Allergy
Consultant	Merck: Clinical Area- Sublingual Immunotherapy for Dust Mites Alk-Abelló– Data Monitoring Committee: Clinical Area– Sublingual Immunotherapy for Dust Mites Gerber Nutritional Institute– Advisory Board: Clinical Area- Solid Foods for Prevention of Food Allergy

The following have no significant relationship to disclose:
Erin Allen, MS, RD, LDN (RD reviewer)
Victoria Anderson (medical writer)
Heather Marie Jimenez, FNP (nurse reviewer)

Annenberg Center for Health Sciences

Staff at the Annenberg Center for Health Sciences at Eisenhower have no relevant commercial relationships to disclose.

The ideas and opinions presented in this educational activity are those of the faculty and do not necessarily reflect the views of the Annenberg Center and/or its agents. As in all educational activities, we encourage practitioners to use their own judgment in treating and addressing the needs of each individual patient, taking into account that patient's unique clinical situation. The Annenberg Center disclaims all liability and cannot be held responsible for any problems that may arise from participating in this activity or following treatment recommendations presented.

This activity is supported by an independent educational grant from **Mead Johnson Nutrition**.

This activity is an online enduring material. Successful completion is achieved by reading and/or viewing the material, reflecting on its implications in your practice, and completing the assessment component.

The estimated time to complete the activity is 1.5 hours.

This activity was released on May 31, 2019 and is eligible for credit through May 31, 2021.

Contact Information

For help or questions about this activity please contact Continuing Education:

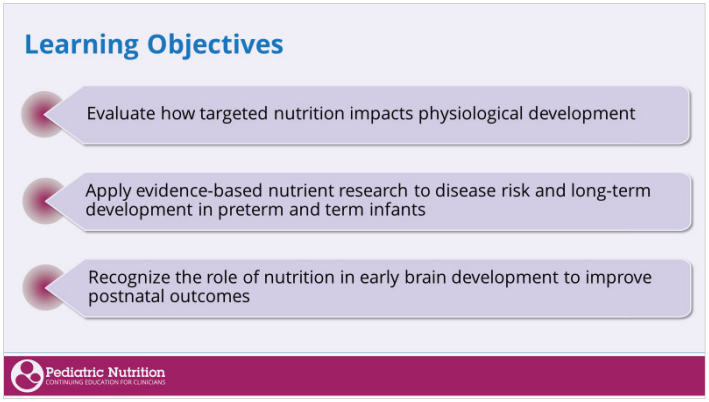
ce@annenberg.net

Editor's Note: This is a transcript of the live presentation from the Annenberg Center for Health Sciences' sponsored symposium presented alongside the Pediatric Academic Societies Meeting, April 28, 2019, in Baltimore, Maryland. It has been edited and condensed for clarity.

Module 1: Introduction

Dr. Camilia Martin: Hopefully, with what you hear today, you will walk away appreciating the value of nutrition for our babies. Specifically, how targeted nutrition impacts physiological development, and then apply evidence-based nutrition research to disease risk, both during the NICU and in the long-term development of our preterm and term infants. And also recognize the role of nutrition in early brain development to improve post-natal outcomes.

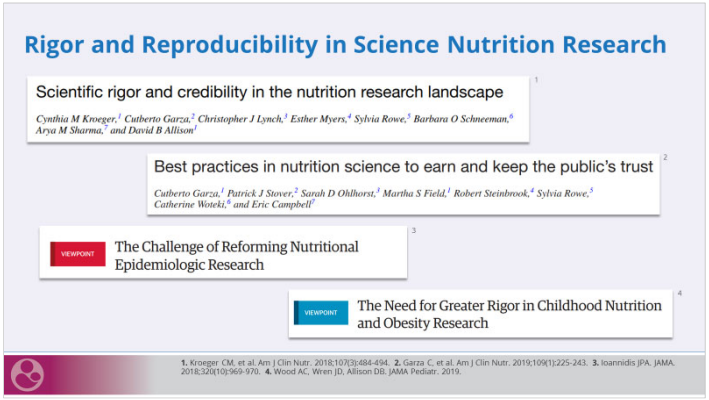
Physiology and Targeted Nutrition in Infants



Slide 1

Here are some brief introductions by both of us: While following the literature, I've noticed the whole concept of rigor and reproducibility in science has been raised. In that the experiments may not be repeatable in somebody else's hands, or different studies asking similar questions, with very different results. I think that's good, keeping us to the task of increasing rigor and reproducibility.

Then recently, I started to see that it's trickled down, specifically, regarding nutrition research. Here are some headlines and titles of recent articles [Slide 2]: "Scientific Rigor and Credibility in the Nutrition Research Landscape"; "Best Practices in Nutrition Science to Earn and Keep the Public's Trust"; "The Challenge of Reforming Nutritional Epidemiologic Research"; and "The Need for Greater Rigor in Childhood Nutrition and Obesity Research."

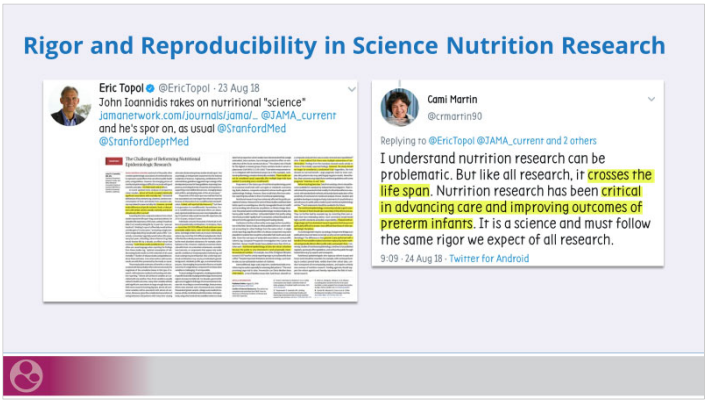


Slide 2

I've been thinking about that, and it didn't quite match with how I think about the research, and what my colleagues are doing, and how we look at nutrition for infants—especially preterm infants.

Eric Topol is a physician who does a lot with genetic medicine, digital medicine, and he always seems to be a month ahead. You read something, and then you see it in the news about a month later. He writes "John Ioannidis takes on nutritional science." It was a *JAMA* article, and he's spot on, as usual. I read it, and it was mainly about adults, but it got me upset because I didn't like the language around nutrition research to be all encompassing like that. To be very specific—and give due credit to what we're learning during our critical stages of preterm infants and newborn development—I had to respond. I said, "Wait. I understand that it can be problematic, but it crosses the lifespan, and nutrition research," I wanted the world to know, "has been critical in advancing and improving outcomes of preterm infants." And I said to myself, "Okay, *phew*, I told the world!" Went to bed. I got 1 "Like"— and that person works with me! And I reflected, *"Ugh*. Who's listening out there?"

So, I have the honor to stand here now, in front of you, and hopefully by the end of this session, you will walk away, and be amazed, and appreciative!



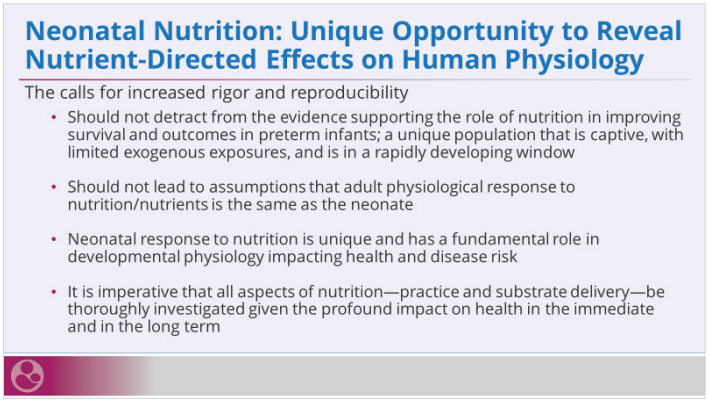
Slide 3

[You will] see what we are doing in this space, and it is amazing, and, I think, humbling, how our early

Physiology and Targeted Nutrition in Infants

actions in the NICU in delivering nutrition *does* impact the course of our babies' lives.

As you hear and see the circulation of those calls of rigor and reproducibility, it should not detract from the evidence supporting the role of nutrition in our preterm infants. It should not lead to assumptions that adult physiologic response to nutrition and nutrients are the same in our infants. *They are not.* They are totally different responses. I also don't appreciate these general headlines saying something was proven ineffective, because I think people walk away, see that, and they don't realize it's different for different folks across their lifespan.



Slide 4

Neonatal nutrition is unique, and it does have a fundamental role, not just in growth, but organogenesis and other important processes we'll talk about. But it is, all along, [important] to remember that all aspects of our nutrition need to be studied.

It does highlight that with nutrition, in general, in a developing [human] being, with so many ramifications, we shouldn't assume safety. We should test it, both in the way we practice and deliver nutrition, but also with its impact on organ development and other items.

Dr. Michael Georgieff: That impact is actually something that has really come to the forefront: What do we mean by impactful nutrition? We all, in the unit, will make nutritional changes; we'll measure something; we call it a biomarker. You see a change in that marker, and you think you've accomplished something. Maybe it's growth, maybe it's a ferritin level—whatever it is, depending on the nutrient you're looking at.

I really encourage you to read an article that was in the *American Journal of Clinical Nutrition*, called the "Pre-B Project."[1] This isn't in our slides, but just a show of hands, who has heard of "Pre-B Project?" [audience responds] So good, quite a few people.

This was a group that got together from NIH (National Institutes of Health), from USDA (United States Department of Agriculture), from the American Academy of Pediatrics, and from the AND (Academy of Nutrition and Dietetics) to try and ask the question: What do we know about the rigor of the evidence in what we do for preterm baby nutrition?

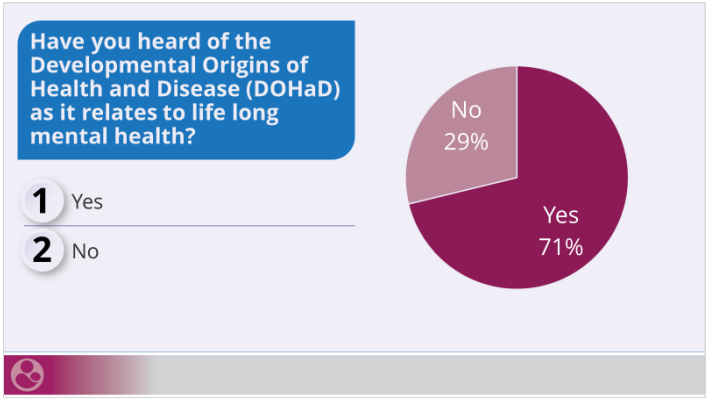
I'm sure everybody can say 120 kcal/kg/d for them, or 4 g of proteins, or how much iron you give, but what is the evidence behind that? As we went through that exercise of what do we know about the evidence of how much nutrition, how much do you give of each nutrient, how do you deliver that nutrition, what about special circumstances? [For example] surgery kids, kids with sepsis… we came to understand that any of the changes we make, and any responses we get in the babies, are only relevant if they affect a longer-term outcome. Because, after all, if everything just gets better anyway, who really cares if you fell off your growth curve? But if there are long-term ramifications, then we are talking about having societal implications. I think that goes much more to what you [Dr. Martin] were talking about: Why we should care about this.

Physiology and Targeted Nutrition in Infants

What are the relevant health outcomes that are out there that you would want to influence by putting kids on a good developmental trajectory?

Neurodevelopment would be one, but metabolic health—risk of cardiovascular disease, diabetes and obesity, later on. Immune health: how good will you be at fighting infection later in your life? Your risk for cancer, your risk for bone disease. Those would be just 5, and you can probably name others. What's inherent to that discussion is this idea of the developmental origins of health and disease.

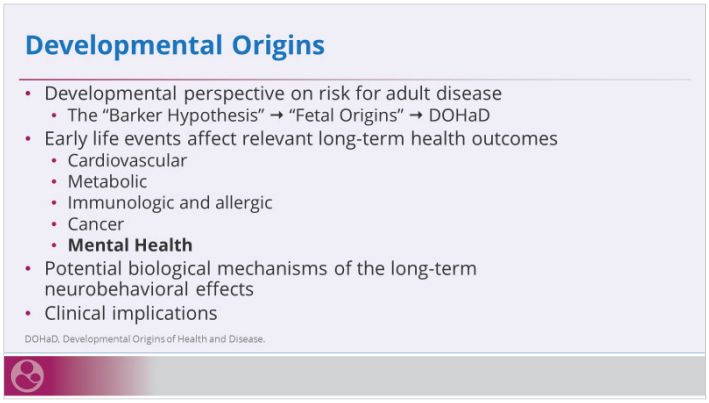
How many of you have heard of the Developmental Origins of Health and Disease (DOHaD), as it relates to lifelong mental health? [audience responds]



Slide 5

Good. So, at least this crowd is more in tune with it than others. I will tell you that I ask this question of pediatric residents. I ask this of medicine pediatric residents, where there's combined programs, and I'm actually stunned at the number of people who have not heard of this concept. Now, if you've been coming to PAS, I'm sure you've heard about this. It's probably more in the context of cardiovascular disease than mental health.

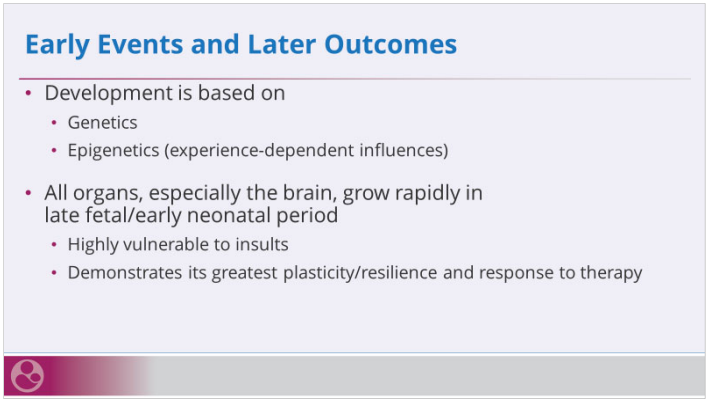
I think many of you know that this idea of developmental origins, this perspective on the risk for adult disease, was originally called the Barker Hypothesis. It was named after David Barker, a British physician, who noticed that the risk of cardiovascular disease in some population in England... that their risk of cardiovascular disease as 60–80-year-olds was a function of their birth weight. Which, when I first heard of this I thought, well, that makes sense. If you're a big chubby baby, you've probably got more fat cells, you're more likely to have heart disease later. No, that wasn't it at all! It was actually the small-for-dates [gestational age] babies who had the higher risk. And that started a set of experiments in areas of investigation as to what would be the pathophysiology behind it.



Slide 6

How were things being set early in life, to affect long-term outcomes? Like cardiovascular, metabolic, immune, cancer, and more recently, mental health, across the lifespan. I think we get comfortable with this idea of developmental trajectories and setting our kids on good developmental trajectories. Being able to assess when they are off trajectories and devise nutritional interventions that get them back on trajectory. So, as we go through my talk, which will be the second part, we're going to talk about the clinical implications. What can you do to assess and put kids back on trajectory?

Physiology and Targeted Nutrition in Infants



Slide 7

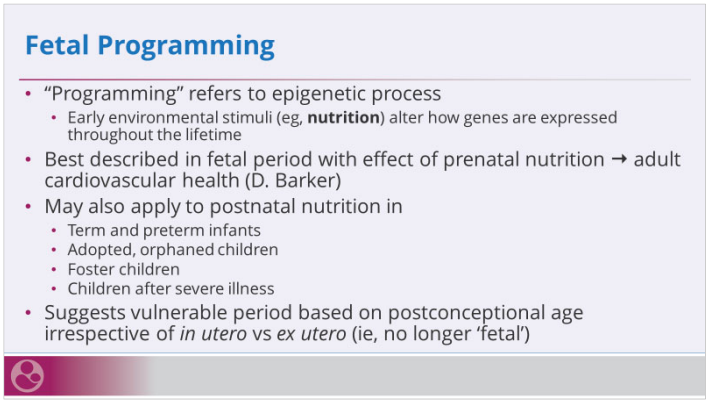
We know that early events do have an effect on later outcomes. We know that babies are not neurologically complete when they are born. They go through a process of development. That development is based on genetics—what the patient brings to the table—and epigenetics, which is how our environment, including nutrition, which actually has some powerful epigenetics influences, sculpt the genetics to give us the ultimate phenotype.

All organs, and especially the brain, grow rapidly in that late fetal and early neonatal period. Any organ growing rapidly is highly vulnerable to the lack of nutrients. So, you were talking about nutrition, and one of the things I tell people is, stop calling it nutrition, and think of it as metabolism. **What metabolic substrates do you need in all the pathways to get these organs to develop during this rapid phase of development?**

I'm going to show you later, especially for the brain, how it's built early on determines how every other piece of the brain gets built later in life. You're setting a scaffold... you're setting a groundwork for later development. There are 2 things that go with that. When you're rapidly developing, you are highly vulnerable—if you don't provide the substrate—you're building it wrong. But fortunately for the babies, they also demonstrate the most plasticity at this point—the most recoverability. I think all of us would agree, and it's been codified in statements, that vulnerability outweighs the plasticity. Meaning, do it right in the first place. Don't fall off the curve and expect catch-up growth, or catch-up nutrients, to save the day. [It's] much better to build things right first.

You've probably heard the term "fetal programming." Programming is probably the wrong word, and—as I'm going to show you at the end of this slide—it's probably also not fetal. But it was noticed in the fetal context that the size-for-dates determines, in some ways, what happens later in life. That was Barker's hypothesis.

This programming often refers to an epigenetic process. That is, early environmental stimuli, like nutrition, altering how genes are expressed across the lifespan. That's described by Barker. But we've now discovered, in multiple labs across the world, that this idea of early patterning, this idea that you're setting your metabolism—whether it's in the brain or in the body—for life, applies in term and preterm infants, not just the fetus; [it] applies in adopted and orphaned children; applies to foster children, and even children who have severe illness and growth restriction because of that, and then have a definitive procedure that puts them back on track.

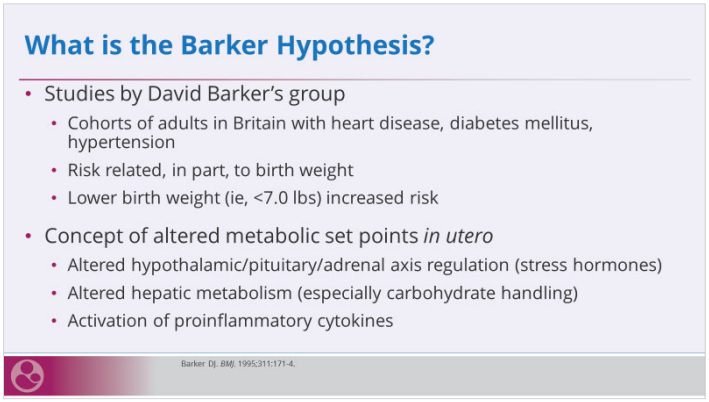


Slide 8

The idea of this vulnerable period now has expanded from fetal nutrition, which has

implications about how you treat the mother, but also in the neonatal period, [and] in the post-natal period. To be honest, we really don't know where the far end of that plasticity ends. There's some evidence that it may even be out into the teenage years. It's pretty exciting that your deck of cards isn't set just at the time of birth.

To show you a little of Barker's data... [let's look at] the cohorts of adults in Britain, looking at their risk of heart disease and diabetes and hypertension, related to their birth weight. And their birth weight, less than 7.0 lbs... actually more specifically, less than 6.2 lbs, increased that risk.[2]

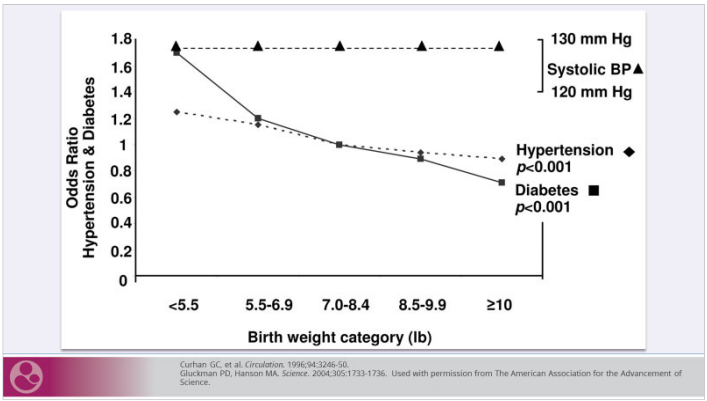


Slide 9

The physiology that was worked out, and continues to be worked out, shows this is a concept of altered metabolic set points in utero. That, in essence, the fetus and the early neonate is anticipating what their nutrient status, what their metabolism should be for the lifespan.

So, if you're malnourished as a fetus, chances are you'll be malnourished later in life; you'll be malnourished as a child and malnourished as an adult. Set your metabolism in a way that is thrifty, so you can utilize what little metabolic substrate you've got. This means changing how your liver works, how your pancreas works, and so on.

Here's some of the data [Slide 10]. You can see on the x-axis, birth-weight category and the risk, particularly of hypertension and diabetes, increasing as the birth weight dropped.[3,4]



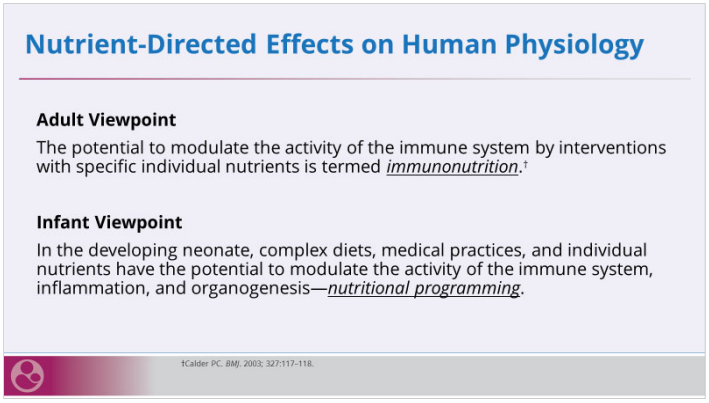
Slide 10

We're going to let Cami [Dr. Martin] talk about the specifics of nutritional programming in the early postnatal period and the risk of neonatal morbidities, and then I'm going to talk about how that relates to brain development.

Module 2: Nutritional Programming in the Early Postnatal Period and Risk of Neonatal Morbidities

Dr. Camilia Martin: Thank you, Michael [Dr. Georgieff]. So, nutrient-directed effects on human physiology. Again, the adult world has explored this. An adult viewpoint in the references below is that it means it has the potential, this nutrient, to modulate the activity of the immune system by interventions with specific individual nutrients, terming it "immunonutrition."[5] That piqued my interest, but again, thinking back to what we've learned over time with our babies in the developing neonates; there's complex diets, medical practices, individual nutrients, all having the potential to modulate the activity of the immune system, inflammation, and most importantly, organogenesis.

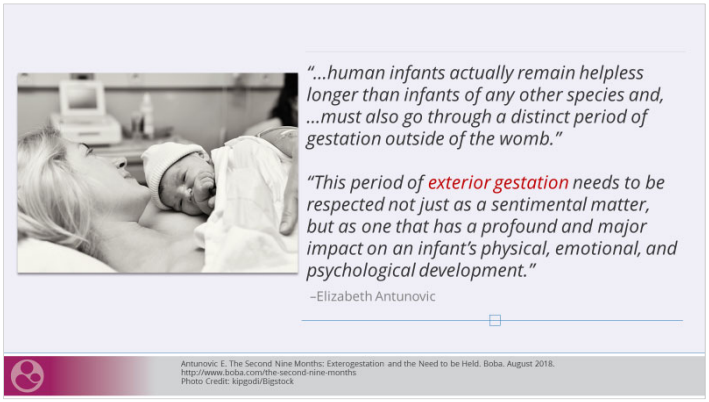
Physiology and Targeted Nutrition in Infants



Slide 11

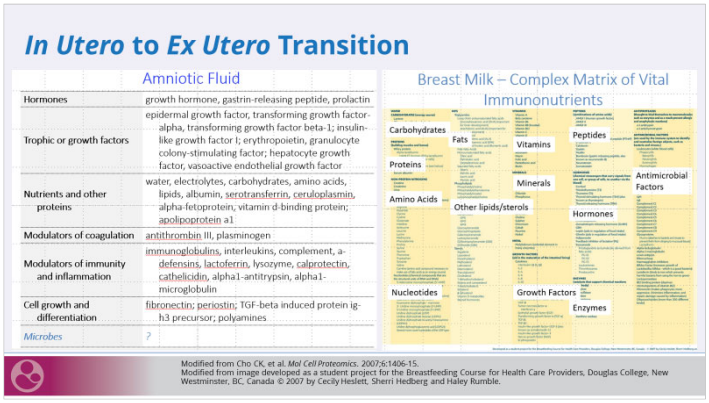
I was thinking that more appropriate for our viewpoint, for the infant, is nutritional programming. But listening to Michael [Dr. Georgieff], I'm going to say maybe it should be nutrient-directed metabolic programming. [It's] even a little bit more specific, but the point is that *it is* all encompassing. It does have all of these roles for our babies.

In another article I was reading, I really liked how this was phrased, talking about that postnatal period. "Human infants actually remain helpless longer than infants of any other species and must also go through a distinct period of gestation outside the womb." I liked thinking about that as another period of gestation. This period of exterior gestation needs to be respected, not just as a sentimental matter, but as one that has a profound and major impact on the infant's physical, emotional, and psychological development.



Slide 12

Teleologically, when you look at what's present in amniotic fluid, and driving the fetal development and the fetal programming, and then you look at what we know is available in breast milk, and all those complex different bioactive molecules, you can see the goal, the ultimate mission and goal for that in utero period and exterior gestation, is very similar.[6] They're very aligned.



Slide 13

Mom's milk—as we've learned—and nutrition, is critical in this period of *exterior gestation*. We're also taking care of babies, where it's even more true for the preterm infants who haven't even completed their first gestation.

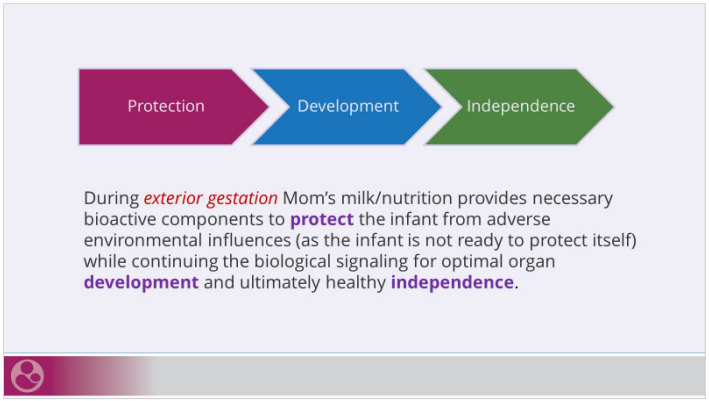
Slide 14

The goal in delivering nutritional care to our babies, is that we're facilitating this. During the exterior gestation, mom's milk and nutrition provides the necessary bioactive components to protect the infant from any adverse environmental influences.

Physiology and Targeted Nutrition in Infants

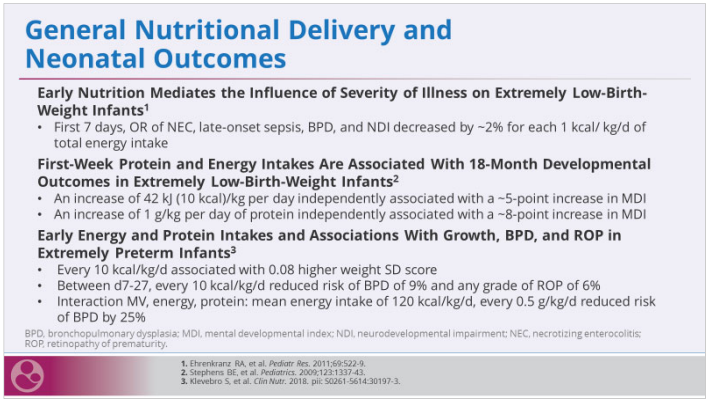
For us, it could be anything they are experiencing in the NICU, while continuing to support biological signaling for optimal organ development that didn't allow to complete during that last gestation. It's still going through an additional period, during the exterior gestation. Again, for optimal organ development and ultimately health independence. That's what we're all trying to achieve with our babies in the NICU.

The first few slides I want to go over, in general, [are] on total nutritional delivery. What's the evidence we have that it matters? That it matters to our health outcomes and our babies. I want to specifically again focus on the early postnatal period, within even sometimes the first 7 days.



Slide 15

I'm highlighting these 3 studies, the first by Dr. Ehrenkranz showing that during the first 7 days, the odds of necrotizing enterocolitis (NEC), late-onset sepsis, bronchopulmonary dysplasia (BPD), and neurodevelopmental impairment decreased by 2% for every increase of 1 kcal/kg/d of total energy.[7]

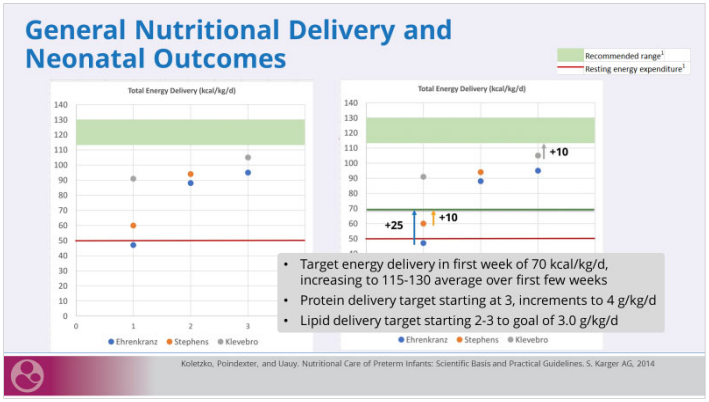


Slide 16

The second study, by Stephens, showing that an increase in 10 kcal/kg/d, or an increase of 1 g/kg/d of protein, both associated with a 5–8-point increase in mental development index (MDI) scores.[8] Finally, Klevebro showed that 10 kcal/kg/d for every 1 of those, associated with better weight gain, and during the latter 3 weeks, every 10 kcal/kg/d increased energy, reducing the risk of BPD and retinopathy of prematurity (ROP).[9] Providing that additional extra nutrition does seem to matter, but how do you apply that in the NICU? How do you know you're not already providing more? Where do you go with this?

I did some quick math. It may not be 100% accurate, but I think it gives us an idea of how we can translate those studies to what we do in the NICU. On the left is a graph on total energy delivery [Slide 17]. The y-axis is the total kcals/kg/d. The x-axis is the week of life, and each dot is [one of] the studies I just mentioned. The red 50 line is what we think is our resting energy expenditure. You want to, at least, meet that. And of course, any target above that, you're hopefully storing, instead of utilizing, storing to help with overall growth.

Physiology and Targeted Nutrition in Infants



Slide 17

The green line, or the green box, is the recommended total energy intake for our babies at 115–130 kcal/kg/d. You can see with these studies that we're not quite there. In the first week, that seemed to be paramount, to add additional [energy] over what we were giving. The blue is Ehrenkranz, the orange is Stephens—just meeting resting energy expenditure—and the gray, the Klevebro group, did better at almost 90 kcal/kg/d.

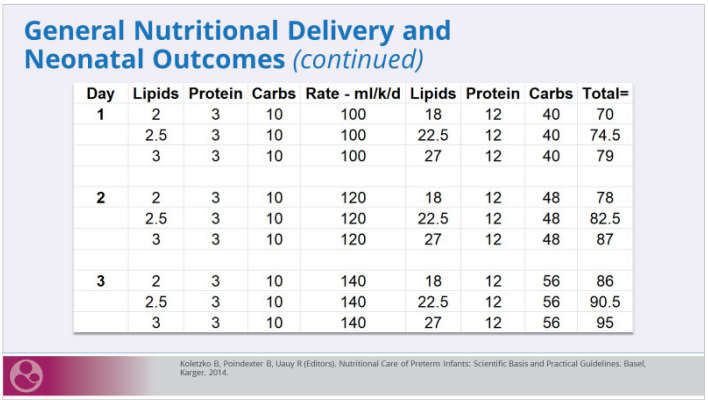
Then at 2 weeks, that incremental increase, and even by the third week, [they're] not getting into that box of the recommended range. Looking back and saying, "Okay, what were their conclusions for their linear modeling about how much to add to at least start to reduce some of these morbidities?" That's the second graph [Slide 17]. If I wanted to cut some of the morbidities by half in the Ehrenkranz study, I would have to give an additional 25 kcal/kg/d.

If I wanted to do it from the Stephens' study, it would be an additional 10 kcal/kg/d. Then for Klevebro, later, because their findings were during the first 3 weeks and another additional 10 kcal/kg/d. For the first week, you see that with the additional 25 and 10 kcal/kg/d, we're getting to about 70 kcal/kg/d, but we are now above—consistently above—the 50 kcal/kg/d, at about 70 kcal/kg/d.

If you increase the 3 weeks by 10 kcal/kg/d in the Klevebro study, you see that we're just getting into the minimum of the 115–130 kcal/kg/d. But at least it starts to paint that picture, that maybe the first-week target we know we can't get to—or can't right now. Happy to be challenged to get to that 115 and 130 kcal/kg/d immediately, but maybe this starts to tell us how to gradually get there. Maybe what our target should be in week 1, and what our target should be at week 3, and hopefully we're going in a linear fashion towards that target.

In summary, the recommendation target-energy delivery in the first week of 70 kcal/kg/d, increasing steadily to get to 115–130 kcal/kg/d by the first few weeks. Protein delivery targets—just to round out the macronutrients (I'm not presenting that data)—starting at 3 g/kg/d with increments to 4 g/kg/d, and lipid delivery starting 2–3 g/kg/d, with increments to a goal of 3 g/kg/d.

I continued to do my math and asked, "What would my orders then look like?" Because it's hard, sometimes, [with] the orders we use in the first couple of days, we wouldn't meet that total of 70 kcal/kg/d. So, I said, "I want to make sure we're meeting the total of 70 kcal/kg/d as a minimum bar," and then asked what each of the advancements would be.



Day	Lipids	Protein	Carbs	Rate - ml/k/d	Lipids	Protein	Carbs	Total=
1	2	3	10	100	18	12	40	70
	2.5	3	10	100	22.5	12	40	74.5
	3	3	10	100	27	12	40	79
2	2	3	10	120	18	12	48	78
	2.5	3	10	120	22.5	12	48	82.5
	3	3	10	120	27	12	48	87
3	2	3	10	140	18	12	56	86
	2.5	3	10	140	22.5	12	56	90.5
	3	3	10	140	27	12	56	95

Slide 18

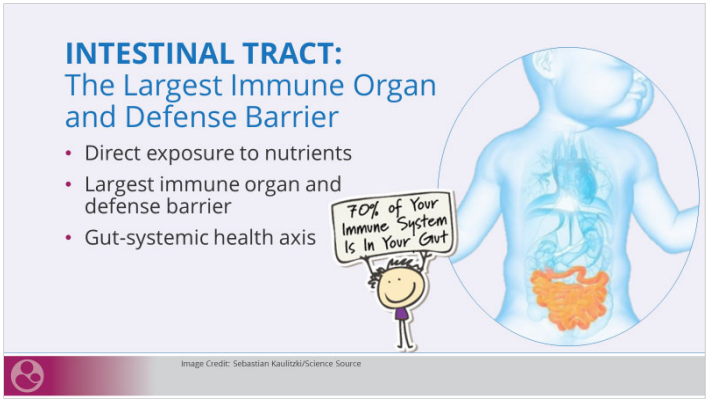
You see that for protein, I set at 3 g/kg/d. I think with their starter PN [parenteral nutrition], everyone's pretty comfortable with starting with 3 g/kg/d. I kept the carbs at 10 g/kg/d. The only thing I fluctuated on was a stepwise fashion. Even though there's not a lot of data on the advancement of lipid emulsions—what's safe or not—but the general expert

consensus is starting at 2 g/kg/d is probably safe. I decided, okay, you're somewhere between 2, 2½, and 3 g/kg/d. And the only other change was changing the total volume each day.

With this, everything is on a per-kilo basis, so the weight doesn't matter. With this, you're at least getting to the minimum of 70 kcal/kg/d. It may be a little bit more. Theoretically, it's possible to target that, so by the 7-day mark you are getting at least 70 kcal/kg/d, probably more, if you continue to increase incrementally in a stepwise fashion, as we do in an infant who's tolerating everything well. Which I know is the caveat, that sometimes our babies seemingly don't tolerate. This would be the recommendation to get closer to what our consensus recommendations would be, and align with those studies I just discussed, that showed these values started to minimize or reduce the risk of common diseases we see in the NICU.

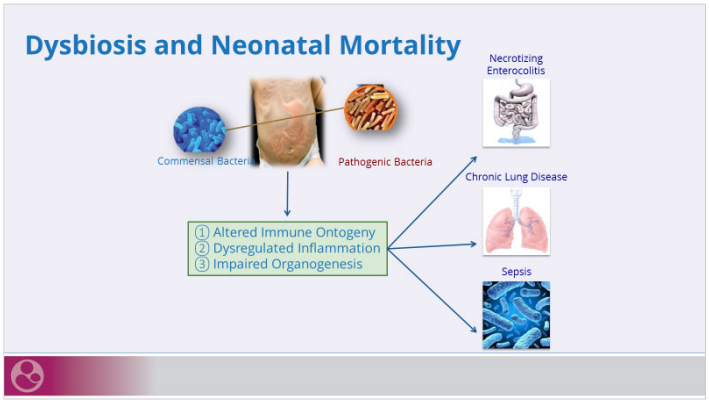
Lung and Gut

Now, I want to dive a little deeper and talk about the lung and the gut. I'll start with the gut. I think the gut, the intestine, is a fascinating organ. It's our largest immune barrier, our immune organ and defense barrier. It takes care of all the digestion and absorption of the nutrients that we provide. So, it's multifunctional, and it's 70% of our immune system.



Slide 19

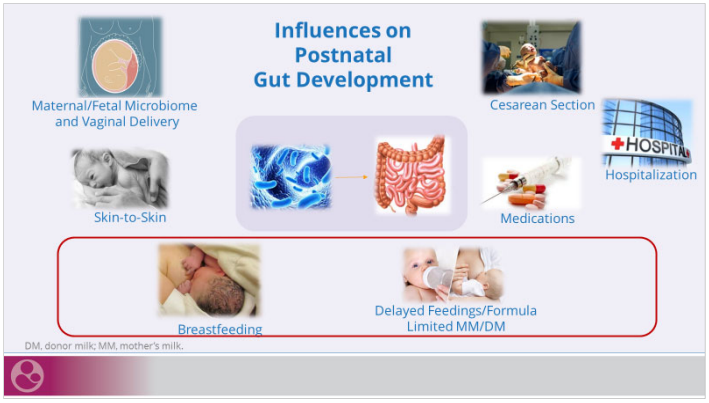
The microbiome. I think it is important to highlight how our practices, and especially how our nutrition, is impacting the microbiome and the microbiome's relevance to health. The concept of dysbiosis and abnormal microbial colonization were, perhaps, more colonized with pathogenic vs commensal bacteria. We show (there have been multiple papers) that this dysbiosis has altered immune ontogeny, dysregulated inflammation, and impaired organogenesis. There are several papers linking it to each of these individual outcomes, which we see in the NICU.



Slide 20

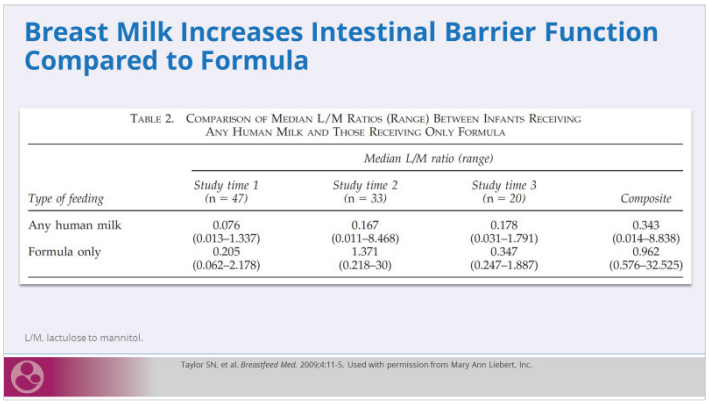
The microbiome has been an important mediating effect on the nutrients. It's what is driving some of that colonization, in addition to other factors, which we'll review. It's also critical to how we're absorbing and digesting those nutrients and creating the metabolites that then go on to further enhance, not only intestinal health, but systemic health.

The influences [on postnatal gut development]: route of delivery, hospitalization, and indigenous organisms, exposure to medications, skin-to-skin contact with mom... But one of the most important drivers, even in adults, as well as infants, is the diet. So often we talk about probiotics, and other measures that are important, but **the most profound influence on what's happening with your microbiome is your diet.**



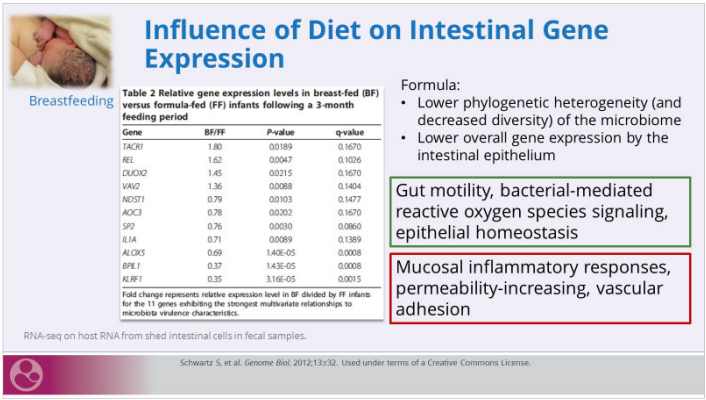
Slide 21

What's happening with breast milk in the intestinal development? This was an early study by Sarah Taylor, et al [Slide 22],[10] who looked at infants who were exclusively given human milk or exclusively given formula. They wanted to look at the effect on intestinal development, and they wanted to look at the intestinal barrier, specifically. They used the lactulose-to-mannitol study, where mannitol small is expected to move through; lactulose is a little bit bigger. If a lot of it moves through, then you have a high lactulose-to-mannitol ratio (L:M), suggesting a leaky gut—an impaired intestinal barrier. This study, in the early 2000s, showing that with formula consistently compared to human milk, you had a higher ratio of the L:M, indicating an impaired intestinal defense all the way through the composite, almost 3-fold difference.



Slide 22

I thought this study by Rob Chapkin, et al, was fantastic [Slide 23]. He was able to take intestinal cells that were shed normally in the stool, then look specifically at the host transcriptomic expression of genes. For the first time, in a noninvasive way, he looked at how diet impacts intestinal gene expression. He compared 3-month-old and full-term infants. The study showed that, yes, there was a microbiome difference, where lower heterogeneity, less diversity of the microbiome, was in formula-fed infants, resulting in a lower overall gene expression. That gene expression—when detected, evaluated, and compared—in breast milk-fed babies, showed a little bit better gut motility, markers for epithelial homeostasis, whereas formula advanced or increased the expression of genes. This involved inflammatory responses, increasing permeability, which goes along with Sarah Taylor's data, and increasing vascular adhesion. [This is] one of the first studies looking at diet-to-host interaction and the difference between breast milk and formula.

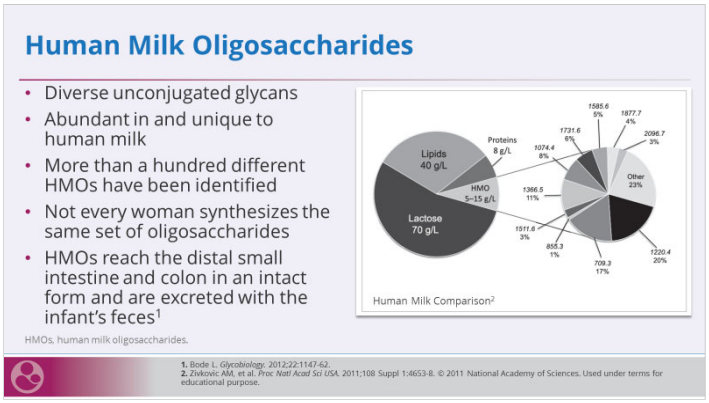


Slide 23

Human milk oligosaccharides (HMO) is another component within human milk. Unconjugated glycans readily pass through our gut and are very abundant in human milk. We probably have the most diverse species, but more importantly are the number of different species of HMOs. More importantly, every mother is unique. We now know, through the unique signature of that HMO,

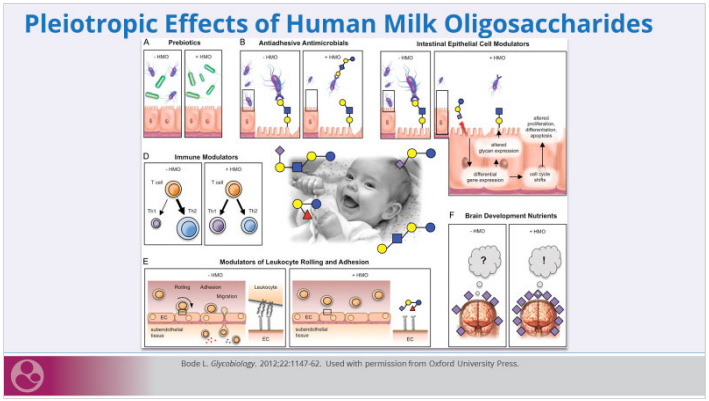
Physiology and Targeted Nutrition in Infants

distribution determines and helps influence the baby's health risk.



Slide 24

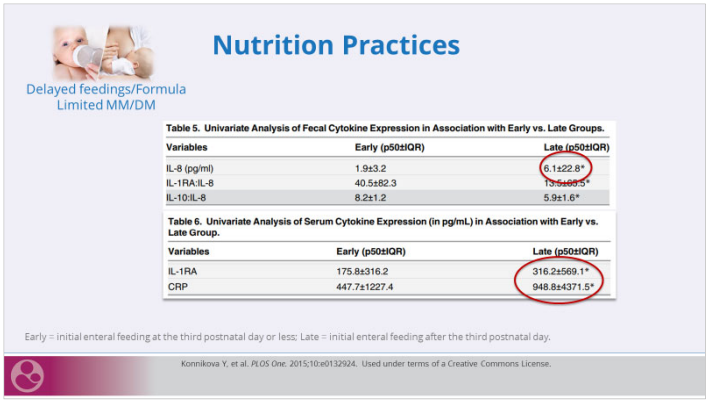
In a summary [article] by Lars Bode, which we should all read,[11] [this slide is] showing pleiotropic effects on manipulating the microbiome, serving as antimicrobials, modulating intestinal epithelial signaling, and immune modulators.



Slide 25

I've shown you—and I'll dive into some nutrients—the complex diet of breast milk vs formula, how differentially impacted the baby can be in its host and intestinal development, and why that is so.

Nutrition practices: I said at the beginning, that not only what we provide, but how we provide it, should be studied. This was a project [Slide 26] with a fellow who was with me one year. We wanted to look at what happens with delayed enteral feedings.[12] Does it matter if we start within day 1 or 2 or 3 or after? We took fecal samples at 2 weeks of life. This was a period where almost all of the babies (130 babies) were already on full-enteral feedings, doing well, seemingly healthy. We looked at the fecal lysates of the stool samples and evaluated it for inflammation and cytokine expression. We found that whether you've started to feed before 3 days or after 3 days, even remotely at 2 weeks of life, it impacted your intestinal, your inflammatory environment, in the gut, where delayed feedings had elevations of interleukin-8 (IL-8), and then with the elevations, you saw systemic changes in IL-1 and CRP [C-reactive protein].



Slide 26

This is 2 weeks out, in a seemingly healthy population, full-enteral feeds. This is a clinical study. We did our best to adjust for all potential confounders, but the decision on when to start enteral feeds impacted the inflammatory environment of the gut.

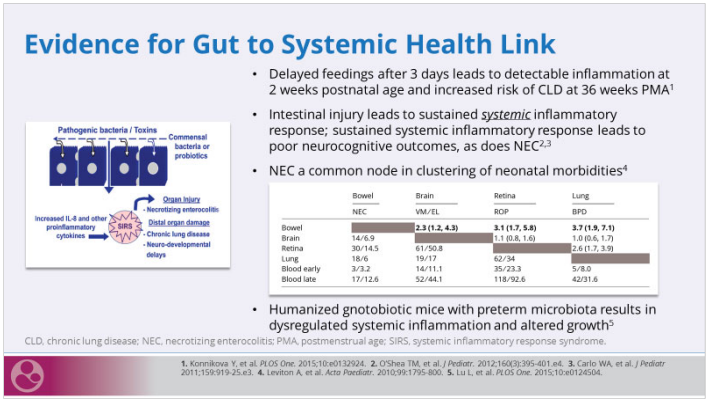
Why it matters and why we wanted to embark on it, is that we're learning more and more that **the gut is the systemic gateway to health**. There's an intestinal liver access, an intestinal brain access. All of these gut-liver, gut-brain... after they talk to each other.

I mentioned [the gut] is the largest immune barrier. How that signaling begins, in the immunity begins in the distal signaling, does matter in outcomes, and with the inflammation. A local inflammation translating to a systemic inflammation, and I showed you that just with delayed enteral feedings.

Physiology and Targeted Nutrition in Infants

We see evidence of that intestinal compromise in systemic health, as well, when we look at NEC, especially those that go on and need surgery. This was a correlation of morbidities based on the ELGAN study [Slide 27], which was over 1500 infants of extremely low gestational-age newborns.

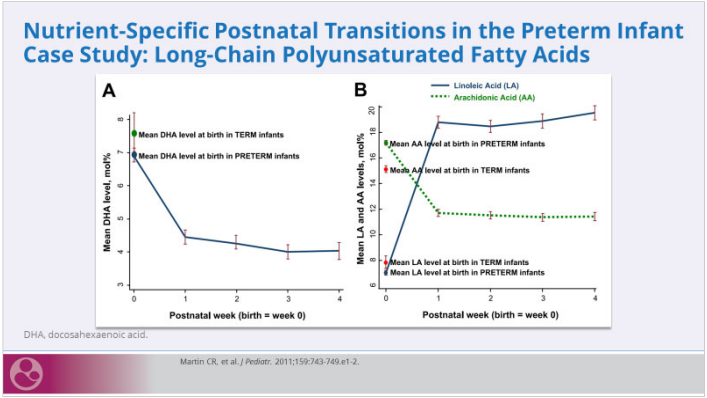
You see in this section [Slide 27, table] that if you had severe intestinal injury, the odds of having brain injury, ROP, and BPD went up. [It] was not explained by just the level of maturity. You would think, maybe they all correlate together because you're looking at the highest risk. That did not explain these correlations. So, is that systemic or intestinal inflammation, that nidus, that's what's going on in the rest of the body? To me, I think it does partly explain that, and why we have to think about methods, early feedings, and what we're feeding, to protect the gut during this transition, this early postnatal period.



Slide 27

DHA and Arachidonic Acid

Now, to dive further into a specific nutrient. So, [this is] my favorite nutrient I like to study within the lipid family and fatty acid biology. In one of the first studies we did, we looked at what happens with systemic fatty acid levels after birth.[13] On the left you see the curve for DHA [Slide 28], docosahexaenoic acid, and on the right, you see the curve for arachidonic acid and linoleic acid.

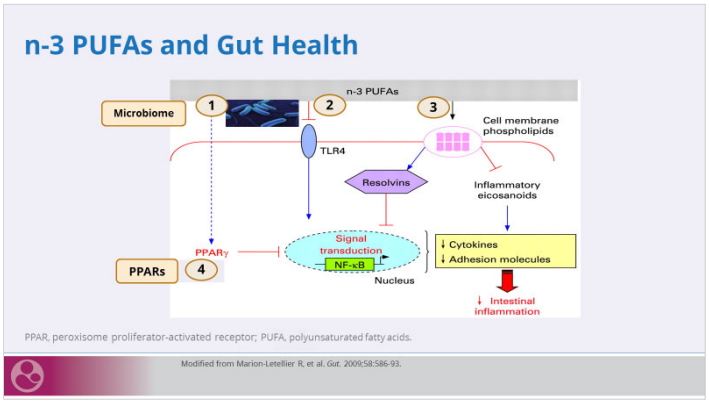


Slide 28

At day 0, at birth, all of these levels are right in tandem with that of a full-term infant. Within 1 week, based on our current practices, but other vulnerabilities of the preterm infant, DHA goes down by almost half. Arachidonic acid goes down by almost half; linoleic acid triples. Within 1 week, we're reversing what's happening in utero. [We] found that for every 1 mol% drop DHA, you are [at a] 2½-fold increased risk of lung disease. Every 1 mol% drop of arachidonic acid, you're at a 40% increase in nosocomial sepsis. Again, adjusting the best we can for all the other clinical factors. So, it seems to matter. We wanted to see, if we start to manipulate these relationships, and how we feed the babies, and with what nutrients, the composition of the lipids, can we help protect the gut?

An adult graphic about the different mechanisms by which n-3 driven PUFAs [polyunsaturated fatty acids] may help gut health [Slide 29]. We know that fatty acids and lipids, in general, actively modulate what microbiome is laid down. They can inhibit TLR4 [toll-like receptor 4] receptors, which is one of the primary mechanisms in our neonatal intestinal inflammation and NEC. It incorporates into phospholipids and signaling and inhibiting inflammatory eicosanoids, while helping with resolvins, pro-resolving terminators, the good family. And it also interacts with PPARs [peroxisome proliferator-activated receptors], which is important

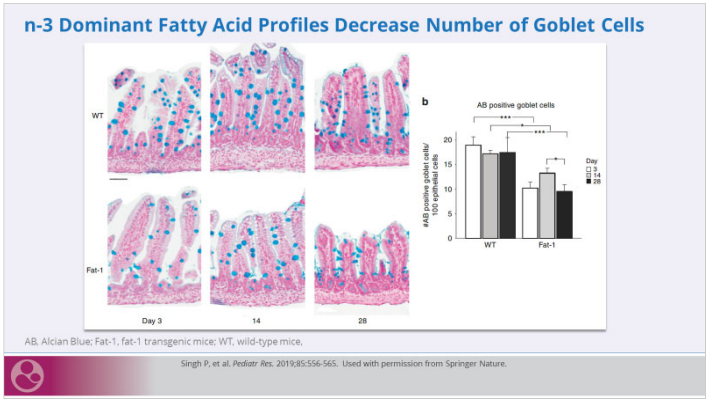
in intestinal cell-health proliferation/differentiation. So, there's some precedent that if we can adjust what we see with the fatty acids, and what we see in the nutrients, can we adjust postnatal intestinal development? I'm going in with the idea that, yes, let's see if we can induce an n-3 dominant profile.



Slide 29

We took these fat-1-transgenic mice, who can eat anything and convert it to DHA and EPA (eicosapentaenoic acid)—they can have McDonald's, and it's DHA and EPA.[14] They don't need salmon. It's great. Then we compared it to wild-type [mice], which do convert n-6s to n-6s and n-3s to n-3s, on the same diet. They're both different in the sense that the wild-type reflected, perhaps, a little bit of what we see in the newborn period now. The fat-1 had elevated DHA, because you were pushing that n-3 pathway, and lower arachidonic acid, because as you push n-3 delivery, you're going to come down reflexively for other counterregulatory mechanisms and bring down your n-6s. I thought it was a good comparison to see... if as a strategy, is this a strategy we should employ?

On the top panel [Slide 30], we did some intestinal morphology studies after a period of this diet. Over time, we did day 3, day 14, day 28 to see a longitudinal trend of what happens with changing, specifically, the fatty acid profile, and we think only that.

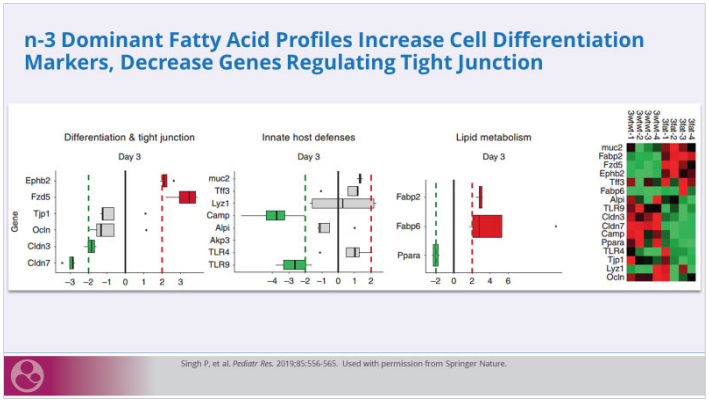


Slide 30

The top panel is the wild-type [mice], and the bottom panel is the fat-1. [We were] a little surprised that as we push the n-3, at each of these days (the blue staining is the goblet cells), the number of goblet cells were definitely lower than that of the wild-type. Is that good? Goblet cells produce mucin, [which is] part of that innate defense of the gut.

We went on and said, "Well, let's look at what's happening with gene expression." And we took genes that were involved in differentiation and tight junctions—those involved within host defenses and lipid metabolism [Slide 31]. Red means an increased expression. Green means a decreased expression. We see that the red, there's an increase in expression of markers related to cell differentiation and proliferation. So, good, but it decreased in those genes that are important in the intestinal barrier, in the intestinal permeability, and also decreased in some inflammatory regulating genes, dampening the immune response.

Physiology and Targeted Nutrition in Infants



Slide 31

Slide 32

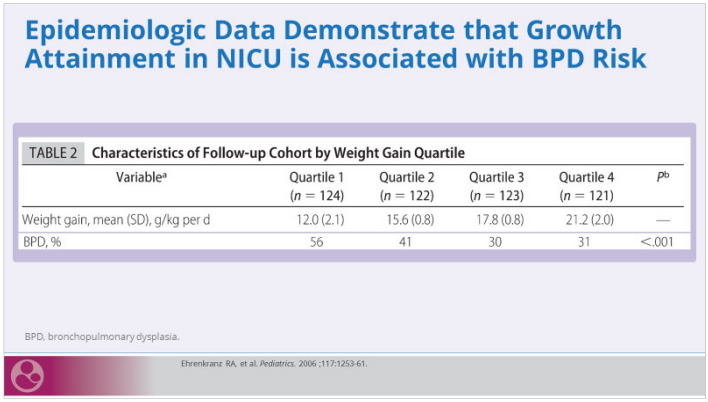
To me, this illustrates exactly why we need to do these studies. Is that good? You almost have a little bit of a mixed effect. You have a positive effect in promoting differentiation, proliferation, but you're reducing goblet cells. You're reducing genes important for the intestinal barrier, and you're dampening the immune response. Is that good or is that bad? And that's the next step, to then put it in a situation where maybe you induce intestinal inflammation and see how they respond. But I've seen a practice where we're adopting these n-3 strategies without understanding this balance, and how they can differentially affect important mediators of health that we don't fully realize, yet.

I'm not so sure increasing that n-3 is what we should be doing, yet. As a package of care then, we know it directly impacts intestinal environment. How fast you deliver and start enteral feeds (breast milk vs formula), but we also have shown you that it's not just local for the gut—it has systemic ramifications.

Manipulating nutrients, to me, is like almost the same as manipulating a potential drug because of how highly bioactive our nutrients are in regulating these processes, these physiologic processes. We should not presume safety. We should not presume no harm. We should understand dose, balance, and windows of opportunity. And that study on the gut gives you a window of how complex it can be.

Nutrition and Lung Development

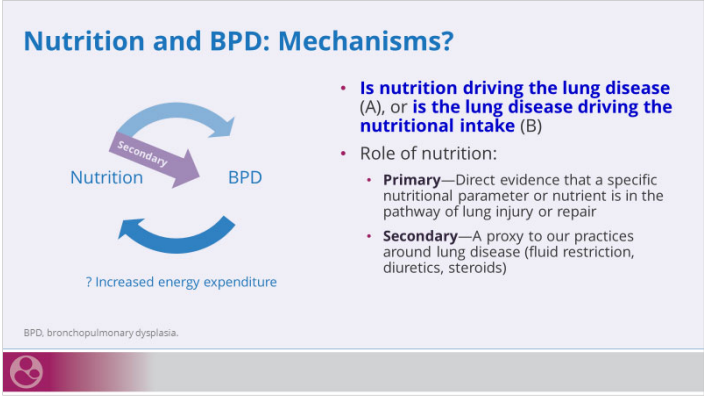
Our babies who have BPD are at risk for impaired neurodevelopment. Through Richard Ehrenkranz, et al's study—the risk of bronchopulmonary dysplasia seems to be related to overall growth while in the NICU.[15] In this study, Dr. Ehrenkranz and his coauthors divided growth into 4 quartiles [Slide 33]. Quartile 4 being highest growing kids at 21 g/kg/d, stepwise down, quartile 1 at 12 g/kg/d. You can see the risk of bronchopulmonary dysplasia increased. The less they were able to grow, less efficiently, they were able to grow while in the NICU.



Slide 33

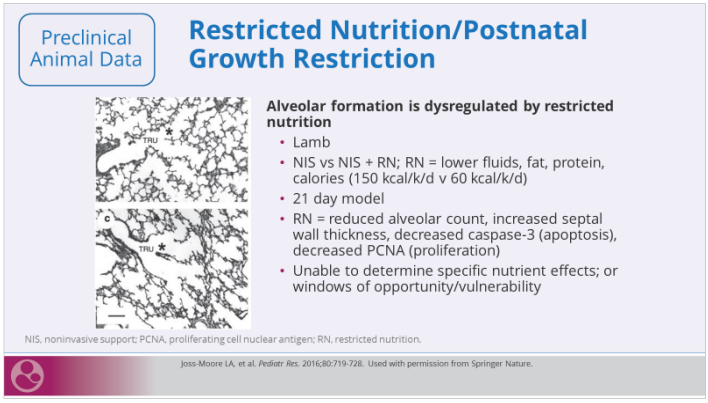
Are nutrition and lung disease related? Are there mechanisms in which it can be impacted to help reduce this? It's always a difficult argument. Is there something specific about nutrient delivery that's interfering with lung development? Or is it that these kids are just really sick, and that perception of

severity of illness changes the way we provide nutrition? Is it a primary effect or is it secondary? I think it's probably both, but to understand what those nutrient-driven pathways are, [we have] modifiable targets in our practice.



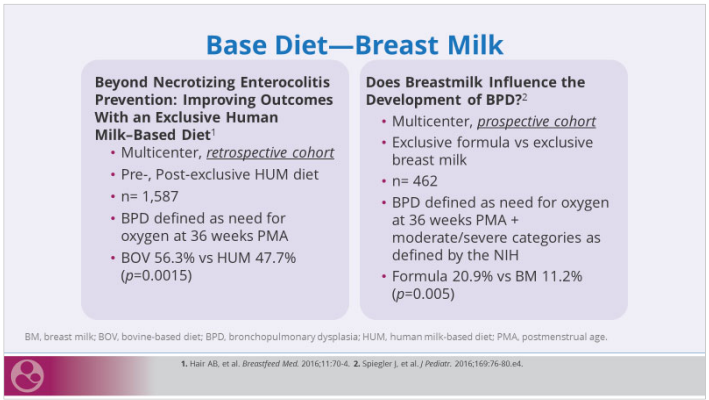
Slide 34

I love this study. This study is a lamb study out of the group from Utah [Slide 35], [led by] Lisa Joss-Moore, PhD.[16] Once the lambs were born, they were maintained in a noninvasive respiratory support. Then, one half had regular nutrition at 150 kcals/kg/d, and the other half had restricted nutrition at 60 kcals/kg/d. So, truly not unlike what we put our babies [through]. I just showed you those graphs, and where they are in energy delivery—not unlike what's happening there in that first week. They did this over several weeks, and they showed [in the] morphology of the lung, which compared a non-restricted nutrition and restricted nutrition, there was reduced alveolar counts. You have, instead of the small units of alveoli, you have bigger ones that coalesce, called alveolar simplification. You had increased septal wall thickness; an imbalance between apoptosis and proliferation. I think [this is] a nice illustration that restricted nutrition alone impacts lung development. Now, is it total energy? Was it a lack of some lipids or protein they were not able to determine? But overall, restricted energy delivery mattered.



Slide 35

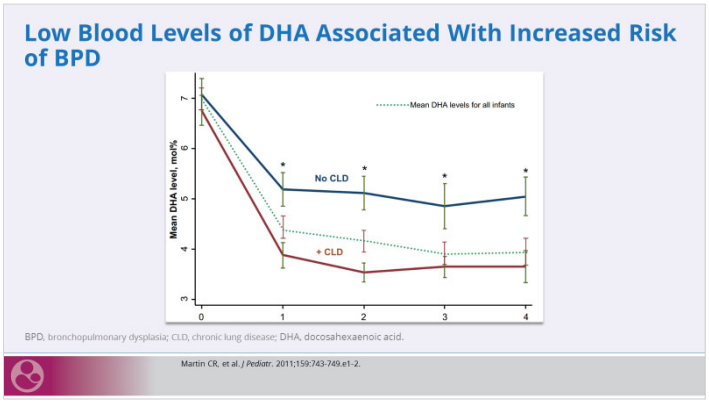
On the clinical end, [here are] a couple [of] breast milk studies, fairly large: one a retrospective cohort, the other a prospective cohort [Slide 36]. One a pre-, post- and using exclusive human [milk-based] diet.[17] The other using exclusive formula vs exclusive breast milk.[18] The bottom line is moving towards a more dominant human-milk diet vs either historical controls or formula, reducing the risk of BPD quite significantly. Especially on the right, going from 20%–11%, so the formula vs breast milk had the biggest difference. Then, on the left, with Dr. Hair's study, from 56%–47%. So, restricted nutrition matters. The complex diet you use matters.



Slide 36

Let's talk about a specific nutrient. Again, going back to the fatty acids. I had mentioned that all our babies dropped their DHA and created this deficit by 1 week of life. We also saw a separation and what that curve looked like, whether the infants

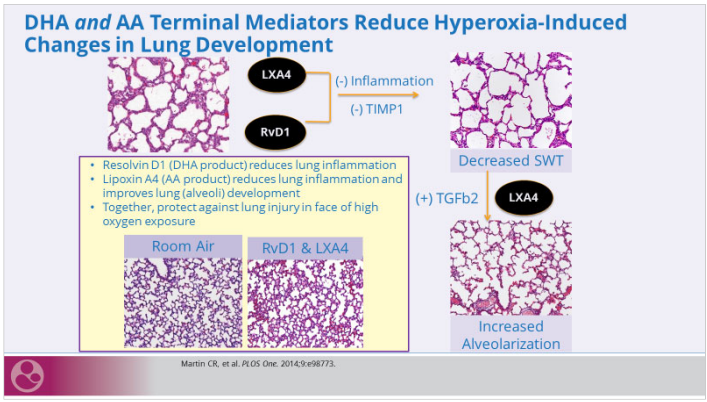
developed chronic lung disease or not, BPD or not. Those who did not had a higher level overall of DHA compared to their peers. So, we took that back to the lab to understand it. Is there a mechanism behind this that can explain it? We used the well-established hyperoxia-induced lung injury model, and we also knew it was probably going to be pretty hard to manipulate DHA and AA, and we know it is, now, in the nursery.[19]



Slide 37

Instead of giving *those* [DHA and AA], we gave the terminal metabolites. DHA metabolizes to resolvin D1 (RvD1). That was the resolvin I mentioned before, which is anti-inflammatory, or pro-resolving of inflammation. And then arachidonic acid produces something called lipoxin A4 (LXA4). This is your room air, nice small alveoli [Slide 43]. This is what happens to your lung when you're exposed to high levels of oxygen; that's no change in magnification. You see that alveolar septal, a simplification of the alveologenesis, and you see the increased septal wall thickness. Individually, they've both decreased septal wall thickness. With lipoxin, increased alveolarization reduced that alveolar simplification.

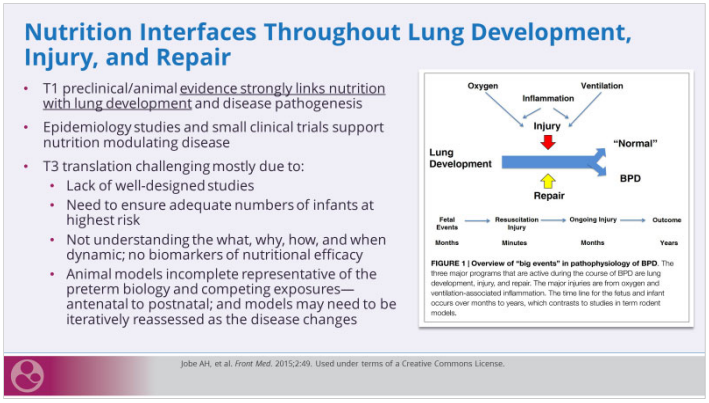
This is when I began to truly appreciate fatty acids aren't just mediating inflammation, as we know in adults—as I say, we can't compare them—but in our developing babies, who are still developing organs, it's mediating alveologenesis. We already know it's important in eye and brain, [it's] also important in lung, and I showed you with the gut, [it's] also important in the gut. Together, combined, you completely ameliorated any hyperoxia-induced lung injury.



Slide 38

Nutrition *does* interface through lung development. We have very strong preclinical data. For example, the lamb study, and then what I just showed you, which links nutrition with lung development and disease pathogenesis. We have good epidemiology in some small clinical trials. The 2 I mentioned about breast milk that show the impact overall: risk of lung disease based on diet.

We also all know that there are challenges moving that forward to get it to the bedside.[20] And so, the large clinical trials and some of these nutrition-directed strategies have not been overwhelmingly effective. I think it's because we don't have well-designed studies. We don't have the number of infants, but our animal models probably lack a little in completely replicating the preterm experience.



Slide 39

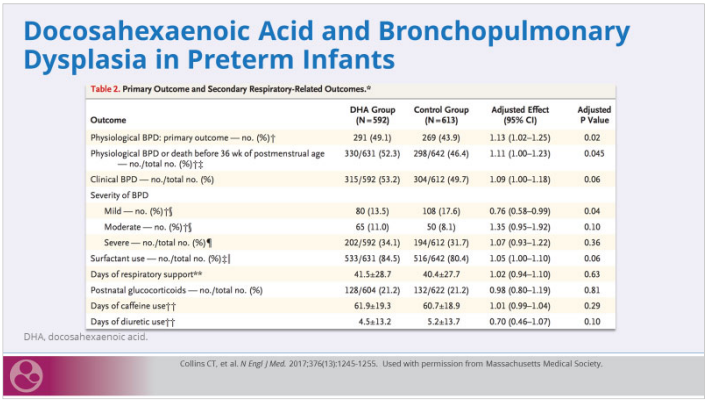
Lipid-Derived Nutrients

Lipids, I think, constitute an example. You have to consider those fatty acids when you're looking at nutrient-driven or nutrient-targeted therapies. To bring it back to the introduction, we do have to think about the rigor and reproducibility. Because given in isolation, one fatty acid alone, giving it at the wrong time or the wrong dose may have no effect and has nothing to say about the nutrient itself. We may just have the wrong information to deliver it or it can potentially cause harm. Everything is not couched in safety when you think about potential nutrients and nutrition.

Slide 40

An example of that is this study [which] came out in 2017, in the *New England Journal of Medicine* [Slide 41].[21] This group looked at providing DHA, very early, for the primary goal of reducing bronchopulmonary dysplasia. What they found was that it did not reduce it. It may have slightly increased the risk of physiologic BPD—physiologic BPD combined outcome of BPD and death. What happened? Why didn't this work? Well, it's all speculation, but it was a single-driven DHA product.

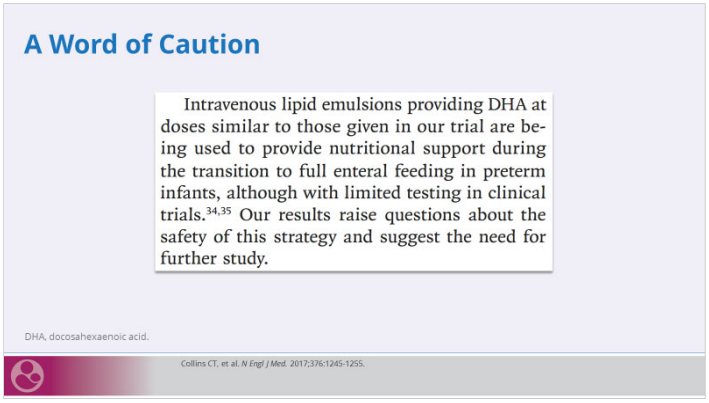
I just mentioned how arachidonic acid was also important for inflammation, immunity, and especially alveologenesis. And when you provide a single fatty acid, like DHA, you're going to drop your arachidonic acid. Was that happening at the metabolic level? I don't know. But this may be an example of going to the clinic without fully realizing the ramifications of some of these nutrient-driven targets.



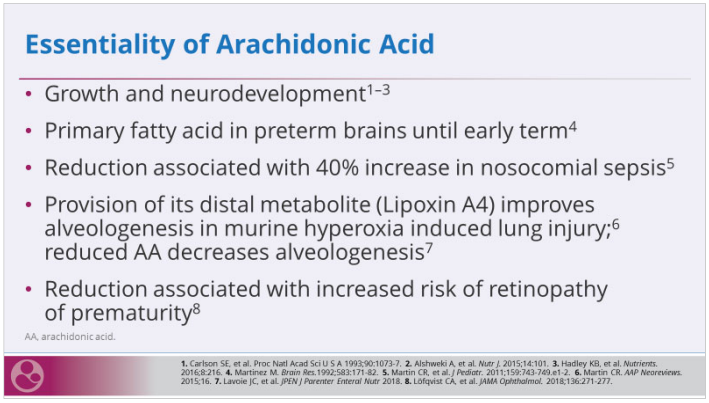
Slide 41

They themselves, to their credit, had to reflect and say, "You know what? What we just did is the same as what we do at the bedside, adopting fish-oil lipid emulsions without fully understanding what's happening metabolically. As a result, our results raise that question and the safety of this strategy and needs further study."

Physiology and Targeted Nutrition in Infants



Slide 42



Slide 43

Essentiality of Arachidonic Acid

The essentiality of arachidonic acid. Why do I bring that up? I brought that up several times now. Did this happen in the Makrides study[21] at the tissue level? Seeing it's important in our lung study with the alveologenesis, and seeing it with the sepsis. I think we can't forget that often our nutrients are in a community. Just like the microbiome is in an ecosystem—in a community—so are our nutrients. We know from historical literature, if you feed just DHA, [as in] Carlson's formula studies,[22] you don't grow.

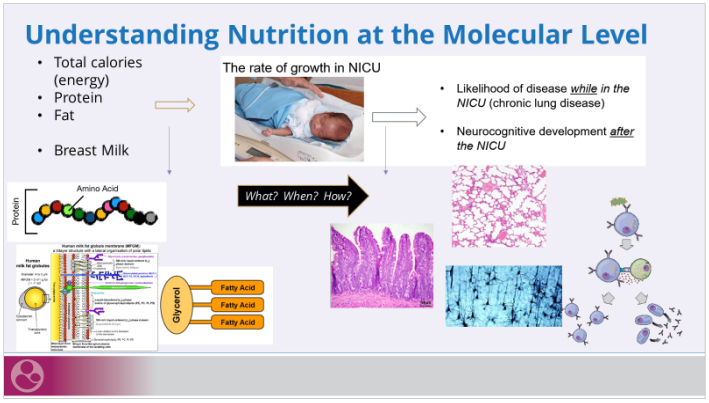
It took getting in the arachidonic acid to show adequate growth—neurodevelopment. It's the primary fatty acid in brains until almost near term. We think it's DHA, but it is arachidonic acid. The reductions [were] associated with a 40% increase in sepsis, which was clinical, as I had shown you. The lipoxin A4 [improves] alveologenesis; that was in the mouse. There's now a guinea pig model showing the same thing.[23] There was also a primary study in Sweden looking at small vs intralipid for the prevention of retinopathy of prematurity, and they saw no difference.[24] Then they unblinded it, ungrouped it, just looked at fatty acid profiles, and showed that the **reduction of arachidonic acid over time, in the course of the event, increased the risk of ROP.**

As I've moved forward in this fatty acid world, trying to disentangle this complexity, I was trying to relate to you that there are relationships to each other. There are nutrient-driven effects that can go in opposite directions. We need to study this, iteratively, repeatedly, until we understand exactly what it's doing before we go back into the bed, using these highly bioactive nutrients, to manipulate outcomes in our babies.

We know at the macro level nutrition is very important. I've talked about total calories, protein, fat, breast milk, influencing the rate of growth—growth impacting not only our short-term morbidities in the NICU, but even the long-term neurodevelopmental outcomes. As we move forward to realize the goal of this session, the physiology in targeted nutrition, we needed to disentangle it.

Let's take out what's exactly in this. What are in these components in these complex diets? What are those nutrients, and then what is it doing at the tissue level? How is it affecting gut development, lung development, the risk of sepsis, immune ontogeny? Because understanding that is going to give us answers to what's important, when is it important, and how should we deliver it to ultimately use nutrient-driven targeted strategies to allow the best outcomes of infants, both preterm and term.

Physiology and Targeted Nutrition in Infants

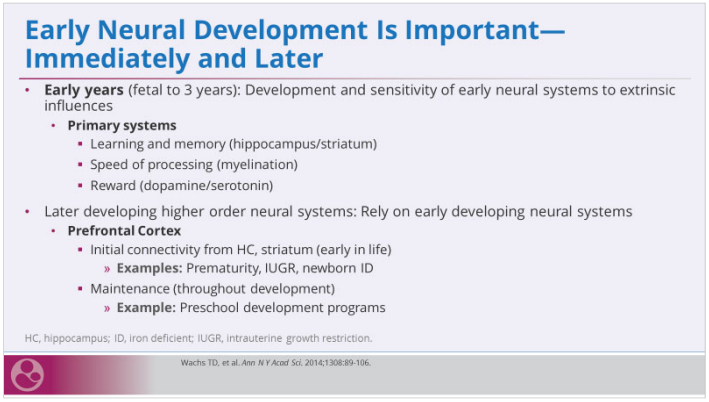


Slide 44

I concentrated on the early postnatal period. Dr. Georgieff is going to speak about the developmental origins and the brain.

Module 3: Developmental Origins and the Brain

Dr. Michael Georgieff: I think you could tell from Dr. Martin's talk, how any number of organ systems that are involved in lifetime health can be affected by the nutrition we give. I really liked that idea of the exteriorized fetus that... even term babies, if you think about, especially in [the] brain, how helpless they are in their first 3 or 4 months before they become cortical creatures. It's almost like they should have had another trimester in there. That's the time our kids are in our hands for us to try and influence a major health outcome, and that's the brain.



Slide 45

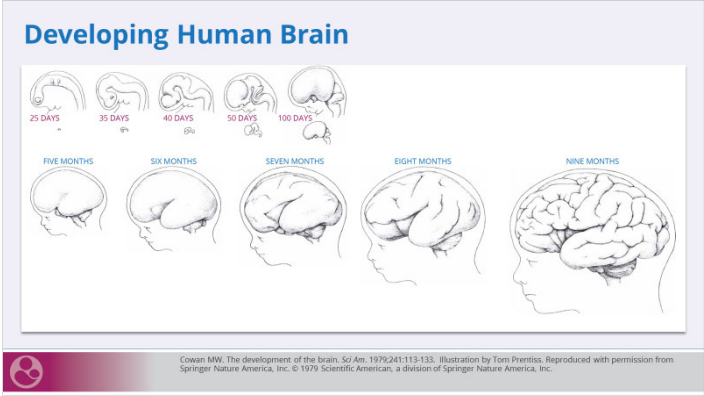
Early neurodevelopment is important, obviously—immediately. We can change nutrients. We can change brain function based on those nutrients. In the early years—our public health policies are focused on those first 1000 days, or 0–3 [years]—it's really to develop and build these systems that are primary systems from which [come] scaffolding of the more complex systems that give us the complex behaviors we see then in older children, adolescents, and adults. Failure to build these primary systems, for example, [diminishes] learning and memory system, the hippocampus and the striatum, which are rapidly developing in this perinatal period. Myelination is also rapidly ramping up. I'm going to show you a map of brain development in a couple of slides. And the reward system, the neurotransmitters, particularly dopamine and serotonin and glutamate, [are] all being shaped in terms of their regulation in this late fetal, early neonatal period.

These higher developing systems, your prefrontal cortex—the things that allow you to multitask, to have attention, to have speed of processing, and so on, in language—those are going to develop later. They're not really developing very much in our hands in the NICU, but they're highly reliant on these primary systems.

Some examples where we have problems in these higher order systems, for example, attention problems, language problems, are linked epidemiologically to events that are happening in the newborn period. For example, prematurity, intrauterine growth restriction (IUGR), newborn iron deficiency.

Why are we in such a vulnerable position as practitioners? Because the brain is in such a vulnerable position. Here's your brain [Slide 46], or here's a fetal brain at 5 months gestation [upper left]. So, that's about the limit of viability, right? ...of where we're saving babies. Now, that brain looks like a coffee bean, right? It's smooth, it's bilobed, and that's it. There's nothing else there. Here [Slide 46, lower right], we progress through gestation up to 9 months, and now it looks much more like a

walnut. That look is because we have all these gyri and sulci that have been formed. If this brain was smoothed out, it would be the size of this room. Obviously, we don't want heads that big, so we compact it by having sulcation and gyration.

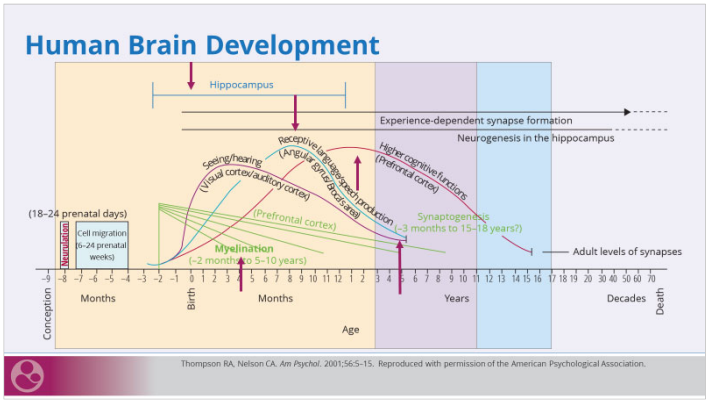


Slide 46

This reflects an enormous amount of brain development and an enormous amount of substrate, protein, energy, PUFAs, iron, and so on, to build that brain and make that happen. It's truly one of the more remarkable things about human existence. That we, in the NICU, can get a baby with a brain like that, have them in a completely different environment than they were expecting to be in, infuse all sorts of yellow stuff and white stuff into their veins, and then stick little tubes down their noses and slowly work up their feeds, and still end up with a pretty good looking brain and very functional children afterwards. Truly, a remarkable feat.

What's going on inside there? I showed you that anatomic change. What's going on inside the brain? Here you can see a map of brain development across the lifespan [Slide 47].[25] First thing you're going to notice, is that the x-axis is really distorted. There's a ton of space devoted to these months leading up to birth, so our preemies would be about here, in the months after birth, leading to about age 3. And then the rest of life happens.

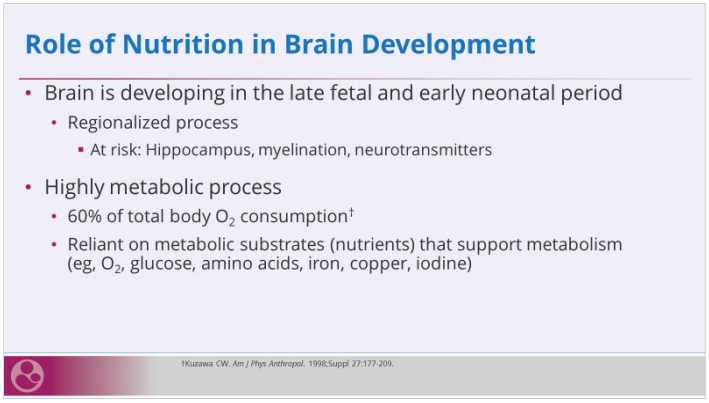
You'll notice that much of brain development takes place and is ramping up in this early period. Myelination starting at about 32-weeks' gestation and going forward. Synaptogenesis, your brain hooking itself together, starting at about 3-months gestation, and of course, going through the lifespan with that rapid hippocampal development—your basic learning and memory system—all vulnerable to things that might be happening in this perinatal period, including nutritional management.



Slide 47

Now, for those of you who have teenage kids, you'll notice everything comes to a grinding halt around 15 or 16 years of age. For those of you who are a bit older, you'll notice that we still have the ability to learn. We still have neurogenesis in the hippocampus across the lifespan, and we shape our synapsis across the lifespan. That's how we learn.

Here's another slide to emphasize that the brain is rapidly developing in that late fetal and early neonatal period [Slide 48]. It's a regionalized process, and that's going to be important in terms of understanding nutritional effects and the behavioral phenotypes you get from that later on.



Slide 48

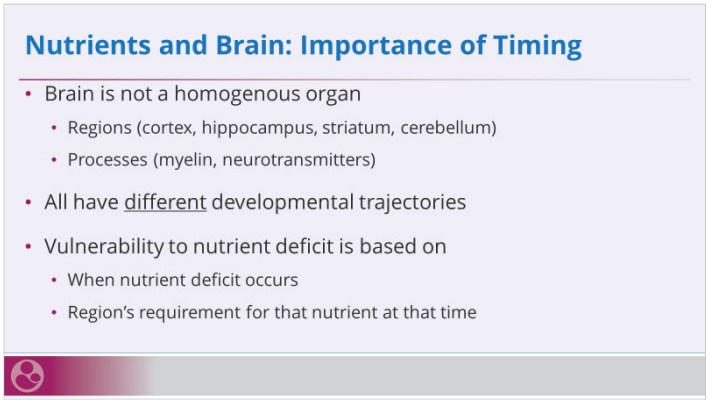
Brain Growth

The brain is a highly metabolic process.[26] That shaping of the brain from that coffee-bean-like looking thing to that walnut-looking organ. You all know from physiology, that your total caloric needs for the day is the sum of all of your organs put together—the oxygen consumption of all of your organs put together.

As you're sitting there awake, your brain is using about 20% of the caloric [expenditure] of your total oxygen consumption. It's pretty big. The brain is one of the bigger oxygen consumers in the body—it and the heart. In a baby, that number is 60%. Sixty percent of the calories you give those babies, 60% of the oxygen consumption of that baby is for brain metabolism and brain growth. It's an enormous amount. It's reliant on those substrates (ie, nutrients) that support that metabolism: oxygen—and oxygen is a nutrient—glucose, amino acids, iron, copper, and iodine.

We sit and talk about the brain, and the brain really is not a single organ. The brain is actually made up of regions and processes. Talking about the brain as a single organ as a recipient of nutrition is like talking about the thorax or the abdomen. There are many things in there, in each of those compartments—they all talk to each other.

Same idea in the brain. The brain has regions—like the cortex, the hippocampus, the striatum, the cerebellum—has processes that are brain-wide, like myelin and neurotransmitters. Importantly, as I showed you on that map, they all have different developmental trajectories. That means that the vulnerability to any nutrient deficits, any substrate deficit, is going to be based on when it's likely that that nutrient deficit occurs, and the region, any of these regions' requirement for that nutrient at that time.

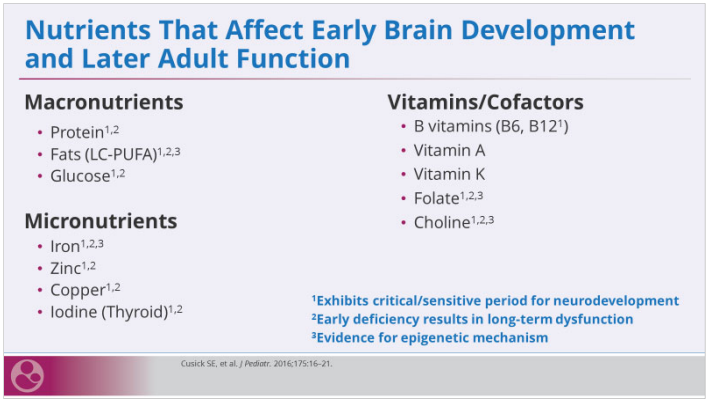


Slide 49

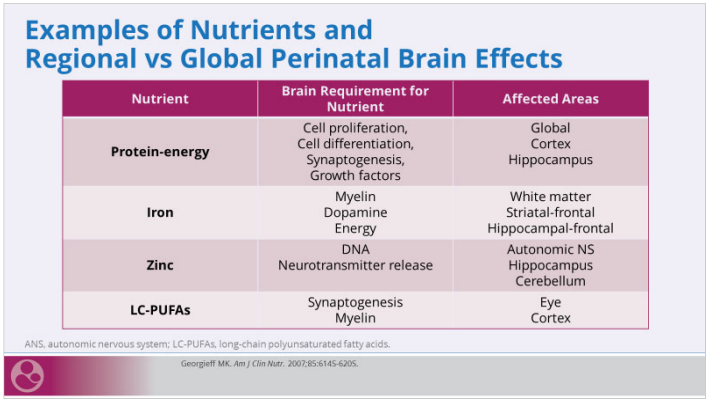
If those 2 things don't happen together, you're not going to get a deficit. If you don't need a certain nutrient, and you don't provide it, I guess that's okay. But for the most part, nutrient requirements are very high in the perinatal period, hence you get large regional effects on the brain.

What I've done is to list out the nutrients that affect early brain development in later adult function. All nutrients are important; they run your cells, basically. All nutrients are important for brain function, but there are certain nutrients that seem to be more important in terms of… if they are shorted in the early developmental time, they affect later functioning.[27] You can see [Slide 50], they fall into the categories of macronutrients, micronutrients, vitamins, and cofactors. I've superscripted these to show you where, either in clinical studies or in preclinical models, a particular nutrient exhibits a critical or sensitive period for neurodevelopment.

Physiology and Targeted Nutrition in Infants



Slide 50



Slide 51

I've superscripted with a "2" those that show when you have an early deficiency, you are at risk for long-term dysfunction. And in a very few of them—and we'll go through this list more thoroughly later—where the evidence for those long-term effects resides in epigenetic modification of chromatin. That may be part of the explanation for why we have long-term effects. A lot of this is under current research protocols to try and figure out how that happens.

This is just another way of saying what I showed you [Slide 51], except to now put it in the context that there are regional effects, and there are also global effects of perinatal nutrition. Protein and energy, for example… here's the brain's requirement for protein and energy; what it does with protein and energy. The effects can be global, or they can be very specific in areas that are rapidly developing in the perinatal period.[28] You can see the same thing for iron, its effects on myelin and dopamine and energy metabolism, and then what the effects are from that.

What's the clinical evidence for long-lasting effects of early nutritional iron status on brains in humans? These are predominantly either clinical studies or epidemiologic studies in humans [Slide 52]. Probably the 3 areas we have the most information on is outcomes of intrauterine growth restriction, which is a total malnutrition, right? That's got to be protein, energy, as well as micronutrients. Then specific micronutrient effects, particularly iron, probably one of the better studied, or more completely studied deficiencies; and then, also effects of supplementation. You would expect that if there are negative effects of a deficiency, then one could study whether providing nutrients to a population at risk for deficiency improves outcomes.

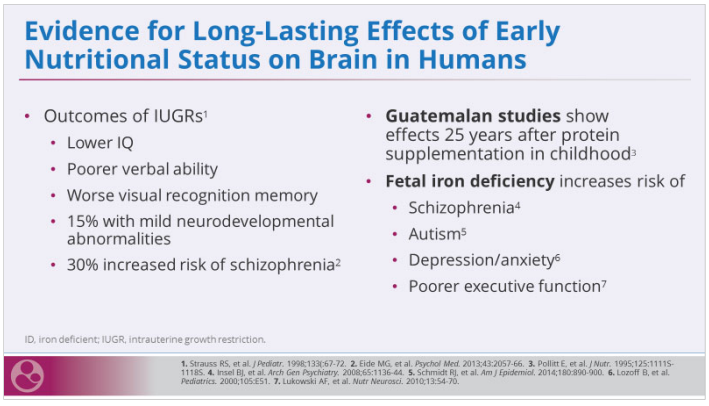
Outcomes of IUGR include children with lower IQs, poor verbal ability, worse visual recognition and memory.[29] Then there's [the] little thing called 15% with mild neurodevelopmental abnormalities. Here, we're talking about attentional problems, hyperactivity problems. They're considered minor because they're not the major handicaps (ie, cerebral palsy, mental retardation), but these are not minor in terms of tripping up kids in school.

An interesting finding is there's a 30% increased risk of schizophrenia if you are intrauterine growth restricted.[30] Fetal iron deficiency has similar effects: increases the risk of autism, increases the risk of schizophrenia,[31] and increases the risk of

depression and anxiety, and other dopamine-mediated pathologies.[32] These are interesting because they have to do with the timing of the iron deficiency.

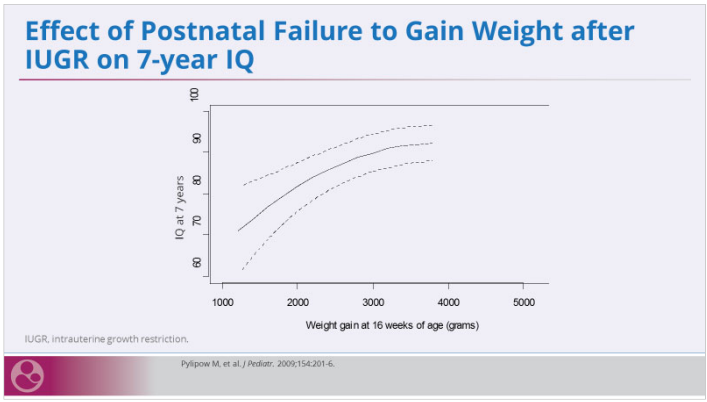
Autism is linked to poor maternal iron intake in the first trimester. Schizophrenia is an effect of the second trimester maternal iron intake,[31] whereas depression and anxiety and poor executive function is more related to perinatal and then postnatal iron deficiency.[32]

Do interventions matter? Well, I think Cami [Dr. Martin] showed you that interventions certainly appear to matter. Probably the definitive study on this is a study that was done by Ernesto Pollitt, et al, in Guatemala,[33] where children in neighboring villages—one village got food, as usual, the other village got [food] supplemented with extra calories and protein. Effects can be seen 25 years and later, on the effect of protein supplementation in childhood. It's a positive effect of nutritional intervention.



Slide 52

For growth restriction, not only do you have effects of malnutrition to the brain prenatally, but you potentially also have growth failure afterwards, and this just shows that additive effect [Slide 53]. This is from the collaborative perinatal database, a study done between 1959 and 1976.[34] It's a rich source for neurologic outcomes because they followed these children until at least 7 years of age and looked at their growth and neurodevelopment. What you see is IQ at 7 years of age, having been growth restricted to start with, having been IUGR to start with: IQ at 7 years as a function of how much weight was gained just in the first 4 months after birth.

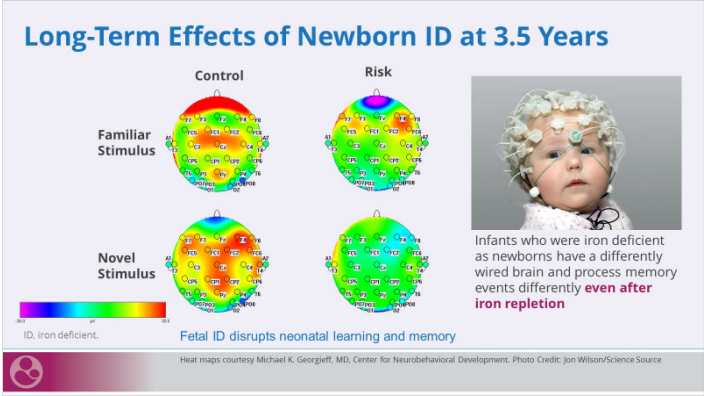


Slide 53

The better the babies gained weight—up to about 3,800 g—the higher the IQ was. Or, turn that upside down: if you have IUGR, and then you fail to grow postnatally—does this sound familiar to what happens in the NICU—you start chipping away at the IQ at 7 years of age. Now, this one was not in preterm infants; this was in term infants, but the principles probably apply to us [neonatologists]. The importance of getting nutrition into our preterm babies, 20%–30% of whom have already experienced IUGR, that critical period narrows, and you need to get that nutrition in those kids earlier.

Here's an example of how early iron deficiency affects processing in the brain [Slide 54]. In this study, these babies were either iron sufficient at birth or iron deficient at birth. They resolved their iron deficiency very quickly, certainly by 9 months of age. [They] brought these kids in at 3½ years of age and asked them to do simple memory tasks: differentiating a familiar stimulus from a novel stimulus, and then we mapped what the brain activity was, using this cute little cap that children just love to have on their heads. You can do these studies in any age baby, other than a 12-month old. A 12-month old would just simply rip that right off their heads. But we can do this in babies, actually.

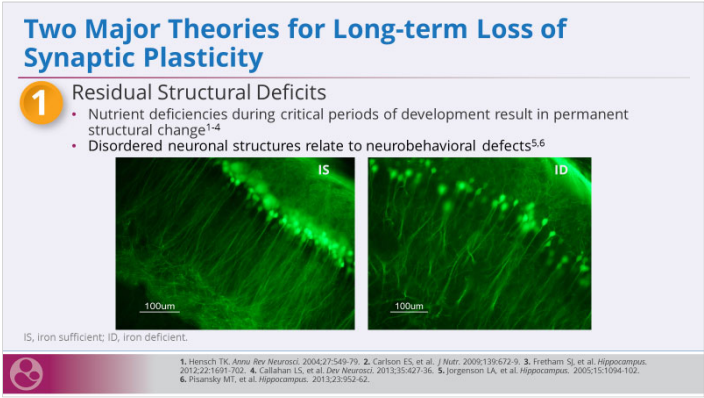
You can see that babies process—these kids process—familiar and novel stimuli differently. That means they recognize the difference. They're showing you a different pattern of recognition.



Slide 54

Now, the risk babies also have different patterns, which you can see how differently wired their brains are, 3½ years after having been iron deficient. We followed these kids into the early teens and found they have difficulty with planning, difficulty with attentional things. Again, that early nutrition really makes a big difference.

How does that happen? How do these early nutritional events shape the brain where we see these residual effects? There are really 2 theories, and we're going to go through both of them, as to how that happens. They're not mutually exclusive. They actually work together.



Slide 55

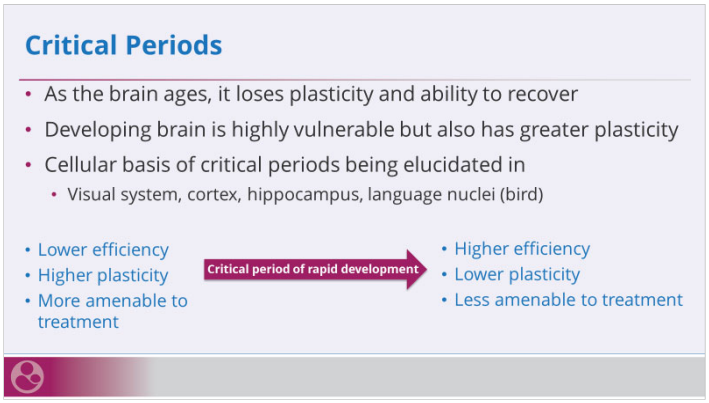
The first one is residual structural deficits [Slide 55]. This is also known as a "critical period hypothesis." I showed you how there are waves of activity in brain development, and how, during the period of rapid development, the brain is more vulnerable. Those periods of rapid development are termed "sensitive" or "critical" periods.[35,36,37,38] Nutrient deficiencies during those critical periods can result in permanent structural change, and then the neurobehavioral deficits that we see are a function of those disordered structures.[39,40] If you look in the brain, you look at that disordered hippocampus. This person, or this mouse, is going to have trouble on recognition memory tasks.

What I'm showing you is the beautiful organization [Slide 55, left image]—in this case of a mouse—of a hippocampus that is iron sufficient. This is the area where recognition memory is thought to happen. Synapses are forming here in an area called CA1. You can see how nicely organized these cells are, how regular the dendrites are, and what an easy target that is for axons to connect and talk to.

This is your brain on iron deficiency [Slide 55, right image]. This amount of iron deficiency is about the amount we see, that has been documented in babies, about a 40% reduction in brain iron. You can see how disorganized the cells look, and you can see how disorganized the structure is, not unlike what Cami [Dr. Martin] showed you, in terms of lung development. Missing a critical nutrient, like iron, in the perinatal period, ends up with this disordered structure in adulthood. These are adult animals; they're no longer iron deficient.

What is it with these critical periods? As the brain ages, we actually lose plasticity and [the] ability to recover. You know that. If an adult has a stroke, they're usually going to be left with a fair residual. Even with physical therapy, you might get 20% or 30% of function back. Yet we have babies who have strokes—term babies who have strokes—and their recovery is actually quite remarkable. Lots of these kids in the follow-up clinic, I cannot even tell on

which side the stroke happened. There's this remarkable plasticity.



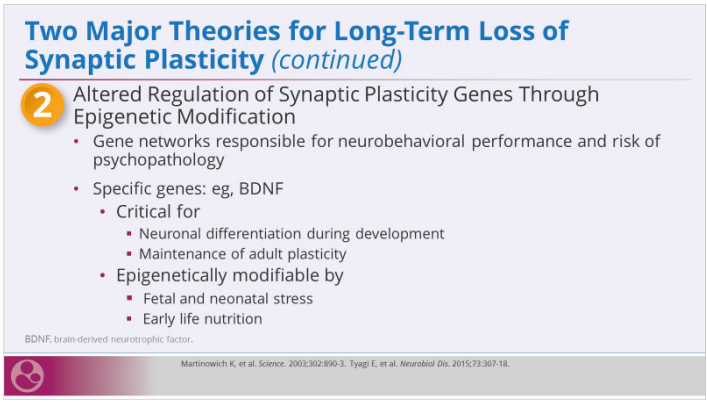
Slide 56

Babies are not very efficient. They can't do math equations for us, but they have tremendous plasticity and are very amenable to treatment. As they proceed, or as these brain regions proceed through this critical period of rapid development, they become highly efficient. They support the behavior much better. The trade-off is that they lose their plasticity and are less amenable to treatment.

Pay attention to when the critical periods are when we're taking care of babies. There are critical periods for myelination, critical periods for dopamine, and critical periods for hippocampal structural development, probably cerebellum, as well. Those are at risk for our nutrient deficits.

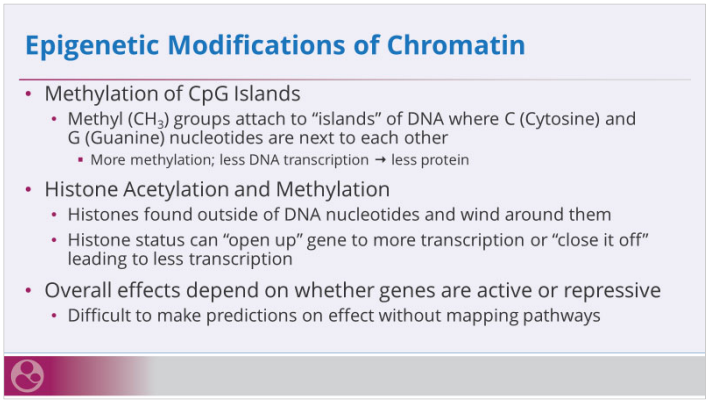
The other possibility is theories related to altered regulation of synaptic plasticity through epigenetic modification. These are gene networks that are responsible for neurobehavioral performance and risk of adult mental-health problems, psychopathology.

There are specific genes you can look at, and there are gene networks you can look at. One of the genes that is a common target for study is the brain-derived neurotrophic factor, BDNF. Now there are clinical studies that show that cord blood BDNF from babies correlates with developmental outcomes, correlates and is driven by risk factors to the brain from maternal gestation. The BDNF is important for neuronal differentiation and for maintaining adult plasticity. It is epigenetically modifiable, both by fetal and neonatal stress, and more importantly, by early life nutrition.[41]



Slide 57

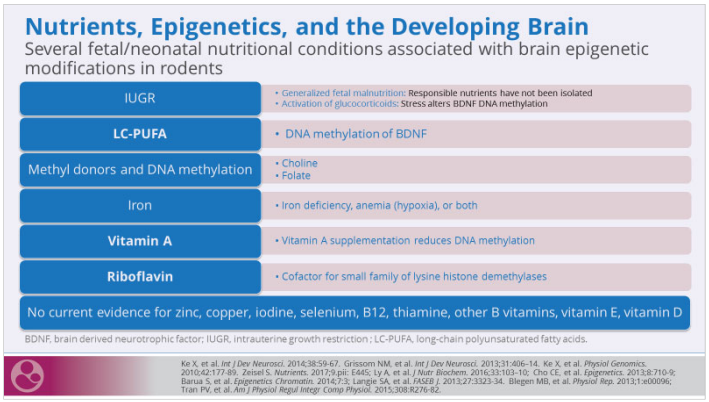
I'm not going to go through all the ways in which environment and nutrition can epigenetically modify chromatin. You've heard, of course, of DNA methylation. The idea is that more methylation results in less DNA transcription, meaning the methylation changes make the DNA less accessible for transcription; and therefore, you get less protein.[42] But there are also histones, and they are acetylated and methylated. There's a second step in DNA methylation called hydroxy methylation. All of these give you a landscape of epigenetic modification.



Slide 58

I'm going to show you the nutrients that have been proven to affect this landscape. The overall effects

of epigenetic modification really depend on whether the genes are active or repressive genes, and how they end up mixing in the end, and how they map onto pathways.



Slide 59

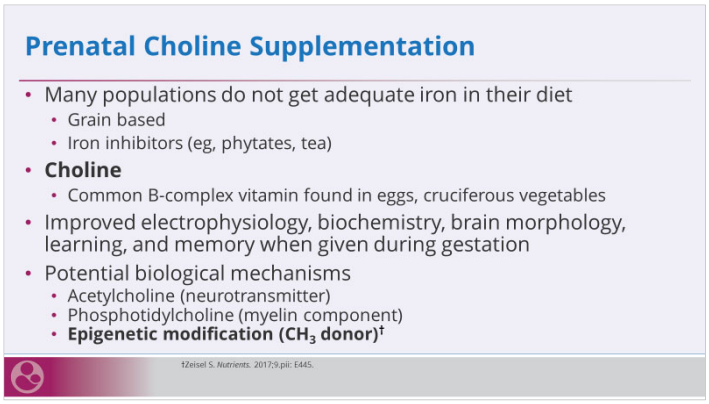
Nutrients, Epigenetics, and the Developing Brain

Which nutrients have been studied in this way? These are those [Slide 59] that were superscripted "3" in the table I showed you earlier. Intrauterine growth restriction affects DNA methylation, particularly of BDNF. LCPUFAs, not surprisingly, affect brain development, and one way they do it is through DNA methylation and BDNF.[43,44,45,46,47]

Methyl donors: we think about a methyl diet, choline, betaine, folate. All of those have been shown to affect not just DNA methylation, but also histone methylation and demethylation.[48] Iron deficiency has a mild effect on DNA methylation but has a major effect on iron-dependent histone demethylases.[49,50] Vitamin A, riboflavin, and then there's a bunch that have not been shown, yet. It's not clear to me from the literature whether that's because they haven't been looked at or whether they really don't have much epigenetic potential.

I'm going to show you an example where nutrients can work for and against an epigenetic landscape. First, I need to tell you about prenatal choline supplementation. It was found back in the late 1980s that increasing the amount of choline in the maternal diet—this is in rats—improved the electrophysiology, the biochemistry, the morphology, and the learning and memory behavior when it was given [at] a very specific time during gestation. Again, implying a critical period, mid-gestation in the rat, and then a second period in the neonate during the period of rapid hippocampal development.

People do not know why this happened. You can think of some choline-based mechanisms by which it happened, but most people are now favoring the fact that choline can act as a methyl donor.[51] Now, that's important because choline is actually commonly available in food.

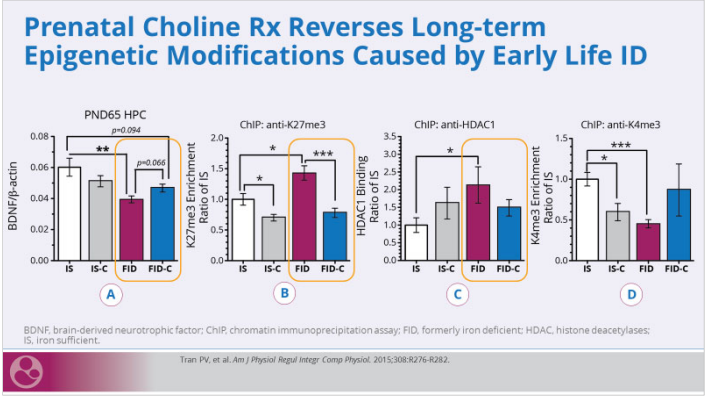


Slide 60

I'm going to show you the interaction of 2 nutrients. Iron deficiency, it turns out, decreases the expression of that important neurotrophic factor: brain-derived neurotrophic factor. We have a pretty good idea of how it does that. In this study [Slide 61], these are animals that were deficient, these are rats that were deficient in the newborn period, but then were allowed to become sufficient. They were repleted in the newborn period, and now they're adults. We're going to take a look at their hippocampus and see what the epigenetic landscape looks like and what the expression of BDNF looks like.

Here are animals that were always iron sufficient (IS) [Slide 61, plots marked IS], here are animals that were iron deficient as pups but are now iron sufficient, so they are FID (formerly iron deficient) [Slide 61, plots marked FID]. You can see the BDNF

level, this important neurotrophic factor for synaptogenesis is significantly decreased, remains suppressed in adulthood.[50] So, this is a lifespan type of effect. If these animals got choline in that newborn period, you actually have some recovery of those levels.

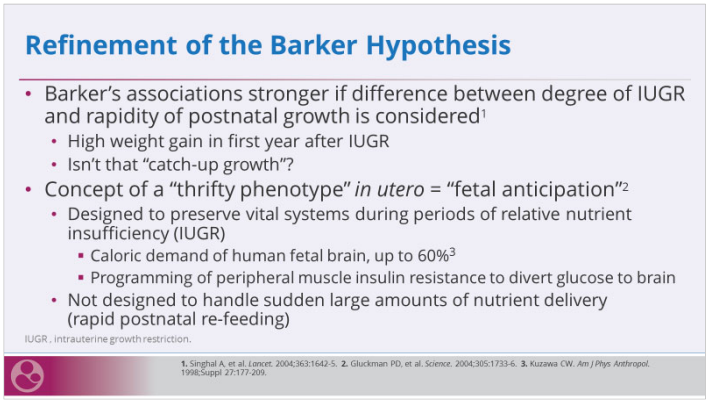


Slide 61

If you ask the question, through what mechanism, you can see it in the histones. This K27 methylation mark is a suppressive mark; it reduces transcription of BDNF. In the formerly iron-deficient animals, we see persistent repression of this K27 mark. Having given them choline, which is the blue bar, they come right back down to normal. And you can see that with the activation, the reverse with the activation mark over here in the D-panel, where the formerly iron-deficient animal has low activation of BDNF of this mark, and then recovery with choline. You can see how these 2 might work together. So, [these are] powerful effects of nutrients in the newborn period.

I want to get back to the Barker Hypothesis. If you remember, we talked about how the Barker Hypothesis tells you that low weight gain in the neonatal growth restriction results in long-term risk of cardiovascular disease.

It was learned over the next 20 years or so, that it really wasn't how small you were at birth, but if you were small, and then grew very rapidly, that really magnified the risks of a cardiovascular disease.[52] This was thought to be due to a mismatch between what the fetus had set itself for: "Gee, I'm going to be malnourished, I'd better be thrifty in the way I set my metabolism." And then all of a sudden, you've got all of this food to work with, or all this substrate to work with. It turns out that is enormously taxing; [it] activates cortisol, activates pro-inflammatory cytokines, which are very toxic to the developing cardiovascular system.

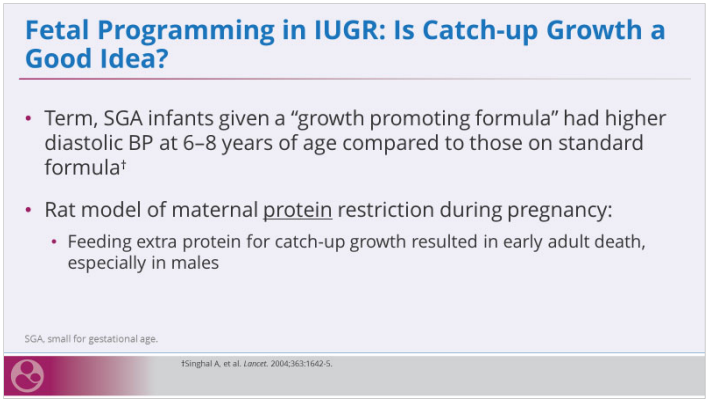


Slide 62

The question we wanted to know was, does that happen in the brain, as well? These are studies that were done in small and term SGA [small for gestational age] infants, who were given a growth-promoting formula. Sure, they grew better, but the cost of that growth appears to be a higher diastolic blood pressure at 68 years of age.

You can model that in rats. If you do a rat model of maternal protein restriction, and you feed extra protein for catch-up growth, you get early adult death, particularly in males. I think Cami [Dr. Martin] mentioned that context is very important, and the matrix of how the nutrients are delivered is very important.

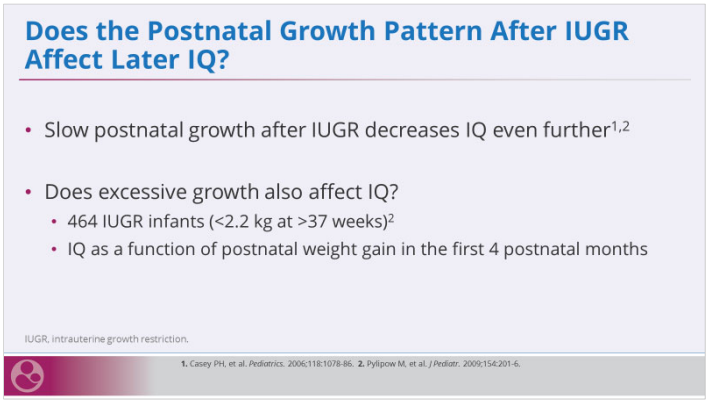
> *"Nutrients are not good or evil. They don't have a moral compass. They work simply in the context of supply and demand, and what's necessary at the time."*



Slide 63

Nutrients are not good or evil. They don't have a moral compass. They work simply in the context of supply and demand, and what's necessary at the time.

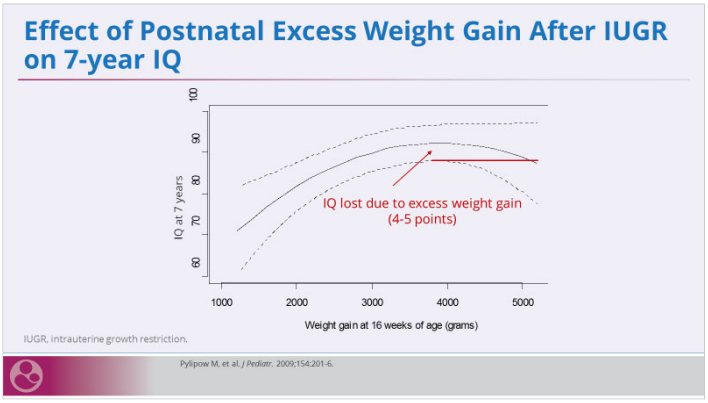
We have to ask the question—we know that slow postnatal growth, after growth restriction, decreases the IQ (I showed you that graph). What happens if you have excessive growth? We know in the Barker data that it increases the risk of cardiovascular disease. Does it do anything to the IQ? In that data set, we had 464 growth restricted babies. These were small babies, less than 2.2 kg at 37 weeks. And we looked at their IQ at 7 years, again, as a function of that postnatal weight gain in the first 4 months.[34]



Slide 64

Here's what we saw [Slide 65]. Remember, we saw that if you didn't grow well after being growth restricted, you had a low IQ. The better you grew, the higher your IQ. But what's with this [Slide 65, red highlight]? If you had excessive growth, more than 3800 g gain, and that's greater than 95th percentile of weight gain in this time period, you actually started to lose IQ points.

Like many things in nutrition, there's a Goldilocks effect. Just because some is good, more is not necessarily better. There's a sweet spot in-between, and our job is to find the biomarkers to monitor in the [NICU], to know when we are hitting that sweet spot.



Slide 65

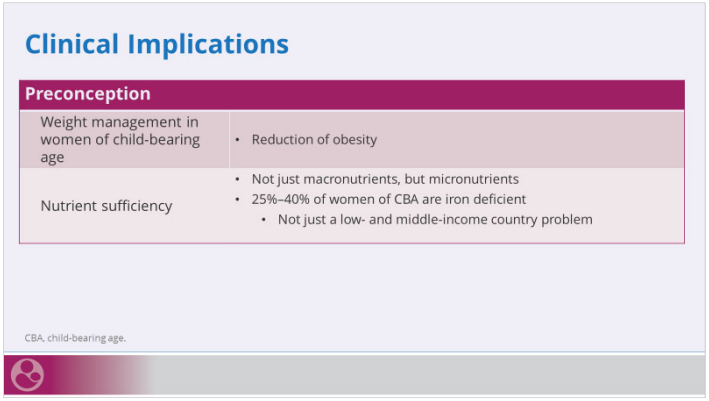
Another example of how over-nutrition, in this case, maternal obesity, affects the offspring's mental health and some of the mechanisms through which it may work. Fetal obesity or maternal obesity is associated with a pro-inflammatory state. We know inflammation is not good for brain development.

Slide 66

Clinical Implications

In these last 3 slides, what are the clinical implications? What can we do differently? Well, I think one thing—and I realize we're at pediatric meetings, and therefore we're not generally obstetricians—is that the conversation, in terms of neonatal brain health and long-term brain health, is both a conversation for obstetricians, in terms of keeping moms healthy, and our care, then, of the subsequent offspring. For the moms, and this may even get us into adolescent health, we want moms who are entering pregnancy to be in the best possible health.

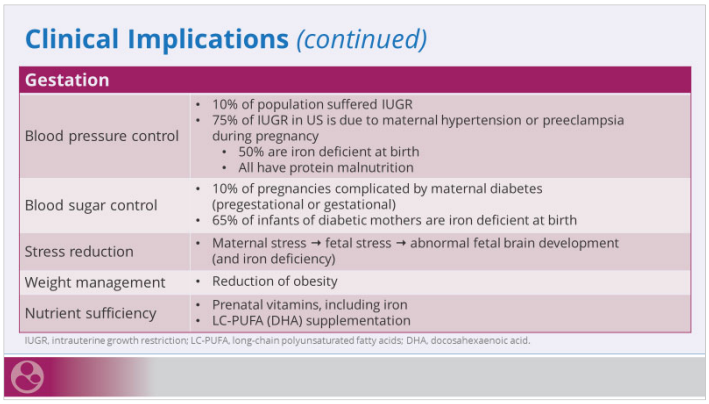
The 2 things we really need to work on is reduction of pro-inflammatory states, like obesity, and reduction of nutrient deficiencies that are common in women of childbearing age (CBA). That's what CBA is. Twenty-five to 40% of women of childbearing age are iron deficient in the United States. That number rises tremendously in low- and middle-income countries.



Slide 67

During gestation, delivery to the fetus and proper loading of the fetus—you knew this from IUGR right? That's an improper loading of protein and energy. It applies to iron, LCPUFAs, and other nutrients that affect brain development. So, maintaining maternal blood pressure under control. Fifty percent of IUGR babies are iron deficient at birth. My guess is they're also deficient in many other nutrients that just simply haven't been looked at.

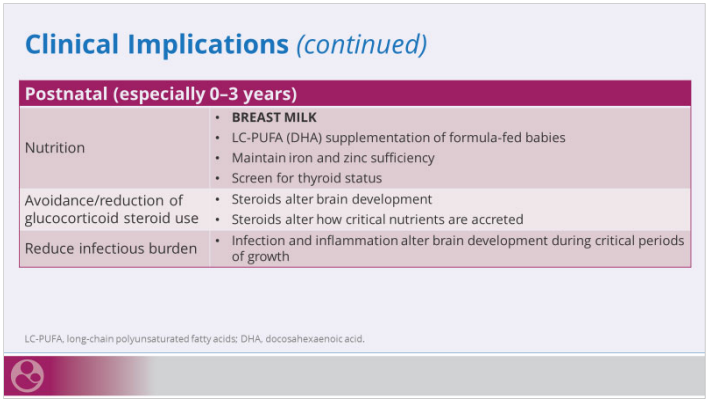
Blood sugar control effects 10% of pregnancies now complicated by maternal diabetes. Sixty-five percent of those kids are going to be iron deficient at birth. Reduction of maternal stress: because maternal stress affects fetal stress, which results in abnormal brain development and also diversion of nutrients from their appointed rounds. Iron gets sequestered when you have stress. Weight management, to again reduce that inflammation. Nutrients sufficiency, including PUFA supplementation during pregnancy.



Slide 68

Finally, in the postnatal period, and this is more for term babies: breast milk, breast milk, and breast milk. And then in formula-fed babies: DHA supplementation; screening, and maintaining iron sufficiency—and especially before kids even become anemic; screening for thyroid status; and again, reduction of stress, and infectious burden, because those really divert the nutrients.

Physiology and Targeted Nutrition in Infants



Slide 69

Abbreviations

AA	arachidonic acid	**IS**	iron sufficient
ANS	autonomic nervous system	**IUGR**	intrauterine growth restriction
BDNF	brain-derived neurotrophic factor	**LC-PUFAs**	long-chain polyunsaturated fatty acids
BM	breast milk	**L:M**	lactulose to mannitol ratio
BOV	bovine-based diet	**MDI**	mental developmental index
BPD	bronchopulmonary dysplasia	**NDI**	neurodevelopmental impairment
CBA	childbearing age	**NEC**	necrotizing enterocolitis
ChIP	chromatin immunoprecipitation assay	**NIS**	noninvasive support
CLD	chronic lung disease	**PCNA**	proliferating cell nuclear antigen
DHA	docosahexaenoic acid	**PMA**	postmenstrual age
DOHaD	Developmental Origins of Health and Disease	**PNGR**	postnatal growth restriction
EPA	eicosapentaenoic acid	**PPAR**	peroxisome proliferator-activated receptor
FID	formerly iron deficient	**PUFA**	polyunsaturated fatty acids
HC	hippocampus	**RAC**	radial alveolar count
HDAC	histone deacetylases	**RN**	restricted nutrition
HMO	human milk oligosaccharides	**ROP**	retinopathy of prematurity
HUM	human milk-based diet	**SGA**	small for gestational age
ID	iron deficient		

1. Raiten DJ, Steiber AL, Carlson SE, et al. Working group reports: evaluation of the evidence to support practice guidelines for nutritional care of preterm infants-the Pre-B Project. *Am J Clin Nutr.* 2016;103(2):648S-678S. doi:10.3945/ajcn.115.117309
2. Barker DJ. Fetal origins of coronary heart disease. *BMJ*. 1995;311(6998):171-174.
3. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation*. 1996;94(12):3246-3250.
4. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science*. 2004;305(5691):1733-1736.
5. Calder PC. Immunonutrition. *BMJ*. 2003;327(7407):117-118.
6. Cho CK, Shan SJ, Winsor EJ, Diamandis EP. Proteomics analysis of human amniotic fluid. *Mol Cell Proteomics*. 2007;6:1406-1415.
7. Ehrenkranz RA, Das A, Wrage LA, et al. Early nutrition mediates the influence of severity of illness on extremely LBW infants. *Pediatr Res.* 2011;69(6):522-529. doi:10.1203/PDR.0b013e318217f4f1
8. Stephens BE, Walden RV, Gargus RA, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. *Pediatrics*. 2009;123(5):1337-1343. doi:10.1542/peds.2008-0211
9. Klevebro S, Westin V, Stoltz Sjöström E, et al. Early energy and protein intakes and associations with growth, BPD, and ROP in extremely preterm infants. *Clin Nutr.* 2019 Jun;38(3):1289-1295. Epub 2018 May 29. doi:10.1016/j.clnu.2018.05.012
10. Taylor SN, Basile LA, Ebeling M, Wagner CL. Intestinal permeability in preterm infants by feeding type: mother's milk versus formula. *Breastfeed Med.* 2009;4(1):11-15. doi:10.1089/bfm.2008.0114
11. Bode L. Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology*. 2012; 22(9):1147–1162.
12. Konnikova Y, Zaman MM, Makda M, D'Onofrio D, Freedman SD, Martin CR. Late enteral feedings are associated with intestinal inflammation and adverse neonatal outcomes. *PLOS One.* 2015;10(7):e0132924. doi:10.1371/journal.pone.0132924
13. Martin CR, Dasilva DA, Cluette-Brown JE, et al. Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. *J Pediatr.* 2011;159(5):743-749.e1-2. doi:10.1016/j.jpeds.2011.04.039
14. Singh P, Ochoa-Allemant P, Brown J, Perides G, Freedman SD, Martin CR. Effect of polyunsaturated fatty acids on postnatal ileum development using the fat-1 transgenic mouse model. *Pediatr Res.* 2019;85(4):556-565. doi:10.1038/s41390-019-0284-0
15. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics*. 2006;117(4):1253-1261.
16. Joss-Moore LA, Hagen-Lillevik SJ, Yost C, et al. Alveolar formation is dysregulated by restricted nutrition but not excess sedation in preterm lambs managed by noninvasive support. *Pediatr Res.* 2016;80(5):719-728. doi:10.1038/pr.2016.143
17. Hair AB, Peluso AM, Hawthorne KM, et al. Beyond necrotizing enterocolitis prevention: Improving outcomes with an exclusive human milk-based diet. *Breastfeed Med.* 2016;11(2):70-74. doi:10.1089/bfm.2015.0134
18. Spiegler J, Preuß M, Gebauer C, et al. Does breastmilk influence the development of bronchopulmonary dysplasia? *J Pediatr.* 2016;169:76-80.e4. doi:10.1016/j.jpeds.2015.10.080
19. Martin CR, Dasilva DA, Cluette-Brown JE, et al. Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. *J Pediatr*. 2011;159(5):743-749.e1-2. doi:10.1016/j.jpeds.2011.04.039

20. Jobe AH. Animal models, learning lessons to prevent and treat neonatal chronic lung disease. *Front Med (Lausanne).* 2015;2:49. doi:10.3389/fmed.2015.00049
21. Collins CT, Makrides M, McPhee AJ, et al. Docosahexaenoic acid and bronchopulmonary dysplasia in preterm infants. *N Engl J Med.* 2017;376(13):1245-1255. doi:10.1056/NEJMoa1611942
22. Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tolley EA. Arachidonic acid status correlates with first year growth in preterm infants. *Proc Natl Acad Sci U S A.* 1993;90(3):1073-1077.
23. Lavoie JC, Mohamed I, Nuyt AM, Elremaly W, Rouleau T. Impact of SMOFLipid on pulmonary alveolar development in newborn guinea pigs. *JPEN J Parenter Enteral Nutr.* 2018;42(8):1314-1321. doi:10.1002/jpen.1153
24. Löfqvist CA, Najm S, Hellgren G, et al. Association of retinopathy of prematurity with low levels of arachidonic acid: A secondary analysis of a randomized clinical trial. *JAMA Ophthalmol.* 2018;136(3):271-277. doi:10.1001/jamaophthalmol.2017.6658
25. Thompson RA, Nelson CA. Developmental science and the media. Early brain development. *Am Psychol.* 2001;56(1):5-15.
26. Kuzawa CW. Adipose tissue in human infancy and childhood: an evolutionary perspective. *Am J Phys Anthropol.* 1998;Suppl 27:177-209.
27. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days." *J Pediatr.* 2016;175:16–21. doi:10.1016/j.jpeds.2016.05.013
28. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. *Am J Clin Nutr.* 2007;85(2):614S-620S.
29. Strauss RS, Dietz WH. Growth and development of term children born with low birth weight: effects of genetic and environmental factors. *J Pediatr.* 1998;133(1):67-72.
30. Eide MG, Moster D, Irgens LM, et al. Degree of fetal growth restriction associated with schizophrenia risk in a national cohort. *Psychol Med.* 2013;43(10):2057-66. doi:10.1017/S003329171200267X
31. Insel BJ, Schaefer CA, McKeague IW, Susser ES, Brown AS. Maternal iron deficiency and the risk of schizophrenia in offspring. *Arch Gen Psychiatry.* 2008;65(10):1136-1144. doi:10.1001/archpsyc.65.10.1136
32. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics*. 2000;105(4):E51.
33. Pollitt E, Gorman KS, Engle PL, Rivera JA, Martorell R. Nutrition in early life and the fulfillment of intellectual potential. *J Nutr.* 1995;125(4 Suppl):1111S-1118S. doi:10.1093/jn/125.suppl_4.1111S
34. Pylipow M, Spector LG, Puumala SE, Boys C, Cohen J, Georgieff MK. Early postnatal weight gain, intellectual performance, and body mass index at 7 years of age in term infants with intrauterine growth restriction. *J Pediatr.* 2009;154(2):201-206. doi:10.1016/j.jpeds.2008.08.015
35. Hensch TK. Critical period regulation. *Annu Rev Neurosci.* 2004;27:549-79.
36. Carlson SE. Early determinants of development: a lipid perspective. *Am J Clin Nutr.* 2009; 89(5): 1523S–1529S.
37. Fretham SJ, Carlson ES, Wobken J, Tran PV, Petryk A, Georgieff MK. Temporal manipulation of transferrin-receptor-1-dependent iron uptake identifies a sensitive period in mouse hippocampal neurodevelopment. *Hippocampus*. 2012;22(8):1691-1702. doi:10.1002/hipo.22004
38. Callahan LS, Thibert KA, Wobken JD, Georgieff MK. Early-life iron deficiency anemia alters the development and long-term expression of parvalbumin and perineuronal nets in the rat hippocampus. *Dev Neurosci.* 2013;35(5):427-436. doi:10.1159/000354178
39. Jorgenson LA, Sun M, O'Connor M, Georgieff MK. Fetal iron deficiency disrupts the maturation of synaptic function and efficacy in area CA1 of the developing rat hippocampus. *Hippocampus*. 2005;15(8):1094-1102.
40. Pisansky MT, Wickham RJ, Su J, et al [Georgieff MK]. Iron deficiency with or without anemia impairs prepulse inhibition of the startle reflex. *Hippocampus*. 2013;23(10):952-962. doi:10.1002/hipo.22151

41. Tyagi E, Zhuang Y, Agrawal R, Ying Z, Gomez-Pinilla F. Interactive actions of Bdnf methylation and cell metabolism for building neural resilience under the influence of diet. *Neurobiol Dis.* 2015;73:307-318. doi:10.1016/j.nbd.2014.09.014
42. Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun YE. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science*. 2003;302(5646):890-893.
43. Ke X, Xing B, Yu B, et al. IUGR disrupts the PPARγ-Setd8-H4K20me1and Wnt signaling pathways in the juvenile rat hippocampus. *Int J Dev Neurosci.* 2014;38:59-67. doi:10.1016/j.ijdevneu.2014.07.008.
44. Grissom NM, Reyes TM. Gestational overgrowth and undergrowth affect neurodevelopment: Similarities and differences from behavior to epigenetics. *Int J Dev Neurosci.* 2013;31:406–414. doi:10.1016/j.ijdevneu.2012.11.006
45. Ke X, Schober ME, McKnight RA, et al. Intrauterine growth retardation affects expression and epigenetic characteristics of the rat hippocampal glucocorticoid receptor gene. *Physiol Genomics.* 2010;42(2):177-189. doi:10.1152/physiolgenomics.00201.2009
46. Zeisel S. Choline, other methyl-donors and epigenetics. *Nutrients*. 2017;9.pii: E445. doi:10.3390/nu9050445
47. Ly A, Ishiguro L, Kim D, et al. Maternal folic acid supplementation modulates DNA methylation and gene expression in the rat offspring in a gestation period-dependent and organ-specific manner. *J Nutr Biochem*. 2016;33:103–110. doi:10.1016/j.jnutbio.2016.03.018
48. Langie SA, Achterfeldt S, Gorniak JP, et al. Maternal folate depletion and high-fat feeding from weaning affects DNA methylation and DNA repair in brain of adult offspring. *FASEB J.* 2013;27(8):3323-34. doi:10.1096/fj.12-224121
49. Blegen MB, Kennedy BC, Thibert KA, Tran PV, Georgieff MK. Multigenerational effects of fetal-neonatal iron deficiency on hippocampal BDNF signaling. *Physiol Rep*. 2013;1(5):e00096. doi:10.1002/phy2.96
50. Tran PV, Kennedy BC, Lien YC, Simmons RA, Georgieff MK. Fetal iron deficiency induces chromatin remodeling at the *BDNF* locus in adult rat hippocampus. *Am J Physiol Regul Integr Comp Physiol*. 2015;308(4):R276-282. doi:10.1152/ajpregu.00429.2014
51. Zeisel S. Choline, other methyl-donors and epigenetics. *Nutrients*. 2017;9.pii: E445. doi:10.3390/nu9050445
52. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet*. 2004;363(9421):1642-1645.