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TRANSCRIPT

Liver-Gut-Microbiome Axis and Fatty Acid Absorption in Preterm Infants

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I've been at Texas Children's since 2009, so a lot of what I'm going to share we've studied over time at our Children's Hospital. I have no disclosures other than that I'm a Georgia Bulldog, and these are my funding sources. And I have 30 minutes, so some of these slides I'm going to go through a little faster than I'd like to. At our nutrition program at Texas Children's we have a 200-bed NICU and we also have a large inborn population including a program for 22- and 23-week infants. We also take care of babies in the cardiac ICU, so nutrition spans all of these babies.

We're a big team, so it's not just me. I have a great team of dieticians and other colleagues as well. We couldn't do anything without our dieticians helping us in rounds and also helping us make our protocols and make sure every year they're up to date. We also have a robust research team. I'm very thankful to have a lot of help; it allows us to do great research.

I've been at Texas Children's for 16 years. I came to do my fellowship. I actually didn't think I'd stay on as faculty, but I did, and I've been there since. And over the years, we've published many papers looking at postnatal growth failure, how to best grow our babies and how to optimize the use of the human milk diet. I will try to minimize talking about human milk today. Dr. Stansfield's going to come up soon and talk to you guys about mother's milk and donor milk, but we've had a lot of experience with that. And we're learning over the years, human milk is not just nutrition. We know there's important immune components, important enzymes, hormones, other factors that we probably can't even test for at this point and so there's been a lot of focus on mother's own milk and how do we optimize that for preterm babies.

And here, this is from Misty Good's group, but just showing you some of the immune modulating components of human milk, such as lysozyme, lactoferrin, IgA and these are important components that help our preemies, help the intestine. And so, in 2012, it's very interesting that American Academy of Pediatrics--at the time in 2012, not many NICUs were using pasteurized donor human milk--the AAP came out with this statement recommending that all babies less than 1,500 g birth weight should receive pasteurized donor milk if their mom's milk is not available. So, always mom's milk first, donor milk second. And ESPGHAN, which is the European guideline committee, came out with similar recommendations.

I would love to talk, at some point in the future, about innovations in human milk. Luckily, there are some industry companies, as well as different researchers across the world, really trying to look at components of human milk and how do we capture those and give those to our preterm babies. So, for example, here is a picture of human milk oligosaccharides. These are carbohydrates that are in milk, but they also are very important for good bacteria in the intestine of the babies and they're immune-modulating. And so how do we maybe localize some of these human milk oligosaccharides and give them to preterm infants? Also, the invention of the human milk analyzer, so Miris. I have this example up here because it's the only FDA-approved device here in the US to analyze human milk for preterm infants. And then we're starting to learn more about human milk fat globules. I'm not going to talk about that today, but actually there are these globules in the milk. They're fat, but they have all of these kind of immune appendages and immune function attached to them and I think we're going to hear about that more in the future.

I'm going to tell you a little bit of a story, I promise we're going to get to the liver-gut-microbiome very shortly, but just telling you a story of how we got to this research and how we got into this field. Early in my career we were worried about babies not growing well on donor human milk. They'd receive mom's milk, they'd get supplemented with donor milk, but we had a proportion of babies that were receiving only donor human milk and that's because their moms weren't able to breast-feed or provide milk. And we really struggled with trying to grow these babies. And then, over the years, more research has come out, even at our own institution, realizing that mother's milk and donor milk are variable in calories, especially protein and fat. So, we're sitting here ordering, or if you're still doing handwritten orders which I remember before we had electronic medical records, but you're writing a prescription for nutrition, enteral nutrition, for the baby for the day and you think you're giving great calories and great protein, but if you're not analyzing the milk, meaning getting the calories, the protein, the fat—the macronutrients—if you're not analyzing, you actually don't know what you're giving. We're basically guessing.

And that's okay if the baby's growing. I think we're fine with that. But if the baby's not growing, what do you do with that information? Also, most moms, at least in the US, that donate

their milk for it to be pasteurized for preterm babies, they have babies that are born on time and so this is term milk. It's usually, these moms donate at about 8 to 9 months of lactation and we know it's lower in protein. And we know babies, the second they're born, they have a protein deficit. So, we're already giving them a base milk that is low in protein.

And I'll let Dr. Stansfield talk about this. This is a paper we worked on together, but I just want to, because I'm talking about protein, highlight this work we did looking at preterm milk. Preterm milk is featured here in the red and the blue, green is donor milk and this is protein, the amounts of protein. You can see that donor milk here in green is much lower in protein than preterm milk at day 7 or day 28. And so we need to keep this in mind when we're trying to calculate our nutrition recipes and the macronutrients our babies are receiving.

I'll also briefly go through this. This is a table we put together, just pasteurization—Holder pasteurization—there's heat applied to donor milk. And the problem with that is it denatures enzymes. It denatures some of these immune components and they're not functional. And I'm going to talk about enzymes, so that's why I wanted to bring it up.

We also, through some of the studies in our center, again started testing mothers' milk, started testing donor milk and there was so much variety. Trying to figure out how do we grow babies, but how do we also address this variability and the calories and especially protein of human milk. We did this study a while ago, but what we were trying to do in this study—this is an older analyzer and they told me recently they're discontinuing it—but we also have a Miris, so we'll be okay. In this study we were analyzing all the milk the babies were receiving every single day and one group was getting standard diet, the other group was getting a supplemented donor milk fat or cream if the milk calories were less than 20 calories per ounce. And so this was a way to kind of even the bar, raise all the calories to 20 calories per ounce base before adding fortifier. And we actually found babies that got this donor milk supplement had better growth in weight and length.

Then, I took it a step further. This study is not published yet and it's not published because we didn't really find the full results that we expected. So we're going back through the data trying to put it together for publication. We thought, well, we'll analyze all the milk the baby's receiving every day and if they're low in protein, we already add fortifier, a donor human milk dried fortifier, but we'll add extra fortifier, if needed, for protein. And we did one level of protein vs a little bit higher level and we actually found no difference between the groups. So, we're currently sorting through this. But this was a 3-year study and, at this point, I decided, well, I'm not getting all the answers. We should be getting all the answers by analyzing mom's milk and doing targeted fortification. We're missing something. We know growth for babies is multifactorial. There's the genetic component, there's other components we can't account for, but there are some modifiable factors and what are those? And I think we're missing those and so that's what I'm going to talk about moving forward.

How do address these other pieces of the puzzle that haven't been well studied? And here's my slide reminding me to tell you that we're missing something and part of that is fat absorption. There's not a lot of physiology studies in our preterm babies when it comes to the liver, when it comes to fat absorption in the intestine. There are some studies from probably 20, 25 years ago, but those babies were about 1,800 g. Now we're taking care of 300-, 400-, 500-g babies and we don't understand their physiology. And then a 22-, 23-week baby born early is completely different. So, what do we need to learn to be able to fix this problem?

This is some work by Cami Martin who I am working with on this project and she found early on—I would say she's a fatty acid expert—that the minute babies are born, meaning they don't get any more nutrients from the placenta, there is a drop in DHA and AA, arachidonic acid. So, these are 2 long-chain fatty acids, they're very important for eye development and brain development and other functions in the body. And we know that the minute a preterm baby is born, their levels drop. And even with our best nutrition, we can't raise those levels very well and it's a problem.

Also, some of Cami's work looking at . . . down here you can see these are these long-chain fatty acids, the carbon links are longer, that's why it's called long-chain fatty acids. And looking at absorption at 2 weeks and 6 weeks of age, you can see here, this is breast milk, this is formula. With babies, for the most part, the coefficient of absorption—basically the percent fat they can absorb—is okay for breast milk, still not great, it's not 100%. And then formula is really bad. The question is what about donor milk? We have no idea! That hasn't been studied. I'm studying it right now, but we don't know that and so we're giving all of these preterm infants, we've been using donor milk at my hospital since 2009, and we don't have this baseline physiology data.

And we thought to look at a population of babies less than 1,250 g because that's our cut-off for using a diet of mother's milk supplemented with donor human milk and fortified with a donor human milk-derived fortifier. And we wanted to look at fatty acid levels. These were actually blood levels, not stool, looking at percent absorption, but we wanted to see what do their blood levels look like? And again, I could go through all these studies a lot longer, but the short version was that babies that received this all-human milk diet, if you want to call that,

maintained their DHA levels from birth. So, instead of this major drop down, they kind of were steady. That's great! The problem is arachidonic acid still had a major drop. We weren't able to correct for that. So, there's more to this story. Sometimes it's not just about DHA; it's about all these other fatty acids working together in combination, and some of that needs to kind of be pieced out and evaluated.

On the same method again, worrying about growth failure in our preterm babies and thinking we're giving this great nutrition. I mean, honestly, the best we can give from enteral nutrition to preterm babies, and they're not growing. Probably 30% to 40% to 50% of them, depending on what gestational age you look at. Again, what else are we missing? Well, we know in babies with cystic fibrosis that they have pancreatic insufficiency. Well, what about preterm babies? There's been some studies on this, but not large numbers, not looking at modern nutrition. And so we looked at a cohort of babies and evaluated fecal elastase. I'll explain that in a minute. But we know that babies develop over time, their pancreas, the minute they're born it starts to kind of develop and produce the enzymes it needs to, but not initially. It takes weeks. And so we tested fecal elastase-1 via ELISA. And this—I couldn't find a better picture, I'm sorry, this is the best I have—is showing us the pancreas. Here's the intestine and this is elastase, and then it makes its way down into the stool and it's called fecal elastase. But we tested for that and it was about 30 babies and we quantified it at early and late. We couldn't do a bunch of different measurements because actually it takes a lot of resources and a grant. Maybe in the future, we can do a bigger study, but we basically checked levels at 2 weeks of age and then at about 2 months of age and looked at this progression. And for the most part, if you look at this, these are lines. This is the early time point. This is the late time point. And you can see most of these fecal elastase levels increased over time. There's a couple that go down and that's against what we would expect, so I'm not sure what's going on with those babies, but overall it increases. So, that's good, but I don't think we can wait 'til the baby's 2 months of age for them to start growing. We want them to start growing within the first few weeks of life, especially once they come off parenteral nutrition.

So, what we found is that, over time, fecal elastase increases and, early on, most preterm babies aren't making enough of these enzymes and so they have kind of this developmental pancreatic insufficiency. And also, we thought, well babies, if they had kind of less pancreatic insufficiency, maybe they had better growth. We didn't find that. It's a small cohort, so we need to expand our population. What we did find is that babies that had pancreatic insufficiency needed more calories for growth, which kind of makes sense. They would need more.

Going back to our challenges. Babies who still aren't growing despite modern nutrition. We're concerned about fatty acid absorption and then I haven't even talked to you yet about the liver. So, we know babies, all their organs, head to toe, are premature when they're born, but there haven't been that many studies about the liver. Before I got into this research, I didn't think about liver much. Don't tell my pediatric GI colleagues that, but it's not something we necessarily think of. And then what about the role of the microbiome?

We looked at a study, it's probably eight years ago at this point, using 16S sequencing. This is a high-level view looking at the bacteria that are in the intestinal microbiota. We took stool, we did the 16S sequencing. I'm not going to go through the entire study, but the short version is we compared babies that received mothers' milk to those that received donor milk and what did the bacteria look like in the stool. And we also looked at this over time, from the first week of life, all the way to week 6. The white boxes are mother's own milk, and what we found is that, over time, this is your diversity. Babies that received mother's own milk had greater bacterial diversity when we tested it compared to donor milk. And it appeared from our data, donor-milk fed infants, if they aren't exposed to mother's milk at all from birth, it takes an additional 2 weeks for their microbiome to match that of mother's-milk fed babies. And that was interesting. And I have more sequencing and more data to show you in a minute, but this is when we came up with the whole idea: how does the liver, the microbiome and the intestine all work together in relationship to fat absorption?

This is a picture from the adult literature. They're actually really figuring this out in the adult world. It's much easier to study, to obtain stool, to study it and figure these things out, but what's very interesting is that there is a component related to fatty acids here. There are microbial metabolites, basically the bacteria there make certain metabolites that actually interact with the entire liver, intestine system. They contribute, and that's very interesting because I don't think we usually think the bacteria make metabolites. Actually, they signal in the body for the body to have certain functions. And one of those is how to digest fat and how to absorb it.

This is one of my big says, this is what you do when you do a big study. You get together a bunch of experts to help you. I'll tell you, I love nutrition and human milk, but I'm not a microbiome expert, I'm not a fatty acid expert like Cami, but we're all learning this together and Ken Setchell here, he is most well-known for bile acid. What I'm going to be talking about in this whole fatty acid absorption, microbiome picture, is bile acids are needed to digest fat and to allow it to be absorbed. If you don't have bile acids, you're in trouble, the babies are in trouble. They can't absorb their fat.

And here's a diagram that we're studying. We think this is what the picture looks like, but we'll see when we're done with the study and see if this is the same. But I'm talking to you guys about the babies' microbiome that is immature, so it's not fully developed. We also know that the liver's immature and the liver is what helps with the bile acids which helps with fat digestion. You've got these 2 immature pieces. And then if the babies don't have the right microbes in their intestine, they can't make these metabolites. They can't make these bile salt hydrolase enzymes that digest fat. And it's a really interesting process that we need to figure out because we know long chain fatty acids, you can see my picture at the bottom, they're important for the brain, I left out the lungs, sorry I know we had a BPD talk yesterday, and the eyes. And so, one of the things we're looking at is these microbial bile acid-modifying genes, the components that the bacteria there make that contribute to fat digestion and absorption.

We're collecting a bunch of stool samples. I will tell you to look at fat absorption, you're actually supposed to look at all of the diet a baby receives over 72 hours and every stool a baby has over 72 hours and then you compare them. That's been a challenge. If you can imagine, it's like 1 stool gets thrown out and then we have to start the whole collection process over. But it will give us a lot of information. Someone asked me, Amy, why are you doing this 72-hour collection? Well, that's what the gold standard is in the literature. Hopefully, through this study, we'll be able to find a test for clinicians, like us at the bedside, an easy way to test for this so that we don't have to do these long collections.

Kind of moving on in this story, looking at the relationship between the gut microbiome and growth failure in preterm babies, we did a meta-analysis because there's actually been many papers looking at this in preterm babies, growth and the microbiome. We know that in the microbiome development, of course, nutrition's related, maternal factors, babies being born early. There's actually a genetic component and antibiotics and medication exposure. And there's actually some more microbial products that I won't talk about today that contribute to growth here on the right. I'm not going to go through this, but the point of this picture is we took all the studies that have studied growth and microbiome. Guess what? None of them overlap. Whether it's a positive association with growth or a negative association with growth. They're all different and part of that is because every paper defined growth failure differently. So, it's really hard to compare literature when this person's defining growth failure this way, someone else is defining it a different way. But it was very interesting. We thought surely, and even last night when I was looking at this slide again, I was like surely there's complete overlap somewhere. There's not.

We put this heat map together. What this is supposed to show you is I came up with, I would say the 12 most popular definitions and the literature for growth failure, whether you're—most of it's based on weight—but if you're looking at length and head circumference. And these are all the different bacteria that have been found in the microbiome. Red means negative association; blue means positive. We also looked at, over time, the postmenstrual age when the sample was obtained and the week of life the baby was when the sample was obtained. And, as you can see, I'm not going to go through it, but it makes no sense. I mean, it's all over the place. So, I think the most important thing that we took away from this paper was that everyone needs to define growth failure the same when they're looking at microbiome papers. If you see a paper published and they say this bacteria causes growth failure, that's great, but look at their definition of growth failure and see if it's something we would use clinically in preterm babies.

We did a second study. We compared babies that were not just preterm babies, but preterm babies compared to preterm babies that had liver problems and cholestasis, meaning their conjugated bilirubin was high and they had trouble with bile acid excretion. And I will just highlight a few things. One, this is different sequencing. This is newer generation, whole genome, meta-genomic sequencing. It's the best you can do. So, it not only tells you the exact bacteria that are present in the sample, but what are the microbes and the factors, their metabolites they make. It gives you a functional idea; not just what's there, but what's it doing, what's the bacteria doing there. And we found, similar to our previous data I showed you, that, over time, there is an increase in diversity over age. There's an age component. Again, it takes time for the microbiome of these preterm babies to develop. And then, down here, I talked to you about bile acids. There is an enzyme made by clostridium, I know we don't like clostridium, we worry about it with NEC, but there's evidently a good clostridium out there that helps digest fat. It makes bile salt hydrolase and that converts primary to secondary bile acids and you can see here in this figure, over time, as a baby matures in gestational age or postmenstrual age, the secondary bile acid synthesis which basically means the ability to digest fat increases. The good news is if you can make it to 30 . . . if you can keep a baby supported on nutrition to 32 weeks, they will start growing because part of that, we think, is that the bile acid synthesis kicks in and this is a similar depiction in another way.

Here's another picture, if you like pie charts and color. I do! These are control babies. These are babies that have the liver disease and the cholestasis and the problem with bile that I talked about. And you can see, over time, at 25 weeks postmenstrual age to up to 40 weeks postmenstrual age, it definitely changes. So, if your secondary bile acid synthesis is working, you will have more unconjugated bile acid. This is just

showing that, over time, you have this increase, but then in cholestatic babies who have their entire liver, bile, fat digestion system messed up, you don't have it. And so that was a very interesting finding, kind of helping us figure out what's going on to help fat digestion in the body. And then we also looked at babies who received cream or did not receive cream, I had talked about the initial study we did where we added donor milk fat to some babies' diets to help with growth, and again the study's much longer, but the short version here is babies that received the cream supplement to their diet, their microbes had up-regulation. Remember I keep talking about metabolites, things they make, up-regulation of lipid metabolism. Someone asked me, "Well Amy, what does that mean. I'm a neonatologist at the bedside, what does this mean?" What it suggests, and again, this is just what's there so we'd have to go back and test the functionality of it, but it makes us think that, you're giving additional fat to the baby and the microbes are recognizing that and they're up-regulating fat digestion from that. So, that's a stretch. We'd have to go back into the basic science lab and do mechanistic studies, but this is one of the first studies to show what we see babies actually affects their overall metabolism from a microbiome standpoint. And this is another picture depicting that.

Going back to all these challenges we have . . . and I'm running out of time so I apologize, but we're making some headway. We're figuring out some things. My goal in the next 5 years is to have this bigger study done and then trying to translate it back to the bedside. So, what can we do to fix this problem? And in going back to this, one of the main things we can do that we know of so far is to optimize mother's own milk use. Breast milk. Giving babies more breast milk, allowing the dose to be more. When you add liquid fortification to human milk or mom's milk, there's a displacement ratio. We need to deliver as much mom's milk as possible. I'm not sure I emphasized this earlier, but mom's milk has fresh enzymes, lipase, all these pancreatic enzymes. It predigests the milk for the baby. We need to be focusing on that until we can figure out a different target. I know we're hearing about probiotics next, so until we can figure out how do we populate the microbiome to help better digest fat or until we figure out a way to supplement bile acids to help with fat digestion, we can optimize mom's milk. That's the best thing we can do. However, some of our moms have medical conditions or they can't lactate and so how do we work around that and help them?

These are some of the studies, the ABSORB study is the one I talked about looking at fatty acid absorption. We're also participating in a trial called LET-FEED where basically trophic feeds vs no trophic feeds, so trying to feed babies faster. And then we're part of the NEC biorepository. If you're interested in necrotizing enterocolitis, the NEC Society was started by Jen Canvasser who lost her baby to NEC and it has a lot of resources. And we have a research group that meets once a month and goes over important topics. If you go online, you can sign up and get information if you're interested. And I couldn't do this without my team and I thank my funding sources and all of my team members that work really hard. And I do have to give a shout-out, you mentioned the Dr. Diane Anderson Neonatal Conference. I took over this conference in 2020 when Dr. Anderson passed away and we are now a virtual conference and it's online. We try to keep the price down, so everyone can attend and learn about nutrition. So, just consider that. Registration will be open soon, but we try to address the hot topics in neonatal nutrition and have speakers from all over the world.

And this is a picture of Texas Children's which keeps growing. Actually, this is an old picture. We have a new building since then. But thank you for your time. I know that was a lot for 8:00 AM, but I hope at least I've emphasized that we're on the path to figuring out more about fatty acid absorption, the microbiome and the liver.

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