THE ESTABLISHMENT OF A HEALTHY INFANT MICROBIOME & THE ROLE OF HUMAN MILK OLIGOSACCHARIDES

Featuring:
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Transcript

Maura Bowen: Hello and welcome to the Abbott Nutrition Health Institute 'Power of Nutrition' podcast. I'm Maura Bowen and I'm here today virtually with Dr Akshay Batra, a Consultant Paediatric Gastroenterologist at Southampton Children's Hospital. Dr Batra is actually dialing in from his office in the hospital today, so we are especially grateful he is able to carve out a few minutes to speak with us. And, of course, we also have Jane Schlezinger, a Paediatric Specialist Dietitian and Medical Science Liaison from Abbott in the UK. Together, Jane and Dr Batra will discuss the infant microbiome; including how it develops, why it is important for infant health and how specifically human milk oligosaccharides impact on gut health and beyond.

Now, in a moment, I'll hand this discussion over for Jane to lead. She will conduct the interview with Dr Batra. But first, it's important to note an infant's microbiome is influenced by multiple genetic and environmental factors including the type of early feeding, mode of delivery, use of antibiotics, maternal genetics and exposure to human milk oligosaccharides, particularly 2'-fucosyllactose. Now, the science is rapidly evolving in this exciting area, and we're delighted to be able to discuss it further, so Dr Batra and Jane, thank you both for joining us. Jane, I will hand over the discussion for you to get started.

Jane Schlezinger: Thanks Maura. It's a pleasure to be here, and thanks again to Dr Batra for joining us. I know you have a particular interest in the field, and we are keen to learn more from you and your expertise. Perhaps you can start by introducing yourself and explaining your background and interest in this topic?

Dr Batra: Thank you both for inviting me to talk about this topic. I am a Paediatric Gastroenterologist, and I have a special interest in nutrition. I find this topic extremely interesting because it has a huge impact in our day to day practice. And we see a fair number of patients in our practice where there is emerging evidence that altering the gut microbiome can improve symptoms and in some cases, change the course of the disease. I've also been involved in some research looking at gut microbiome in children with inflammatory bowel disease. I find the emerging research in this area extremely fascinating.

There has been a lot of interest in this field for a few years now, and I feel we are in a good position where we understand the differences in the microbiome in disease and health. What we need to do more is to understand whether these differences are a causation or only an association. If you take a condition like necrotizing enterocolitis or inflammatory bowel disease in children. We know that the microbiome of a healthy child will be different to somebody with inflammatory bowel disease. What we don't yet know is whether this is the cause of their condition, or this is just an association because of an inflamed bowel. That is the information we need moving on for further research in this area.

Jane Schlezinger: So from your work, you clearly see first-hand the importance of the infant microbiome and the impact of the different factors that contribute to its development from the outset., Can you explain a little more about the gut microbiome overall; what it is and why it's important to infant health and development?

Dr Batra: Our gastrointestinal tract starting from the mouth, all the way down to the colon, is full of bugs everywhere. These include bacteria, fungi, viruses and protozoa and together we combine it and call it microbiota. Interestingly, there are more than three quarters of our genetic material is contributed by these microflora, making us slightly less human than we would like to believe and more of bacteria! The small intestine mainly has the Lactobacillus and Proteobacteria and that is mainly because of the abundance of disaccharides like lactose in the milk and weaning foods. Colon on the other hand, because it contains more undigested oligo and polysaccharides, promotes the growth of Bacteroidales and Clostridiales, especially in the early infancy. In the first month of life, Lactobacillus is the main dominant bacteria within the gut, with gradual proliferation of Bifidobacterium and Bacteroides over the next few months.

The microbiome plays a very important role in the working of the human body, and they contribute to preventing infections, regulating the immune system, and also in aiding digestion and absorption of nutrients. They protect us against infections by competing for the limited space and food that is available within the bowel, and inhibiting the pathogenic bacteria from growing because of that. This is something called direct colonisation resistance, but also bacteria like Bifidobacterium for example stimulate the host to secrete a mucus protective layer which stops the bacteria from invading the intestinal lining. They can work in other ways, for example, Lactobacillus digests the disaccharides to produce acid, and making the environment very acidic inhibits the growth of certain pathogenic organisms as well. That is the direct impact that the microbiome has on stopping the bacteria, but it also works indirectly by stimulating the body’s immune system.

The gut microbiome stimulates the body’s immune system and helps develop appropriate responses to immunological stresses. And, I want to stress on the word "appropriate" because it doesn't just educate the immune system to amplify responses to pathogens, but it also dampens responses like autoimmune or allergic triggers. And they do this by attaching to receptors on the immune presenting cells and

increasing the signaling either within the gut or in the blood stream because of low leakage of these bacteria into the circulation.

Jane Schlezinger: What type of factors affect the microbiome's development? Does one factor play a more important role than others for example?

Dr Batra: In a way it is a slightly difficult question to answer because we don't as of yet know what factors influence the microbiome. We know it's a very complex process, and the order and timing by which the gut is colonised has a lasting impact on the adult microbiome. To a point that within the first year, most of the bacteria that we have as adults are developed, and after three years of age there is hardly any, if any variation within the microflora. And there are a lot of factors that we are yet to understand that closely interplay to the development and maintenance of the gut flora.

The quality as well as the diversity of the organisms are very important. And this journey starts from the time of birth. The microflora in babies born by vaginal delivery is different to those that are born by caesarean section. This is because the vaginal delivery allows introduction of maternal microflora into the baby's gut, promoting the start of a healthy microbiome. If there was one factor that was most important in the development of the microbiome, I would say it is breast feeding because there is direct introduction of the bacteria from breast milk. And, even though the bacterial load of the maternal breast milk is low, it mimics that of the maternal flora. Also, the process of breast feeding allows contact with skin bacteria, which again helps colonise the baby's gut.

If they are breast-fed, then the microbiome of a breast-fed baby born by caesarean section becomes similar to that born by a vaginal delivery by six months of age. This unfortunately is not replicated in formula-fed infants. Because we have evolved over the years and symbiosis with this gut microbiome, the bacteria have adapted very well to using the substrate in breast milk as their food. The main carbohydrate in breast milk is lactose and this acts as a food substrate for Lactobacillus and helps grow the Lactobacillus which is the main bacteria in the first month to two months.

The other major carbohydrates are the human milk oligosaccharides, which are small chain carbohydrates that are not digested or absorbed within the bowel and provide food for the bacteria in the colon, especially bacteria like Bifidobacterium which is the most abundant bacteria in the colon. Another factor which is an increasing cause of dysbiosis in early life is the use of medication within the first six to twelve months of life. Quite understandably we know that antibiotics adversely affect the gut flora, so much so that use of antibiotics within the first month of life is associated with altered gut bacteria even as an adult. But, there are other medicines which we do not always associate with this, things like medicines we use for reflux, like proton pump inhibitors or acid suppressants and alginates which are very commonly used but have a negative impact on the gut bacteria and promote growth of pathogenic organisms.

Jane Schlezinger: If the diversity of the microbiome is not promoted during the first months of life, what impact can this have on the health of an infant and the developing immune system?

Dr Batra: The reduced diversity and dysbiosis increases risk of infections and we talked about how they can protect us from infections. There is emerging evidence that promoting the growth of beneficial bacteria through use of pro and pre-biotics can reduce other conditions like necrotising enterocolitis in pre-term infants. The gut microbiome also influences our metabolic responses, and there is evidence to suggest that individuals who have depleted microbial diversity have an increased lifelong risk of increased fat, insulin resistance and dyslipidaemia which could be contributing to the growing epidemic of obesity, diabetes and heart disease in adults.

Jane Schlezinger: We hear a lot about 'beneficial bacteria' in the microbiome - how can the growth of these be promoted? And, how does breast feeding, and the contents of breast milk play a role?

Dr Batra: Coming back to the association-causation bit, we know that bad bacteria are seen more in disease. And we think that they trigger certain diseases. What we yet don't know is what we can do to positively change the gut bacteria and even if we do that, if we can reverse or alter the course of disease. There is some evidence supporting a positive impact of interventions like using probiotics, and all journals are full of articles suggesting the beneficial effect of probiotics in changing the gut bacteria, and also changing the course of diseases. Though I have to say we are not at the stage where we can definitely say that is the case.

Especially because we know that the gut bacteria are very protective about their environment, and even if we add some probiotics into the gut, that doesn't mean that those bacteria would be allowed to start living there. Because there would be the competitive inhibition for both space and food by the current bacteria. But it is believed that the probiotics as they are passing through the gut, the 24-36 hours that they are there, they change the gut environment, tipping the favour for beneficial bacteria. But the bigger thing to consider is to look at what we eat and pre-biotics, which would have more of an impact on the gut bacteria.

We know that carbohydrates act as substrate for gut bacteria and sugars that promote the growth of healthy bacteria like Lactobacillus and Bifidobacterium can improve the gut flora. In early life, it's an easy question to answer, because we know promoting breast feeding is the most important intervention that we can do to promote healthy bacteria within the gut. That is because breast milk contains hormones, growth factors, cytokines, immunoglobulins, all of which regulate the development and function of the neonatal immune system and help develop the gut microbiome. This is by both direct and indirect antimicrobial activity on the bad bacteria. They also secrete immunoglobulin A, which is a very important immunoglobulin for the gut lining, which helps guide the gut to colonise the healthy bacteria and inhibit the pathogenic bacteria. But other bio active components like lactoferrin and lysozyme and very importantly, human milk oligosaccharides, which are in breast milk, promote the growth of good bacteria.

There are certain other cytokines as well which protect neonates and guide the immunity like interferons and tumor necrosis factor. In early life I think it is easy to say that we can have a huge impact on the gut bacteria by simple measures like avoiding medication as much as possible and promoting breast feeding. Later in life, we are still not in a position to say whether changing bacteria would be as beneficial as changing it as an infant.

Jane Schlezinger: That is a great answer, very informative. You've mentioned human milk oligosaccharides as a main element of breast milk, and we know their levels are around 5-20 g/l in breast milk, and the levels often exceed the concentration of even protein in breast milk. Can you explain more about these in terms of their structure, different types, and what affects their concentration levels?

Dr Batra: They are one of the main carbohydrates in breast milk. Obviously the most important nutritive carbohydrate is lactose which provides the main calorie source. But following this the other significant carbohydrate is human milk oligosaccharides. The HMO structure starts with a lactose unit which forms with a linkage of galactose and glucose together, and then there are other monosaccharides that get added on, making these glycans. The three major types of HMOs are the fucosylated, non fucosylated and sialylated HMOs. The most prominent of these is the 2-fucosyllactose which from here on I'm going to call 2'-FL, and it forms about a third of the total HMOs within the breast milk.

They are all non-nutritive but they are bioactive factors, which help provide a food substrate for growth of the beneficial bacteria. Equally importantly, it is not just they are feeding beneficial bacteria there, but the fact they are digested and metabolised by bacteria within the gut. They change the environment within the colon to help the growth of good bacteria and stop the pathogenic organisms from growing.

The composition of HMOs within the milk varies and is dependent on different factors. There are more HMOs early on during lactation compared to later in life; colostrum has the highest number of these oligosaccharides. It also changes with maternal nutrition, but maternal genetics plays a huge part as well. There are certain genes which promote secretion of HMOs in the breast milk – Lewis gene and a Secretor gene, both of which decide the concentration of HMOs within the breast milk, but also the type of HMOs - they are being looked at more and more as a major factor within the breast milk which promote the development of a healthy microbiome.

Jane Schlezinger: And, yes, a very exciting area of infant nutrition. How do human milk oligosaccharides differ from prebiotics such as fructo-oligosaccharides and galacto-oligosaccharides, known more commonly as GOS and FOS?

Dr Batra: Even though they are similar in composition, there still isn't enough evidence to say they are as beneficial as the oligosaccharides present in breast milk. The main difference is that HMOs are the original food substrate for these good bacteria. And, we don't know if substituting them with artificially produced oligosaccharides would have the same effect on selectively promoting the growth of good

bacteria. There is some evidence to say they have an impact on the stooling, but not yet enough to say that they have the same beneficial impact on the gut microbiome as human milk oligosaccharides or human milk would.

Jane Schlezinger: Following on from that, what is the most abundant human milk oligosaccharide and what specific bacteria do human milk oligosaccharides help to encourage in an infant's microbiome?

Dr Batra: So, there are three main types of HMOs - there are the neutral, acid and neutral N containing HMOs. The neutral HMOs are the most abundant and they make up about half of the HMOs within breast milk and 2'-FL forms the biggest proportion of the neutral HMOs; it's about a third in concentration. Neutral HMOs, especially 2'-FL promote the growth of bifidobacterium within the colon and inhibit bacteria like Escherichia and E.coli or Peptostreptococci. This is the main contributing reason why the gut microbiome over the first six months of life moves from predominately Lactobacillus based to the addition of other bacteria like Bifidobacterium and Clostridiales.

Jane Schlezinger: As the most abundant human milk oligosaccharide, can you tell us more about the role of 2'-FL and its multiple beneficial functions on infant health?

Dr Batra: 2'-FL obviously does not work in isolation. It is part of the whole multitude of bioactive compounds within breast milk, but it is the most abundant HMO, and we know that human milk oligosaccharides have a role in development of the immune system. It helps inhibit the invasion of pathogenic bacteria by stimulating mucin production by intestinal cells - which is something that 2'-FL is specifically is very good at. And the increased mucin barrier reduces the invasion of pathogenic bacteria into the body. There is also production of by-products like acids, organic acids which all change the environment to reduce the pathogenic bacteria within the gut.

There is some role of HMOs directly impacting the immune system as well. So, we know there is a degree of translocation of these oligosaccharides across the intestinal wall and they are able to exert some direct immunomodulatory effects, mainly in the form of binding to receptors to increase the signaling and production of cytokines, which help promote immune responses against pathogens. But, possibly some evidence to suggest reduce the allergic responses.

Jane Schlezinger: What evidence base is there that you are aware of for the role of HMOs and 2'-FL?

Dr Batra: There is a lot of research both in labs and in real clinical practice as well. So, in labs there is increasing evidence that HMOs influence which bacteria they allow to grow, and which they don't, like we just talked about, in a lab they have been able to demonstrate that they would selectively help the growth of certain bacteria We have just talked about the maternal genetics and how that can impact the HMOs. That's been used quite effectively in trying to understand the role of HMOs as part of breast milk

in promoting health and comparing mothers who secrete less HMOs to those who secrete more. There are some demonstrable changes within the gut microbiome that we can see. It is safe to say that HMOs are an important part of breast milk and do have an impact on the development of the immune system and maintaining health in infancy and in children.

Jane Schlezinger: Human milk oligosaccharides could also have an impact beyond the gut. This is an exciting area in research at the moment. And, a clear role in the developing immune system. Can you explain this further for the listeners?

Dr Batra: In terms of the HMOs, they do get into the blood stream by translocation, and they do directly protect breast-fed infants against microbial infections as well. To give some examples, the HMOs that get translocated into the systemic circulation promote and at the same time inhibit the growth of regulatory T cells. What that means is that it helps streamline the regulation that T cells provide, which means that the immune system works more effectively in fighting infections and reduces the auto immune responses. Acidic HMOs especially induce production of interferons and interleukins by upregulating the immune responses to infection, which has a huge part to play in preventing infections but also reducing the severity of illness with an infection.

At the same time, acidic HMOs are again very good at downregulating the autoimmune pathway as well. The fucosylated HMOs also kind of work by inhibiting infections by binding to certain pathogens, especially to E-coli, and there is increasing evidence that increased numbers of fucosylated HMOs specifically reduce the production of E-coli because of its binding to the cell wall and not allowing it to be able to invade the intestinal lining.

The other very important thing HMOs do, is that they change the gut environment to make it less conducive for pathogenic bacteria to grow. They do this by producing more mucin like we just said, or producing more organic acids, which don't allow the bad bacteria within the gut.

Jane Schlezinger: Taking what we've discussed so far into consideration then, would you say there are major differences between the microbiome of infants who are breast-fed versus those who are formula-fed?

Dr Batra: The biggest difference is the predictability of the gut microflora. So, there is very little variation between individuals who are breast-fed within the gut microbiome. So, most of them have a very similar microbiome. It is quite a diverse microbiome which is quite stable. In formula-fed babies, the inter-individual variation within the first six months is a lot higher than breast-fed babies. But also, there is less variety and the microbiome changes very rapidly in formula-fed babies within the first year. In breast-fed babies, there is a definite abundance of bacteria like Lactobacillus, Bifidobacterium, all of which we know are the most prominent bacteria within our microflora and are supposed to be very beneficial. Whereas

the formula-fed infants have more of Enterococci and Clostridiales, which aren't as beneficial as the others.

Even after weaning, there are some changes within the gut microbiome. But babies who are breast-fed, especially with complementary feeding up to one year, tend to have a very stable microbiome that lasts into adulthood, which is different to those who are formula-fed, where the bacteria can be quite variable after weaning and after three years of life.

Jane Schlezinger: From the research that I've read it seems that HMOs seem to be a specific substrate for Bifidobacteria, increasing the levels of Bifidobacteria that you don't see compared to the microbiome of formula-fed infants for example. That I find incredibly interesting as we know the advantages of Bifidobacteria in terms of infant health and reductions in pathogenic infections etc. What do we need in terms of future research do you think regarding biosynthesized 2'-FL? And other innovations in infant nutrition?

Dr Batra: What we need to know, as the most important question is one, with the synthetic molecules; if we are able to achieve the same effects as the HMOs in breast milk. We know that it isn't just the oligosaccharides in the breast milk which contribute to the improving gut microbiome and improving health. But they are a contributing factor. What we need to be able to demonstrate is that artificial 2'-FL can achieve that. But, more importantly is whether as an adult the risks associated with not being breast-fed are to some degree minimised with the use of 2'-FL. So, what we need to be able to show is not just a change in microbiome in the first year of life, but equally reduced risk of autoimmune diseases, reduced risk of metabolic problems like obesity and diabetes. That is what we are hoping to be able to demonstrate for them to be effective at all. If we can't achieve the same benefits in the long term, then clearly this isn't a true substitute.

Jane Schlezinger: What would be your main takeaway messages about HMOs, and their role in the development of the infant microbiome? As our last question.

Dr Batra: One of the main things to understand is the gut flora is like any other vital organ in our body. It is absolute essential and disruption of it is associated with poor health. We still do not fully understand all factors that influence our gut microbiome, but we know breast feeding promotes the development of healthy microflora. We know that it does that by the presence of many pro and pre-biotics and we know HMOs play an important role in growth of healthy bacteria within the gut. But also, a direct impact on stimulating and educating the immune system.

Jane Schlezinger: Thank you, Dr Batra, for sharing your knowledge and wealth of experience with us today. I'll now hand back to Maura to close our discussion, but it's been great to talk to you and thank you so much for fitting us into your busy schedule today.

Dr Batra: Thank you very much for asking me to talk. It was very good to be able to talk to you about this.

Maura Bowen: Well thank you Dr Batra and thank you Jane. This was a very informative conversation and we appreciate what you have shared with us today so much. Listeners, if you are interested in this topic and would like to learn more about human milk oligosaccharides, as well of a whole host of other subjects, please visit ANHI.org/UK. Also, ANHI's 'The Power of Nutrition' podcast series is on Spotify now where you can find more than 40 episodes highlighting the latest nutrition science, presented by some of the World's leading experts in this space. Be sure to subscribe and share us with your colleagues.

Thanks for listening!