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The Gut Microbiome and Childhood Obesity: Connecting the Dots

An interview with Noel Theodore Mueller, PhD, MPH,
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The field of study on the gut and the microorganisms living there could reshape what we know about human health. A seminal article on the topic describes it thusly: Human health can be thought of as a collective property of the human-associated microbiota.[1] *Noel Mueller, PhD, MPH, of Columbia University Medical Center in New York City, here describes the known landscape of the microbiota in infants and children and how these microorganisms can affect obesity risk.*

Childhood Obesity: Please provide a brief overview on the influence of gut microbiota on childhood obesity?

Dr. Mueller: Our bodies inside and out are teeming with microbial life. The ensemble of microbial genes, known as the human microbiome, outnumbers the genes in our own genome on the order of 100 to 1. Our gut is the most dynamic microbial ecosystem in our body and one of the most complex on earth. Of the tens of trillions of microorganisms inhabiting our gut, collectively referred to as gut microbiota, bacteria dominate.

The majority of the gut microbiota are commensal (living in a non-harmful coexistence with the host) or symbiotic (involved in a mutually beneficial relationship with the host). Many gut microbiota have been shown to play an integral role in determining various states of health and disease in humans. The upside for those interested in improving public health is that, unlike our genetic code, the human microbiome can be modified and cultivated by lifestyle and environmental factors.

So, where does our microbiome come from? While for many years it has been thought that the microbial world begins at birth, recent studies have reported evidence of bacterial DNA in the placenta, in fetal membranes, and in the meconium of babies, suggesting that there may be maternal-fetal exchange of bacteria *in utero*.[2] But this area of research is still in an embryonic phase, and more work is needed to determine whether or not the bacteria found in the intrauterine environs is living or merely the phagocytic remnant of a fight between invading microorganisms and the immune system.

Regardless of when the first encounter with microbes occurs, the largest primary exposure to the microbial world comes at birth. And to prepare for this exposition, the microbial communities in our mothers' vaginas and intestinal tracts change dramatically over the course of pregnancy. These changes to the maternal microbiome during pregnancy are likely adaptive to promote the growth of the fetus and to provide vaginally delivered babies with the bacteria needed to break down certain complex carbohydrates, such as oligosaccharides, found in breast milk. These earliest exposures to the microbial world are also paramount for educating the newborn immune system, developing their organs, and also potentially programming their metabolic function.

Many, if not most, of the bacteria colonize the intestines intestines colonize through vertical transmission from the mother to the newborn at birth. These are mostly nonpathogenic and play a critical role in digestion and metabolic signaling, among other processes. Gut dysbiosis, broadly defined as an imbalance of gut microbiota, has been associated with obesity and other metabolic disorders in humans. For example, seminal research from Jeff Gordon's lab out of Washington University in St. Louis has demonstrated that transplanting obese fecal microbiota into germ-free mice cause these mice to become obese compared to germ-free mice inoculated with fecal microbiota from normal weight individuals.[3–5] Importantly this translational research showed that the observational association between gut microbiota and obesity might indeed be causal.

It is also important to note that beyond obesity, there is now a vast literature base showing that gut microbiota are associated with myriad infectious, autoimmune, and metabolic diseases. In fact, it is within the realm of possibility that microbiota play a role in most human pathologies.

Childhood Obesity: Could you discuss what is known about the microbiota in infants and the association with childhood obesity?

Dr. Mueller: There are few published studies that have measured the gut microbiota of infants and then followed

these infants forward in time to observe how early colonization by these bugs associates with weight gain. One study, recently presented at the 2015 Experimental Biology conference in Boston, found that higher prevalence of the phylum Firmicutes in early infancy was associated with greater weight gain later on.[6] Another study—the KOALA cohort study—reported that higher *Bacteroides fragilis* was associated with greater childhood weight gain.[7] These two longitudinal studies are a couple of stronger ones to date, though, on face value, their findings do not appear entirely consistent. These inconsistencies may be due to differences in sampling, sequencing, in addition to the populations that are under study, as we know that the microbiome is significantly modified by geography and by ethnicity. Thus, I think it is important that future research, longitudinal in nature, drives down to the species level and incorporates microbial gene expression and metabolomics to help understand the functionality of the differences in the microbiota. Such research will be imperative to elucidating the role of the infant gut microbiota in the development of childhood obesity.

Childhood Obesity: Research seems to say at around age 3 years children's microbiota resembles that of their parents. Does this mean the window of birth to 3 is when the microbiota is shaped for people?

Dr. Mueller: A widely cited study that examines participants from Malawi, Amerindians from Venezuela, and the U.S. at different stages in the life course showed that there was relatively high bacterial diversity in the gut up until about the age of 3, at which point the bacterial diversity became relatively stable, resembling a microbial ecosystem characteristic of adult guts.[8] Findings from that study, corroborated by others, suggest that the gut microbiome is more variable and prone to disruption early in life, and therefore that the best time to intervene on factors to alter the long-term ecology of the gut microbiota might be between birth and around 3 years of life. The importance of this critical early-life window in shaping the gut microbiome is the primary reason why my work is currently focused on understanding the determinants of the maternal-offspring exchange of microbiota, and how we can leverage knowledge generated from this research to reduce practices that perturb the natural assembly of the gut microbiota. In this vein, we are conducting research to understand how C-section delivery,[9] early-life antibiotic use, and formula feeding disrupt natural assembly of the gut microbiome, and how these exposures may affect trajectories for weight gain and metabolic disorders in childhood.

I think it is important to note that while the greatest impacts on the assembly of the gut microbiome may occur between zero and 3 years of life, there is still incredible potential to procure a healthy microbiome after that time. In the future, I hope to examine the modifying effect of lifestyle exposures, such as diet, later in life. One question that needs to be addressed is how, as adults, we can introduce and propagate new, potentially healthful bacterial colonies, such as those found in kefir and kimchi, to our already established gut microbiota—like any complex, mature ecosystem, our gut microbiota may resist newcomers unless there has been a major disturbance or you introduce the new-coming bacteria with the resources needed for it to survive.

This line of inquiry ongoing research into the health effects of synbiotics, drives: the combination of probiotics with prebiotics. Prebiotics such as polysaccharides or complex carbohydrates, promote the growth of either the bacteria you are introducing probiotics or certain types of bacteria in the gut. Prebiotics can be found naturally in fruits, vegetables, beans, nuts, seeds, and most other plants high in fiber. Consuming a prebiotic-rich diet is one avenue to cultivating the healthy intestinal microbiota in the robust microbial ecosystem that is our gut.

Childhood Obesity: Could you discuss diversity in microbiota and its impacts on health and obesity?

Dr. Mueller: As I noted earlier, the gastrointestinal tract is one of the most complex microbial systems on earth. The microorganisms that inhabit the gut can be divided into prokaryotes, which are bacteria and archaea, bacterial phages, or the viruses that infect prokaryotes, eukaryotic viruses, and finally meoifauna, including fungi and protozoa. But of these different types of microorganisms, bacteria have been the most studied in relation to human health.

Greater bacterial diversity means more types of bacteria spread across the phylogenic tree. Many studies in adult populations have found that greater bacterial diversity of the gut is associated with protection from various diseases, including those that are autoimmune in nature, like asthma, and those that are metabolic in nature, like obesity. It seems, based on preliminary research, that the gut, not unlike other complex ecosystems, is in its best state of health when there is greater ecological diversity. Yet, the mechanisms for how greater bacterial diversity in the gut is associated with different states of health and disease are still being elucidated through ongoing research.

One important question in this research space is: How can we cultivate bacterial diversity in our gut? One seemingly simple approach is to consume a diet rich in nonprocessed, nonrefined whole foods, including a variety of high-fiber plants containing resistant starch, such as seeds, legumes, and unprocessed whole grains, and plants that contain high amounts of soluble fiber, like root vegetables and onions. Such plants provide polysaccharides, which, because of their indigestibility in our small intestine, make their way to our colon, where they are fermented by a diverse array of microbiota. Feeding the colon with an eclectic mix of polysaccharide substrate cultivates the various colonic microbiota so that no one species is

overrepresented. Thus, a dietary pattern rich in varied high-fiber plants promotes a robust microbial ecosystem able to maintain a strong gut epithelium and prevent gut permeability. It is believed that increased gut permeability can lead to immune-mediated inflammatory cascades that ultimately result in obesity and other metabolic diseases.

Childhood Obesity: Could you expand on the idea of preventing gut permeability?

Dr. Mueller: There are certain types of gut bacteria that produce short-chain fatty acids via fermentation of indigestible polysaccharides. The short-chain fatty acids produced by these microbiota provide energy to the colonocytes, thereby strengthening colonic mucosa and reducing transfer of bacterial lipopolysaccharides from the intestinal lumen to the blood. It is important to control the bacterial lipopolysaccharide efflux into the blood because they can cause a low-grade inflammation that may lead to myriad chronic diseases.

Childhood Obesity: Some recent studies[10] point to breastfeeding as preparing the infant's gut microbiota for solid food and overall positive health outcomes. Could you discuss this idea further?

Dr. Mueller: We recently published a paper, ''The infant microbiome development: Mom matters,''[2] that reviews Cesarean section (C-section) delivery, antibiotic use early in life, and breastfeeding and how those are important factors for determination of the infant gut microbiome and likelihood for the future health of the infant. Human breast milk contains oligosaccharides, which are a type of complex carbohydrate that act as a prebiotic by cultivating the growth of bifidobacteria in the infant gut. As vaginal delivery is one of the primary avenues by which bifidobacteria colonizes the infant gut, the prebiotic benefits of human breast milk may be modified by mode of delivery, but this hypothesis remains to be tested. But germane to your question: I think the jury is still out on whether or not breastfeeding prepares the infant gut microbiota for solid food, making this an intriguing area of active research. To the best of my knowledge, debate remains about whether or not the changes to the infant microbiomes occur before introduction of solid food, in preparation for the solid foods, or whether the introduction of solid food itself induces the changes to the infant gut microbiota. In this light, I think more longitudinal studies are needed to determine the temporality of changes to the infant gut microbiota and the introduction of solid foods.

Childhood Obesity: Your and coauthors' recent research[11] found "Cesarean sections and exposure to antibiotics in the second or third trimester were associated with higher offspring risk of childhood obesity." But it did not conclude that the changes in neonatal gut microbiota were the reason for increased risk. Could you explain further and discuss what you would like to see in future studies?

Dr. Mueller: In this study, we examined factors previously shown to disrupt the assembly of the neonatal or infant gut microbiome, particularly C-section delivery and antibiotic use by the mothers late in pregnancy, and we found these factors were associated with childhood obesity after controlling for a host of confounding factors, including, importantly, maternal overweight or obesity. However, one limitation to this study was that we did not collect fecal samples from the offspring, so we were unable to demonstrate the association was indeed due to differences in the infant gut microbiota. But other studies, including our own,[9] have found differences in the gut microbiota from babies born by C-section versus vaginal delivery, and difference between babies who were exposed versus not exposed to antibiotics. So, we know these factors perturb the assembly of the microbiome in the infants.

We are now interested in exactly *how* these factors disrupt the microbial assembly and whether these perturbations mediate the development of obesity, and, we are in the process of conducting longitudinally studies to answer these questions. Eventually, we hope to leverage findings from our research to guide creation of policies to prevent overuse of C-section and antibiotic use and restore the microbiome in infants after they are exposed to these medical practices. Restoration approaches may hold promise for preventing childhood obesity in the future.

One potentially restorative approach that my colleague, Dr. Maria Gloria Dominguez-Bello, from the New York University School of Medicine, has debuted (www.asmonlineeducation.com/php/asm2014abstracts/data/papers/I-741.htm) is the inoculation of babies born by C-section with the vaginal microflora of their mother. We are currently trying to obtain funding for a large trial in which we would inoculate C-section babies after surgery, and then follow these babies forward in time to compare the assembly of their gut microbiota to that of babies born vaginally and by C-section without inoculation.

Childhood Obesity: If there is a disturbance to the microbiome, what do we know about our ability to fix it?

Dr. Mueller: In addition to manually introducing microbiota from the birth canal to the baby after birth, also known as the ''gauze in the vagina technique,'' which still needs to be proven, breastfeeding the baby very early after birth is a restorative approach that may attenuate the differences in the microbiome that we see between C-section-delivered babies and vaginally delivered babies, and between babies exposed versus not exposed to antibiotics at birth. Other potential approaches to restoring the gut microbiota after early-life disturbances include introducing probiotics and prebiotics to infants through formula; however, these have been less studied and I am skeptical

that they can mimic the effects of natural human breast milk. So, when possible, breastfeeding remains the idyllic method for nourishing the infant and their microbiome.

Childhood Obesity: How much do we know about the composition of gut microbiota during early life and the specific microbes' effects on obesity prevention? Is there a missing link that you would like to see filled in through research?

Dr. Mueller: As I mentioned above, there are now a couple prospective studies that have identified microbial phylotypes associated with weight gain. However, this research is really nascent and it seems clear that we need more longitudinal studies in diverse populations that are large in sample size so we can not only determine temporality of associations, but also rule out confounding and start to understand interactions with genetic and environmental factors, as there are many modifying factors involved in the development of our microbiota and its effect on health and disease.

For example, we may want to consider stratifying analyses for babies born vaginally versus those born by C-section. We need to better understand how long differences in the microbiome between vaginally delivered and C-section-delivered babies persist, whether there are critical windows in which microbiome differences might imprint long-term health consequences, and whether restorative approaches can help alleviate these downstream consequences. To address these questions, we need large, prospective cohorts that longitudinally sample the mother and their offspring at several body sites and time points, starting before or during pregnancy and continuing forward. Such studies will allow us to really disentangle when the changes in the microbiome occur in the mother and the infant and whether or not we can identify microbial markers during pregnancy or early in the life of the newborn that can be targeted for intervention to prevent childhood obesity and other diseases.

Childhood Obesity: Are there other thoughts you would like to share on the future study of the microbiome and childhood obesity?

Dr. Mueller: There is a lot of interest in the intergenerational association between maternal obesity and childhood obesity. And much of the focus thus far has been on shared genetics between the mother and the infant, because the child, of course, has 50% of their mother's genetic makeup. But there has not been a lot of emphasis on the sharing of microbes from the mom to the baby, and how this may contribute to the intergenerational obesity association. We recently demonstrated at the 2015 Experimental Biology conference[12] that mother-to-newborn transmission of obese bacteria occurs at birth in vaginal deliveries. As such, preventing maternal overweight and obesity and maternal excess weight gain during pregnancy may obviate transmission of obese bacteria and therefore, in theory, reduce the risk of childhood obesity. The critical period around the time of birth is a fascinating window to explore, because of the modifiability of microbiota.

Yet, above all, it is important to point out that this area of research is still very young—we are just beginning to scratch the surface in our understanding of the microbial world and how it relates to different aspects of human health. As technology and our computing bandwidth continue to advance, so, too, does our ability to hone in on definitions of good and bad bacteria and the ideal microbial ecosystem for human health. The hope is that, one day in the near future, we can better leverage the knowledge we are gaining from this research to provide unique solutions to pressing public health problems, like the epidemic of childhood obesity.

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