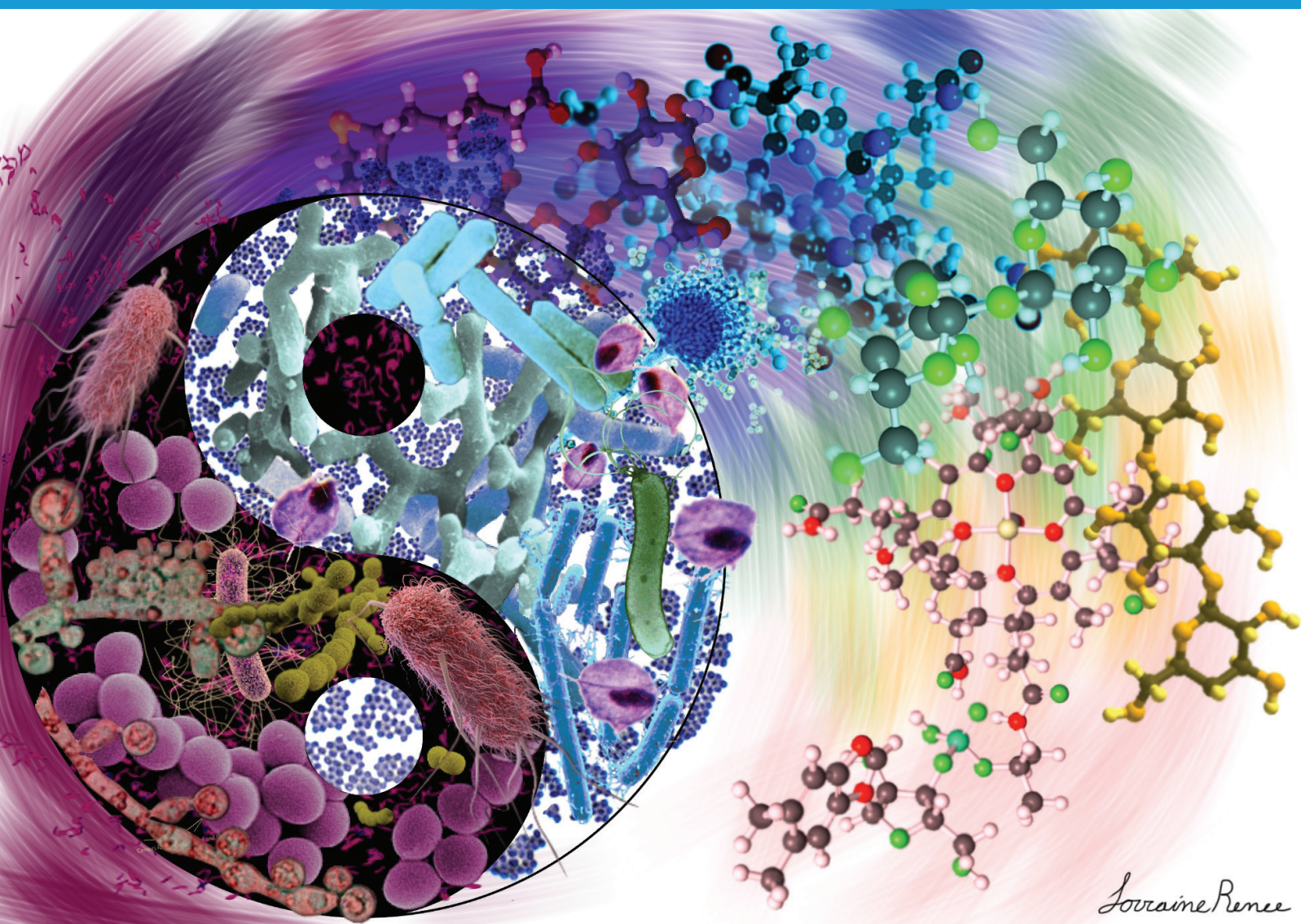


# The 116th Abbott Nutrition Research Conference

April 19-20, 2017

Columbus, Ohio, USA

*The Maternal Microbiome & Perinatal Colonization*



*Lorraine Renee*

# Welcome

New research continues to stress the importance of gut bacteria across the lifespan. The first 1,000 days of life—conception to two years—is a critical time in human development, in part because of the lasting effects of these tens of trillions of symbiotic and sometimes pathogenic microorganisms living within us. Recognizing the emerging science and vital implications for maternal and infant health, Abbott Nutrition convened an expert interdisciplinary group of scientists from nutrition, molecular genetics, neonatology, and neurology to present their scientific contributions.

Topics included the maternal microbiome’s possible influences on an infant’s immune response, metabolism, neurodevelopment, birth weight, and gestation time. Researchers also discussed the biochemistry of human milk, benefits of human milk in infant development, and the role of the microbiome in nutritional status.

Although many studies presented associations and not causation, agreement among the scientific community exists that additional maternal and pediatric microbiome research is necessary to improve human health in areas such as lowering asthma and allergy incidence, reducing obesity and diabetes incidence, and preventing preterm births and complications.

The health implications of the human gut microbiota are seemingly boundless, and the journey of intervention discovery is accelerating with anticipation of practical application. We offer the proceedings from this research conference to present the most recent global maternal and infant gut microbiome research that may benefit clinical practice to improve health, prevent chronic diseases, and reduce the impact of acute illnesses.

Manager Area Scientific Affairs .....Sean M. Garvey, PhD  
Abbott Nutrition Research & Development, Global Scientific Affairs

Global Medical Director ..... Larry W. Williams, MD  
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# The Maternal Microbiome & Perinatal Colonization

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## 116th Abbott Nutrition Research Conference Faculty

Left to right, front: Dr Catherine Stanton, Dr David Sela, Dr Marloes Dekker Nitert, Dr Tamar Gur, Dr Deborah Sloboda  
Left to right, back: Dr Michelle (Shelley) McGuire, Dr Mark Underwood, Dr B. Brett Finlay

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# The Maternal Microbiome & Perinatal Colonization

The 116th Abbott Nutrition Research Conference was held at Ross Park, headquarters of Abbott Nutrition Research & Development in Columbus, Ohio, USA on April 19-20, 2017. This Report contains summaries of presentations given by the following contributors.

## Keynote Address

*Let Them Eat Dirt: Raising Children with Their Microbes*

B. Brett Finlay, PhD  
University of British Columbia  
Michael Smith Laboratories, Department of Microbiology and Immunology  
Department of Biochemistry and Molecular Biology  
Vancouver, British Columbia, Canada

We now realize that the eradication of microbes over the past century with the hygiene battle has unexpected consequences. Our war on microbes is having unforeseen consequences in increased rates of inflammatory bowel disease, mental health disorders, childhood obesity, and asthma. Dr Finlay reveals how asthma is associated with a specific gut microbiome signature at 3 months of age.

He further provides animal model evidence that both a malnourished diet, low in protein and fat, and specific microbes combine to contribute to the etiology of environmental enteropathy, a subclinical chronic inflammatory disease of the small intestine impacting the persistence of childhood malnutrition worldwide. Dr Finlay concludes that we must rethink our relationship with our microbes and establish a balance between hygiene and exposure to beneficial microbes.

## Early Life Complications

*The Role of the Intestinal Microbiota in Necrotizing Enterocolitis and Neonatal Sepsis*

Mark A. Underwood, MD  
The University of California Davis School of Medicine  
Division of Neonatology  
Sacramento, California, USA

Dr Underwood discusses the pro-inflammatory nature of the infant gut microbiota of preterm infants. He provides evidence that intestinal dysbiosis, an alteration in the microbiota associated with a disease process, increases the risk for necrotizing enterocolitis (NEC) in the premature infant. Human milk bioactives and probiotics partially correct gut dysbiosis and decrease the risk of NEC in preterm infants.

## The Maternal Microbiome

### ***Perinatal Programming of Disease Risk: Maternal, Microbial and Metabolic Influences***

Deborah Sloboda, PhD  
McMaster University  
Department of Biochemistry and Biomedical Sciences  
Hamilton, Ontario, Canada

Evidence shows that the mother's gut microbiota educates the fetal immune system during pregnancy. Adverse events occurring during critical developmental windows *in utero* and during infancy may increase the risk of developing chronic diseases later in life. Dr Sloboda explains that such adversity includes poor maternal or infant nutrition, stress, or exposure to maternal disease.

She presents research findings in animal models of prenatal caloric restriction that have shown associations between fetal growth restriction and offspring obesity, insulin resistance, leptin resistance, and altered appetite. She discusses how gut microbiota associated with maternal obesity can also predict childhood obesity as well as obesity and metabolic diseases later in life. Evidence continues to emerge on the relative impact of the prenatal environment on microbiota composition and later health outcomes.

### ***The Gut Microbiota Regulates Metabolism & Blood Pressure in Pregnancy***

Marloes Dekker Nitert, PhD  
The University of Queensland  
School of Chemistry and Molecular Biosciences  
Brisbane, Australia

Pregnancy is a state of significant change in the metabolic and cardiovascular systems of the mother, which not only affects the infant but also the mother. Dr Dekker Nitert discusses how these changes in some mothers can present a higher risk for developing gestational diabetes, pre-eclampsia and Cesarean section delivery in the short term, and developing type 2 diabetes and cardiovascular disease later in life. The infant of a mother with gestational diabetes has an increased risk of being born large for gestational age and with hypoglycemia, and an increased risk for obesity and metabolic disease later in life.

Dr Dekker Nitert identifies the gut microbiota as a key regulator of vitamin and short-chain fatty acid metabolism, cholesterol metabolism, and the immune system. She further illustrates that the gut microbiota changes in pregnancy may contribute to the regulation of glucose and lipid metabolism through affecting the levels of metabolic hormones. In early pregnancy, her research shows a negative correlation between the abundance of *Odoribacter* in the gut microbiota and blood pressure. Manipulation of the composition of the maternal gut microbiota could therefore be a new target for the prevention of pregnancy complications.

### ***Prenatal Stress and the Microbiome: Relevance to Neurodevelopment***

Tamar L. Gur, MD, PhD  
The Ohio State University College of Medicine  
Institute for Behavioral Medicine Research  
Columbus, Ohio, USA

Microbes are an essential part of the gut-brain axis, and evidence supports microbes as an important contributor to mental health. Dr Gur's research lab now has animal model evidence that prenatal stress alters maternal microbiota, placental microbes, and leads to dysbiosis in adult female and male offspring. In placentas of female origin, their findings report significant increases in cytokines and chemokines in prenatal stress, as well as a significant decrease in brain derived neurotrophic factor, a protein involved in synapse formation and neurodevelopment. They also found a concomitant increase in anxiety-like behavior and decrease in cognitive ability in females exposed to prenatal stress. Exposure of the male fetus to prenatal stress alters exposure to corticosterone *in utero* and continued alterations in the hypothalamic-pituitary-adrenal axis, oxytocin, microbiome, neuroinflammation and social behaviors in adulthood.

Dr Gur concludes that gaining an understanding of how alterations in the placental microbes influence inflammation and neurodevelopment may support use of prebiotics or probiotics during pregnancy to improve mental health outcomes in offspring.

## **The Human Milk & Infant Gut Microbiomes**

### ***The Human Milk Microbiome – What's Normal, and Possible Factors Mediating Variability***

Michelle (Shelley) K. McGuire, PhD  
Washington State University  
School of Biological Sciences  
Paul G. Allen School for Global Animal Health  
Pullman, Washington, USA

Human milk composition is not only critical to understanding optimal nutrition during infancy, but also to understanding optimal nutrition throughout the lifespan. Although milk produced by healthy moms was once thought to be sterile, emerging research shows that it contains a diverse and live microbial community. Dr McGuire explains that understanding the variability in microbe sources (e.g., the mouth of the suckling infant) and the health implications of these microbes to both mothers and infants are a focus of current investigation.

Dr McGuire's latest research shows that maternal intake of many dietary components is associated with variation in the human milk microbiome worldwide. Differences are likely due to a complex interplay among maternal genetic variation, cultural and behavioral differences, environmental conditions, and pathogen risk.

### ***Human Milk Interactions with the Developing Infant Microbiome***

David A. Sela, PhD  
University of Massachusetts Amherst  
Department of Microbiology  
Amherst, Massachusetts, USA

The interconnectedness of the gut microbiome with our physiology provides a tractable target to improve human health. Dr Sela's research group investigates the interactions between human milk bioactives, such as human milk oligosaccharides (HMOs), and the early establishment and function of the infant gut microbiome.

He reviews the health and metabolic benefits of HMOs in the developing neonatal microbiome, and possible implications for prebiotic strategies to specifically enrich targeted bifidobacterial populations within the infant gut.

### ***Gut Microbiota in Developing Neonates***

Catherine Stanton, DSc, PhD, MSc, BSc  
APC Microbiome Institute & Teagasc Moorepark Food Research Centre  
Cork, Ireland

The gut microbiota of full-term vaginally-born, exclusively human milk-fed infants, with no previous exposure to antibiotics, can be considered the “gold standard” of gut microbiota in early life. Dr Stanton further explains that the composition of the gut microbiota is initially in a state of flux and stabilizes by 2-3 years of age.

Dr Stanton shares results from the INFANTMET study of microbiome development during the first 24 weeks of age, and confirmed that delivery mode and gestational age at birth both have significant effects on early neonatal microbiota development. *Bifidobacterium* was found to be a major component of the infant gut microbiota.

Dr Stanton concludes that little is known about how early the gut microbiome is positively influenced through nutrition. Understanding the optimal nutritional regimen to support optimal development of the gut microbiota is an appealing strategy to promote health and reduce disease risk.

# Contents

## Keynote Address:

- Let Them Eat Dirt: Raising Children with Their Microbes*..... 10  
B. Brett Finlay, PhD

## Early Life Complications:

- The Role of the Intestinal Microbiota in Necrotizing Enterocolitis and Neonatal Sepsis* ..... 14  
Mark A. Underwood, MD

## The Maternal Microbiome:

- Perinatal Programming of Disease Risk: Maternal, Microbial, and Metabolic Influences*..... 18  
Katherine Kennedy, MSc, Johanna Selvaratnam, PhD, and Deborah Sloboda, PhD
- The Gut Microbiota Regulates Metabolism and Blood Pressure in Pregnancy* ..... 24  
Marloes Dekker Nitert, PhD
- Prenatal Stress and the Microbiome: Relevance to Neurodevelopment* ..... 27  
Tamar L. Gur, MD, PhD

## The Human Milk and Infant Gut Microbiomes:

- The Human Milk Microbiome – What’s Normal, and Possible Factors Mediating Variability*..... 31  
Michelle (Shelley) K. McGuire, PhD
- Human Milk Interactions With the Developing Infant Microbiome*..... 35  
David A. Sela, PhD
- Gut Microbiota in Developing Neonates*..... 38  
Catherine Stanton, DSc, PhD, MSc, BSc



# Keynote Address:

*Let Them Eat Dirt: Raising Children with Their Microbes*

B. Brett Finlay, PhD



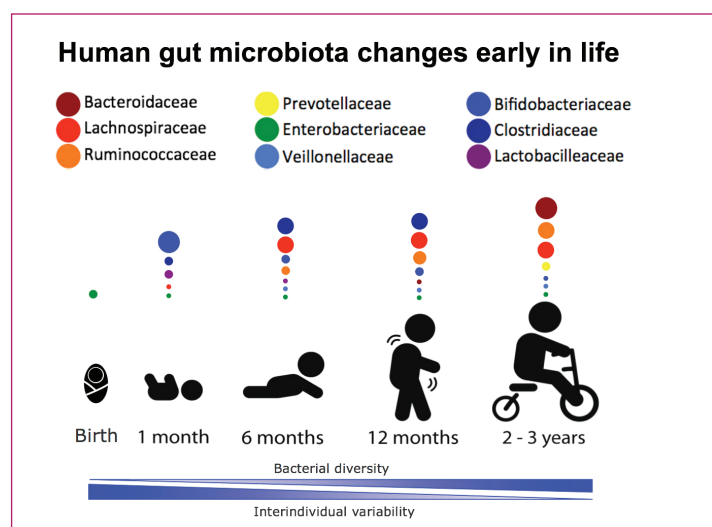
# Let Them Eat Dirt: Raising Children with Their Microbes

B. Brett Finlay, PhD

Ever since Robert Koch and Louis Pasteur showed that germs cause infectious diseases 125 years ago, society has been at war with microbes. Sewer and sanitation systems were developed and garbage collection established. Life-saving antibiotics became available near the end of the Second World War. Vaccines were developed to prevent many common childhood diseases. Society went on a campaign against microbes using sanitation and other hygienic methods. Collectively, this major hygiene campaign did a wonderful job of ridding the developed world of disease-carrying germs: “Cleanliness is next to Godliness.” “The only good microbe is a dead one.” “Cover your mouth when you cough.” This remarkable hygiene battle resulted in spectacular increases in life expectancy, and infant mortality plummeted over the past century, dropping from about 1 in 10 to 1 in 200.

However, we now realize that this eradication of microbes has unexpected consequences. Asthma rates have gone from about 1% to over 10% of all children.<sup>1</sup> In the past five years, we have witnessed unprecedented growth in childhood obesity, in addition to the continuing adult obesity epidemic. The rates of inflammatory bowel diseases, mental health disorders such as depression, anxiety, and even autism continue to increase rapidly. While the rates of nearly all infectious diseases continue to decline, the opposite is occurring in the non-infectious diseases. We haven’t changed genetically in 50 years. It turns out that our war on microbes is having unforeseen consequences as collateral damage.

There are at least as many microbes in and on you as human cells, and they encode greater than 93% of the DNA in and on us.<sup>2</sup> We are more microbe than human: *H. sapiens* DNA comprises less than 7% of the total genes in a human being! Even precision medicine is in trouble; humans are more than 99.9% identical genetically, yet we each have a unique set of microbes, sharing less than half with any other person.<sup>2</sup> We are colonized at birth, and even that first birthday present—fecal and vaginal microbes from Mom—is critical for setting us up for life (Fig 1).<sup>3</sup> If you are born by Cesarean section (C-section), as over one quarter of Canadian and American children now are, you will miss out on these important microbes and this increases one’s chances for allergies and asthma by 20% later in life.<sup>4</sup> This 20% difference in allergy and asthma risk is also observed in those who were breastfed versus bottle-fed, and those who live on a farm versus in a city.<sup>4</sup> These factors result in exposure to



**Fig 1. Stages of microbial colonization of the infant and child intestine.**<sup>3</sup>

## Let Them Eat Dirt: Raising Children with Their Microbes

different microbes. Even owning a dog drops the chance of getting asthma by 20% (cats have no effect).<sup>4</sup> With all this fascinating epidemiology comes the need to do more science.

Using a murine asthma model we were able to show that the gut microbiota has a profound influence on asthma.<sup>5</sup> Further studies showed that there was an early life period which was critical for later asthma susceptibility.<sup>6</sup> This effect seemed to be mediated by affecting how the immune system developed, including affecting regulatory T cells. This work identified a ‘critical window’ early in life where gut microbial changes (dysbiosis) are most influential in experimental asthma.

In a more recent study, we compared the gut microbiota of 319 subjects enrolled in the **Canadian Healthy Infant Longitudinal Development (CHILD)** Study, and showed that infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life.<sup>7</sup> The relative abundances of the bacterial genera *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* were significantly decreased in children at risk of asthma. This reduction in bacterial taxa was accompanied by a reduced fecal production of acetate (a short-chain fatty acid [SCFA]) and dysregulation of enterohepatic metabolites. Inoculation of germ-free mice with these four bacterial taxa ameliorated airway inflammation in their adult progeny, demonstrating a causal role of these bacterial taxa in averting asthma development.<sup>7</sup>

Most recently, we compared the bacterial and eukaryotic gut microbiota of 97 infants from the coastal community Las Esmeraldas, Ecuador at 3 months of age by 16S and 18S sequencing (manuscript submitted). Bacterial metagenomes were predicted from 16S rRNA data using PICRUSt (**P**hylogenetic **I**nterpretation of **C**ommunities by **R**econstruction of **U**nobserved **S**tates), and categorized by function using KEGG Orthology (**K**yoto **E**ncyclopedia of **G**enes and **G**enomes). The concentration of fecal SCFAs was determined by gas chromatography. This study found marked bacterial and fungal dysbiosis of the gut of 3-month-old babies at a high risk of asthma in a coastal Ecuadorian population. The alterations were taxonomically different yet functionally similar to the dysbiosis previously found in Canadian babies. This study again strongly supports the importance of an early critical window of microbial dysbiosis in the context of allergy and asthma.

Environmental enteropathy (EE) is a subclinical chronic inflammatory disease of the small intestine, and has a profound impact on the persistence of childhood malnutrition worldwide. However, the etiology of the disease remains unknown but animal models were lacking, hindering mechanistic studies. We recently showed that early-life consumption of a moderately malnourished diet low in protein and fat, in combination with oral exposure to commensal *Bacteroidales* species and *Escherichia coli*, remodels the murine small intestine to resemble features of EE observed in humans.<sup>8</sup> We also found profound changes on the small intestinal microbiota, metabolite and intraepithelial lymphocyte composition, along with the susceptibility to enteric infection. These findings provide evidence indicating that both diet and microbes combine to contribute to the etiology of EE, and that this novel murine model can be used to elucidate the mechanisms behind this understudied disease.

## Let Them Eat Dirt: Raising Children with Their Microbes

Collectively, we must rethink our relationship with our microbes and establish a balance between hygiene and exposure to beneficial microbes. Each generation gets cleaner, but the diversity and kinds of microbes in and on our bodies are decreasing rapidly. We are depriving the body of a critical part of its normal function, and the results are reflected in both health and disease. Ironically, because of our assault on microbes, some microbes could become endangered species, yet they have been an essential part of our evolution for millennia. We can't return to the wider range of microbes (and infectious diseases) of our great grandparents. We can, however, embrace microbes for our own good, as we rebalance hygiene with healthy microbes.

What can we do about this? We should think about how our communities, playgrounds, and time at school are designed to allow children access to ground-level play and outdoor learning. Antibiotics should be reserved for infectious diseases that clearly require treatment, and not squandered on colds, flus, and other conditions for which they are of no benefit. Attention to this is required ecosystem-wide: in medical care, dentistry, veterinary medicine, and agriculture. Finally, serious research is required to get us past our current superficial approach to probiotics. We need to understand which microbes are of health benefit, and how and when they can be successfully introduced to best benefit health. The future of health, wellness, and medical care will include our microbes, providing new tools to hopefully increase our health and longevity, long after major threats from germs have been mostly banished.

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# Early Life Complications:

*The Role of the Intestinal Microbiota in Necrotizing Enterocolitis and Neonatal Sepsis*

Mark A. Underwood, MD





# The Role of the Intestinal Microbiota in Necrotizing Enterocolitis and Neonatal Sepsis

Mark A. Underwood, MD

**Definitions:** The microbiota is the community of micro-organisms inhabiting an anatomic niche, such as the small intestine, the mouth or the vagina. Dysbiosis means an alteration in the microbiota associated with a disease process. The word microbiome has been used both as a synonym for microbiota and more specifically to refer to the genes expressed by the microbiota. Probiotics are dietary supplements that contain live organisms that confer a health benefit. Prebiotics are dietary supplements that are not digested by the host (e.g., the infant), but stimulate the growth of desirable commensal bacteria. Sepsis occurs when pathogenic microbes invade the tissue or blood causing symptoms of infection. Bacterial translocation occurs when intestinal bacteria leave the lumen of the intestinal tract and penetrate the single layer of enterocytes to arrive in the lamina propria of the small intestine (in close proximity to blood vessels and lymphatics). Necrotizing enterocolitis (NEC) is an intestinal disease that predominantly affects premature infants; the hallmark is a sudden onset of abdominal distention, bloody stools, abnormal abdominal radiographs and sometimes intestinal perforation. NEC is the leading cause of death in very premature infants from 2 to 8 weeks of age. Late onset sepsis (LOS) is a bacterial bloodstream infection that occurs at greater than 48-72 hours. In this abstract we will focus predominantly on bacteria, as the role for viruses, archaea, and fungi are much less clear.

**Introduction:** The micro-organisms that inhabit skin and mucosal surfaces interact constantly with the host's immune system. In the term infant, these host-microbe interactions have effects over the short term (e.g., neonatal sepsis and infant colic) and over the life span (e.g., altering the risk for autoimmune diseases such as type 1 diabetes, allergic diseases such as food allergies and eczema, and adult diseases such as obesity and metabolic syndrome).<sup>1,2</sup> In the preterm infant, the skin, gastrointestinal tract, and the innate and adaptive immune systems are immature and function only marginally often resulting in prolonged hospital stays and exposure to a variety of medications, medical surfaces, and instruments. As a result, preterm infants develop intestinal dysbiosis increasing the risk of sepsis and NEC. Among the most exciting discoveries in neonatology of the last decade, is evidence that altering the intestinal microbiota of the infant can decrease the risk of these short and long term outcomes (Table 1).<sup>3</sup>

**Table 1. The Preterm Infant Gut Microbiome and Risk for NEC Onset<sup>3</sup>**

Preterm Infant Microbiome	NEC Risk Factors	LOS
<ul style="list-style-type: none"><li>Breastfed term infants in developing countries are dominated by bifidobacteria and <i>Bacteroides</i></li><li>In many developed countries there is a loss of bifidobacteria</li><li>Premature infants are dominated by Proteobacteria (especially from 28-33 weeks)</li></ul>	<ul style="list-style-type: none"><li>Intestinal dysbiosis precedes NEC<ul style="list-style-type: none"><li>Increased Proteobacteria</li><li>Decreased Firmicutes and Bacteroidetes</li></ul></li><li>Human milk and probiotics decrease NEC risk</li><li>Prolonged antibiotics and H2 blockers increase NEC risk</li></ul>	<ul style="list-style-type: none"><li>The bacteria identified in late onset sepsis (&gt;48-72 hours of age) often originate in the gut</li><li>Funisitis predisposes to gut dysbiosis which predisposes to LOS with differing organisms</li><li>Human milk, probiotics and lactoferrin may be effective at preventing LOS</li></ul>

NEC=necrotizing enterocolitis, LOS=late onset sepsis, H2=histamine 2

## The Role of the Intestinal Microbiota in Necrotizing Enterocolitis and Neonatal Sepsis

**What is the evidence linking the microbiota of the neonate to NEC and sepsis?** Perhaps the most compelling example of dysbiosis and neonatal sepsis is infection with Group B streptococcus (GBS). Prior to the 1990s, GBS sepsis was the most common cause of neonatal sepsis and was often fatal. The understanding that GBS is a common colonizer of the maternal genitourinary tract, that it generally does not cause symptoms in the mother, and that acquisition of GBS prior to or during delivery often led to neonatal GBS sepsis prompted universal screening of pregnant women and prophylactic antibiotics during labor resulting in a dramatic decrease in the incidence of GBS sepsis.<sup>4</sup> While GBS sepsis is much less common now, the same pattern of colonization of the neonate with maternal bacteria in the perinatal period occurs with *E. coli* sepsis (the most common cause of neonatal sepsis in the first days of life since the institution of GBS prophylaxis). In premature infants, the most common cause of sepsis after the first few days of life is *Staphylococcus epidermidis*. *S. epidermidis* is a common colonizer of both the skin and the intestinal tract. Detailed studies have demonstrated that *S. epidermidis* reaches the bloodstream through defects in the skin, but even more commonly through translocation of this bacterium across the mucosal surface of the intestine.<sup>5</sup>

The evidence that intestinal dysbiosis increases the risk for NEC in the premature infant includes the following: 1) animal models of NEC demonstrate dysbiosis and the central role of recognition of bacteria by the immature host triggering a poorly modulated inflammatory response,<sup>6-9</sup> 2) medications commonly administered to premature infants such as antibiotics and acid-blockers cause intestinal dysbiosis and increase the risk of NEC,<sup>10-12</sup> 3) human milk partially corrects intestinal dysbiosis and decreases the risk of NEC,<sup>13</sup> 4) probiotics partially correct dysbiosis and decrease the risk of NEC in human trials and animal models,<sup>3</sup> and 5) human milk components, including lactoferrin, epidermal growth factor, and human milk oligosaccharides, decrease the risk of NEC in human studies and/or the incidence of NEC in animal studies.<sup>14-16</sup>

The impact of NEC and LOS is significant. While NEC affects a relatively small number of infants, the mortality rate is high and the costs for survivors are significant including the costs of prolonged hospitalization, surgeries, and the long term complications of NEC including poor growth, home total parenteral nutrition (TPN), and neurodevelopmental delays. The cost of NEC in the U.S. has been estimated at up to \$1 billion per year. LOS also has associated mortality and increased length of hospitalization with the increase in costs for a single episode of LOS estimated at \$10,000.<sup>17</sup>

## The Role of the Intestinal Microbiota in Necrotizing Enterocolitis and Neonatal Sepsis

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# The Maternal Microbiome:

## *Perinatal Programming of Disease Risk: Maternal, Microbial, and Metabolic Influences*

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# Perinatal Programming of Disease Risk: Maternal, Microbial, and Metabolic Influences

Katherine Kennedy, MSc, Johanna Selvaratnam, PhD, and Deborah Sloboda, PhD

It is now well established that the environment within which we develop as embryos, fetuses and infants, sets the stage for health later in life. Adverse events occurring during these critical developmental windows shape and mold primordial cells and systems in a manner that may increase risk of developing disease. Such adversity includes poor maternal or infant nutrition (malnutrition, caloric restriction or caloric excess), stress, or exposure to maternal disease. Many pre-clinical and clinical studies have shown that developmental responses to these early life events are inextricably linked to chronic diseases later in life. This framework of perinatal disease risk programming, termed the *Developmental Origins of Health and Disease (DOHaD)*, has its foundations in early epidemiological studies conducted by Dr. David Barker who, over three decades ago, demonstrated an association between weight at birth and mortality due to ischemic heart disease in adulthood.<sup>1</sup> This initial observation led to the formation of the “*Barker hypothesis*”—a hypothesis founded on the concept that adverse environmental stimuli that occur during prenatal life induce developmental adaptations that later result in the increased risk of what we otherwise assumed were lifestyle-associated diseases, including glucose intolerance, hypertension, and type 2 diabetes.<sup>2</sup> A marker of an adverse developmental environment, low birth weight, has since been associated with increased risk of many adult disorders including glucose intolerance,<sup>3</sup> insulin resistance,<sup>4</sup> and obesity.<sup>5</sup>

Adversity during developmental critical windows requires the fetus to make adaptations to maximize its survival postnatally. The developing fetus processes cues or signals from the maternal environment via the placenta, in order to predict which adaptations are most beneficial for postnatal survival. This concept—predictive adaptive response<sup>6</sup>—proposes that the degree of mismatch between the pre- and postnatal environments is a major determinant of subsequent disease. While these changes in fetal physiology may be beneficial for short term survival *in utero*, they may be maladaptive postnatally, contributing to poor health outcomes. For example, in the case of maternal malnutrition (or caloric restriction) during pregnancy that results in fetal growth restriction and low birth weight, fetal metabolic function is adapted to a nutrient-poor environment and therefore more susceptible to the effects of nutrient excess postnatally. Epidemiological data from the Dutch Hunger Winter also demonstrate this effect, linking prenatal undernutrition to increased risk of obesity,<sup>7</sup> hypertension,<sup>8</sup> diabetes, and coronary heart disease later in life.<sup>9</sup>

Pre-clinical studies have shed some light on mechanistic signaling pathways that underpin developmental programming. In rodents, maternal caloric restriction during pregnancy results in drastically reduced locomotor activity and increased caloric intake in offspring, and when combined with a hypercaloric postnatal diet, these effects are exacerbated.<sup>10</sup> Many animal models of prenatal caloric restriction have also shown associations between fetal growth restriction and offspring obesity,<sup>11,12</sup> insulin resistance, leptin resistance, and altered appetite.<sup>13</sup> Interestingly, similar effects on offspring disease risk have been shown in models of maternal diet-induced obesity. Administration of a high fat diet during pregnancy results in



## Perinatal Programming of Disease Risk: Maternal, Microbial, and Metabolic Influences

decreased birth weight but increased adiposity, leptin resistance, and insulin resistance in the offspring, independent of postnatal diet.<sup>12</sup>

Despite great advances in acknowledging the relationship between maternal nutritional adversity and offspring health and disease risk, including risk for obesity and type 2 diabetes, the mechanisms behind this relationship remain unclear. Recently, the role of the gut microbiota in obesity<sup>14</sup> has become a target of intense investigation. In non-pregnant individuals, obesity is associated with a shift in the gut microbiota, characterized by decreased abundance of Bacteroidetes and increased abundance of Firmicutes.<sup>15,16</sup> The emerging concept of an “obese gut microbiota” is supported by weight loss interventions, such as caloric restriction and bariatric surgery that are associated with a shift in the gut microbiota back to a more favorable abundance of *Bacteroides*.<sup>17</sup> Since healthy pregnancy is accompanied by metabolic adaptations that mirror those seen in obesity, including insulin and leptin resistance and increased adiposity, maternal gut microbiota became a target of study. In a landmark 2012 paper, Koren et al showed that the maternal gut microbiome may directly mediate maternal metabolic adaptations during pregnancy.<sup>18</sup> Colonization of germ-free mice with the gut microbiota of women late in pregnancy was shown to induce increased adiposity and insulin insensitivity.<sup>18</sup> This finding established the gut microbiota as a critical (and possibly adaptable) novel organ involved in pregnancy-related metabolic function. Since both pregnancy and obesity are associated with microbiota capable of altering host metabolism, increased interest has been directed at the role of the gut microbiota in obese pregnancy which may serve as a new target of therapeutic intervention.

Despite National Academy of Medicine (formerly Institute of Medicine) guidelines regulating gestational weight gain during pregnancy, more than half of all women enter pregnancy obese or gain more weight than is recommended.<sup>19</sup> Obesity is accompanied by low-grade systemic inflammation,<sup>20</sup> contributed to in part by increased gut permeability and translocation of bacterial fragments, such as lipopolysaccharide (LPS),<sup>21</sup> into circulation. LPS in turn, binds to toll-like receptors that activate expression of proinflammatory cytokines. Obesity and pregnancy independently produce shifts in the gut microbiota, and in combination, maternal obesity is associated with an even more distinct gut microbiota.<sup>22</sup> This has direct relevance to the developmental programming of obesity in children, since maternal obesity is one of the most significant predictors of childhood obesity<sup>23,24</sup> as well as obesity and metabolic disease later in life.<sup>25</sup> Thus, due to its role in mediating metabolism, the maternal gut microbiota has been implicated in poor maternal metabolic adaptation to pregnancy, linking maternal obesity to compromised metabolic function in offspring.<sup>26,27</sup>

In order to meaningfully investigate the mechanisms linking maternal obesity to childhood obesity risk, we use a mouse model of maternal diet-induced obesity to study gut microbial shifts and placental function at mid-gestation (embryonic day [E]14.5) and term pregnancy (E18.5). We have shown that pregnancy alone results in a shift in maternal microbiota characterized by increased levels of the genera *Bifidobacterium* and *Akkermansia*. These increases are amplified in the presence of maternal diet-induced obesity, and are associated with a predicted increase in fatty acid, vitamin B6, and ketone metabolism.<sup>28</sup> These shifts in the composition of the gut microbiota may impact maternal metabolism through altered production of bacterial metabolites, including short-chain fatty acids (SCFAs). In addition to their role as a fuel source for both microbes and human cells, SCFAs may signal through G-coupled protein receptors (GPR41, GPR43),

## Perinatal Programming of Disease Risk: Maternal, Microbial, and Metabolic Influences

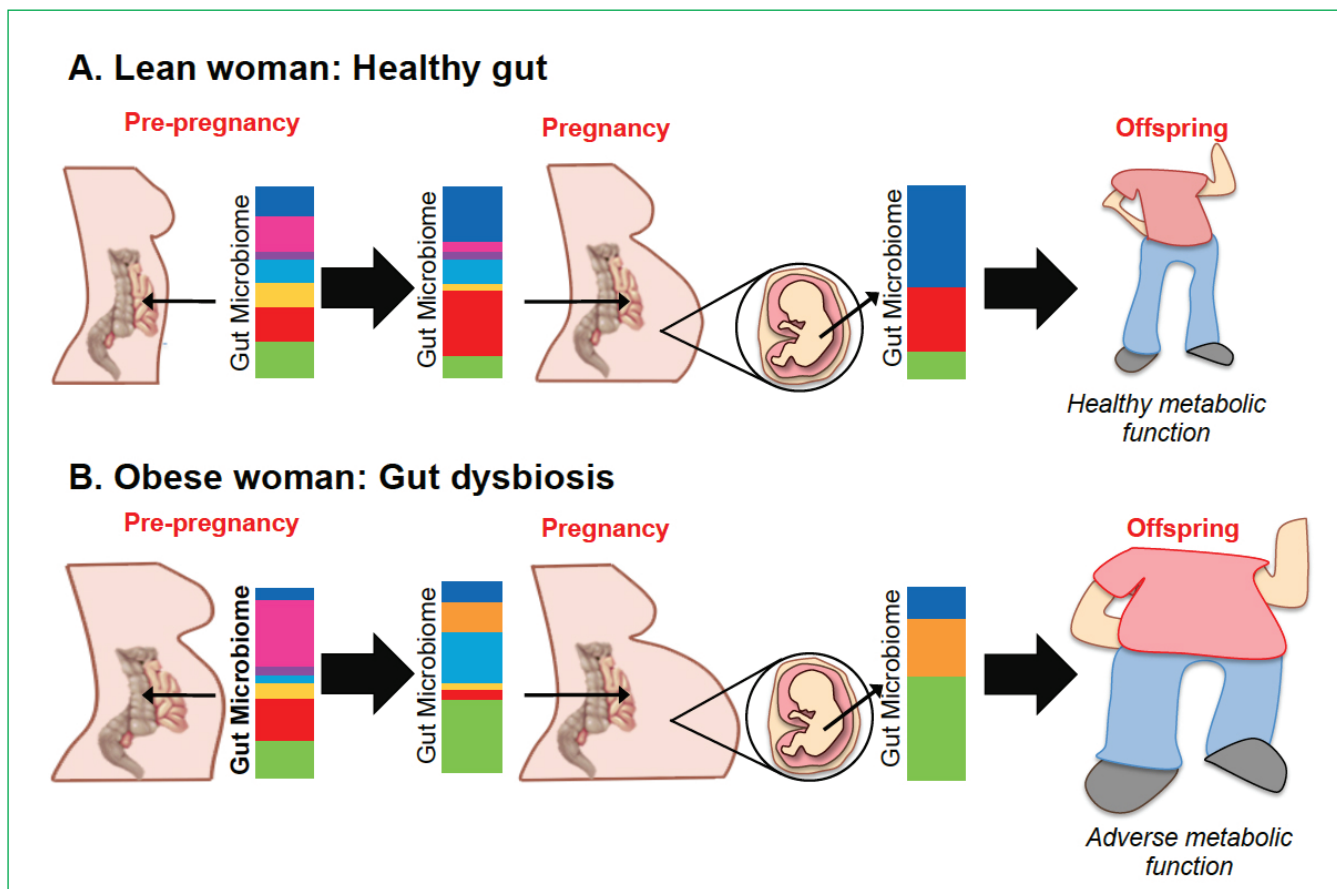
influence epigenetic regulation through inhibition of histone deacetylases (HDACs),<sup>29</sup> and suppress LPS-induced inflammation<sup>30</sup> and improve intestinal barrier function.<sup>31</sup> We have shown maternal diet-induced obesity to be associated with decreased levels of key microbial producers of SCFAs in pregnancy.<sup>28</sup>

Maternal gut microbiota may influence fetal development not only via modulation of maternal metabolic adaptation and inflammation, but also directly through colonization and release of bacterial products *in utero*. It has long been proposed that under normal circumstances, the newborn acquires a bacterial inoculation at birth, after exposure to maternal fecal and vaginal microbes. Large-scale sequencing and computational analyses of metagenomics data suggest that the developing fetal gut may be seeded before birth. Bacterial populations associated with the maternal gastrointestinal tract have been isolated using both culture-dependent and independent techniques, from meconium, fetal membranes, and umbilical cord blood of healthy neonates.<sup>32</sup> In pre-clinical work, translocation of maternal gut bacteria to mesenteric lymph nodes (MLN) has been identified during pregnancy; while during lactation, these bacteria were no longer present in the MLN but largely isolated to mammary tissue.<sup>30</sup> Although this has not been formally tested in humans (apart from the presence of bacteria in mammary tissue), these observations do suggest that gut microbes might travel from the maternal gut to extra-intestinal tissues during pregnancy and lactation. Dendritic cells have been proposed to sample gut bacteria prior to translocating to the mammary glands during lactation<sup>33</sup> but formal studies have not verified this hypothesis.

Recent work has shown that perinatal immune development is also influenced by the maternal microbiota.<sup>34</sup> In innovative pre-clinical studies using germ-free mice, Gomez de Agüero et al showed that prenatal colonization increased postnatal gut immune cell numbers (monocytes, leukocytes but not T or B cells) in offspring, as well as gut expression of genes linked to cell division, differentiation, mucus, metabolism, and antimicrobials. In these studies, maternal microbiota-derived compounds were transferred to maternal and offspring extra-intestinal tissues, and maternal antibodies enhanced the retention and transmission of microbial molecules; in which maternal microbial molecules were bound to maternal IgG and transferred to offspring via the placenta and through intestinal uptake from milk.<sup>34</sup>

Evidence is still emerging on the impact of the prenatal environment on microbiota composition throughout life.<sup>35</sup> It has yet to be proven empirically whether the relationship between commensal bacterial transmission and obesity risk is direct or indirect. Emerging evidence, though, supports the role of maternal systems, including nutrient uptake and utilization and inflammatory-mediated changes in gut function, in driving the ‘transfer’ of obesity between a mother and her offspring.<sup>36</sup> Future research will help shed light on microbial-mediated metabolic disease transmission (Fig 1), and provide new insights into microbe x host immunity in (high risk) pregnancies and uncover new avenues toward therapeutic interventions.

## Perinatal Programming of Disease Risk: Maternal, Microbial, and Metabolic Influences



**Fig 1. Maternal gut microbiota composition changes over the course of pregnancy.<sup>37</sup>**

A. Lean woman during pregnancy with stable, healthy gut microbiota. B. Obese woman during pregnancy with disrupted gut microbiota.

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## Perinatal Programming of Disease Risk: Maternal, Microbial, and Metabolic Influences

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# The Gut Microbiota Regulates Metabolism and Blood Pressure in Pregnancy

Marloes Dekker Nitert, PhD

**P**regnancy is a state of large changes in the metabolic and cardiovascular systems of the mother. The placenta releases many hormones and growth factors, which not only affect the infant but also the mother. Placental release of human placental lactogen and growth hormone induces insulin resistance in the mother, which ensures adequate glucose supply to the developing fetus. Women with higher pre-pregnancy levels of insulin resistance, which includes women with high pre-pregnancy body mass index (BMI) and those who are genetically predisposed, are at higher risk of developing gestational diabetes mellitus (GDM). GDM is associated with adverse outcomes for mother and infant, both perinatally and later in life.<sup>1</sup>

For the mother, these include a higher risk in the short term for developing pre-eclampsia and delivery by Cesarean section, and in the future, type 2 diabetes and cardiovascular disease. The infant of a mother with GDM has an increased risk of being born large for gestational age and admittance to the neonatal intensive care unit for hypoglycemia. In later life, the infants of mothers with GDM face an increased risk of obesity and metabolic disease.

Prevention of GDM is therefore of key importance and reducing pre-pregnancy obesity would be ideal. However, up to half of all pregnancies are unplanned and strategies including altering diet and physical activity have not been proven to be consistently successful.<sup>2</sup> Furthermore, the substantial barriers to pregnant women engaging in these high intensity interventions, and the costs associated with implementing them in the general population, have been well described.<sup>3</sup> Alternative strategies are needed to prevent GDM and its complications.

## The gut microbiota, metabolism and immune function

*Short-chain fatty acids (SCFA).* In recent years, the gut microbiota—the composite of all bacteria in the gut—has been identified as a key regulator of metabolism. The gut microbiota not only synthesizes vitamins but also ferments indigestible carbohydrates into SCFA.<sup>4</sup> Intestinal cells can use SCFA, such as butyrate, as an energy supply or facilitate transport of SCFA into the general circulation where they serve as signaling molecules to the liver, the kidney, adipose tissue, and endothelial cells.<sup>5</sup>

*Cholesterol.* The gut microbiota can also regulate cholesterol metabolism. Some of the bacteria in the gut microbiota can bind cholesterol to their outer membranes. Other bacteria express enzymes that convert cholesterol to coprostanolol, which cannot be absorbed by the body. Lastly, some bacteria metabolize bile acids to form secondary bile acids. All three of these mechanisms lead to lower cholesterol uptake from the gut.<sup>6</sup>

*Immune function.* The gut microbiota also regulates the immune system through altering the release of cytokines from the cells lining the intestinal wall.<sup>5</sup> Finally, the gut microbiota can alter the permeability of the intestinal wall, thereby enabling leakage of bacterial products across the intestinal wall, which can lead to inflammation.

## The Gut Microbiota Regulates Metabolism and Blood Pressure in Pregnancy

### Changes to the gut microbiota in pregnancy

The composition of the gut microbiota is determined by host and environmental factors including genetic make-up, age, diet, obesity, disease states, and medications. In pregnancy, the composition of the gut microbiota changes over gestation: the gut microbiota of women in the third trimester of pregnancy has a higher proportion of bacteria belonging to the phylum Proteobacteria. This phylum is associated with a pro-inflammatory state. In elegant experiments where germ-free mice were transplanted with the gut microbiota of women in late pregnancy, an increase in body weight and insulin resistance were observed.<sup>7</sup> These changes mirror the changes seen in pregnant women and provide tantalizing indications that the gut microbiota may actually contribute to the physiological changes observed in pregnancy.

### The gut microbiota regulates metabolism in pregnancy

Only a limited number of studies have addressed the role of the gut microbiota in regulating metabolism in pregnancy in humans. We recently published a study that showed that a higher abundance of the genus *Collinsella* is positively correlated with higher levels of fasting insulin, C-peptide, and triglycerides in overweight and obese pregnant women at 16 weeks gestation.<sup>8</sup> The anorexigenic hormone leptin, which is secreted from adipose tissue and the placenta, is positively correlated with the abundance of the family *Lachnospiraceae*. *Lachnospiraceae* abundance increases with a higher intake of animal protein and lowers SCFA production.<sup>9</sup> Furthermore, levels of the incretin hormone gastric inhibitory polypeptide (GIP) are positively correlated with the abundance of the SCFA-producing genus *Coprococcus*. These results indicate that the composition of the gut microbiome may contribute to the regulation of glucose and lipid metabolism through affecting the levels of metabolic hormones.

### The gut microbiota regulates blood pressure in pregnancy

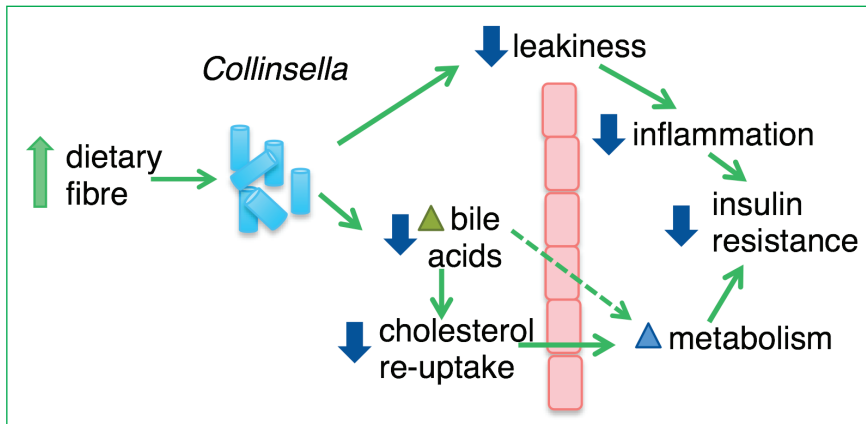
Outside of pregnancy, there has been some evidence that the composition of the gut microbiota is altered in people with hypertension.<sup>10</sup> In early pregnancy, we have reported a negative correlation between the abundance of the SCFA-producer *Odoribacter* in the gut microbiota and blood pressure.<sup>11</sup> A potential mechanism by which higher SCFA levels can lower blood pressure is by lowering the release of inflammatory markers such as plasminogen activator inhibitor-1 (PAI-1).<sup>11</sup> However, whether the gut microbiota regulates blood pressure later in pregnancy and is altered in women who develop hypertensive disorders of pregnancy needs to be determined.

### Altering the composition of the gut microbiota—a new strategy to improve pregnancy outcomes?

These early studies indicate that the gut microbiota is indeed a regulator of metabolic and cardiovascular health in pregnancy. Manipulation of the composition of the gut microbiota could therefore be a new target for the prevention of complications of pregnancy. Strategies for altering gut microbiota composition include altering dietary intake, especially dietary fiber (Fig 1), as well as probiotic and prebiotic supplementation to increase the abundance of SCFA producers. These approaches may prove to be successful. However, only if

## The Gut Microbiota Regulates Metabolism and Blood Pressure in Pregnancy

we get a deeper understanding of the complex interactions between the bacteria within the gut microbiota and their human host, can we hope to design successful strategies to improve pregnancy outcomes for mother and infant.



**Fig 1. Model for how dietary fiber affects *Collinsella* abundance and metabolism.**

**Source:** ©Marloes Dekker Nitert

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# Prenatal Stress and the Microbiome: Relevance to Neurodevelopment

Tamar L. Gur, MD, PhD

Mental illnesses are highly heritable, and while transmission is partially genetic, genetics do not underlie the entire contribution. Maternal stress and illness during pregnancy also exert influence on the developing infant and factor into the mechanisms underlying psychopathology. Indeed, infants exposed to antenatal stress and maternal anxiety demonstrate increased risk of developing altered stress response, anxiety disorders and depression in adulthood.<sup>1</sup> Mechanistic understanding of how stress alters the intrauterine environment and affects the developing nervous system is lacking.

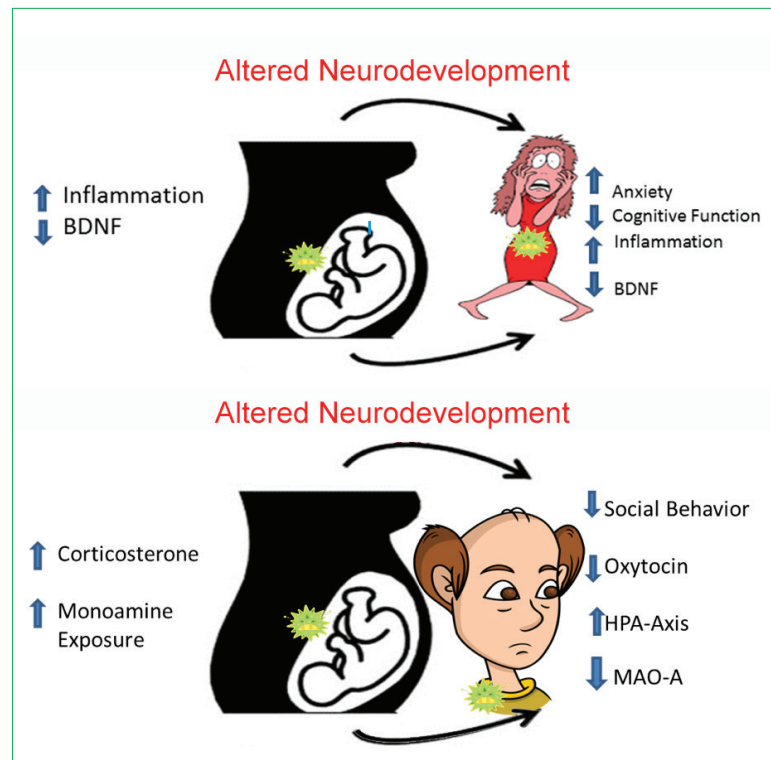
Adverse prenatal events, including maternal stress, have the capability of negatively influencing the neurodevelopment of the fetus, with long term cognitive and behavioral implications.<sup>2</sup> The interplay between intrauterine growth factors, hormones, and the immune system is dynamic and integral to healthy development in the fetus. Recent studies have reported that the placenta harbors a unique microbial population,<sup>3</sup> though this remains a controversial and open question. Microbes are an essential part of the gut-brain axis, which has been gaining momentum and evidence over the past several years as being an important contributor to mental health. Stress is known to lead to gut microbiota dysbiosis, though whether this plays a role in transmission of stress from mother to offspring is largely unknown.

Therefore, we decided to test the hypothesis that prenatal stress alters the maternal microbiome, leading to dysbiosis, inflammation, and changes in growth factors *in utero* and into adulthood. In order to test this, fecal and placental samples were collected from mouse dams and adult offspring. Microbial diversity was assessed using the Illumina MiSeq® platform, for targeted 16S ribosomal RNA gene sequencing. We now have evidence that prenatal stress alters maternal microbiota, placental microbes and leads to dysbiosis in adult female<sup>4</sup> and male offspring. Furthermore, in placentas of female origin we report significant increases in cytokines and chemokines, as well as a significant decrease in brain derived neurotrophic factor (BDNF). BDNF is a critically important trophic factor involved in synapse formation and neurodevelopment. We also found a significant decrease in BDNF, and an increase in cytokines, in the adult female hippocampus, a brain region important in memory and mood regulation. We therefore performed several experimental behavioral paradigms in the female offspring, to see whether the dysbiosis, inflammation, and change in BDNF were associated with aberrant behavior. Indeed, we found a concomitant increase in anxiety-like behavior and decrease in cognitive ability in females exposed to prenatal stress. Together, these data suggest that prenatal exposure to stress leads to alterations in microbiome, cytokines and BDNF *in utero*, and this continues into adulthood, and is accompanied by increased anxiety-like behavior and changes in cognition in female offspring (Fig 1).

In regards to males, while they had dysbiosis in adulthood, the subsequent biological consequences differed from those found in females. In male-derived placentas, we found a significant increase in corticotropin-releasing hormone (CRH), and a significant decrease in 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2), which breaks down the stress hormone corticosterone. Thus, the male fetuses appear to be exposed to altered corticosterone, an important stress hormone, *in utero*. They also demonstrate a

## Prenatal Stress and the Microbiome: Relevance to Neurodevelopment

significant alteration in their hypothalamic-pituitary-adrenal axis (HPA Axis) into adulthood, along with decreases in oxytocin receptor levels. Associations with alterations in the HPA Axis and autism have been described in the human literature in regards to individuals with autism spectrum disorder (ASD).<sup>5,6</sup> In addition, we found a significant reduction in monoamine oxidase A (MAO-A), an enzyme responsible for breaking down monoamines like serotonin, in male-derived placentas. This suggests that male fetuses were developing in a milieu with altered levels of monoamines, which could directly impact neurodevelopment. The decrease in MAO-A continued into adulthood. Adult male brains also demonstrate increased levels of cytokines IL-6 and IL-1 $\beta$  (interleukin). Both alterations in MAO-A<sup>7</sup> and neuroinflammation<sup>8,9</sup> have been tied to social behavior. We therefore examined behavior in a social approach paradigm, which measures the preference of a test mouse to a stimulus mouse, in comparison to an object. Male offspring exposed to prenatal stress showed a significant reduction in social behaviors when compared to controls (manuscript in preparation). Together, these data suggest that exposure of the male fetus to prenatal stress alters exposure to corticosterone *in utero* and continued alterations in the HPA Axis, oxytocin, microbiome, neuroinflammation and social behaviors in adulthood (Fig 1).



**Fig 1. Impact of prenatal stress on neurodevelopment.**

BDNF= brain derived neurotrophic factor, HPA-Axis=hypothalamic-pituitary-adrenal axis, MAO-A= monoamine oxidase A

It has been well established that there are pronounced sex differences in the frequency and severity of psychiatric disorders, with depression and anxiety more prevalent in females, whereas psychosis, autism spectrum disorders and externalizing disorders more prevalent in males.<sup>10-13</sup> These studies begin to establish that some of these sex differences could originate from differential effects of the microbiome on male and female offspring as they are influenced by maternal stress. Pinpointing the contribution of the microbiome and subsequent immune response and trophic factor alterations to modifications in neurodevelopment in the offspring are an essential part of increasing the fund of knowledge regarding the impact of prenatal stress on neurodevelopment. Gaining a mechanistic understanding of how alterations in the placental microbes influence inflammation and neurodevelopment will support use of prebiotics or probiotics during pregnancy to improve mental health outcomes in offspring.



## Prenatal Stress and the Microbiome: Relevance to Neurodevelopment

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# The Human Milk & Infant Gut Microbiomes:

*The Human Milk Microbiome – What’s Normal, and Possible Factors Mediating Variability*

Michelle (Shelley) K. McGuire, PhD

*Human Milk Interactions With the Developing Infant Microbiome*

David A. Sela, PhD

*Gut Microbiota in Developing Neonates*

Catherine Stanton, DSc, PhD, MSc, BSc



# The Human Milk Microbiome – What’s Normal, and Possible Factors Mediating Variability

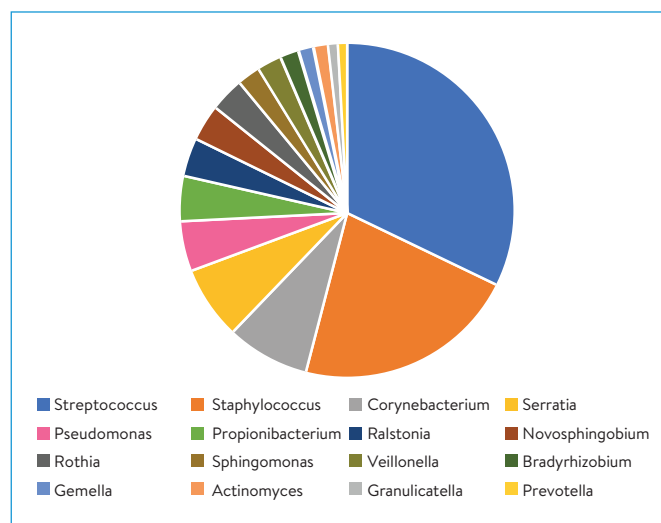
Michelle (Shelley) K. McGuire, PhD

**H**uman milk is inarguably the only food designed by Mother Nature intended solely for the nourishment of humans. As such, carefully characterizing human milk composition is not only critical to understanding optimal nutrition during infancy, but likely also to understanding optimal nutrition throughout the human lifespan.

Since the advent of “germ theory” in the mid-19<sup>th</sup> century (and likely before), milk produced by healthy women has been assumed to be sterile. Indeed, the presence of culturable bacteria in milk has long been considered a sign of breast infection or sample contamination. A classic example of this belief can be gleaned from a study conducted some years ago by Wyatt and Mata<sup>1</sup> designed to analyze milk produced by impoverished Guatemalan women. The authors concluded, “The presence of Enterobacteriaceae in human colostrum and milk reflects the low levels of personal hygiene and environmental sanitation in the population studied.” It now appears that these bacteria were likely neither a consequence of poor sanitation nor environmental contamination. Instead, they were likely ubiquitously present in milk produced by these at-risk women living in a difficult environment. Even the researchers behind the now-famous Human Microbiome Project did not consider including milk as a biological specimen of sufficient interest in terms of identifying its microbial content.<sup>2,3</sup>

However, using both culture-dependent and culture-independent (molecular) methods, myriad independent investigators have now identified and sometimes quantified a rich and diverse microbial community in human milk.<sup>4-8</sup> As illustrated in Figure 1, the most abundant genera found in milk tend to be *Streptococcus* and *Staphylococcus*, followed by *Corynebacterium*, *Serratia*, *Pseudomonas*, and *Propionibacterium*.<sup>9</sup> As such, attention has shifted from a focus on the pathogenicity of microbes in milk to understanding variation in milk’s microbial profiles with the eventual hope of delineating the sources and health implications of these microbes to both mothers and infants.

For instance, several investigators have explored whether milk’s bacterial profiles shift over the postpartum period. Results are mixed in this regard, but generally indicate that changes are minor if any.<sup>7,10,11</sup> Delivery mode has also been examined, again with mixed findings. For instance, Cabrera-Rubio and colleagues<sup>10</sup> reported decreased diversity in milk produced by



**Fig 1. Relative abundance of bacterial genera identified in human milk samples.<sup>9</sup>**

Left to right, Highest abundance (%) to lowest abundance (%) of bacterial groups comprising  $\geq 0.5\%$  of total sequence reads in human milk samples (n=79)

**Source:** Williams JE et al. *J Hum Lact.* 2017;33(3):540-551. Adapted by permission of Authors.

## The Human Milk Microbiome – What’s Normal, and Possible Factors Mediating Variability

women delivering via surgical intervention compared to those delivering vaginally. In addition, they reported that the microbiome of milk produced by women undergoing emergency Cesarean deliveries was comparable to that of women delivering vaginally; but milk produced by women having elective Cesarean deliveries was different in composition than that of the other two groups. Khodayar-Pardo and colleagues<sup>12</sup> found greater amounts of bacteria in women delivering via Cesarean. Conversely, Urbaniak and coworkers<sup>13</sup> found no differences in the milk microbiome associated with delivery mode. Kumar and colleagues<sup>14</sup> also found differences related to delivery mode, but this was modified by the woman’s country of origin. Clearly, additional studies will need to better understand the potential of delivery mode (and all that goes with it, like antibiotic use) to influence the microbes in a woman’s milk.

Maternal nutritional status has also been studied as a possible mediator of the human milk microbiome. Again, like time postpartum and delivery mode, findings are inconsistent. For instance, Cabrera-Rubio and colleagues<sup>7</sup> reported that milk from obese mothers tended to contain more bacteria (although it was less diverse) than that produced by healthy-weight women. Higher maternal body mass index (BMI) was also related to greater relative abundances of *Lactobacillus* and *Staphylococcus* in colostrum and mature milk, respectively, and lower relative abundance of *Bifidobacterium* in milk produced at 6 months. Davé and coworkers<sup>15</sup> also found that prepregnancy BMI was inversely correlated with *Streptococcus* ( $r = -0.67$ ), and positively associated with microbial diversity ( $r = 0.77$ ) in milk produced by Mexican-American women ( $P < 0.05$  for both). These data, however, are difficult to assess in terms of causality because BMI is completely confounded by chronic maternal nutrient intake (both energy-yielding and non-energy-yielding nutrients).

Recent studies from our group, however, have found that usual maternal intake of many dietary components is associated with variation in the milk microbiome.<sup>9</sup> For instance, relative abundance of Firmicutes is highest in milk produced by women consuming the highest levels of energy-yielding macronutrients; higher relative abundance of Firmicutes in milk is also related to increased maternal consumption of long-chain, polyunsaturated, omega-3 fatty acids. Relationships between milk microbes and maternal consumption of amino acids, vitamins, and minerals were also noted. Whether these relationships are causal in nature, occur due to shifts in the mother’s gastrointestinal microbiome, or are due to direct effects of maternal diet on variation in milk nutrient content will require further study, including randomized controlled intervention trials.

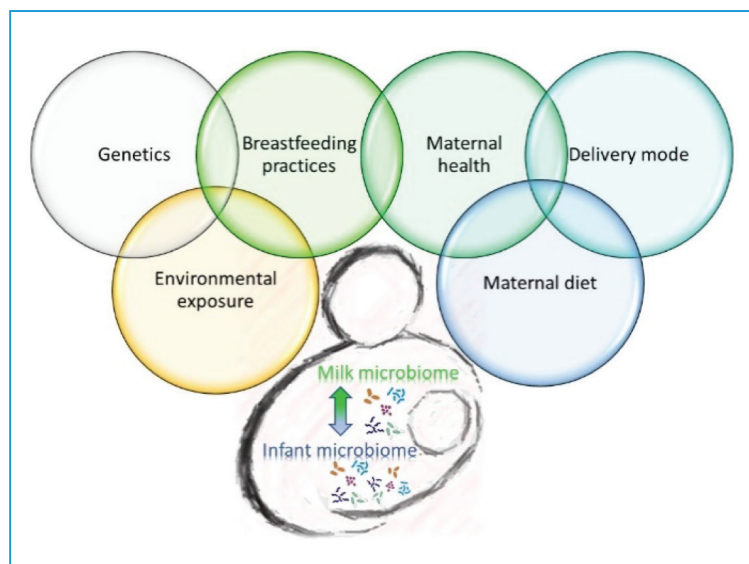
Aside from variation in maternal nutritional status, several studies have provided convincing evidence that bacteria found in local fermented foods and probiotic supplements can be coordinately found in milk produced by women consuming the food or supplement.<sup>16,17</sup> Additional controlled intervention trials will be needed to understand how maternal nutritional status and dietary intake influence (or do not affect) the milk microbiome. Included in these studies should be analysis of the milk for micronutrient and macronutrient content, and analysis of the maternal fecal microbiome.

A limited number of studies have also investigated the possibility that milk constituents, other than microbes, might be related to its microbial community structure. For instance, we have shown that the concentration of human milk oligosaccharides is positively associated with relative abundance of

## The Human Milk Microbiome – What’s Normal, and Possible Factors Mediating Variability

*Staphylococcus* and that this relationship is likely causal in nature.<sup>9,18</sup> Variation in immune cell profiles in milk is also associated with variation in milk microbial communities,<sup>9</sup> and this appears to be unique to each woman. Again, whether these relationships are causal in nature, and if so their directionality, will need further longitudinal study.

Regardless of its source, emerging research provides solid evidence supporting considerable variability in the human milk microbiome around the world.<sup>14</sup> We hypothesize that these differences are likely due to a complex interplay among maternal genetic variation, cultural and behavioral differences, environmental conditions, and pathogen risk (Fig 2). Indeed, it is likely that human milk has been “designed” over the millennia to fit the unique nutritional and immunologic needs of infants living in a particular culture and location, a process we refer to as *eco-homeorhesis*.<sup>19</sup> If this is true, then there likely is no ‘one-size-fits-all’ construct when it comes to the milk microbiome and even optimal infant nutrition around the globe.



**Fig 2. Factors influencing variability in the human milk microbiome.**

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# Human Milk Interactions With the Developing Infant Microbiome

David A. Sela, PhD

Organisms have evolved to cooperate in nutritional feeding strategies. We see evidence of this in the animal kingdom, such as marine birds that loiter around whales while they acquire food from the ocean. The same principle has evolved in microbial communities that colonize humans, often referred to collectively as the gut microbiome. Nutrition interventions seek to extend this ecological concept toward enhancing human health through optimizing joint feeding strategies within the gut microbiome. Ultimately nutrition provides a set of solutions to achieve one's full health potential, including optimal growth and development during childhood and maintenance of quality of life with advancing age. Optimal nutrition precisely tailored to the individual's genome, lifestyle, and even microbiome all have the potential to mitigate pathological states and bolster prophylactic strategies. The interconnectedness of the gut microbiome with our physiology provides a tractable target to improve human health.

Our group specifically researches the interactions between molecules dissolved in human milk and the early establishment and function of the infant gut microbiome. At one point, human milk was solely characterized as providing a benefit through direct nutrition and modulation of the immune system, in addition to panoply of benefits to the infant. However, we now know that the infant microbiota is influenced by, and interacts with, human milk molecules that are not fully hydrolyzed in the upper gut and arrive at the lower gastrointestinal tract.<sup>1</sup> Human milk oligosaccharides (HMOs) are produced in the mammary gland at a significant energetic cost to the mother and remain intact once ingested. Upon transit to the colon, prominent bifidobacterial populations within the infant gut consume HMOs. Bifidobacteria are often enriched within the infant gut, and formula-fed infants exhibit greater diversity within the entire gut bacterial microbiome.<sup>2</sup> The differences between breastfed and formula-fed infants are also evident in the differential metabolites produced by the microbiota depending on feeding mode.<sup>3</sup> Interestingly, more amino acids and fewer amino acid degradation products were found in circulation in formula-fed infants, compared to breastfed controls, although impact to host physiology remains speculative.<sup>3</sup> Benefits that bifidobacteria provide to the developing infant include pathogen inhibition, production of molecules that modulate host processes, and metabolic benefits.<sup>4</sup>

Approximately 200 distinct HMO structures have been identified across human milk samples, and each mother is capable of producing around 60 different HMOs.<sup>5</sup> HMOs comprise up to five monosaccharide residues, with a variety of combinations, glycosidic linkages, and degrees of polymerization. An example of a simple yet abundant HMO is 2'-fucosyllactose (2'-FL), a trisaccharide comprised of glucose, galactose, and fucose (Fig 1). HMOs are generally

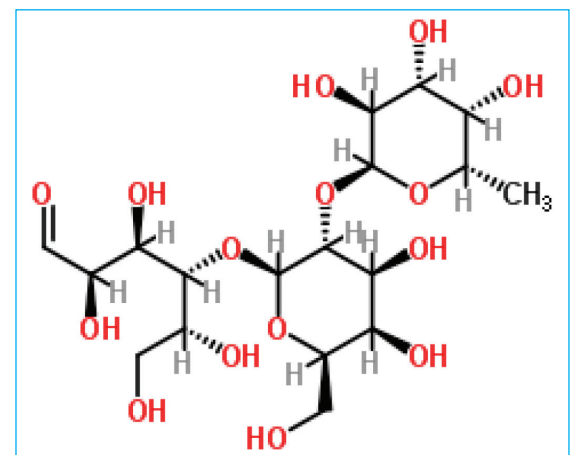


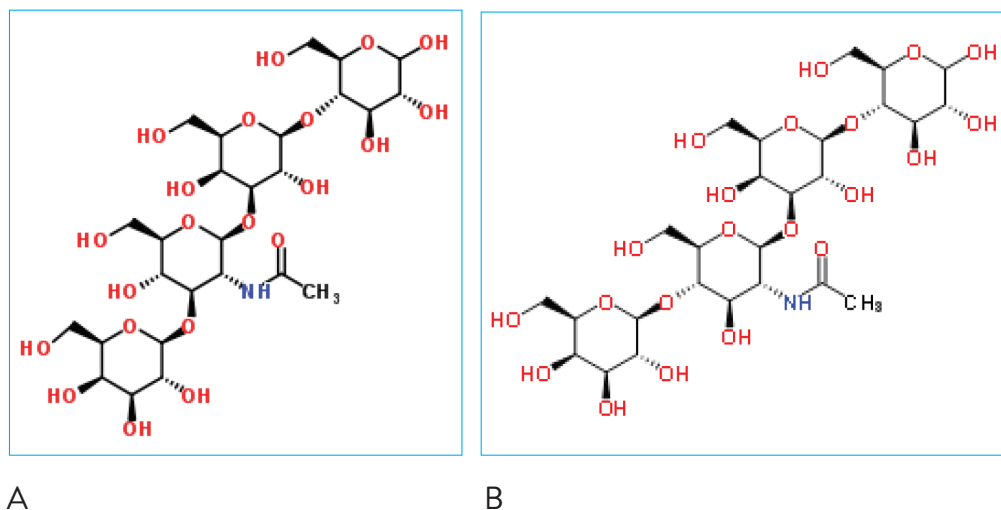
Fig 1. 2'-fucosyllactose (2'-FL).

## Human Milk Interactions With the Developing Infant Microbiome

planar in molecular orientation and may have linear or branched structures. That there are only ~200 different HMO species identified suggest a functional constraint that has coevolved between the mother and infant mediated by the microbiome. In general, infant colonizing bifidobacteria utilize HMOs to a greater extent than adult-associated taxa such as *Bifidobacterium adolescentis*. This is evident in the *in vitro* growth phenotype and the identification of the specific HMOs consumed by *Bifidobacterium longum* subsp. *infantis* (*B. infantis*).<sup>1,6</sup> These growth assays are conducted with HMO as a sole carbohydrate source to determine if the bacterium is capable of utilizing it as energy. *B. infantis* is the archetypical HMO consumer that serves as the experimental model for this physiological phenomenon. In general, *B. infantis* consumes the most abundant HMO species that are also the lowest in molecular weight and degree of polymerization. This provides several implications for the judicious modulation of the developing neonate microbiome.

The *B. infantis* genome sequence encodes a 40 kbp gene cluster that enables the bacterium to capture small mass oligosaccharides from the extracellular environment and metabolize it within the cytoplasm.<sup>7</sup> This gene cluster contains all the glycosyl hydrolases and transporters that are predicted to interact with HMOs. Subsequent research has supported this model by characterizing several molecular mechanisms involved in HMO utilization.<sup>8,9</sup> Moreover, *B. infantis* exhibits a global gene expression profile that varies when consuming specific HMO structures and across time.<sup>10</sup> In addition, early HMO consumption *in vitro* induces a gene expression profile distinct from later timepoints. Moreover, gene expression while *B. infantis* consumes lactose is more similar to HMO utilization than 2'-FL. This is surprising as lactose is structurally similar to 2'-FL as the latter is identical with the exception of an additional fucosyl group. This may have implications for prebiotic/synbiotic approaches as pre-culturing carbohydrate choice (e.g., lactose or 2'-FL) may be used to optimize probiotics prior to delivery to the consumer. Moreover, there are distinct differences between the utilization of lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose (LNnT). This is significant as these two tetrasaccharide HMO species differ by only one beta linkage between the terminal galactose and *N*-acetylglucosamine (Fig 2). This is consistent with observed strain-dependent utilization of LNT versus LNnT. Bifidobacteria isolated from non-human primates are capable of utilizing both LNT and LNnT in contrast to bacteria isolated from the pig gut (unpublished data). This suggests that the bifidobacteria that colonize

phylogenetically similar hosts to humans (i.e., primate) exhibit a similar



**Fig 2. (A) Lacto-*N*-tetraose (LNT); (B) lacto-*N*-neotetraose (LNnT).**

## Human Milk Interactions With the Developing Infant Microbiome

HMO utilization pattern than more divergent hosts. The ability to utilize both HMOs is consistent with the repertoire of glycosyl hydrolase genes present in the primate microbiome. Furthermore, strains exhibit growth responses that are distinct depending on the HMOs that are utilized (unpublished data). This potentially has implications for prebiotic strategies to specifically enrich targeted bifidobacterial populations within the infant gut.

The goal of this research is to understand the fundamental physiology of bifidobacterial utilization of HMOs. This will extend not only to bifidobacterial populations as well as interactions with other members of the developing infant microbiome. For example, other non-bifidobacteria secrete butyrate that bifidobacteria do not synthesize. This short-chain fatty acid provides energy to colonocytes and possesses anti-inflammatory properties. Ultimately, translation of these findings requires testing hypotheses in infants to conclude there is indeed a benefit to infant health.

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# Gut Microbiota in Developing Neonates

Catherine Stanton, DSc, PhD, MSc, BSc

The first 1,000 days of human life represents a crucial period in early development when organs and tissues are rapidly developing. This period offers a critical window for influencing long term health via optimum nutrition, be it *in utero* during pregnancy and following childbirth. Starting from birth, a diverse and complex microbial ecosystem begins to develop in the infant gastrointestinal tract, a process which undoubtedly influences health and disease status – not just in early life but more likely throughout life.

The population of bacteria on babies at the time of birth reportedly resembles mother's vaginal microbiota if the baby is born vaginally or that of maternal skin, if born by Cesarean section (C-section).<sup>1</sup> In addition to delivery mode, factors reported to influence the development of the infant gut microbiome include: gestational age, host genetics, feeding regimen, and perinatal antibiotic usage. Thus, the microbiota of full-term vaginally-born, exclusively human milk-fed infants, with no previous exposure to antibiotics, can be considered the “gold standard” of gut microbiota in early life. The gut microbiota composition is initially known to be in a state of flux and consequently stabilizes by 2-3 years of age, to more closely resemble that of an adult.

In the INFANTMET study, we compared gut microbiota development of full term and preterm (<35 weeks gestation) infants that were either spontaneous vaginally delivered or delivered by C-section. These babies were followed from birth to two years of age, in initially breastfed infants (n = 199). We have recently reported on the microbiome development in this cohort during the first 24 weeks of age (Fig 1),<sup>2</sup> and confirmed that mode of delivery and gestational age at birth both have significant effects on early neonatal microbiota development.

Vaginally-delivered infants had a more diverse microbiota at just one week of age, which remained relatively stable over the first six months compared to infants born by C-section, whose initial microbiota was very different with lower Actinobacteria and Bacteroidetes at one week of age. Indeed, there was relatively little change in the microbiota composition of vaginally-delivered infants throughout the first 24 weeks of life. In contrast, it took eight weeks for infants born by C-section to develop a similar composition to the former group. At 24 weeks, all infants whether preterm or full-term and born vaginally or by C-section had similar though distinct gut microbiota compositions. *Bifidobacterium* was found to be a major

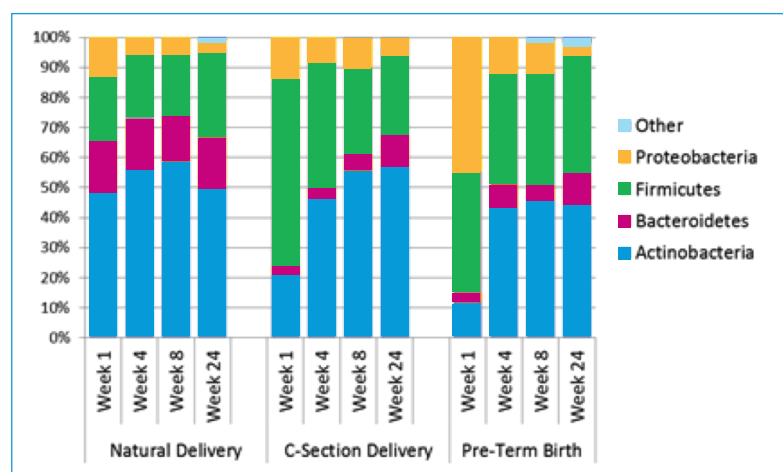


Fig 1. INFANTMET Microbiota to Week 24.<sup>2</sup>

## Gut Microbiota in Developing Neonates

component of the infant gut throughout this period and represented up to 50% of entire fecal microbiota. Interestingly, prolonged breastfeeding (> 4 months) was found to impact the gut microbiota of C-section-delivered but not vaginally-delivered infants.

An interesting outcome of the INFANTMET study concerned 10 twin sets contained within the sample population. We found that twins' microbiota were more similar to one another than to random infants, reflecting the influence of host genetics and environment on early microbiota composition.<sup>2</sup> Indeed, in a separate study, we reported on the microbiota composition of monozygotic twins within a dichorionic triplet set.<sup>3</sup>

Dichorionic triplets pose a unique informative study design as they contain monozygotic twins and a naturally-occurring fraternal triplet as control with similar pre- and postnatal environmental factors. By one month of age, the gut microbiota was observed to be dominated by bifidobacteria in all three babies with a lower level of diversity at the genus level observed in the fraternal twin and greater similarity between the monozygotic twin pair. The colonization pattern converged by 2 and 3 months of age and while at 12 months microbial diversity was increased in all three infants, there appeared to be no greater similarity in the microbiota profile of the monozygotic twins versus the fraternal control. Principal coordinate analysis (PCoA) of the microbiota composition showed that the monozygotic twins grouped together compared to the fraternal triplet at months 1 and 2. However, at 3 months and particularly by 12 months of age, the microbiota composition appeared equally dissimilar among all three. It is tempting to suggest from this observation that host genetics plays a role in shaping the microbiota at a very early stage of development, the long term health implications of which are unknown.

It is our belief that interference with the natural development of the infant microbiota as a result of C-section delivery mode, preterm birth and early antibiotic exposure has long term implications for microbial diversity/stability and consequent health. A number of disease states are reportedly associated with lower intestinal microbial biodiversity,<sup>4</sup> indicating that C-section combined with aseptic conditions and antibiotic treatment, although often necessary for treating/preventing infection, may not be the optimum start in early life.

The preschool years (1–5 years of age) is a time of rapid and dramatic postnatal brain development and neural plasticity, and thus of fundamental acquisition of cognitive development (i.e., working memory, attention and inhibitory control). Ensuring optimal nutrition during this period is essential for long term health into adulthood, but currently little is known about how early the gut microbiome is positively manipulated through nutrition. However, understanding the optimal nutritional regimen required for the beneficial development of the gut microbiome composition/functionality is an appealing strategy to promote health and reduce disease risk.

While human milk is the optimum first food for microbiome development following birth, alternative feeding regimens, based on infant formula require further research to develop and confirm the efficacy of microbiome-modulating ingredients, including prebiotics and probiotics, to mirror the benefits of human milk.

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