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.[1] 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.[2] A marker of an adverse developmental environment, low birth weight, has since been associated with increased risk of many adult disorders including glucose intolerance,[3] insulin resistance,[4] and obesity.[5]

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[6]—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,[7] hypertension,[8] diabetes, and coronary heart disease later in life.[9]

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.[10] Many animal models of prenatal caloric restriction have also shown associations between fetal growth restriction and offspring obesity,[11,12] insulin resistance, leptin resistance, and altered appetite.[13] 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

ANHI ABBOTT NUTRITION HEALTH INSTITUTE
www.anhi.org
116th Abbott Nutrition Research Conference: The Maternal Microbiome & Perinatal Colonization

171320/October 2017 LITHO IN USA
Abbott

decreased birth weight but increased adiposity, leptin resistance, and insulin resistance in the offspring, independent of postnatal diet.[12]

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[14] 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.[15,16] 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.*[17] 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.[18] Colonization of germ-free mice with the gut microbiota of women late in pregnancy was shown to induce increased adiposity and insulin insensitivity.[18] 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.[19] Obesity is accompanied by low-grade systemic inflammation,[20] contributed to in part by increased gut permeability and translocation of bacterial fragments, such as lipopolysaccharide (LPS),[21] 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.[22] 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[23,24] as well as obesity and metabolic disease later in life.[25] 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.[26,27]

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.[28] These shifts in the composition of the gut microbiota may impact maternal metabolism through altered production of

171320/October 2017 LITHO IN USA

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

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.[32] 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.[30] 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[33] but formal studies have not verified this hypothesis.

Recent work has shown that perinatal immune development is also influenced by the maternal microbiota.[34] 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.[34]

Evidence is still emerging on the impact of the prenatal environment on microbiota composition throughout life.[35] 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.[36] 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.

ANHI ABBOTT NUTRITION HEALTH INSTITUTE

www.anhi.org
116th Abbott Nutrition Research Conference: The Maternal Microbiome & Perinatal Colonization

171320/October 2017 LITHO IN USA

Abbott

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

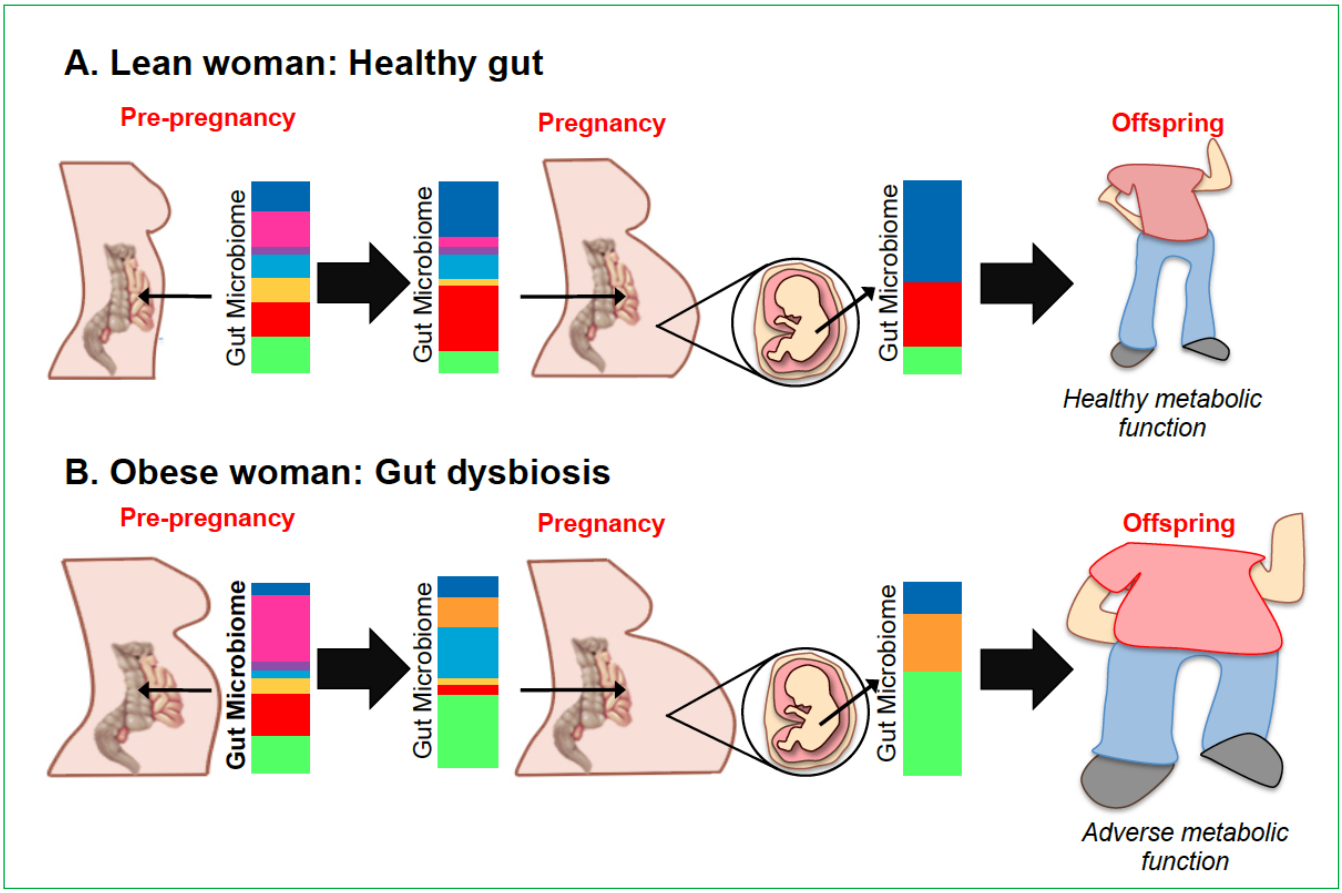

Fig 1. Maternal gut microbiota composition changes over the course of pregnancy.[37]

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

References

1. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet*. 1989;2(8663):577–580.

2. Barker DJ. The fetal and infant origins of adult disease. *BMJ*. 1990;301(6761):1111.

3. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*. 1991;303(6809):1019–1022.

4. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. *Diabetologia*. 1994;37(2):150–154.

171320/October 2017 LITHO IN USA

5. Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr.* 1999;70(5):811–816.

6. Gluckman PD, Hanson MA. *The Fetal Matrix: Evolution, Development and Disease*. Cambridge University Press;2004:272.

7. Stein AD, Zybert PA, van de Bor M, Lumey LH. Intrauterine famine exposure and body proportions at birth: the Dutch Hunger Winter. *Int J Epidemiol.* 2004;33(4):831–836.

8. Gilbert JS, Nijland MJ. Sex differences in the developmental origins of hypertension and cardiorenal disease. *Am J Physiol Regul Integr Comp Physiol.* 2008;295(6):R1941–R1952.

9. Kahn HS, Graff M, Stein AD, Lumey LH. A fingerprint marker from early gestation associated with diabetes in middle age: the Dutch Hunger Winter Families Study. *Int J Epidemiol.* 2009;38(1):101–109.

10. Vickers MH, Breier BH, McCarthy D, Gluckman PD. Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. *Am J Physiol Regul Integr Comp Physiol.* 2003;285(1):R271–R273.

11. Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am J Physiol Endocrinol Metab.* 2000;279(1):E83-87.

12. Howie GJ, Sloboda DM, Kamal T, Vickers MH. Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *J Physiol.* 2009;587(4):905–915.

13. Desai M, Babu J, Ross MG. Programmed metabolic syndrome: prenatal undernutrition and postweaning overnutrition. *Am J Physiol Regul Integr Comp Physiol.* 2007;293(6);R2306–2314.

14. Bäckhed F, Ding H, Wang T, et al.The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA.* 2004;101(44):15718–15723.

15. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006;444(7122):1027–1031.

16. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature.* 2006;444(7122):1022–1023.

17. Furet JP, Kong LC, Tap J, et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes.* 2010;59(12):3049–3057.

18. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell.* 2012;150(3):470–480.

19. Dudenhausen JW, Grünebaum A, Kirschner W. Prepregnancy body weight and gestational weight gain-recommendations and reality in the USA and in Germany. *Am J Obstet Gynecol.* 2015;213(4): 591–592.

20. Tzanavari T, Giannogonas P, Karalis KP. TNF-alpha and obesity. *Curr Dir Autoimmun.* 2010; 11:145–156.

21. Cani PD, Delzenne NM. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. *Curr Opin Pharmacol.* 2009;9(6):737–743.

22. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr.* 2008;88(4):894–899.

ANHI ABBOTT NUTRITION HEALTH INSTITUTE

www.anhi.org
116th Abbott Nutrition Research Conference: The Maternal Microbiome & Perinatal Colonization

171320/October 2017 LITHO IN USA

Abbott

23. Patro B, Liber A, Zalewski B, Poston L, Szajewska H, Koletzko B. Maternal and paternal body mass index and offspring obesity: a systematic review. *Ann Nutr Metab.* 2013;63(1-2):32–41.

24. Starling AP, Brinton JT, Glueck DH, et al. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start study. *Am J Clin Nutr.* 2015;101(2):302–309.

25. Rooney BL, Mathiason MA, Schauberger CW. Predictors of obesity in childhood, adolescence, and adulthood in a birth cohort. *Matern Child Health J.* 2011;15(8):1166–1175.

26. Collado MC, Isolauri E, Laitinen K, Salminen S. Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. *Am J Clin Nutr.* 2010;92(5):1023–1030.

27. Mueller NT, Whyatt R, Hoepner L, et al. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. *Int J Obes.* 2015;39(4):665–670.

28. Gohir W, Whelan FJ, Surette MG, Moore C, Schertzer JD, Sloboda DM. Pregnancy-related changes in the maternal gut microbiota are dependent upon the mother's periconceptional diet. *Gut Microbes.* 2015;6(5):310–320.

29. Tan J, McKenzie C, Potamitis M, Thoburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* 2014;121:91–119.

30. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci.* 2014;111(6):2247–2252.

31. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr.* 2009;139(9):1619–1625.

32. Rautava S, Collado MC, Salminen S, Isolauri E. Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. *Neonatology.* 2012; 102(3):178–184.

33. Donnet-Hughes A, Perez PF, Doré J, et al. Potential role of the intestinal microbiota of the mother in neonatal immune education. *Proc Nutr Soc.* 2010;69(3):407–415.

34. Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T, et al. The maternal microbiota drives early postnatal innate immune development. *Science*. 2016;351(6279):1296–1302.

35. Azad MB, Konya T, Persaud RR, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG*. 2016;123(6):983–993.

36. Steegenga WT, Mischke M, Lute C, et al. Maternal exposure to a Western-style diet causes differences in intestinal microbiota composition and gene expression of suckling mouse pups. *Mol Nutr Food Res.* 2017;61(1). doi: 10.1002/mnfr.201600141

37. Gohir W, Ratcliffe EM, Sloboda DM. Of the bugs that shape us: maternal obesity, the gut microbiome, and long-term disease risk. *Pediatr Res.* 2015;77(1):196–204.

ANHI ABBOTT NUTRITION HEALTH INSTITUTE

www.anhi.org
116th Abbott Nutrition Research Conference: The Maternal Microbiome & Perinatal Colonization

171320/October 2017 LITHO IN USA

Abbott