



Obesity and Gestational Diabetes Mellitus Pathways for Programming in Mouse, Monkey, and Man—Where Do We Go Next? The 2014 Norbert Freinkel Award Lecture

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Obesity and gestational diabetes mellitus continue to increase worldwide and span the spectrum of age, race, ethnicity, and socioeconomic status. Alarming, 1 in 10 infants and toddlers is obese, and 1 in 5 youths is both obese and at risk for metabolic syndrome prior to puberty. The mechanisms underlying how poor maternal health imparts risk for future metabolic disease in the offspring are beginning to emerge in deeply phenotyped human and nonhuman primate models. Maternal diet and obesity impact fuels, hormones, and inflammation with powerful effects on fetal metabolic systems. These are accompanied by persistent changes in the infant microbiome and epigenome and in offspring behavior. These results suggest that gestational and lactational dietary exposures are driving health risks in the next generation. Whether maternal diet can prevent changes in the womb to alter infant life-course disease risk is still unknown. Controlled, mechanistic studies to identify interventions are sorely needed for a healthier next generation.

In the late 1980s, Dr. David Barker used historical birth records from Hertfordshire, England, to pioneer the concept that the origins of adult disease could be strongly associated with fetal environmental exposures in pregnancy that resulted in low birth weight and predicted increased risk for cardiovascular morbidity and mortality in the offspring (1,2). Over the past several decades, the Developmental Origins of Health and Disease (DOHaD) hypothesis has been validated epidemiologically and mechanistically in both human and animal models. Data from animal models of nutritional constraint and uteroplacental insufficiency suggest that the gestational milieu influences the postnatal phenotype to cause susceptibility to childhood obesity and metabolic disorders through changes in tissue and organ development and metabolic reprogramming (3–7). DOHaD has taught us about the role of a mismatch between a constrained prenatal and a plentiful postnatal environment in the pathogenesis of obesity, i.e., the “thrifty” pathway, likely operating in populations undergoing rapid transition.

Another developmental pathway to obesity and its comorbidities, likely more important in Western societies, is developmental overnutrition. In fact, Norbert Freinkel, in his Banting Lecture in 1980 (8) may have been the first to conceptualize the overnutrition hypothesis in utero as a contributor to long-term offspring metabolic complications. He discussed this in the context of diabetes in pregnancy

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leading to excess fuel exposure to the fetal-placental unit, not only from glucose but also from excess lipids and amino acids (AAs). He termed this “fuel-mediated teratogenesis,” defined as alterations occurring subsequent to organogenesis during the differentiation and proliferation of fetal cells, and wrote “such changes could cause long-range effects upon behavioral, anthropometric, and metabolic functions” (8). This pathway reflects the effects of hypernutrition during fetal life and sets the stage for further amplification of the pathophysiological effects of encountering an obesogenic environment postnatally. More than 30 years later, emerging data suggest that in fact maternal diet, potentially amenable to manipulation, may be a key determinant of the fetal epigenotype and resulting phenotype (9–17).

Animal models, including nonhuman primates (NHP), sheep, rodents, and even flies and zebrafish, have shown that fetal growth and development are vulnerable to changes in nutrition in all three phases of development. These include 1) early gestation during implantation, placentation, and subsequent embryogenesis when placental nutrient transport may be set; 2) midgestation when number, growth, and function of critical organs like pancreas, brain, kidney, and skeletal muscle develop; and 3) during the late third trimester when fetal growth (adiposity) accelerates and regulatory set points in the brain, neuronal-metabolic feedback loops, and mitochondrial function may be impacted. While animal models may have vastly different patterns of gestation and placental structure, they have been critical in the discovery of potential molecules and mechanisms leading to early-onset obesity. Despite these discoveries, there is lack of knowledge about the impact of environmental exposures on biochemical and molecular processes that govern metabolic risk, particularly in utero on infants born to mothers who are obese or have gestational diabetes mellitus (GDM).

The focus of our research group is on understanding the role of maternal nutrition and the intrauterine environment on molecular, endocrine, and epigenetic origins of childhood obesity. This involves developing novel animal models of GDM and obesity (transgenic

mice, NHP) together with longitudinal invasive human clinical investigation in vivo and in vitro utilizing myocytes, adipose tissue, and, more recently, umbilical-derived mesenchymal stem cells (MSCs) obtained from infants born to obese women with and without GDM. More recently, we have begun investigating the role of the microbiome (MB) in mothers and their infants with a goal of understanding how maternal nutrition in pregnancy and breast milk composition can influence infant microbial function and the trajectory of weight gain, adiposity, and development of nonalcoholic fatty liver disease (NAFLD), the leading cause of liver disorders in children and adults. The work of myself and my colleagues over the past 20 years has been due in no small part to my association and collaboration with Dr. Patrick Catalano (Case Western Reserve University) and Dr. Linda Barbour (University of Colorado Denver), who continue to teach me about the important clinical problems encountered in the diagnosis, treatment, and underlying pathophysiology of normal, obese, and GDM pregnancies. They have also taught me about the power of collaboration and the value of true friendship.

MATERNAL INSULIN RESISTANCE IN OBESITY AND GDM: CELLULAR MECHANISMS THAT IMPART INSULIN RESISTANCE, CONSEQUENCES OF EXCESS FETAL NUTRIENT EXPOSURE, AND POSTPARTUM RISK FOR PROGRESSION TO TYPE 2 DIABETES

Maternal insulin resistance (IR) is a normal part of human pregnancy and is critically important to maintain the maternal fuel supply to support the growing fetus, particularly during the third trimester. However, women with obesity or a history of GDM enter pregnancy with preexisting IR that worsens with advancing gestation (18). In type 2 diabetes and GDM, the maternal metabolic environment is characterized by IR and inflammation, and both conditions shunt excess fuels to facilitate fat accretion and fetal growth (19). Emerging data suggest that excess maternal IR may be a more important predictor of neonatal adiposity than prepregnancy BMI (20,21). In a study of 301 infants of women with GDM, mild glucose intolerance, or normal glucose tolerance,

maternal IR by the Matsuda index predicted offspring weight gain and adiposity from 0–12 months, but prepregnancy BMI did not (20). Maternal IR has the capacity to shunt not only glucose but all nutrients to the fetus, including glucose, triglycerides (TGs), free fatty acids (FFAs), and AAs, all of which can be used for fetal fat accretion and excess fetal growth. Furthermore, the placental transcriptome is a target of the altered environment of diabetic and obese pregnancies. Genes for lipids and AA transport are upregulated in the placenta of women with GDM more than those for glucose, as are genes for inflammatory pathways (22,23).

Mechanistically, we demonstrated that insulin-stimulated glucose transport in human skeletal muscle fibers from obese women is suppressed in late pregnancy and more so in skeletal muscle of women who develop GDM (24). In transgenic mice, and later in human pregnancy, we showed that human placental growth hormone is a major driver of the normal IR of pregnancy by suppressing the IRS1-associated PI3-kinase insulin signaling cascade in skeletal muscle (25,26). Further, we showed in GDM subjects that reduced IRS1 in skeletal muscle and adipose tissue, along with decreased insulin receptor activation, may be responsive to overnutrition as well as inflammatory changes during late pregnancy (27). In addition, using proteomic analysis in skeletal muscle of obese and GDM subjects, we found reduced mitochondrial function and increased oxidative stress in both obese and GDM patients, controlled in part by the mitochondria deacetylase SIRT3 (28). In GDM subjects, there are additional unique changes in calcium binding proteins associated with reduced mitochondrial function, suggesting these might underlie the reduced oxidative capacity and ultimately lower exercise tolerance, particularly evident in women with GDM (29). Last, when we followed a series of obese women with GDM longitudinally postpartum we found that specific defects in IRS1 and p70S6K in human skeletal muscle may persist up to a year later and may underlie the chronic IR and future risk for type 2 diabetes (19,30), beyond simple obesity. While there are complex mechanisms underlying the IR in skeletal muscle and adipose tissue, given that fetal growth and adiposity increases with even

modest changes in glucose (31), FFAs, and TGs (32), the IR pathways uncovered here may be extremely important. Furthermore, they are highly relevant in explaining why obese women, whom we have shown to have elevated 24-h glycemic profiles as well as FFAs and TGs compared with normal-weight women (32), are at an increased risk for delivering infants with increased adiposity.

IMPACT OF A WESTERN-STYLE DIET AND MATERNAL OBESITY ON METABOLIC SYSTEMS IN THE NEXT GENERATION: LESSONS LEARNED FROM NHP

Maternal obesity and GDM are important risk factors for obesity in the next generation. Whether this is due to specific changes in maternal diet, the maternal phenotype (IR), or a combination of the two has remained poorly understood. In order to study the impact of specific changes in maternal diet and obesity on fetal pathophysiology and future health risks, in 2007 Dr. Kevin Grove at the Oregon National Primate Research Center and I began a collaboration to develop an NHP model for studying maternal obesity and its impact on the development of fetal metabolic systems. Our group has spent the past decade developing and studying a sophisticated NHP Japanese Macaque model of chronic consumption of a maternal high-fat/calorie-dense Western-style diet (WSD) starting early in the reproductive years that has critically important developmental and physiological similarities to humans. The importance of the NHP model is that the placenta, brain, liver, skeletal muscle, and pancreas structures are similar to humans, and it is the only natural model that develops the full spectrum of metabolic diseases as in humans. Consequentially, the developmental changes in vital organs are highly similar to humans, including complex psychosocial behaviors that can be studied longitudinally in the offspring. These qualities make the NHP model uniquely powerful and critically important.

We have shown that consumption of a maternal WSD causes placental dysfunction, tissue-specific changes in the offspring mitochondria, widespread inflammation, hepatic steatosis, and broad developmental changes in the liver, skeletal muscle, brain, and pancreas, as outlined below. These alterations are accompanied

by significant and persistent changes in the epigenome, the MB, and offspring behavior. Importantly, many of these abnormalities persist even when NHP offspring are weaned to a healthy diet after lactation, suggesting that gestational and lactational dietary exposures are significant and possibly permanent contributors in the initiation and development of pathways that drive health risks in the next generation.

One of the most striking early findings we discovered in the NHP model is the development of fetal NAFLD. NAFLD is a general term used to describe a broad spectrum of liver abnormalities, ranging from simple, uncomplicated hepatic steatosis to nonalcoholic steatohepatitis (NASH), with different degrees of inflammation and fibrosis. NAFLD is the most common liver disease in children and adults (33,34) and the leading cause of liver transplantation. Prevalence estimates of NAFLD in children range from 3–10% in Western societies, approaching 55% in children who are obese (35). When we performed cesarean sections in the early third trimester (G130) in

mothers fed a WSD, we discovered the fetuses had a dramatic increase in liver lipids (Fig. 1) (13). Importantly, we found that all fetuses had elevated liver lipids whether their mothers developed obesity or not on the WSD. Fetuses at this stage of development have very little adipose tissue to store lipids, suggesting that exposure to a WSD resulted in excess lipid availability to the fetus that accumulates in the liver during gestation. The livers also stained for a marker of oxidative stress (4-hydroxynonenal), suggesting lipid overload may have effects on protein functions, or so-called lipotoxicity.

During the next breeding season, we performed a diet reversal, whereby obese mothers were switched from a WSD to a healthy control diet. Although the mothers remained obese, they manifested less IR. In these G130 fetuses, liver TGs were significantly improved but had not returned to control levels, suggesting that maternal obesity, even on a healthy diet, contributes to fetal hepatic steatosis. To examine if these

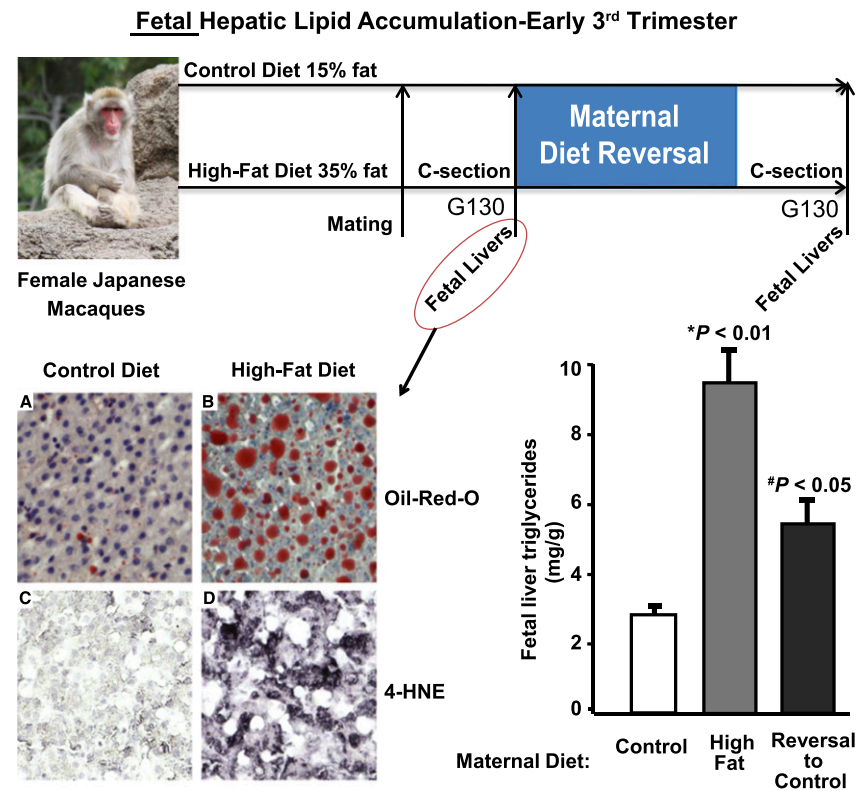


Figure 1—Liver steatosis in the fetal liver during the early third trimester of NHP on a high-fat diet. Female Japanese Macaques were maintained on high-fat or chow diet for a minimum of 2 years prior to mating. In a subsequent pregnancy, obese females were switched to a chow diet prior to mating and cesarean sections (C-section) were performed. Livers were stained for neutral lipid (Oil-Red-O) and 4-hydroxynonenal (4-HNE), a marker of oxidative stress (data are from McCurdy et al. [13]).

changes persisted postnatally, a separate cohort of obese mothers on a WSD were allowed to give birth and the offspring were weaned to a healthy diet at 6–7 months of age. At 12–14 months of age, the livers from the juvenile animals showed increased TGs and increased liver macrophage cell numbers and the liver macrophages were hyperresponsive to fatty acids in vitro, producing high levels of cytokines (36). Overall, a WSD coupled with maternal obesity was associated with long-term consequences, increasing the risk for progression from uncomplicated hepatic steatosis to NASH. This includes inflammation via Toll-like receptors on the resident macrophages (Kupffer cells), immune cell activation, and priming for recruitment of additional immune cells from the bone marrow (Fig. 2). Importantly, these changes occurred prior to the development of juvenile obesity in offspring born to obese mothers with high IR on a WSD. The placentas from the obese mothers on a WSD showed evidence of oxidative

stress, inflammation, reduced placental blood flow, and hypoxia (9). These findings suggest that chronic WSD and maternal obesity may be the “first hit” in the progression of simple steatosis to inflammation and NASH in later life.

To determine whether this early liver steatosis is present in human neonates who have more subcutaneous fat to store lipid than NHP, we used magnetic resonance spectroscopy to show that maternal obesity and GDM predict neonatal hepatic fat storage (37). We measured intrahepatocellular lipid in 2-week-old newborns born to both normal-weight and obese mothers with GDM and showed a 68% increase in liver fat in the infants of obese GDM mothers. In another cohort, Modi et al. (38) reported an 8.6% increase in intrahepatocellular lipid content for each 1-point increase in prepregnancy BMI. The positive correlation between offspring intrahepatocellular lipid and maternal prepregnancy BMI held for the entire population of obese and normal-weight mothers. Other variables, such as

gestational weight gain and early postnatal weight change, did not influence intrahepatic fat storage in this cohort of 25 mother-infant pairs. Importantly, increased fetal hepatic fat storage appeared to be independent of overall adiposity in the newborn, suggesting that the drivers of hepatic fat storage and subcutaneous adipose fat storage may be different during fetal life. In another infant cohort, liver fat doubled between birth and 2 months, regardless of breast-feeding (39), raising the question of whether excess liver fat storage at birth may have long-term consequences, particularly in obese teenagers (40,41). Notably, NAFLD increases the risk of cardiovascular events eightfold and type 2 diabetes threefold and is a strong risk factor for hepatocellular carcinoma (42).

A second striking finding in the NHP studies is the long-term behavioral changes noted in the offspring exposed to maternal WSD during pregnancy and weaning. When these animals were switched to a healthy diet at weaning (6–7 months of age) and studied at 12–14 months of age as juveniles, the male animals displayed an increase in repetitive behaviors (pacing stereotypy), while the females displayed increased anxiety-like behavior (10). Offspring of both sexes displayed impairments in social behavior. A potential mechanism for these behavioral changes is the suppression of serotonin synthesis by the raphe nuclei noted in these juveniles (10), suggesting that the systems that govern anxiety and depression may be hardwired by the maternal diet.

There is strong evidence that obese children tend to consume more dietary fat than nonobese children as a function of total calories (43), thus contributing to higher BMI. In addition, fats tend to deregulate control mechanisms for body weight regulation (appetite and activity) associated with changes in the hypothalamus, dopaminergic pathways, and other areas of the brain for reasons that are not completely understood (44). Whether maternal obesity and/or diet can modify the neurocircuitry in utero and whether it undergoes further modification during lactation and early feeding resulting in food preferences that occur prior to the development of obesity remain unknown. In our studies of juvenile NHP offspring exposed to a maternal WSD during pregnancy and lactation and then weaned to healthy diets

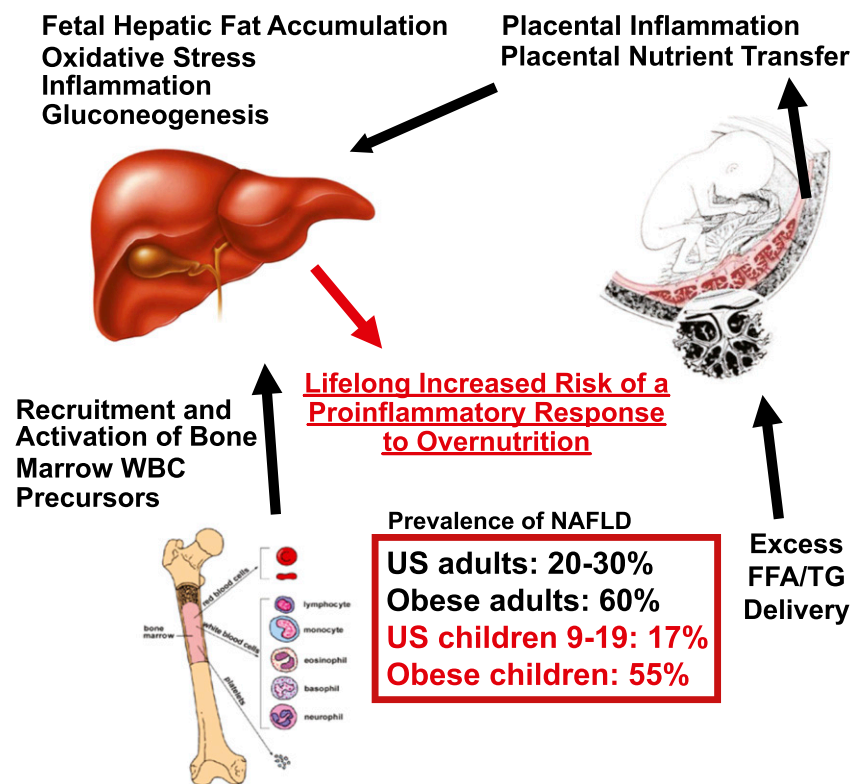


Figure 2—Consequences of maternal overfeeding on fetal liver and the evolution of NAFLD. Exposure to excess lipids triggers placental inflammation, oxidative stress, and liver steatosis prior to birth. After weaning, a combination of recruited hepatic macrophages and alternatively activated resident immune cells, together with IR and obesity, is a significant risk factor for inflammation and progression of NAFLD. Prevalence of NAFLD is shown. WBC, white blood cell.

postnatally, there was already a strong preference for selection of high-energy (high-fat) foods over all other sources of calories. Of note, these changes took place prior to the emergence of obesity in the animals. Although not all animals were equally affected, when combined with the social/behavioral abnormalities noted above, the results suggest that early changes in brain development and behavior may be one of the most compelling concerns for the DOHaD hypothesis and its impact on children in modern society. Whether these changes are governed by exposure in utero to higher fuels, cytokines, or oxidative stress noted in the fetuses of WSD-fed mothers; postnatally by higher breast milk n-6:n-3 fatty acids (12); or by other bioactive and appetite regulatory components such as leptin or insulin remain to be determined. Interventional studies in NHP obese mothers and the study of longer-term brain epigenetic modifications in their 3-year-old offspring are currently under way.

MATERNAL OBESITY AND THE INFANT MB: THE UNDISCOVERED COMMUNITY WITHIN

The gut MB plays a significant role in both maternal and infant gut health and child development. The intestinal microbiota is referred to as our “second genome” and is acknowledged as 10 times the size of our own genetic repertoire, involved in the susceptibility of many disorders as diverse as obesity, type 1 diabetes, inflammatory bowel disease, allergies, autism, cancer, and asthma. The term “gut microbiota” represents a complex bacterial community within the small and large intestine, capable of affecting health by contributing to energy retention and appetite, preventing colonization of the host by pathogens, and influencing the development and maintenance of the immune system. In human pregnancy, there are temporal shifts in both the diversity within a microbial community, known as α -diversity, which can be measured by phylogenetic diversity and species richness, and in the diversity shared among different communities, known as β -diversity, which can be measured by changes in microbial abundance (45). The microbiota in late pregnancy has reduced α -diversity (richness) but higher β -diversity (abundance of certain species) compared with nonpregnant women or women in early pregnancy (46).

Remarkably, when germ-free (gnotobiotic) mice were transplanted with fecal microbiota from healthy women in their first or third trimester of pregnancy, those receiving the third trimester microbiota had increased intestinal cytokines, gained more weight, and were more glucose-intolerant than those receiving the first trimester microbiota (46). This striking result implies that the third trimester microbiota have the ability to induce an alternative metabolic state in mothers, associated with greater energy extraction and inflammation. Such a transformation would serve to potentiate maternal IR for fuel transfer to the fetus and ultimately to transfer the capacity for increased energy retention to the newborn, ensuring the survival of the next generation. For example, in the third trimester, there is greater representation of lactic acid bacteria, which are highly prevalent in the infant gut, whereas butyrate-producing bacteria (*Faecalibacterium*, *Blautia*, and *Ruminococcus*), which dominate the gut in adulthood, are enriched in early pregnancy (46). The acquisition of increased maternal lactic acid bacteria by the third trimester may be an adaptation to transfer these organisms during the perinatal period to the infant to take maximum advantage of the main energy source for the child, lactose in mother's milk. Although the newborn acquires its microbiota from the mother during delivery, there is some limited evidence for microbial presence in the placenta, amniotic fluid, and meconium in full-term pregnancies without overt infection (47–51). However, if and to what extent early tolerance of the fetus by the antepartum acquisition of any bacterial species toward colonization of the mouth and gastrointestinal tract may impact the health and development of the newborn remain to be seen.

In newborn infants, gastrointestinal microbes introduced through dietary exposures are noted for their ability to serve as direct inducers/regulators of the infant immune system during breast-feeding through alterations of the infant gut microbiota (52,53). Diet is a powerful driver of the MB; however, the specific molecular factors and mechanisms by which the mother's diet influence development of the infant microbiota in the offspring are unknown.

Further, the role of the MB in obesity and GDM or how these states alter the neonatal MB remains largely unknown. Differences in the gut MB in infants experiencing different life events, such as breast-feeding versus formula feeding, cesarean versus vaginal birth, and environmental exposure to antibiotics, have been well documented. Epidemiological studies have shown that antibiotic treatment during the first 6 months of life (54,55) or disrupted colonization from cesarean delivery (56,57) may increase the risk of being overweight later in life. These two interventions have no direct contribution to host caloric intake or metabolism (58) but have large effects on the MB (59,60). The mechanisms by which the MB may affect newborn weight gain or adipose tissue development remains unknown but could be due to immune signaling, toxin release, nutrient utilization, or regulation of appetite. Our groundbreaking studies in NHP have shown that a WSD in obese mothers leads to decreased diversity of offspring intestinal MB at 1 year and increased liver steatosis and inflammation (36,61). Importantly, these deleterious outcomes occur prior to the onset of obesity and are not reversed by switching to a healthy diet after weaning. This suggests that the maternal influence to modify the microbial ecosystem in infants may be driven by the pioneering bacteria acquired at birth and during lactation. In humans, we recently discovered that a proinflammatory profile of increased n-6 relative to n-3 fatty acid in human milk, along with increased human milk insulin and leptin at 2 weeks of breast-feeding, predicts changes in the infant microbiota and the infant bacterial metagenome (B.E. Young and M.C. Rudolph, unpublished data). Importantly, the normalization of the n-6:n-3 ratio in transgenic mice fed a high-fat diet during pregnancy has been shown to prevent excess weight gain and fatty liver in the next generation (62).

Despite these suggestive data, there are no published studies directly linking maternal diet to altered microbiota-derived metabolites and durable changes in the developing infant immune system or adipose tissue development. The composition and metabolism of the adult gut MB are known to be rapidly influenced by diet; however, these changes are often

transient (63–65), and there is tremendous interindividual variation (so-called “responders and nonresponders” [66]), suggesting that host genetics can shape the composition of the gut MB (67). Conversely, the gut MB can also modify dietary exposures in ways that are beneficial or detrimental to the human host. For example, the SCFAs acetate, butyrate, and propionate, which are formed by microbial metabolism of fiber, resistant starches, and nonstarch polysaccharides in the distal colon, may be metabolically consequential both at the site of production and at distal tissues. The major product of microbial fermentation, acetate, is a substrate for hepatic cholesterol and TG synthesis and increases hepatic expression of genes involved in fatty acid metabolism and lipogenesis (68). Conversely, exposure of adipose tissue to propionate suppresses the expression of proinflammatory cytokines, upregulates GLUT4 expression, and stimulates leptin (69–71). In a study of pregnant women with obesity, serum acetate levels were associated with maternal weight gain and maternal adiponectin levels (72). Propionate, on the other hand, was inversely correlated with maternal leptin (72). Butyrate largely serves to support the energy needs of the colonic epithelial cells to proliferate and differentiate (73). However, butyrate is also an epigenetic regulator (a histone deacetylase inhibitor) that promotes early anti-inflammatory immune cell development (74) as well as adipose tissue metabolism (75) through the SCFA receptors GPR43 and GPR41 in adipose tissue and liver.

While the core human gut microbiota may contribute to the developmental origins of disease by modifying metabolic pathways in maternal and infant tissues, it may also participate as an epigenetic modifier. Recently, Kumar et al. (76) classified eight well-matched pregnant women into two groups based on their dominant microbiota, i.e., *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Deep sequencing of DNA methylomes revealed a clear association between bacterial predominance and epigenetic profiles. The genes with differentially methylated promoters in the *Firmicutes* group were linked to genes specifically involved in lipid metabolism, obesity, and the inflammatory response. This is one of the first studies that highlights the association of the predominant bacterial phyla

in the gut with methylation patterns. While these studies are simple correlations, longitudinal studies identifying microbial species or metabolites prior to health consequences may give us a deeper insight into the molecular mechanism of such epigenetic modifications. It should be noted, however, that despite the wealth of 16S sequencing data and metatranscriptomic data emerging based on shotgun sequencing methods, the functions of most bacterial genes from the mammalian microbiota remain poorly understood. Whereas 16S sequencing has proven extremely useful in terms of typing and determination of the number of phyla, metagenomic sequencing has provided an increasingly high depth of data based on homology with known microbial genes and may shed light on how microbiota affect metabolic pathways. There is an incontrovertible need to identify the functional roles of these bacteria and the bioactive molecules that affect human health.

FUTURE OF DOHAD RESEARCH: PLAUSIBLE INTERVENTIONS TO INTERRUPT THE VICIOUS CYCLE

While maternal genetic, epigenetic, and dietary factors contribute to the development

of obesity and metabolic syndrome in the next generation, the determination of how and when to intervene during pregnancy and postnatally in human infants is a complex problem. The lack of compelling evidence necessary to justify the huge efforts that would be required to modify the maternal diet of high-risk women with obesity and GDM is a significant challenge to this field. Our own data in NHP demonstrate that maternal diet has a significant impact on gene expression in the fetus and 1 year later prior to the development of obesity in the offspring (16,17,36). Recent human studies suggest that maternal nutrition and the intrauterine environment can alter DNA methylation in umbilical cord blood (77,78), umbilical cord tissue (79,80), and buccal cells (81). However, most clinical studies examining the role of maternal nutrition and infant epigenetics are retrospective; there are no studies investigating maternal nutritional interventions and epigenetic changes in the infant. Furthermore, the tissue-specific nature of epigenetic control adds to the complexity of such studies and remains a concern.

A relatively new research tool in pregnancy is the study of infant-derived MSCs. In utero, MSCs give rise to specific

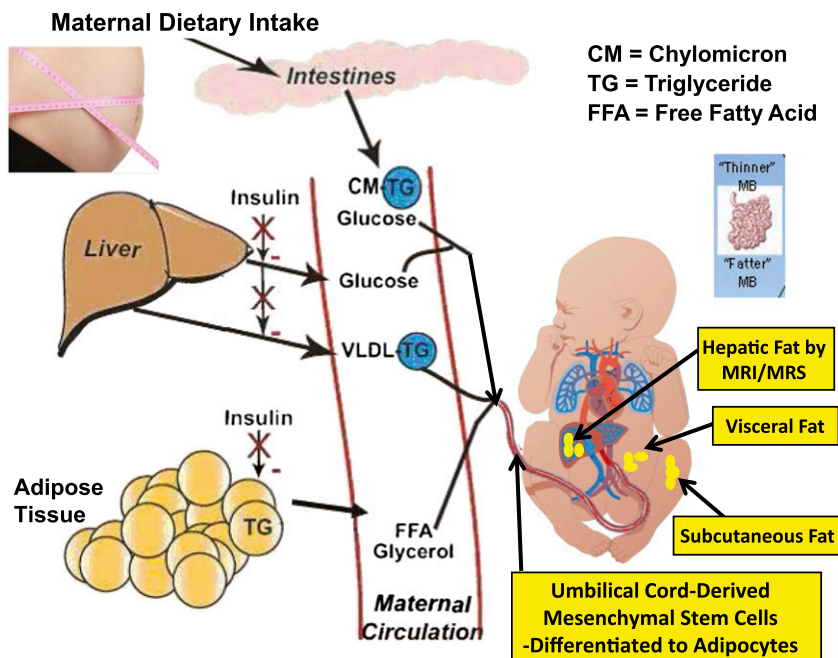


Figure 3—Fetal adaptations to maternal obesity and GDM. Maternal obesity, estimated to affect about one in four pregnant women, and GDM and type 2 diabetes in pregnancy, the rates of which are rapidly growing, increase circulating glucose and lipids due to increased lipolysis, hepatic glucose production, and dietary (chylomicron-derived) lipids. Newborns show increased hepatic lipids, subcutaneous fat, and adipogenesis in infant-derived umbilical cord MSCs. MB changes in infants born to obese and GDM mothers are under study. MRS, magnetic resonance spectroscopy.

tissue types, including fat, skeletal muscle, and bone. In amniotic-derived MSCs from obese women, there was a shift in MSC commitment to the adipocyte lineage during fetal development (82). Our own preliminary studies point to newborn adiposity as a stronger phenotypic biomarker of adipocyte differentiation and lower mitochondrial energy metabolism in the MSCs from infants born to obese women, suggesting both factors could contribute to lineage changes in infant adiposity and low energy expenditure. More mechanistic epigenetic and metabolomic analysis of these cells and longitudinal analysis of these infants are under way as part of the Healthy Start Study (83) and may give critical translational insight into pathways underlying developmental origins of obesity.

Although the transmission of metabolic risk in infants born to mothers with obesity or GDM may be mediated by excess fuels, inflammation, oxidative stress, and other metabolic risk factors, altering the maternal diet is one of the few modifiable and potentially potent manipulations. Dietary intake in human pregnancy is often poorly measured, if at all, and may be a very fundamental source of variability in metabolic pathways influencing maternal and fetal fat accretion, requiring further investigation. Recently, we have shown in highly controlled diet studies that women with GDM randomized to a eucaloric higher-complex carbohydrate/low-fat (CHOICE) diet compared with the conventional low-carbohydrate/higher-fat diet resulted in normoglycemia and appeared to reduce maternal IR (84). Furthermore, after extending the diets to delivery with all meals provided, the subjects on the CHOICE diet showed decreased adipose cytokine expression, increased maternal adipose tissue insulin sensitivity, and a trend for lower infant adiposity (85) compared with those on the conventional diet. These outcomes suggest the metabolic actions of lowering fat intake on maternal tissues are a promising target for reducing IR and controlling excess fuel transfer to the fetus. Our current studies are aimed at exploring how this diet alters maternal glycemic profiles and lipids, adipose tissue metabolism, placental nutrient transport and inflammation, and mechanisms underlying infant growth, including

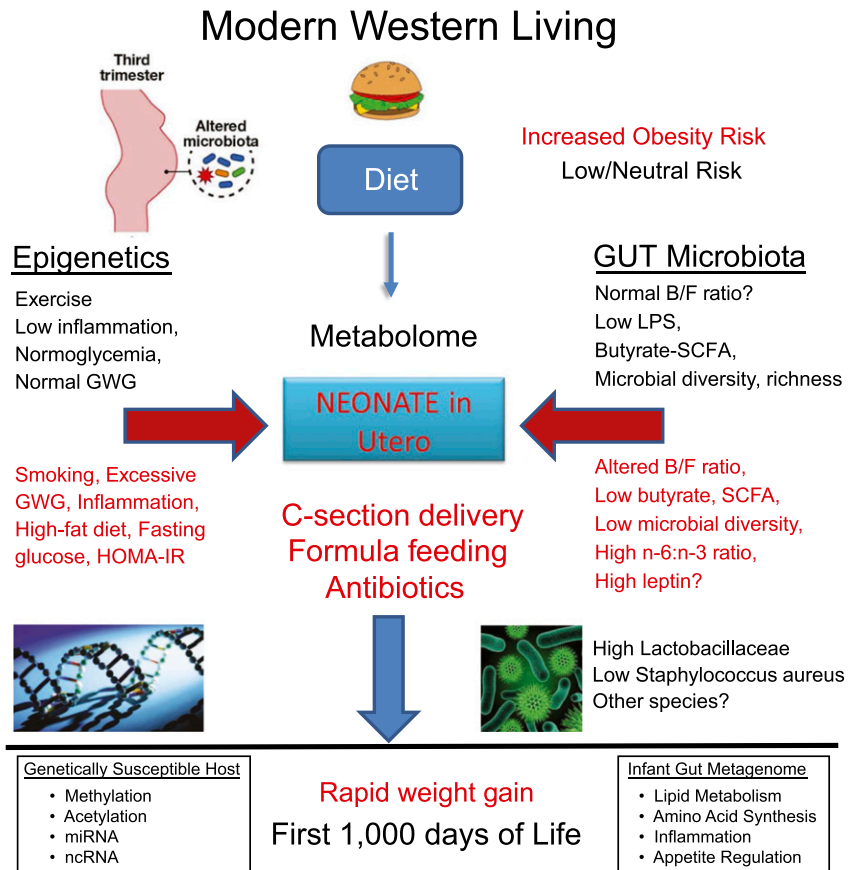


Figure 4—The impact of maternal diet on changes in obesity risk includes epigenetic and MB changes during the first 1,000 days of life. Metabolomic changes in mothers and babies may be associated with exposure to changes in gut microbes, dietary fatty acids, inflammation, excess insulin and leptin, along with antibiotics, and they may impact the host epigenome to increase infant weight gain. B/F, bacteroidetes/firmicutes; C-section, cesarean section; GWG, gestational weight gain; LPS, lipopolysaccharide; miRNA, microRNA; ncRNA, noncoding RNA.

changes in infant liver steatosis and the MB (Fig. 3).

SUMMARY AND FUTURE DIRECTIONS

Fundamentally, fetal developmental programming by maternal nutrition may occur in two ways: first, by gene-environment interactions, such as diet, that may produce persistent epigenetic events and second, by impacting normal organ development to impart risk for developing chronic disease(s). Most of the changes in adiposity in offspring born to obese or GDM women occur within the normal range in birth weight, suggesting that programming likely involves subtle effects on metabolic regulation during development. The fact that many outcomes are modifiable by diet in the first 1,000 days of life suggests that maternal diet can be a powerful intervention to modify the transgenerational risk of obesity by modifying

organ growth and development, fat acquisition, appetite/behavior, and epigenetic risk in the offspring, as shown in Fig. 4. For example, it is possible that altered maternal microbiota and mucosal immunity might directly influence maternal metabolism and as a result influence the pathogenesis of IR and perhaps placental function. While these observations of changes in the MB and inflammation are highly provocative and there is evidence for increased circulating lipopolysaccharide levels in pregnant obese patients (86), they suggest a hypothesis for future experiments, including the development of novel pre/probiotics for pregnancy. Tying together the transmission of the maternal MB from an obese or GDM mother with antibiotic exposure, mode of delivery, infant energy retention, and immune function that may contribute to predisposition to NAFLD and other immunologic diseases also deserves greater attention. The

development of comprehensive advanced techniques, including deep sequencing of bacteria genomes (the metagenome), epigenetic platforms (methylation, histone modification, microRNAs, and noncoding RNAs), and metabolomic discovery tools, may fundamentally inform our research directions.

While human investigation is inherently variable and large sample sizes are often needed to sort out trends from noise in such data sets, random sampling under controlled conditions can increase their predictive accuracy. Metabolomic intermediates and biomarkers, including AAs, lipids, and carbohydrate intermediates, can influence the offspring in utero, and data on these biomarkers are being applied to human pregnancy (87–89). However, our understanding of the metabolomic signatures that influence infant growth and development during the first 2 years of life is minimal and gravely needed. Human studies outside of pregnancy suggest that reduced dietary fat intake and improved exercise can slow down the progression of diabetes from GDM (90,91). However, randomized interventional trials in pregnancy that attempt to modify lifestyle in order to favorably affect infant outcomes have been largely disappointing (92). While efforts to address the environmental factors influencing infant health (e.g., exercise, limiting maternal weight gain, promoting exclusive breast-feeding, and administering pre/probiotics) are under way, sizable dietary changes may lead to better outcomes. Although there remains a considerable challenge to deliver these interventions in low-resource settings, the genetic and epidemiological life-course data showing long-term effects of maternal obesity or GDM exposure on the metabolic health of offspring commands our efforts to discern how gestational exposures in the modern environment can be specifically targeted to reduce childhood obesity risk. Primary intervention in utero and in early life that attenuates obesity potential may be one of the most important public health efforts to enhance population health worldwide.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

- Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;2:577–580
- Barker DJ. The origins of the developmental origins theory. *J Intern Med* 2007;261:412–417
- Bhargava SK, Sachdev HS, Fall CH, et al. Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. *N Engl J Med* 2004;350:865–875
- Eriksson JG, Forsén T, Tuomilehto J, Osmond C, Barker DJ. Early growth and coronary heart disease in later life: longitudinal study. *BMJ* 2001;322:949–953
- Knight B, Shields BM, Hill A, Powell RJ, Wright D, Hattersley AT. The impact of maternal glycaemia and obesity on early postnatal growth in a nondiabetic Caucasian population. *Diabetes Care* 2007;30:777–783
- Metzger BE, Lowe LP, Dyer AR, et al; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008;358:1991–2002
- Symonds ME, Gopalakrishnan G, Bispham J, et al. Maternal nutrient restriction during placental growth, programming of fetal adiposity and juvenile blood pressure control. *Arch Physiol Biochem* 2003;111:45–52
- Freinkel N. Banting Lecture 1980. Of pregnancy and progeny. *Diabetes* 1980;29:1023–1035
- Frias AE, Morgan TK, Evans AE, et al. Maternal high-fat diet disturbs uteroplacental hemodynamics and increases the frequency of stillbirth in a nonhuman primate model of excess nutrition. *Endocrinology* 2011;152:2456–2464
- Sullivan EL, Grayson B, Takahashi D, et al. Chronic consumption of a high-fat diet during pregnancy causes perturbations in the serotonergic system and increased anxiety-like behavior in nonhuman primate offspring. *J Neurosci* 2010;30:3826–3830
- Sullivan EL, Smith MS, Grove KL. Perinatal exposure to high-fat diet programs energy balance, metabolism and behavior in adulthood. *Neuroendocrinology* 2011;93:1–8
- Grant WF, Gillingham MB, Batra AK, et al. Maternal high fat diet is associated with decreased plasma n-3 fatty acids and fetal hepatic apoptosis in nonhuman primates. *PLoS One* 2011;6:e17261
- McCurdy CE, Bishop JM, Williams SM, et al. Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. *J Clin Invest* 2009;119:323–335
- Aagaard-Tillery KM, Grove K, Bishop J, et al. Developmental origins of disease and determinants of chromatin structure: maternal diet

modifies the primate fetal epigenome. *J Mol Endocrinol* 2008;41:91–102

- Cox J, Williams S, Grove K, Lane RH, Aagaard-Tillery KM. A maternal high-fat diet is accompanied by alterations in the fetal primate metabolome. *Am J Obstet Gynecol* 2009;201:281.e1–9
- Suter MA, Chen A, Burdine MS, et al. A maternal high-fat diet modulates fetal SIRT1 histone and protein deacetylase activity in nonhuman primates. *FASEB J* 2012;26:5106–5114
- Suter MA, Takahashi D, Grove KL, Aagaard KM. Postweaning exposure to a high-fat diet is associated with alterations to the hepatic histone code in Japanese macaques. *Pediatr Res* 2013;74:252–258
- Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *Am J Obstet Gynecol* 1999;180:903–916
- Barbour LA, McCurdy CE, Hernandez TL, Friedman JE. Chronically increased S6K1 is associated with impaired IRS1 signaling in skeletal muscle of GDM women with impaired glucose tolerance postpartum. *J Clin Endocrinol Metab* 2011;96:1431–1441
- Hamilton JK, Odrobina E, Yin J, Hanley AJ, Zinman B, Retnakaran R. Maternal insulin sensitivity during pregnancy predicts infant weight gain and adiposity at 1 year of age. *Obesity (Silver Spring)* 2010;18:340–346
- Shapiro AL, Schmiege SJ, Brinton JT, et al. Testing the fuel-mediated hypothesis: maternal insulin resistance and glucose mediate the association between maternal and neonatal adiposity, the Healthy Start study. *Diabetologia* 2015;58:937–941
- Radaelli T, Lepercq J, Varastehpour A, Basu S, Catalano PM, Hauguel-De Mouzon S. Differential regulation of genes for fetal placental lipid pathways in pregnancy with gestational and type 1 diabetes mellitus. *Am J Obstet Gynecol* 2009;201:209.e1–209.e10
- Aye IL, Jansson T, Powell TL. Interleukin-1 β inhibits insulin signaling and prevents insulin-stimulated system A amino acid transport in primary human trophoblasts. *Mol Cell Endocrinol* 2013;381:46–55
- Friedman JE, Ishizuka T, Shao J, Huston L, Highman T, Catalano P. Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes. *Diabetes* 1999;48:1807–1814
- Barbour LA, Mizanoor Rahman S, Gurevich I, et al. Increased P85alpha is a potent negative regulator of skeletal muscle insulin signaling and induces in vivo insulin resistance associated with growth hormone excess. *J Biol Chem* 2005;280:37489–37494
- del Rincon JP, Iida K, Gaylann BD, et al. Growth hormone regulation of p85alpha expression and phosphoinositide 3-kinase activity in adipose tissue: mechanism for growth hormone-mediated insulin resistance. *Diabetes* 2007;56:1638–1646
- Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes Care* 2007;30(Suppl. 2):S112–S119
- Boyle KE, Newsom SA, Janssen RC, Lappas M, Friedman JE. Skeletal muscle MnSOD,

- mitochondrial complex II, and SIRT3 enzyme activities are decreased in maternal obesity during human pregnancy and gestational diabetes mellitus. *J Clin Endocrinol Metab* 2013;98:E1601–E1609
29. Boyle KE, Hwang H, Janssen RC, et al. Gestational diabetes is characterized by reduced mitochondrial protein expression and altered calcium signaling proteins in skeletal muscle. *PLoS One* 2014;9:e106872
30. Friedman JE, Kirwan JP, Jing M, Presley L, Catalano PM. Increased skeletal muscle tumor necrosis factor- α and impaired insulin signaling persist in obese women with gestational diabetes mellitus 1 year postpartum. *Diabetes* 2008;57:606–613
31. HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. *Diabetes* 2009;58:453–459
32. Harmon KA, Gerard L, Jensen DR, et al. Continuous glucose profiles in obese and normal-weight pregnant women on a controlled diet: metabolic determinants of fetal growth. *Diabetes Care* 2011;34:2198–2204
33. Pacifico L, Cantisani V, Ricci P, et al. Nonalcoholic fatty liver disease and carotid atherosclerosis in children. *Pediatr Res* 2008;63:423–427
34. Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988–1994 to 2007–2010. *J Pediatr* 2013;162:496–500.e1
35. Alisi A, Manco M, Vania A, Nobili V. Pediatric nonalcoholic fatty liver disease in 2009. *J Pediatr* 2009;155:469–474
36. Thorn SR, Baquero KC, Newsom SA, et al. Early life exposure to maternal insulin resistance has persistent effects on hepatic NAFLD in juvenile nonhuman primates. *Diabetes* 2014;63:2702–2713
37. Brumbaugh DE, Friedman JE. Developmental origins of nonalcoholic fatty liver disease. *Pediatr Res* 2014;75:140–147
38. Modi N, Murgasova D, Ruager-Martin R, et al. The influence of maternal body mass index on infant adiposity and hepatic lipid content. *Pediatr Res* 2011;70:287–291
39. Gale C, Thomas EL, Jeffries S, et al. Adiposity and hepatic lipid in healthy full-term, breastfed, and formula-fed human infants: a prospective short-term longitudinal cohort study. *Am J Clin Nutr* 2014;99:1034–1040
40. Anderson EL, Howe LD, Fraser A, et al. Weight trajectories through infancy and childhood and risk of non-alcoholic fatty liver disease in adolescence: the ALSPAC study. *J Hepatol* 2014;61:626–632
41. Breij LM, Kerkhof GF, Hokken-Koelega AC. Accelerated infant weight gain and risk for non-alcoholic fatty liver disease in early adulthood. *J Clin Endocrinol Metab* 2014;99:1189–1195
42. Armstrong MJ, Adams LA, Canbay A, Syn WK. Extrahepatic complications of nonalcoholic fatty liver disease. *Hepatology* 2014;59:1174–1197
43. Berkowitz RI, Moore RH, Faith MS, Stallings VA, Kral TV, Stunkard AJ. Identification of an obese eating style in 4-year-old children born at high and low risk for obesity. *Obesity (Silver Spring)* 2010;18:505–512
44. Begg DP, Woods SC. Hedonic and homeostatic overlap following fat ingestion. *Cell Metab* 2013;18:459–460
45. Lozupone CA, Knight R. Species divergence and the measurement of microbial diversity. *FEMS Microbiol Rev* 2008;32:557–578
46. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012;150:470–480
47. Bearfield C, Davenport ES, Sivapathasundaram V, Allaker RP. Possible association between amniotic fluid micro-organism infection and microflora in the mouth. *BJOG* 2002;109:527–533
48. Jiménez E, Fernández L, Marín ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr Microbiol* 2005;51:270–274
49. Jiménez E, Marín ML, Martín R, et al. Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008;159:187–193
50. Stout MJ, Conlon B, Landeau M, et al. Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. *Am J Obstet Gynecol* 2013;208:226.e1–7
51. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med* 2014;6:237ra265
52. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. *Nature* 2012;489:231–241
53. Nauta AJ, Ben Amor K, Knol J, Garssen J, van der Beek EM. Relevance of pre- and postnatal nutrition to development and interplay between the microbiota and metabolic and immune systems. *Am J Clin Nutr* 2013;98:586S–593S
54. Ajslev TA, Andersen CS, Gamborg M, Sørensen TI, Jess T. Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *Int J Obes (Lond)* 2011;35:522–529
55. Trasande L, Blustein J, Liu M, Corwin E, Cox LM, Blaser MJ. Infant antibiotic exposures and early-life body mass. *Int J Obes (Lond)* 2013;37:16–23
56. Blustein J, Attina T, Liu M, et al. Association of caesarean delivery with child adiposity from age 6 weeks to 15 years. *Int J Obes (Lond)* 2013;37:900–906
57. Huh SY, Rifas-Shiman SL, Zera CA, et al. Delivery by caesarean section and risk of obesity in preschool age children: a prospective cohort study. *Arch Dis Child* 2012;97:610–616
58. Coates ME, Fuller R, Harrison GF, Lev M, Suffolk SF. A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. *Br J Nutr* 1963;17:141–150
59. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008;6:e280
60. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010;107:11971–11975
61. Ma J, Prince AL, Bader D, et al. High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model. *Nat Commun* 2014;5:3889
62. Heerwagen MJ, Stewart MS, de la Houssaye BA, Janssen RC, Friedman JE. Transgenic increase in n-3/n-6 fatty acid ratio reduces maternal obesity-associated inflammation and limits adverse developmental programming in mice. *PLoS One* 2013;8:e67791
63. Russell WR, Gratz SW, Duncan SH, et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr* 2011;93:1062–1072
64. Smith SC, Choy R, Johnson SK, Hall RS, Wildeboer-Veloo AC, Welling GW. Lupin kernel fiber consumption modifies fecal microbiota in healthy men as determined by rRNA gene fluorescent in situ hybridization. *Eur J Nutr* 2006;45:335–341
65. Finley JW, Burrell JB, Reeves PG. Pinto bean consumption changes SCFA profiles in fecal fermentations, bacterial populations of the lower bowel, and lipid profiles in blood of humans. *J Nutr* 2007;137:2391–2398
66. Korpela K, Flint HJ, Johnstone AM, et al. Gut microbiota signatures predict host and microbiota responses to dietary interventions in obese individuals. *PLoS One* 2014;9:e90702
67. Goodrich JK, Waters JL, Poole AC, et al. Human genetics shape the gut microbiome. *Cell* 2014;159:789–799
68. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* 2006;40:235–243
69. Al-Lahham S, Roelofsens H, Rezaee F, et al. Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *Eur J Clin Invest* 2012;42:357–364
70. Al-Lahham SH, Roelofsens H, Priebe M, et al. Regulation of adipokine production in human adipose tissue by propionic acid. *Eur J Clin Invest* 2010;40:401–407
71. Rumberger JM, Arch JR, Green A. Butyrate and other short-chain fatty acids increase the rate of lipolysis in 3T3-L1 adipocytes. *PeerJ* 2014;2:e611
72. Priyadarshini M, Thomas A, Reisetter AC, et al. Maternal short-chain fatty acids are associated with metabolic parameters in mothers and newborns. *Transl Res* 2014;164:153–157
73. Gamet L, Daviaud D, Denis-Pouxviel C, Remy C, Murat JC. Effects of short-chain fatty acids on growth and differentiation of the human colon-cancer cell line HT29. *Int J Cancer* 1992;52:286–289
74. Park J, Kim M, Kang SG, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* 2015;8:80–93
75. Gao Z, Yin J, Zhang J, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009;58:1509–1517
76. Kumar H, Lund R, Laiho A, et al. Gut microbiota as an epigenetic regulator: pilot study based on whole-genome methylation analysis. *MBio* 2014;5:e02113-14
77. Relton CL, Groom A, St Pourcain B, et al. DNA methylation patterns in cord blood DNA and body size in childhood. *PLoS One* 2012;7:e31821
78. Morales E, Groom A, Lawlor DA, Relton CL. DNA methylation signatures in cord blood associated with maternal gestational weight gain: results from the ALSPAC cohort. *BMC Res Notes* 2014;7:278

79. Godfrey KM, Sheppard A, Gluckman PD, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes* 2011;60:1528–1534
80. Teh AL, Pan H, Chen L, et al. The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes. *Genome Res* 2014;24:1064–1074
81. Ollikainen M, Smith KR, Joo EJ, et al. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Hum Mol Genet* 2010;19:4176–4188
82. Iaffaldano L, Nardelli C, Raia M, et al. High aminopeptidase N/CD13 levels characterize human amniotic mesenchymal stem cells and drive their increased adipogenic potential in obese women. *Stem Cells Dev* 2013;22:2287–2297
83. Crume TL, Shapiro AL, Brinton JT, et al. Maternal fuels and metabolic measures during pregnancy and neonatal body composition: the Healthy Start Study. *J Clin Endocrinol Metab* 2015;100:1672–1680
84. Hernandez TL, Van Pelt RE, Anderson MA, et al. A higher-complex carbohydrate diet in gestational diabetes mellitus achieves glucose targets and lowers postprandial lipids: a randomized crossover study. *Diabetes Care* 2014;37:1254–1262
85. Hernandez TL, Van Pelt RE, Anderson MA, et al. Women with gestational diabetes randomized to a higher complex carbohydrate/low fat diet manifest lower adipose tissue insulin resistance, inflammation, glucose, and free fatty acids. *Diabetes Care* 2014;37:1254–1262
86. Basu S, Haghiaci M, Surace P, et al. Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. *Obesity (Silver Spring)* 2011;19:476–482
87. Sachse D, Sletner L, Mørkrid K, et al. Metabolic changes in urine during and after pregnancy in a large, multiethnic population-based cohort study of gestational diabetes. *PLoS One* 2012;7:e52399
88. Kuc S, Koster MP, Pennings JL, et al. Metabolomics profiling for identification of novel potential markers in early prediction of preeclampsia. *PLoS One* 2014;9:e98540
89. Austdal M, Skråstad RB, Gundersen AS, Austgulen R, Iversen AC, Bathen TF. Metabolomic biomarkers in serum and urine in women with preeclampsia. *PLoS One* 2014;9:e91923
90. Johns DJ, Hartmann-Boyce J, Jebb SA, Aveyard P; Behavioural Weight Management Review Group. Diet or exercise interventions vs combined behavioral weight management programs: a systematic review and meta-analysis of direct comparisons. *J Acad Nutr Diet* 2014;114:1557–1568
91. Schellenberg ES, Dryden DM, Vandermeer B, Ha C, Korownyk C. Lifestyle interventions for patients with and at risk for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 2013;159:543–551
92. Tanvig M. Offspring body size and metabolic profile—effects of lifestyle intervention in obese pregnant women. *Dan Med J* 2014;61:B4893