Published in final edited form as:

Nat Rev Gastroenterol Hepatol. 2017 February ; 14(2): 81–96. doi:10.1038/nrgastro.2016.160.

Developmental origins of NAFLD: a womb with a clue

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Abstract

Changes in the maternal environment leading to an altered intrauterine milieu can result in subtle insults to the fetus, promoting increased lifetime disease risk and/or disease acceleration in childhood and later in life. Particularly worrisome is that the prevalence of NAFLD is rapidly increasing among children and adults, and is being diagnosed at increasingly younger ages, pointing towards an early-life origin. A wealth of evidence, in humans and non-human primates, suggests that maternal nutrition affects the placenta and fetal tissues, leading to persistent changes in hepatic metabolism, mitochondrial function, the intestinal microbiota, liver macrophage activation and susceptibility to NASH postnatally. Deleterious exposures *in utero* include fetal hypoxia, increased nutrient supply, inflammation and altered gut microbiota that might produce metabolic clues, including fatty acids, metabolites, endotoxins, bile acids and cytokines, which prime the infant liver for NAFLD in a persistent manner and increase susceptibility to NASH. Mechanistic links to early disease pathways might involve shifts in lipid metabolism, mitochondrial dysfunction, pioneering gut microorganisms, macrophage programming and epigenetic changes that alter the liver microenvironment, favouring liver injury. In this Review, we discuss how maternal, fetal, neonatal and infant exposures provide developmental clues and mechanisms to help explain NAFLD acceleration and increased disease prevalence. Mechanisms identified in clinical and preclinical models suggest important opportunities for prevention and intervention that could slow down the growing epidemic of NAFLD in the next generation.

NAFLD is a general term used to describe a broad spectrum of liver abnormalities, ranging from simple, uncomplicated hepatic steatosis to NASH, accompanied by different degrees of

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Author contributions

All authors researched data for the article, provided a substantial contribution to discussion of content, wrote the article, and reviewed and edited the manuscript before submission.

Competing interests statement

The authors declare no competing interests.

inflammation and fibrosis. NAFLD is increasing in prevalence, especially in adolescents¹, despite the levelling off of the obesity epidemic from 2003–2004 to 2013–2014 in children and adolescents aged 2–19 years (REF. 2). The most common chronic liver disease worldwide, NAFLD increases the risk of cardiovascular events eightfold and type 2 diabetes mellitus (T2DM) threefold³, and is a strong risk factor for hepatocellular carcinoma (HCC)⁴. At the time of paediatric NAFLD diagnosis, 25–50% of children have NASH and 10–25% have advanced fibrosis⁵. For many decades, it was thought that NAFLD was predominantly driven by obesity in the setting of genetic susceptibility. However, this association with obesity does not explain the variable severity of illness and its outcome⁶, suggesting that NAFLD is a multifactorial disease⁷.

Interest in how exposure to excess nutrition affects disease precursor pathways in fetuses, neonates and infants has intensified in the past decade, owing to recognition that pregnant women with obesity and/or gestational diabetes mellitus (GDM) might transmit this phenotype to their offspring, leading to a vicious cycle of obesity and diabetes over generations. The ‘Developmental Origins of Health and Disease’ paradigm posits that environmental exposures (*in utero* and during early life) can permanently change the body’s structure, physiology and metabolism, leading to increased disease risk and/or disease acceleration even after the exposure has occurred, including across generations^{8,9}. Evolutionarily, maternal transfer of excess nutrients might have contributed to increased survival chances for offspring; however, maternal obesity and diabetes mellitus are among the most powerful predictors of childhood obesity^{10,11} and other adverse health outcomes such as NAFLD, which affects up to 34% of children with obesity (5–19 years of age)¹². A particularly alarming trend is the prevalence of obesity in children, affecting 8% of infants and toddlers (birth to 2 years of age) and 17% of children and adolescents (aged 2–19 years) in the USA². With this high prevalence of obesity, NAFLD is predicted to become the most common aetiology for liver transplantation in the twentyfirst century¹³.

Compelling evidence suggests that the metabolic syndrome (which incorporates abdominal obesity, hypertension, hyperglycaemia and dyslipidaemia) originates, in part, from a suboptimal intrauterine environment^{9,14,15}. Data published in 2016 indicate that maternal obesity in humans promotes umbilical cord mesenchymal stem cells from newborn babies to preferentially differentiate into adipocytes rather than myocytes, correlating with an increase in neonate adiposity measured within 72 h of birth¹⁶. Furthermore, MRI and magnetic resonance spectroscopy in babies born to mothers with GDM who are obese showed a 70% increase in liver fat, with no statistically significant increase in subcutaneous or intraabdominal fat¹⁷, suggesting that fetal exposures during gestation (that is, obesity, GDM or raised levels of fatty acids) increase NAFLD risk in the human neonate. In an MRI study in neonates born to mothers who were obese without GDM, intrahepatic fat was highly correlated with maternal BMI, but not birth weight¹⁸, suggesting maternal obesity might be initiating events in the liver beyond increased adiposity in newborn babies.

As in adults, children with NAFLD can progress to cirrhosis and endstage liver disease¹⁹. Paediatric NASH (the more severe disease) has a multidimensional aetiology for which there is no approved therapy, often resulting in the need for liver transplantation. Potential approaches to treat established NAFLD include behavioural changes, such as altering diet

macronutrients and use of prebiotics and probiotics, and targeted approaches, including farnesoid Xactivated receptor (FXR; also known as bile acid receptor) and G proteincoupled bile acid receptor 1 (TGR5) agonists, endocannabinoid receptor family antagonists, betaine (which acts as a methyl donor for epigenetic modifications), antioxidants and more recently inhibition of Hedgehog signalling components (discussed later). Prevention or treatment in highrisk mothers and their offspring might be warranted, given the increasingly younger age of onset and lifetime consequences of NAFLD in children and teens. Consequently, there is an enormous public health need to understand the underlying dietary or metabolic factors in mothers with obesity or GDM that lead to early obesity and NAFLD risk in the next generation. In this Review, we will discuss known gestational risk factors and early postnatal mechanisms for predisposition to NAFLD (and subsequent NASH) in human infants and animal models, with an emphasis on developmental programming, nutrition, gut microbiota and changes in immunity affecting liver macrophages. We also present novel pathways that might be targets for future interventions.

Developmental programming of NAFLD

Evidence in humans

Human studies have shown that maternal BMI predicts infant intrahepatocellular lipid storage and hepatic fat doubles over the first 10–12 weeks of age, regardless of breastfeeding^{17,18,20}. *In utero* determinations of hepatic lipid content in human fetuses are limited by technical constraints; however, stillborn babies from mothers with GDM have an increased prevalence of histopathological hepatic steatosis²¹, supporting the development of prenatal steatosis. In addition to babies born to mothers with obesity or diabetes, neonates who are born with intrauterine growth restriction also have increased predisposition to develop NAFLD postnatally^{22,23}. Although mechanisms underlying this pathophysiology are poorly understood, studies of stem cells from babies born to mothers with obesity have provided insight. These cells have increased adipogenic capacity¹⁶, and changes in amino acid and fatty acid oxidation metabolites in lipidexposed, adipocytedifferentiated stem cells were predictive of rapid increases in adiposity from birth to 5 months of age²⁴. Notably, biomarkers of mitochondrial dysfunction are elevated in plasma at 48 h of life in neonates born to mothers who are obese²⁵.

Evidence in animal models

In rodents and nonhuman primates (NHPs), a maternal highfat diet, independent of maternal obesity, results in accumulation of triglycerides in fetal liver, hepatic oxidative stress and apoptosis, priming the liver for later development of NASH^{26–30}. These Japanese macaque fetuses from mothers on a chronic highfat diet, regardless of obesity in the mother, have increased levels of hepatic triglycerides and oxidative stress, detectable in the early third trimester²⁸. At 1 year of age, juvenile NHP offspring from this model have persistently increased liver triglycerides, increased expression of lipogenic genes in liver tissue and isolated hepatocytes, and increased activation of inflammatory gene expression in response to lipopolysaccharide (LPS) in hepatic macrophages²⁹. Importantly, this liver phenotype persists despite weaning to a healthy control diet, and is only present in offspring from mothers who developed obesity and insulin resistance on the highfat diet²⁹.

Multiple animal models demonstrate that continued exposure to a highfat diet during gestation and postnatal life, compared with only postnatal highfat diet feeding, rapidly worsens the liver phenotype to fibrosis^{31–33}. In mice, 3monthold offspring from mothers fed a highfat diet during pregnancy and lactation show increased hepatic steatosis and signs of NAFLD, which progresses to NASH when offspring are weaned onto a highfat diet^{32–34}. Lactation also has an independent contribution towards the programming of NAFLD, as offspring born to controldietfed dams yet nursed by obese dams on highfat diet had increased hepatic *Tnf* and *Il6* expression and levels of triglycerides³⁴. In addition to these models of maternal overnutrition, maternal undernutrition increases NAFLD predisposition in rodents with intrauterine growth restriction^{35,36} and postnatal sheep whose mothers were nutrient restricted³⁷. Collectively, these studies in humans, NHPs, rodents and sheep demonstrate that gestational undernutrition as well as overnutrition predisposes the offspring to NAFLD. Postnatal highfat diets, including during the weaning period, probably play an important part in disease acceleration, but more research is needed to understand how breastfeeding and early regulation of the immune system and metabolism, as well as genetic factors, accelerate disease phenotypes.

Sexspecific effects might also contribute to the programming of NAFLD. In humans, men have a higher rate of hepatic steatosis than women³⁸. However, sex differences in offspring of obese pregnancies in animal models are less clear, with some studies showing increased liver triglyceride content in male offspring³⁹, female offspring⁴⁰, or no differences between sexes²⁹.

Intrauterine clues for NAFLD risk

Maternal insulin resistance

Maternal insulin resistance naturally occurs in pregnancy with advancing gestation. However, in pregnancies complicated with diabetes, glucose delivery to the fetus increases, stimulating fetal insulin secretion. Increased fetal insulin concentrations in turn potentiate fetal growth (a concept commonly referred to as the Pedersen hypothesis) and metabolic pathways regulated by glucose and insulin⁴¹. Interestingly, fetal overgrowth and metabolic abnormalities are present even in mothers with diabetes who have good glycaemic control⁴². Furthermore, maternal obesity, independent of glycaemia, is highly correlated with fetal hyperinsulinaemia, growth and neonatal adiposity⁴³. These studies suggest that fuels other than glucose (that is, lipids) in mothers with obesity contribute to excess fetal growth and developmental programming.

Excessive maternal insulin resistance owing to obesity and/or poor diet is one important factor that might underlie *in utero* programming of fetal hepatic metabolic dysfunction and steatosis (FIG. 1). Drivers associated with maternal insulin resistance might include excess fuel supply, inflammation, hormones (for example adiponectin⁴⁴) and fetal hypoxia, as discussed later. In humans, increased maternal insulin resistance, independent of excessive gestational weight gain, mediates fetal adiposity⁴⁵, and increased maternal insulin resistance in mice results in progressive development of postnatal lipid deposition in the offspring⁴⁶. Although insulin produced by the mother does not cross the placenta, excessive insulin resistance during pregnancy might directly increase placental inflammation, altering nutrient

transfer to the fetus^{47,48}. In support of an early driving role for maternal insulin resistance, fetal hepatic steatosis was reduced when NHP mothers on a highfat diet were supplemented with resveratrol, which improved maternal insulin sensitivity and placental function⁴⁹.

The role of the placenta

The placenta might mediate the negative effects associated with maternal insulin resistance on the fetus and fetal liver. Placentas from humans with GDM and placentas from NHPs and rodent mothers with insulin resistance have increased hypoxia, oxidative stress, cytokine production and inflammation^{50–53}, exposing the fetus to factors inducing lipogenic, inflammatory and mitochondrial oxidative stress pathways in the developing liver. In NHPs, placentas from mothers who develop obesity on a highfat diet have increased cytokine expression (including CCL2 (also known as MCP1) and IL1 β), which could contribute to increased cytokine concentrations (including IL1 β , IL2, IL4, IL13, IL16, IL17, MCP1 and TNF) in umbilical blood⁵⁰. Furthermore, in isolated human trophoblasts, treatment with IL1 β inhibited insulin signalling via IRS1 and decreased amino acid transport (via system A), demonstrating insulin resistance⁵⁴.

Placental lipid delivery to the fetus—By the third trimester, most pregnant women have a blood lipid profile that would be considered highly atherogenic in the non pregnant state⁵⁵. This profile is characterized by a threefold increase in plasma triglyceride levels and a 25–50% increase in plasma total cholesterol concentration⁵⁶. In general, fetal cord and neonatal plasma lipid levels are much lower than those in adults^{57,58}. However, circulating triglyceride levels are higher in mothers with GDM or obesity⁴³ and are correlated with maternal insulin resistance⁵⁹. Higher free fatty acid concentrations are found in umbilical cord blood samples in pregnancies from mothers with GDM compared with mothers without GDM⁴². Evidence exists for increased lipid transport across the placenta in human pregnancies complicated by GDM, as isolated placental cells from pregnancies with GDM have increased expression of genes for fetoplacental lipid metabolism and preferential use of fatty acids as lipogenic substrates⁶⁰. Mice with obesity during pregnancy have increased placental expression of fatty acid transporter proteins⁶¹ and data published in 2016 have confirmed the transfer of maternally derived dietary lipids across the placenta to the fetus⁶². Furthermore, increased fatty acid levels signal the placenta, via Toll like receptor 4 (TLR4), to produce a pro inflammatory response and increase nutrient transport^{63–65}. Thus, increased concentration and transport of lipids derived from the mother might be the source of lipids accumulating in the fetal liver.

Placental dysfunction and fetal hypoxia—The severity of liver damage in obesity related NAFLD in children and adults has been associated with concomitant hypoxia and inflammation resulting from sleep apnoea⁶⁶. Interestingly, fetal hypoxia owing to reduced utero placental blood flow and umbilical venous oxygenation is a common pregnancy complication in mothers with obesity or GDM. These fetuses also have increased plasma glucose and lactate concentrations, demonstrating metabolic adaptations in response to hypoxia⁶⁷. Similar reductions in placental blood flow, placental vasculature, fetal oxygenation and neonatal survival have been found in NHP⁵⁰ and rat⁶⁸ models of maternal obesity.

Although the mechanisms for decreased placental function as a result of maternal obesity or GDM are not clear, term placentas from human pregnancies complicated by GDM have increased villus volume and villous immaturity, suggesting decreased transfer capacity^{67,69}. Trophoblast cells from GDM placentas also have decreased serotonin transport as a consequence of defective insulin signalling, which might be a mechanism contributing to decreased uteroplacental perfusion and blood flow⁷⁰. Further, in experimental animal models, *in utero* exposure to hypoxia and nutrient insufficiency increases susceptibility to hepatic steatosis, mitochondrial dysfunction, oxidative stress and NAFLD postnatally^{35,71–75}. These data strongly suggest that fetal hypoxia associated with placental dysfunction is an early clue that the heightened risk of NAFLD can begin *in utero*.

Fetal hepatic lipid metabolism

In the normal fetus, regardless of species, expression of genes for hepatic *de novo* lipogenesis and lipid oxidation is suppressed compared with the postnatal period, as the fetus (and fetal liver) relies primarily on carbohydrates (glucose and lactate) and amino acids for oxidative metabolism, before switching postnatally to lipid based fuels during lactation⁷⁶. However, fetuses of mothers with GDM or obesity have elevated blood glucose and insulin concentrations⁶⁷, which might induce *de novo* lipogenesis. Indeed, fetal and postnatal offspring from obese NHP mothers have upregulated hepatic fatty acid synthase (*FASN*) and sterol regulatory element binding protein 1 (*SREBP1*) mRNA expression^{29,77}, and hepatocytes isolated from juvenile offspring in this model have increased lipogenic capacity when stimulated *in vitro*²⁹. Thus, amplified fetal hepatic lipid accumulation might result from expanded *de novo* synthesis of lipids or increased delivery of maternal lipids across the placenta. Importantly, in the absence of mature hepatic lipid oxidation pathways⁷⁶ or adipose tissue depots⁷⁸ in the fetus before the third trimester, increased intrahepatic lipids probably remain and accumulate in the liver.

Alternatively, fetal hepatic lipid accretion might result from a decrease, or a failed adaptive increase, in lipid oxidation. In humans, progression from NAFLD to NASH is first associated with an adaptive increase in hepatic lipid oxidation^{79,80}. Over time, accelerated lipid oxidation is not sufficient to mitigate steatosis, which worsens, and NASH follows as oxidative stress and decreased mitochondrial oxidation develop⁸¹. In NHPs exposed to a highfat diet, cellular apoptosis is enhanced in the liver³⁰. Hepatocyte apoptosis is now thought to have an important role in the progression to steatohepatitis in patients with NAFLD⁸². Further, an immature antioxidant defence system in the fetal liver compared with adults, combined with increased hepatic lipid load, might also contribute to elevated mitochondrial stress and apoptosis⁸³.

Programmed mitochondrial metabolism

In addition to increased levels of hepatic triglycerides and apoptosis, the livers of mouse offspring from obese, highfat dietfed mothers have decreased electron transport chain activity and increased expression of oxidative stress, fibrosis and inflammation genes^{32,33}, all signs of 'lipotoxicity'. Owing to increased lipid load and lack of an adaptive increase in mitochondrial oxidation, excess fatty acids in the fetus might not be completely oxidized, creating a backup of incomplete acylcoenzyme A species that can impair cell metabolism

via increased cell stress, increased inflammation and decreased mitochondrial function and gene expression^{84,85}. In this context, excess free fatty acids combined with reduced mitochondrial metabolism could limit mitochondrial biogenesis. In fetal skeletal muscle from NHP mothers fed a highfat diet, we demonstrated reduced maximal mitochondrial respiratory capacity and increased oxidative stress *in vitro* and *in vivo* despite an upregulation in activity of electron transport chain components, suggesting these mitochondria are not capable of adapting to excess fuels⁸⁶. Skeletal muscle mitochondria numbers in NHP fetuses are reduced⁸⁶, and liver mitochondria numbers are suppressed in rat livers at weaning⁸⁷ and in mouse livers from adult offspring of mothers fed a highfat diet during pregnancy³². Mitochondrial DNA copy number was also reduced in liver samples from rat offspring of mothers fed a highfat diet and *Ppargc1a* mRNA, a regulator of mitochondrial biogenesis, was decreased only in male offspring⁸⁸. Interestingly, increased *PPARGC1A* promoter methylation and decreased mRNA expression of the gene was associated with decreased mitochondrial DNA content and increased insulin resistance in patients with NAFLD⁸⁹. A major unanswered question is how fuel overload and/or hypoxia mechanistically programmes mitochondrial dysfunction, and whether mismatch between nuclear and mitochondrial genes has a role in mitochondrial damage and reduces the ability to oxidize fuels postnatally. Although correlations exist between excess circulating maternal lipid concentrations, mitochondrial dysfunction and obesity in the offspring, there are no data in human infants, nor are early mechanisms for programming mitochondria before obesity development fully understood.

Epigenetic mechanisms programming NAFLD

Epigenetic mechanisms, including DNA methylation and histone modifications, also contribute to the programming of NAFLD and have been reviewed elsewhere^{90,91}. However, the gene targets and causative epigenetic modifications leading to steatosis and fibrosis are not fully understood. Several studies have used largescale systems biology approaches⁹¹ or gene specific approaches to identify epigenetic modifications that correlate with liver triglyceride accumulation^{39,40,77,89,92}. In fetal liver from highfat dietfed NHP mothers, we observed decreased PGC1 α acetylation²⁸, which suggests increased PGC1 α activity and expression of downstream genes for mitochondrial oxidative stress or control of fatty acid oxidation. In addition, histone H3 Lys14 acetylation (H3K14ac) is increased in fetal liver from highfat-dietfed mothers, but this change is reversed when mothers are switched to a healthy diet⁷⁷; however, the genes regulated by this modification remain unknown. In human pregnancies, increased *PPARGC1A* promoter methylation in umbilical cord samples taken at birth correlated with increased maternal BMI⁹³, suggesting a potential programming mechanism for mitochondrial metabolism. Thus, the programming effects and potential epigenetic effects of maternal obesity on offspring mitochondrial function and metabolic control might not be organ specific, as they are evident in early embryogenesis and even in oocytes before fertilization^{94,95}.

Early postnatal factors

Contribution of the gut microbiota

A possible pathway linking maternal obesity and diabetes with offspring NAFLD susceptibility is through the effects of maternal metabolic status on early microbial colonization of the offspring's gastrointestinal tract. Indeed, animal studies have demonstrated direct roles for the gut microbiota in each of the liver lesions that are observed in NAFLD, including steatosis⁹⁶, NASH⁹⁷ and fibrosis⁹⁸. However, mechanistic evidence showing early changes in liver or gut functions that precede disease manifestation is needed to understand causation (FIG. 2). Emerging evidence suggests that the severity of NAFLD postnatally is strongly associated with gut dysbiosis⁹⁹ and a shift in gut metabolic function, including production and utilization of shortchain fatty acids (SCFAs) and bile acids^{100–103}. The functions of SCFAs and bile acids in NAFLD have been reviewed elsewhere¹⁰⁴.

Extensive research has shown that diet modulates the composition and function of gut microorganisms in humans and other mammals^{105–109}. However, despite the rapid turnover of gut microbes in response to acute dietary changes, longterm dietary habits play a large part in determining gut microbiota composition^{110,111}. In the NHP model, a maternal highfat diet caused persistent (beyond 1 year) intestinal dysbiosis that remained in the offspring even after offspring were weaned on to a healthy diet¹¹². Data published in 2016 showed that highfat diet intake during the third trimester in human mothers was associated with a variation in the neonatal gut microbiota that persisted through 4–6 weeks of age, suggesting that maternal diet can structure the infant gut microbiota¹¹³. In addition to gut microbiota changes resulting from a maternal highfat diet, there are lasting effects on liver inflammation and steatosis in the NHP model²⁹. Mice born to Westernstyle diet fed breeders had immune dysregulation and increased disease susceptibility as well as a heightened colonic inflammatory response, including increased liver LPS levels¹¹⁴. Taken together, these observations suggest that maternal diet driven changes in the offspring gut microbiota can have persistent effects on the host immune system that increase risk for inflammatory diseases in later life.

Alteration of the infant gut microbiota

Although the maternal gut microbiota differs between women who are overweight and those of a normal weight^{115,116}, and differences in the gut microbiota exist in 6 month old infants who later became overweight at 7 years of age compared with infants who did not later become overweight¹¹⁷, whether the gut microbiota in early life establishes obesity remains to be determined. In addition to maternal obesity and diet, the colonization of the infant gut is also powerfully affected by mode of delivery (Caesarean section versus vaginal delivery), antibiotic use and breastfeeding exposure¹¹⁸. An increasing body of epidemiological and animal studies links changes in the infant gut microbiota to allergy, autoimmune disease and obesity risk later in life^{118–120}, suggesting that the early neonatal immune system is programmed; however, pathways between specific microbes and their functions remain elusive. The earliest colonizing species are recognized as 'pioneering microbes' that educate the developing immune system from a state of hyperstimulation to one of immune tolerance. Both *Bacteroides* and *Lactobacillus*, abundances of which are reduced by maternal obesity,

are capable of inhibiting the NF κ B pathway, which could affect inflammatory states in the mother and her offspring, particularly if the offspring inherits this altered microbiota¹²¹. Maternal gut dysbiosis and its accompanying systemic inflammation, if transmitted to the newborn baby, can be reasonably hypothesized to alter the establishment of the nascent infant immune system.

Breastfeeding signals and effects of obesity

Maternal breast milk promotes the colonization and development of the intestinal microbiota in the infant. Breast milk bacterial composition varies by lactation stage¹²², gestational age¹²², maternal health status¹²³ and mode of delivery^{122,123}. Human breast milk contains a lowbiomass microbiota that has been previously hypothesized to be partially derived from the maternal gut^{124,125}. The most abundant genera in the breastmilk microbiota are *Streptococcus*, *Staphylococcus*, *Serratia* and *Corynebacterium*¹²⁵. *Bifidobacterium* and *Lactobacillus* spp. are also found in breast milk and are transferred to the neonatal gut^{126,127}. In addition to bacteria, breast milk contains high levels (the third largest component of human milk) of human milk oligosaccharides, comprising complex sugars with substantial prebiotic effects that serve as a source of energy for desired microbial growth^{128,129}. Thus, breastfeeding provides prebiotics, in the form of oligosaccharides, and probiotics, in the form of live bacteria that contribute to the colonization of bacteria in the infant gut. Formulafed infants are not exposed to the same nutrients that breastfed infants are and thus develop a different microbiota¹³⁰. The breakdown products of human milk oligosaccharides support the growth of the commensal microbiota and promote antiinflammatory effects, and represent a crucial window of opportunity for shaping the infant gastrointestinal and immune systems as well as the adult gut microbiota¹³¹.

In newborn babies and in mice, the immune system gains tolerance to microorganisms based on the timing of colonization. Gammaproteobacteria are a major source of LPS and are considered transient pioneering bacteria important for developing endotoxin tolerance and protection against excessive inflammatory and autoimmune gastrointestinal disorders later in life^{132–134}. A statistically significant 50% reduction in Gammaproteobacteria abundance was found in stool of 2weekold babies born to mothers with obesity, along with increased levels of acetate¹³⁵, which promotes hepatic *de novo* lipogenesis and NAFLD. We have transferred gut microbes from infants born to mothers who are obese into germfree mice; these mice showed signs of elevated cytokines in portal blood and liver endoplasmic reticulum stress (increased gene expression of spliced *Xbp1*) when compared with mice colonized with stool from babies born to mothers of a normal weight (J. E. Friedman, unpublished work). Chronic endoplasmic reticulum stress is associated with hepatic steatosis, insulin resistance, oxidative stress and hepatocyte cell death^{136,137}, and is present in the livers of patients with NAFLD or NASH^{138,139}. Although more work needs to be done, these data suggest that alterations in the neonatal microbiota born to mothers with obesity might produce an early proinflammatory response, resulting in altered liver gene expression and, potentially, persistent immunological changes in the infant. Longitudinally detailed analyses at the strain level are necessary to understand these effects on metabolic reprogramming of the immune system and susceptibility to liver disease in later life.

Bile acids as early programming events for NAFLD

The gut microbiota is essential for the metabolism of primary bile acids into secondary bile acids, yet only in the past few years has this process been considered in NAFLD pathogenesis. In contrast to the toxic effects of excess accumulation of bile acids on hepatocytes, certain hydrophilic, less toxic bile acids such as ursodeoxycholic acid have antiinflammatory effects that might be relevant for NAFLD¹⁴⁰. Bile acids function as signalling molecules through a variety of receptors to regulate their own synthesis as well as other metabolic processes, such as glucose, lipid and energy homeostasis, as reviewed elsewhere¹⁴⁰. In the case of NAFLD and NASH, bile acid-related pathways might be involved given that the microbiota, through defined enzymatic activities (including deconjugation, dehydroxylation and oxidation, among others), is a critical modulator of bile acid pool size and composition and can substantially modify the chemical and signalling properties of bile acids^{100,102,141}. On the other hand, bile acids also shape the intestinal microbiota through direct antimicrobial effects¹⁴². Further, the action of bile acids via the hepatocyte FXR and Kupffer cell TGR5 receptors have been demonstrated to inhibit lipid accumulation and inflammation, respectively^{143–145}. The transplantation of dysbiotic mouse microbiota into germfree mice resulted in increased hepatic fat accumulation through SREBP and CREB, regulators of *de novo* lipogenesis, and elevated hepatic inflammation, such as NFκB activity; these pathways are known to be inhibited by FXR and TGR5 activation^{146–148}. A key question is whether the mechanism by which the gut microbiota influences liver fat includes modulation of bile acid metabolism¹⁴⁹. Compelling human and animal data suggests that *in utero* exposure to excess bile acids during pregnancy results in reprogramming of metabolism and increased risk of the metabolic syndrome in later life¹⁵⁰. Gut microbes can also affect bile acid pool composition¹⁵¹, by conjugating and deconjugating bile acids. Thus, physiological bile acid pool compositions might have homeostatic antiinflammatory effects, altered in the setting of a dysbiotic intestine, and might exert effects that promote macrophage activation^{147,152,153} (FIG. 3).

Several important questions are raised pertaining to both the fundamental biological significance and potential clinical relevance of these results. Are there changes in early infant intestinal microbes that result in metabolic programming across the immune system? Given that the early infant microbiota is unstable and matures over the first 1–2 years of life^{154,155}, would longer breastfeeding (encouraged in all infants) promote a healthier liver and changes in bile acid composition? Work to date has mostly focused on bacterial taxonomic composition of these communities rather than on the functional features they express. Clearly, well-controlled longitudinal studies are critically important to understanding how the early changes in the gut microbiota seed the development of NAFLD in the infant of a mother with obesity.

Hepatic macrophages and NAFLD

Concept of the gut–liver axis

Intestinal permeability is increased in children with NAFLD and correlates with more severe liver disease¹⁵⁶. In newborn babies, permeability of the gut during neonatal life is also increased¹⁵⁷, allowing uptake of nutrients and development of systemic immune tolerance.

However, a shift in microbial colonization, in addition to altering inflammatory stimuli, can expose the neonate liver to harmful microbial products and metabolites that interact with the innate and adaptive immune systems^{158,159}. Owing to its direct anatomical connection to the gut via the portal vein, the liver is exposed to the entire gamut of gutmicrobederived factors (for example endotoxins), gutderived mediators such as cytokines (including IL1 β and TNF) and hormones (fibroblast growth factors), primary and secondary bile acids, and metabolites (derived from both host and microbiota, for example citrulline, SCFAs, bile acids, succinate and lactate) (FIG. 3). Liver macrophages are the first line of defence and have a homeostatic role¹⁶⁰, protecting the host^{161–164} and critically contributing to initiation¹⁶⁵ and resolution¹⁶⁵ of liver injury from pathophysiological alterations in the gut^{165–172}. The intricate functional relationships between the liver, its macrophages and the gut have been conceptualized as the gut–liver axis^{173–175}.

The importance of the gut–liver axis in liver pathology has been substantiated by a host of studies implicating gutderived factors (for example bacterialderived endotoxins) as mediators of the pathogenesis of liver injury, including NASH^{100,176–183}. Gutderived factors are thought to enter the portal vein in increased concentrations because of increased gut permeability, secondary to dysbiosis. Endotoxinactivated liver macrophages then produce proinflammatory cytokines that promote liver inflammation (including IL1 β , TNF and IL6)¹⁸¹. A role for the gut–liver axis is not unique to the pathogenesis of NAFLD or NASH; it also has been demonstrated to contribute to the pathogenesis of cholestatic liver disease associated with total parenteral nutrition¹⁸¹. In addition, various mouse models of liver fibrosis have demonstrated the involvement of increased gut permeability, absorption of gutderived endotoxins and hepatic macrophage activation in its pathogenesis^{176–178,184–186}.

In addition to governing endotoxin tolerance in neonates, studies have identified that increased concentrations of gutderived LPS are necessary for activating macrophages in mouse models of cholestasis, induced by parenteral nutrition with intestinal injury or fibrosis secondary to bileduct ligation^{180,181}. Abrogation of TLR4 signalling or suppression of intestinal microbiota with antibiotics was protective against both macrophage activation and liver injury in these models^{180,181}. Similarly, there is evidence that the pathogenesis of NAFLD and NASH involves activation of hepatic macrophages¹⁸⁷ that might begin with *in utero* exposures to lipid overload or fetal hypoxia as one critical pathway, and potentially by gutderived or metabolic factors other than fat, such as succinate¹⁵⁹ or SCFAs¹⁸⁸, as another critical pathway (FIG. 3). However, considering that alterations in the gut–liver axis alone might be necessary but not sufficient to promote NASH, it will be critical to identify extraintestinal factors and pathways that advance the disease.

Macrophages and tissue homeostasis

How direct interactions between macrophages and local cells, such as stellate cells and fibroblasts, control the pathogenesis of NASH, or how they maintain tissue homeostasis, is an active area of investigation¹⁸⁹. Furthermore, the relative contributions of transcription factor expression, macrophage ontogeny and the hepatic microenvironment to macrophage programming and function in the regulation of liver homeostasis and in the pathogenesis of NAFLD and/or NASH remain to be determined. To minimize damage to inappropriate

targets and preserve tissuespecific functions, the timing and magnitude of macrophage responses must be coordinated¹⁶⁰. Macrophages have also been recognized as critical in promoting resolution when the injury subsides or has been cleared¹⁶⁵. Crosstalk between macrophages and other tissue cells regulates immune responses, from initial inflammatory activation to resolution and finally tissue repair^{160,161,190}. Major unanswered questions include which mechanisms operate in liver macrophages to maintain homeostasis in the face of high microenvironment variability, and which mechanisms antagonize and overturn tissue homeostasis such that liver macrophages become activated or programmed to promote nonresolving and profibrotic liver injury^{191,192}.

Metabolic reprogramming of macrophages

Macrophage programming involves increased expression of arginase 1, downstream of hypoxia-inducible factor 1 (HIF1), and metabolic reprogramming to favour aerobic glycolysis in sterile chronic inflammatory conditions^{190,193}. Mechanistic studies have demonstrated that macrophages can retain immunologic memory (referred to as trained immunity), a phenomenon that involves HIF1-dependent metabolic pathways and a shift to aerobic glycolysis (a prerequisite for macrophage proinflammatory activation) and epigenetic reprogramming^{194–196}. These changes lead to the promotion of transcriptional programmes that alter intracellular signalling and metabolic pathways (for example, increased glycolysis and reprogramming of the tricarboxylic acid cycle)^{190,194,197}. Some of the earliest evidence that macrophages might exhibit memorylike features came from investigations of LPS-induced tolerance. In one such study, genespecific chromatin modifications were associated with silencing of genes coding for inflammatory molecules in response to LPS stimulation, and the priming of other genes coding for antimicrobial molecules¹⁹⁸. These findings suggested that macrophage polarization and activation in response to subsequent activation signals could be primed by LPS^{190,199}. Given the early gut permeability engendered by dysbiotic infant gut microbiota, it is tempting to speculate that certain bacteria and their outputs might trigger programming events in liver macrophages and in progenitor cells in the bone marrow as well. Indeed, evidence has emerged to indicate that innate immune memory can be transferred via haematopoietic stem and progenitor cells that alter the function of their differentiated progeny²⁰⁰, depending upon the composition of the gut microbiota²⁰¹. Furthermore, *in utero* exposures in mothers with obesity, such as fetal hypoxia (FIG. 1), might initiate macrophage epigenetic reprogramming. Whether liver macrophage responses have the capacity to be primed or reprogrammed by the microbiota in babies born to mothers with obesity, or whether the baseline microbes have a role in the future health of the offspring by exerting a newly described type of immunological memory upon further insult, remains to be determined.

Resident and recruited macrophages

Under inflammatory conditions, bonemarrow-derived monocytes are recruited to the liver, where they differentiate into macrophages and are critical for initiation and resolution of liver injury and inflammation^{165,166}. Recruited macrophages are potentially functionally distinct from resident macrophages and probably display different phenotypes in early (proinflammatory) versus late (proresolving) stages of inflammation¹⁶⁵. A limitation to most studies of macrophage immunometabolism is that resident and recruited macrophages are

assessed as a uniform population rather than separately. Accordingly, functional dichotomy in resident versus recruited macrophages at different stages of inflammation might be directly linked to programming of cellular metabolism^{190,202,203}. Resolving the role of tissue macrophage ontogeny, in terms of the relative contributions of yolk sac derived, fetal liver derived and bone marrow derived macrophages to the pool of tissue resident and recruited macrophages, might contribute to better understanding of the mechanisms that control tissue injury in NASH^{204–206}. Additionally, it remains to be determined if and how longlasting functional changes (trained immunity, innate immune memory and epigenetic changes) in recruited and/or resident macrophage populations occur, and if and how these cells have a role in promoting nonresolving chronic inflammation. A study published in 2016 has shown that activation of TLR4 by microenvironmental cues stimulates divergent signalling pathways in macrophages²⁰⁷, suggesting that crosstalk with microorganisms might be functionally relevant for controlling matrix formation and tissue injury.

Macrophage reprogramming before obesity

In addition to being exposed to maternal–placental factors, the prenatal and perinatal liver microenvironment receives signals from the gut microbiota that can affect hepatic macrophage metabolic reprogramming. Dysbiosis has been shown to compromise the intestinal barrier, setting the stage for a pathophysiological gut–liver axis and for programming and activation of liver macrophages towards a profibrotic phenotype^{181,183}. Gut microbiota dysbiosis can increase concentrations of proinflammatory cytokines, metabolites (such as succinate and lactate), bile acids and levels of endotoxins in the portal vein, all of which might affect hepatic macrophage activation^{152,181,208,209}. These alterations could promote pathways that induce lasting changes in the epigenetic landscape of the macrophage, priming a proinflammatory and profibrotic phenotype upon exposure to a second insult, such as a Westernstyle diet (FIG. 3). This profibrotic macrophage phenotype in chronically inflamed and fibrotic tissue can occur in the relative absence of microbes and can be considered a sterile inflammatory pathology¹⁹³.

Considering that the fetal liver has a prominent role as a haematopoietic organ and in fetal macrophage development, and considering the possibility that macrophages in various tissues could be fetal liver derived, prenatal alterations in the liver microenvironment might not only affect programming of liver resident macrophages but also macrophages in other tissues, including adipose tissue and bone marrow^{204,205}. Unlike adult tissues, fetal tissues have a high capacity to heal without scarring or fibrosis²¹⁰. Whether exposure of liver macrophages prenatally to maternal derived factors or perinatally to infant gut derived factors interferes with physiological programming of the tissue such that it aberrantly assumes an adult phenotype and promotes scarring and fibrosis is unclear. Given the severity and rapid progression of paediatric liver disease upon diagnosis⁵, future studies are required to identify the early microenvironmental features that programme liver macrophages, activate specific phenotypes conducive to chronic inflammation, or prevent resolution and promote continuous activation of proremodelling and/or profibrotic pathways that contribute to progression of fatty liver disease.

Targeting the gut–liver axis

Modulating maternal macronutrients

Intervention strategies designed to ameliorate the developmental programming of NAFLD are primarily preclinical; however, clinical interventions have targeted childhood obesity by modulating macronutrients or using drugs or supplements (TABLE 1). Few effective dietary interventions have been identified, which is concerning as early intervention has been shown to have a crucial effect on laterlife health and wellbeing of offspring²¹¹. Studies using the antidiabetic drug metformin, provided to mothers with obesity throughout pregnancy, did not find an effect on neonate birth weights^{212–214}. At 2 years of age, offspring of mothers with GDM exposed *in utero* to metformin had increased subcutaneous fat as compared with insulinexposed counterparts, although overall body fat was the same²¹⁵. Longerterm followups will be required to determine whether children receiving metformin treatment *in utero* develop less visceral fat and, potentially, increased insulin sensitivity. Previous evidence suggests that dietbased interventions are effective in achieving a reduction in gestational weight gain in pregnant women with GDM who are overweight or obese, but result in no substantial effect on birth weight, as reviewed elsewhere^{216,217}. However, a promising approach in women with GDM is the use of a diet higher in complex carbohydrates and lower in fats; a study in pregnant women with GDM showed improved maternal and infant outcomes after only 7 weeks of treatment^{218,219}. Thus, it is likely that a combination of a healthier diet and nutritional or pharmacological supplements will be required to achieve benefits in mother and offspring, taking the GDM status of the mother into account.

Altering the gut microbiota

As reviewed elsewhere by Patterson *et al.*²²⁰, diet is a key modulator of the gut microbiota. NAFLD incidence and disease severity have been linked to gut dysbiosis in a wellcharacterized population of adults with NAFLD, with *Bacteroidetes* abundance independently associated with progression to NASH and *Ruminococcus* linked to fibrotic lesions⁹⁸. Comparison between humans who are lean or obese shows that bacterial species diversity is reduced in obesity^{221,222}; mucindegarding bacteria *Akkermansia muciniphila* and antiinflammatory *Faecalibacterium prausnitzii* are emerging as potential key therapeutic targets²²³, although the mechanisms governing their role in inflammationdriven metabolic disease are yet to be determined. Probiotics and prebiotics have been investigated in both clinical (TABLE 1) and preclinical models; however, as we have reviewed elsewhere¹¹⁸, most probiotic trials administered during pregnancy have had limited success in improving maternal and neonatal outcomes. The probiotic *Lactobacillus rhamnosus* GG, administered in conjunction with *Bifidobacterium lactis*, has been most commonly studied in pregnancy. Shortterm prenatal treatment in women without obesity seemed to affect development of the fetal innate immune system²²⁴, whereas longer perinatal treatment did not affect infant birth weight²²⁵. Interestingly, the probiotic intervention reduced weight gain in the offspring during the first 24 months of life; however, by 4 years of age the intervention was not protective²²⁶, suggesting therapeutic interventions might need to be implemented in multiple time windows throughout development to blunt NAFLD pathogenesis.

Few studies have investigated efficacy of probiotics in paediatric patients. Nonetheless, targets for probiotic therapies in adults have been identified. For example, butyrate-producing bacteria such as *Roseburia intestinalis*, *Eubacterium hallii* and *Faecalibacterium* spp. have been implicated in reducing insulin resistance in humans²²⁷. In a preclinical model of NAFLD in rats, periodic treatment beginning postnatally with a probiotic mixture including *Bifidobacterium*, *Lactobacillus*, *Lactococcus* and *Propionibacterium* was protective against weight gain, improved insulin sensitivity and prevented development of NAFLD in adult animals²²⁸, suggesting these genera include candidates for future prenatal testing. Pyrroloquinoline quinone (PQQ), a bacterial cofactor important in reproductive health, might have probiotic properties. Engineered PQQ-secreting *E. coli* str. Nissle protected against hepatic hyperlipidaemia in a rat model of ethanol-induced liver damage²²⁹, supporting PQQ as a potential novel strategy to reduce risk of NAFLD development. The key to optimizing probiotic or prebiotic approaches are functional maps of both healthy and obese gut microbiota, particularly for infants, which are only just being investigated²³⁰.

Targeting bile acids

Bile acids and their receptors have emerged as potent therapeutic targets in NAFLD. The safety and efficacy of obeticholic acid, a synthetic bile acid analogue and FXR agonist, has been investigated in several adult human studies for treatment of both NAFLD²³¹ and NASH²³² with promising yet mixed results, and clinical trials in adults with other FXR agonists are currently underway²³³. INT767, a powerful dual FXR and TGR5 agonist, exerted an anti-inflammatory response and improved NAFLD in an obese *db/db* mouse model¹⁵²; the drug has now entered phase I clinical studies in healthy human volunteers. Evidence from a human study comparing the post prandial bile acid profiles of patients with NASH and healthy individuals suggests that bile acid hydrophobicity is implicated in progression of hepatic inflammatory disease²³⁴ and bacterial processing of bile acids might be a potential avenue for therapeutic intervention. Additionally, bile acids are cosecreted with phosphatidylcholine, and the choline supply is critically important for both prenatal and postnatal development²³⁵. Supplementing sows during gestation with betaine, a choline derivative, suppressed expression of lipogenic genes in neonatal piglet livers²³⁶. Taken together, modifying the abundance, composition, processing and secretion of bile acids has shown efficacy in ameliorating hepatic fat accretion, at least in preclinical models, and these pathways represent promising candidates for preclinical testing.

Targeting Hedgehog signalling

An emerging understanding of the role of the Hedgehog signalling pathway in the pathogenesis of NAFLD and NASH has led to preclinical and clinical trials of pathway mediators. Hedgehog signalling is inhibited in healthy liver but activated in disease; ballooned hepatocytes produce the Hedgehog ligand sonic hedgehog, which stimulates hepatic stellate cells and fibroblasts, initiating a profibrotic response²³⁷. Inhibiting or eliminating smoothened, a G protein-coupled receptor mediating signalling in the presence of sonic hedgehog ligand²³⁸, resulted in reduced macrophage activation and expression of proinflammatory genes in a mouse model²³⁹. Analysis of liver biopsies from patients with NASH enrolled in the PIVENS trial showed that response to treatment (vitamin E) was inversely associated with levels of sonic hedgehog²⁴⁰. Prenatal supplementation with

vitamin E has not been studied in the context of NAFLD; however, it has not been associated with positive outcomes in other inflammatory models such as allergy^{241,242}. Other mediators of Hedgehog signalling, including natural products such as isoflavones, might have more therapeutic promise, as reviewed elsewhere²⁴³.

Alternative approaches

Antioxidants and bioactive lipids have been proposed as potential treatments for NAFLD and NASH but many have not yet been studied in human pregnancy. Perinatal and postnatal human studies have investigated the effect of treatment with fish oil and ω 3 polyunsaturated fatty acids on various aspects of offspring health and development. Fish oil and fatty acid supplements during perinatal or postnatal periods have not been effective at reducing offspring BMI or fat mass (TABLE 1), although fish and cod liver oil consumption during pregnancy was correlated with higher maternal and offspring docosahexanoic acid levels and increased placental weight²⁴⁴. Treatment with fish and cod liver oil during the first year of life reduced subsequent risk of type 1 diabetes mellitus²⁴⁵ and eczema²⁴⁶, presumably via the antiinflammatory action of docosahexanoic acid and similar longchain ω 3 fatty acids. Our laboratory has used a *Fat1* transgenic mouse model (which can endogenously convert ω 6 fatty acids to ω 3 fatty acids) to test the effect of increasing the maternal ω 3 to ω 6 tissue fatty acid ratio during highfat diet consumption. Wildtype offspring of *Fat1* dams had decreased hepatic lipid accumulation and were protected from highfatdietinduced inflammation²⁷. Thus, increasing the maternal ω 3 to ω 6 fatty acid ratio and reducing placental inflammation might be a promising target for improving outcomes in the fetus of a mother with obesity consuming a highfat diet.

Other antioxidants have shown efficacy for reducing steatosis in adult human or animal studies and might be potential pathways to target in pregnancy models. Silibinin (also known as silybin) is a potent antioxidant extracted from milk thistle. When coformulated with phosphatidylcholine and vitamin E, treatment of adult patients with NAFLD with silybin improved insulin resistance, liver histology²⁴⁷ and indices of hepatic and metabolic function²⁴⁸. In support of antioxidant therapy, restoration of antioxidant balance with vitamin A, E, and C and selenium supplementation during Westernstyle diet feeding in pregnant rats was associated with decreased inflammation and oxidative stress in the preimplantation embryo, fetus and neonate²⁴⁹.

Shortterm resveratrol trials in adult humans with NAFLD have resulted in improved measures of hepatic insulin sensitivity and reduction of inflammatory mediators, although fat accretion was not altered²⁵⁰. A study published in 2016 by Vega *et al.*²⁵¹ demonstrated reductions in body fat and serum triglyceride levels in adult offspring of proteinrestricted dams treated with resveratrol, although hepatic triglyceride concentration was not measured. Our group has previously shown a decrease in fetal hepatic triglyceride levels following maternal supplementation with resveratrol in obese NHPs⁴⁹. Whether the beneficial effects of resveratrol on the fetal liver are direct, or indirect via improvements in the maternal–placental–fetal axis, remains to be determined (FIG. 1)^{49,252,253}.

Resveratrol has also been demonstrated to act in the brain through the endocannabinoid system²⁵⁴. Endocannabinoids have emerged as versatile signalling molecules mediating

critical functions in almost every peripheral organ (reviewed elsewhere^{255–257}). Endocannabinoids and their bioactive oxidative products bind to the cannabinoid receptors, CB1 and CB2, as well as to peroxisome proliferator-activated receptors (PPARs)^{258,259}. Similar to bile acids, endocannabinoids symbiotically interact with the gut microbiota²⁶⁰. In animal models, hepatic CB1 activation results in fatty liver, hyperinsulinaemia, increased endoplasmic reticulum stress and suppression of insulin signalling^{261–263}. CB2 activation has been implicated in protection from liver fibrosis^{264–267}, fatty liver disease²⁶⁸ and acute ischaemic liver injury^{269,270}. Although eliciting promising metabolic results, human clinical trials with globally acting CB1 antagonists have had deleterious central effects²⁵⁵; therefore, increased understanding of the systemic role of endocannabinoid signalling in metabolic disease will be required before modulation of this system can be used during pregnancy.

Conclusions

NAFLD affects >34% of children with obesity¹²; however, the factors that accelerate disease progression to fibrosis, cirrhosis and liver failure are multifactorial. With almost two-thirds of all women in the USA entering pregnancy overweight or obese, and the increased incidence of GDM and other environmental exposures and stressors present, the majority of fetuses are subjected to some form of metabolic or environmental exposure and insult, with detrimental effects on their future health. However, the mechanisms underpinning the link between exposure to an adverse *in utero* environment and metabolic disease in later life remain largely unknown. Moreover, the parameters currently used to assess *in utero* metabolic exposure, including maternal weight gain and infant birth weight, are poor predictors of later metabolic disease, suggesting that programming probably involves subtle effects on organs and tissues during development beyond birth weight. Thus, there is an urgent need to identify early maternal and infant bioenergetic, epigenetic, inflammatory and microbial biomarkers that mark mechanisms underlying metabolic disease in the next generation. Greater emphasis on prevention in high-risk infants could yield substantial results. As William Shakespeare wrote, “We know what we are, but know not what we may be.”

Acknowledgments

The authors are supported by the NIH–National Institute of Diabetes and Digestive and Kidney Diseases (R24 DK090964), the American Diabetes Association (ADA 1-13-GSK-13), and the Colorado NIH Nutrition and Obesity Research Center (NORC; P30 DK048520). We thank R. C. Janssen for help with editing the manuscript.

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Key points

- Exposure to maternal overnutrition or undernutrition increases susceptibility to NAFLD in childhood and hastens progression to NASH across the lifespan, especially when offspring are exposed postnatally to a high-fat (Western-style) diet
- Maternal obesity and insulin resistance acting through the placenta produce intrauterine exposures including increased fetal insulin, lipids, inflammation and possibly hypoxia, which cause developmental programming of hepatosteatosis before birth
- Programming of hepatic pathways including *de novo* lipogenesis, oxidative stress, mitochondrial dysfunction and inflammation persists in juvenile offspring born to and breastfed by mothers with obesity, despite weaning to a healthy diet
- Pioneering microorganisms transmitted to the neonate at birth and microorganisms and bioactive milk products transferred during breastfeeding educate the infant immune system with lifelong implications for disease susceptibility
- Pro-inflammatory and pro-remodelling phenotypes in liver macrophages might be programmed in infancy through alteration of intracellular metabolic and epigenetic pathways, increasing responsiveness to subsequent inflammatory stimuli
- Supplements in preclinical models targeting novel pathways and receptors might be emerging therapies for use in tandem with a dietary shift to more complex carbohydrates during pregnancy

Figure 1. Early *in utero* factors programming NAFLD

During pregnancy, mothers chronically consuming a high-fat or Western-style diet might be obese and insulin resistant, with increased plasma lipid and glucose levels that are transferred via the placenta to the fetus. Maternal insulin resistance and hyperinsulinaemia might additionally impair placenta function, reducing blood flow and decreasing fetal oxygen delivery, resulting in fetal hypoxia. Evidence also exists for increased inflammation and cytokine production in the placenta. Collectively, these exposures to the fetus comprise early factors that promote a pathological offspring phenotype. GDM, gestational diabetes; HFD, high-fat diet; ROS, reactive oxygen species; WSD, Western-style diet.

Figure 2. Postnatal events contributing to steatosis and progression to NASH

Steatosis is present in the fetal liver in offspring from obese pregnancies and remains evident in the neonatal liver. Additional early insults after birth in these offspring include exposures from breast milk, microbiota, maturation of immune pathways, inflammation and impaired mitochondrial function. Each of these exposures furthers the progression of fatty liver disease. Across the lifespan, continued exposure to an unhealthy diet after weaning can represent a ‘second hit’ that accelerates the liver phenotype towards development of NASH.

Figure 3. Early liver microenvironment influences macrophage programming

The prenatal and perinatal liver microenvironment in the offspring of mothers with obesity promotes programming of liver macrophages (recruited and resident) via metabolic and epigenetic alterations, favouring a profibrotic phenotype and expression of pro-inflammatory molecules upon a second postnatal 'hit'. This maladaptive immunity might directly result from alterations in the infant gut–liver axis associated with dysbiosis owing to perinatal colonization of the infant intestine with microbiota from a mother with obesity, and postnatal compromise of the intestinal barrier leading to altered absorption of endotoxins, cytokines, bile acids, SCFAs, metabolites and hormones. Disturbances in macrophage function can lead to aberrant tissue repair, such that uncontrolled production of inflammatory mediators and growth factors, deficient generation of anti-inflammatory macrophages or failed communication between macrophages and stellate cells contributes to persistent liver injury and the development of pathological fibrosis. Arg1, arginase 1; FGF19, fibroblast growth factor 19 (also known as FGF15 in mice); FSP1, fibroblast-specific protein 1 (also known as S100-A4); FXR, farnesoid X receptor (also known as bile acid receptor); HIF1, hypoxia-inducible factor 1; MMP, matrix metalloproteinase; SCFA, short-chain fatty acid; TGFβ, transforming growth factor β; TGR5, G protein-coupled bile acid receptor 1; TIMP, tissue inhibitor of matrix metalloproteinase (also known as Metalloproteinase inhibitor 1); TLR, Toll-like receptor.

Table 1

Human clinical interventions targeting childhood obesity

Study	Intervention	Timeframe	Outcome	Refs
Prenatal interventions				
• Tanvig <i>et al.</i> (2014) • Lifestyle in Pregnancy and Offspring (LiPO)	Dietary advice, coaching, exercise	Pregnancy	No difference in BMI of offspring at age 3 years	271
• Gillman <i>et al.</i> (2010) • Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS)	Treatment for mild GDM with dietary advice and glucose monitoring	Pregnancy	No difference in BMI of offspring at age 4–5 years	272
• Hernandez <i>et al.</i> (2015) • Choosing Healthy Options in Carbohydrate Energy (CHOICE)	Higher complex carbohydrate, lower fat diet for mothers with GDM	31 weeks through delivery	Trend toward lower infant adiposity	218
• Rowan <i>et al.</i> (2008, 2011) • Metformin in Gestational Diabetes (MiG)	Metformin for mothers with GDM	20–33 weeks through to delivery	• No difference in neonatal outcomes • Increased skinfold thickness at age 2 years	214, 215
• Syngelaki <i>et al.</i> (2016) • Metformin in Obese Nondiabetic Pregnant Women (MOP)	Metformin in mothers with obesity without diabetes	12–18 weeks through to delivery	No difference in neonatal birth weight z-score	212
• Chiswick <i>et al.</i> (2015) • Effect of Metformin in Pregnant Obese Women (EMPOWaR)	Metformin in mothers with obesity without diabetes	12–16 weeks through to delivery	No difference in mean birth weight percentile	213
Perinatal interventions				
• Martin <i>et al.</i> (2014) • Kramer <i>et al.</i> (2007) • Promotion of Breastfeeding Intervention Trial (PROBIT)	Promotion of breastfeeding	Pregnancy and continuing 3 months postpartum	No difference in BMI of offspring at age 6.5 and 11.5 years	273, 274
• Wen <i>et al.</i> (2012) • Healthy Beginnings	Home visit diet education	Prenatally through to 24 months	Reduced BMI at 24 months	275
• Navarro <i>et al.</i> (2013) • Maternal-Child Pastoral Program	Home and group visit diet education	Prenatally through to 13–24 months	Reduced BMI at 13–24 months	276
• Hauner <i>et al.</i> (2012) • Much <i>et al.</i> (2013) • Impact of Nutritional Fatty Acids During Pregnancy and Lactation on Early Human Adipose Tissue Development (INFAT)	1200 mg per day ω -3 LCPUFAs as a maternal supplement with a concomitant reduction in arachidonic acid intake	15 weeks gestation through to 4 months postpartum	No difference in fat mass (skin fold thickness) at age 12 months	277, 278
• Luoto <i>et al.</i> (2010) • Pre-, Peri-, and Postnatal Programming and Origins of Disease: Early Targeting the Epidemics of Allergy and Overweight (NAMI)	Maternal supplementation with probiotic <i>Lactobacillus rhamnosus</i> GG	4 weeks prior to delivery through 6 months postpartum	No difference in BMI of offspring at age 10 years	226
Postnatal interventions				
• Lauritzen <i>et al.</i> (2005) • Asserhøj <i>et al.</i> (2009) • Danish National Birth Cohort (DNBC)	Maternal supplementation with fish oil	First 4 months of lactation	No difference in BMI at 9 months or 7 years	279, 280
• Andersen <i>et al.</i> (2011) • Essential Fatty Acids During Complementary Feeding (EFiON)	Infant supplementation with fish oil	9–18 months	No difference in BMI at 18 months	281

GDM, gestational diabetes mellitus; LCPUFA, long-chain polyunsaturated fatty acid.