VOLUME 35 ISSUE 2
APRIL 2022

JHND

Journal of Human Nutrition and Dietetics

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WILEY

THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION

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Journal of Human Nutrition and Dietetics

The Official Journal of the British Dietetic Association

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Production Editor: Divya Pundir (email: jhn@wiley.com)

Journal of Human Nutrition and Dietetics

Volume 35 • Issue 2 • April 2022

DOI: 10.1111/jhn.12988

EDITORIAL

JHND Journal of Human Nutrition and Dietetics BDA The Association of UK Dietitians

Complementary feeding: Should baby be leading the way?

COMPLEMENTARY FEEDING

The introduction of solid foods and drinks other than milk (complementary feeding) is a key developmental milestone that exerts powerful changes in terms of functional changes to the gastrointestinal tract, the immune system and metabolic processes. Diversification of the diet exposes the infant to a greater range of fatty acids and proteins, and these, as well as associated micronutrients, must be absorbed from a more varied food matrix containing complex carbohydrates, and a following meal-based pattern of feeding. Successful complementary feeding must ensure that the requirements of the infant for nutrients are met by the dietary supply because milk is a poor source of iron, zinc, vitamin D and vitamin A, all of which are essential for the maintenance of normal growth and function. The introduction of solid foods also serves to stimulate the development of the reflexes that coordinate biting and chewing with swallowing of food. The World Health Organization has set out guidelines advising parents that all babies should be exclusively breastfed for the first 6 months of life, with no introduction of complementary foods prior to this time.[1] At 6 months, nutritionally adequate complementary foods should be introduced, with continuation of breastfeeding to 2 years of age. The UK Department of Health generally advocates this approach, although their guidelines suggest flexibility by wording the advice as delay until "around 6 months." It has been suggested that babies who can sit up and hold their head steady, have good hand-eye coordination, have lost the tongue thrust reflex and are growing at a rapid rate may benefit from introduction of solids at between 4 and 6 months.

The timing of the introduction of complementary feeding is of considerable importance and so confusion among parents as a result of imprecise guidelines is unfortunate. Late introduction (after 6 months of age) puts infants at risk of malnutrition because their stores of nutrients that have carried over from fetal life will become depleted. Early introduction of solids carries a risk of choking, as well as a risk of overwhelming the capacity of the kidneys and gastrointestinal tract to handle solutes and nitrogenous waste (leading to dehydration), and also elevates the risk of gastroenteritis and allergic sensitisation. Preterm infants represent a special case and the introduction of complementary foods should be based upon their developmental stage rather than their chronological age. Early weaning is more likely in this group of infants and there may be ongoing consequences of doing so. A study of 108 preterm infants in Brazil found that use of inappropriate foods for weaning was also a problem and early introduction of ultra-processed foods, cows milk and wheat-based foods was associated with lower weight-for-age *z*-scores at age 2 years.[2]

The introduction of complementary foods should be accomplished gradually and the full process of weaning will typically take 6 months. Throughout that time, milk should remain a key part of the diet. Feeds of breast milk or appropriate infant formula should continue, with both later being used for drinks and mixing with solid foods. The World Health Organization strongly promotes breast milk and considers that commercial follow-on or growing-up milks are unnecessary.[1] Importantly, formula milks targeted at infants older than 6 months of age are not regulated in the same way as milks for younger children. This enables open marketing and, globally, their use is increasing. Although such products are able to maintain intakes of iron and other micronutrients, when infants are transitioning to solid foods, they increase the risk of waterborne infection and exposure to contaminants, and also commit families to an unnecessary cost. Their use is growing particularly quickly in areas such as the Asia-Pacific region.[3]

BABY-LED WEANING

The introduction of complementary foods is a process that generally involves parents selecting foods that have either been purchased specifically for use in the weaning process (commercially produced pureed foods) or household food items that have been pureed or finely chopped at home before being offered to babies via a spoon. Over time, the child will normally start to be offered unprocessed finger foods as the next step in the transition to normal family meals. The concern has been raised that this parentally driven process can pressure

children to eat rather than to experiment with textures and flavours and hence develop their own food preferences. An alternative approach termed "baby-led weaning" is increasingly popular, particularly among families in high-income countries, including the UK. When the baby-led approach is adopted, infants are provided with finger foods from the initiation of weaning and self-select from the same foods that the rest of the family are consuming.[4] It is claimed that this enables the development of the neural pathways that control satiety and enhances motor skills. It has been argued that freeing the infant from parental pressure to eat encourages self-regulation of appetite and will reduce risk of childhood obesity.[5]

In this issue of the *Journal of Human Nutrition and Dietetics*, two papers explore the impact of baby-led weaning on nutrient intake in infants. A study of 36 baby-led weaned infants compared to 60 traditionally weaned infants found that there were few differences between the groups of infants in terms of exposures to specific food groups,[6] although nutrient intakes were different when infants were between 6 and 8 months of age. The infants in the baby-led group had lower intakes of micronutrients (iron, iodine, zinc, vitamin B12 and vitamin D) and milk provided a greater percentage of their daily energy and saturated fat intake.[6] The smaller study by Brown *et al.*[7] also found that baby-led weaning was associated with a greater proportion of energy and nutrients being delivered by breast or formula milk rather than from solid foods. This was consistent with the baby-led approach providing a slower transition to solids. Low intakes of micronutrients as reported in the current issue of the journal[6,7] have also been reported in previous studies and may be the product of infants self-selecting foods that are sweeter and less nutrient-dense.[5,8] The major concern expressed about baby-led weaning is that it may increase the risk of the infant choking because missing out the soft-food stage of the introduction of complementary food means that large food items may be encountered before the baby has full control over mastication. However, there is no compelling evidence that choking is a particular risk.[9]

There is some support for the idea that baby-led weaning facilitates self-regulation of food intake in infants,[10] although the literature is fatally compromised by bias issues. Participants in studies of complementary feeding and the baby-led approach generally recruit well-educated, highly motivated, predominantly breastfeeding mother–baby pairs, with strong adherence to guidance on exclusive breastfeeding for 6 months. For example, Brown *et al.*[7] reported that 80% of their sample were educated to at least degree level and, although 69% of "traditionally" weaning mothers were breastfeeding at 6 months, 88% were doing so in the baby-led group.[7] Similarly, Pearce and Langley-Evans[6] found that baby-led weaning mothers were more likely (86% baby led vs. 73% traditional weaning) to be breastfeeding beyond 6 months and were better educated than traditionally weaning women. To put this into context, only 75% of women in England and Wales (where these studies were conducted[6,7]) even initiate breastfeeding and only 34% still breastfeed (only 1% exclusively) to 6 months. The clear sample bias issues in the literature make it difficult to dissect out any real effect of baby-led weaning from generally strong maternal health behaviours. Although not definitively demonstrated, the general balance of opinion is that longer-duration breastfeeding in itself protects infants from obesity.[11] This confounds any association between baby-led weaning and childhood obesity[5] and almost certainly other observations that baby-led weaning produces better growth or metabolic outcomes.

THE NEED FOR ENHANCED EDUCATION

The first year of life is unique in that there are no other life stages where there are such specific guidelines on food and nutrition. The World Health Organization has been very clear and directive in terms of breastfeeding advice and statements about when complementary foods should be introduced.[1] Despite this specificity that is echoed by departments of health all over the world, compliance at the population level is remarkably poor and, in most parts of the world, solid foods are often introduced earlier than 6 months of age. It has been estimated that almost 20% of US infants receive solids before 4 months, whereas, in the UK, 75% of babies may start to be weaned by 5 months.[12,13]

The reasons why guidance on complementary feeding is so poorly adhered to are complex in that decisions made by individual parents on how to proceed are shaped by many factors (socio-economic status, infant growth rate, maternal age, infant sleeping pattern), that are further modulated by local social and cultural norms. In the UK, a degree of confusion exists about the timing of weaning because the advice given to parents has been inconsistent. As a result, many parents mistrust the advice of health professionals,[14] and prefer to make use of possibly unreliable sources to make judgements about timing and pattern of introduction of complementary foods. Many of those sources are now Internet-based and are subject to inaccuracy, deliberate misinformation[15] and manipulation by manufacturers of formula milks and other products aimed at very young children. Confusion, lack of understanding and family/socio-cultural expectations drive parents to make decisions that they consider to be in the best interests of babies, but which do not sit well with the available evidence-base. Baby-led weaning may be one example of this. Cutting through the complex web of information and considering what may be effective drivers of appropriate weaning behaviour will require new approaches to engage parents and enhance compliance with feeding guidelines.

Sangalli et al.[16] have demonstrated how a primary care intervention that included training for mothers on the timing of complementary feeding and the types of food that should be used could be rolled out on a large scale in Brazil. Health centres in the intervention arm of the study gave women access to counselling on how to feed babies and children within Brazilian guidelines. At 3 years of age, children in the intervention arm had lower energy, carbohydrate and fat intakes than those in the control arm. At 6 years of age, the children in the intervention arm had smaller waist circumferences and skinfold measurements.[16] This paper shows that that appropriate exposure to professional counselling can have a beneficial impact on the nutrition and growth of young children.

It is clear that the answers to the current problems associated with complementary feeding in high- and middle-income countries do not lie in asking parents to let their babies to lead the way and select their own foods. It is noteworthy that almost 60% of parents who follow baby-led weaning use interactive media sources rather than health professionals to shape their approach to complementary feeding,[17] which means that compliance with weaning guidelines is lower. Guidelines are already in place and have a strong evidence-base, such that the solutions lie in promoting the guidelines, enhancing access to reliable information, improving the training of health visitors so that they are better equipped to answer questions and promote behaviour change, and ensuring that parents of young children can access resources whenever they are required. Smartphone applications have been shown to be trusted and accessible to women in need of support when breastfeeding[18] and perhaps similar eHealth solutions that follow on from breastfeeding promotion tools might prove beneficial in guiding parents through an infant's transition from milk to solid foods. High-quality resources are required to counteract the often poor and unreliable advice that parents extract from social media and Internet sources.

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Received: 15 November 2021 | Accepted: 22 February 2022

DOI: 10.1111/jhn.12999

INVITED REVIEW

JHND Journal of Human Nutrition and Dietetics | BDA The Association of UK Dietitians

Overweight, obesity and excessive weight gain in pregnancy as risk factors for adverse pregnancy outcomes: A narrative review

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Funding information

None

Abstract

The global prevalence of overweight and obesity in pregnancy is rising and this represents a significant challenge for the management of pregnancy and delivery. Women who have a pre-pregnancy body mass index greater than 25 kg m^{-2} are more likely than those with a body mass index in the ideal range (20–24.99 kg m^{-2}) to have problems conceiving a child and are at greater risk of miscarriage and stillbirth. All pregnancy complications are more likely with overweight, obesity and excessive gestational weight gain, including those that pose a significant threat to the lives of mothers and babies. Labour complications arise more often when pregnancies are complicated by overweight and obesity. Pregnancy is a stage of life when women have greater openness to messages about their lifestyle and health. It is also a time when they come into greater contact with health professionals. Currently management of pregnancy weight gain and the impact of overweight tends to be poor, although a number of research studies have demonstrated that appropriate interventions based around dietary change can be effective in controlling weight gain and reducing the risk of pregnancy complications. The development of individualised and flexible plans for avoiding adverse outcomes of obesity in pregnancy will require investment in training of health professionals and better integration into normal antenatal care.

KEYWORDS

gestational diabetes, obesity, pre-eclampsia, pregnancy, stillbirth

Highlights

- Overweight and obesity before pregnancy and excessive gestational weight gain are major determinants of risk for pregnancy loss, gestational diabetes, hypertensive conditions, labour complications and maternal death.
- Pregnancy is regarded as a teachable moment when women are at their most receptive to messages about their health. However, unclear guidance on diet and physical activity, weight stigma from health professionals, inexperience and reluctance among professionals about raising issues about weight, and stretched resources put the health of women and babies at risk.
- Excessive weight gain in pregnancy and post-partum weight retention compromise future fertility and increase risk for future pregnancies.

All authors contributed equally to this work.

- Large randomised controlled trials have had little success in addressing excessive gestational weight gain or antenatal complications. Individualised, culturally sensitive and responsive interventions appear to have greater success.

INTRODUCTION

Modern medical care has made pregnancy and childbirth relatively safe for women who live in developed countries. Across the Global North the maternal death rate is less than 1 per 10,000 births and rates of stillbirth and late fetal death rates are between four and six per 1000 births.[1] Improvements in outcomes since the 1960s have been driven by a range of factors, including high standards of hygiene and sanitation and improved nutrition, but, most importantly, women's control over reproduction.[2] Because approximately 60% of pregnancies are now planned in advance, there is an opportunity for women and their partners to make lifestyle changes that promote better health in pregnancy and reduce risk of poor pregnancy outcomes.[3]

The relative safety of pregnancy and childbirth for women in high-income countries is a major benefit of the medicalisation of pregnancy, characterised by antenatal surveillance and intervention. Of course, this is not true of countries in the Global South where pregnancy and childbirth-related complications remain the major cause of death for young women. Where women benefit from advances in obstetric care, there are concerns that the medical management of pregnancy has become too intrusive and that the benefits of close surveillance and early intervention, particularly in labour, do not justify the associated cost and the loss of autonomy for women.[4–6] Against this background, it is surprising that dietary change and weight management is not a fixed feature of pregnancy care. Dietary advice is loose and in addition to being given a list of things to avoid (potential sources of food pathogens, liver, oily fish, alcohol and caffeine) women are merely advised to consume a healthy 'balanced' diet. Given the low quality of the western diet and the current prevalence of overweight and obesity, this approach is unlikely to have any efficacy. For many women, there is no advice given on weight gain until they are already pregnant and even then, it is lacking in quality. As this review will describe, the avoidance of overweight and obesity should be the highest priority for women who are considering becoming pregnant because excessive body fat is the single greatest risk factor for poor pregnancy outcomes and pregnancy complications.

The prevalence of overweight and obesity is highly variable across the world, with the highest rates in women observed in the Pacific island nations, the Caribbean and the Middle East. It is estimated that, globally, there are close to 39 million pregnancies per year complicated by maternal obesity[7] and, in some countries, the estimated prevalence of overweight and obesity in pregnancy is over 60% (South Africa 64%, Mexico 65%, USA 55%–63%).[7,8] In England, the combined prevalence of overweight and obesity is 35% among 16–24-year-old women, rising to 61% among 35–44 year-olds, highlighting the high level of potential risk among women of reproductive age.[9] The highest rates of antenatal obesity are observed in areas of high deprivation, among older mothers and in minority ethnic groups.[10] Women in the UK who are Black (odds ration [OR] = 1.70, 95% confidence interval [CI] = 1.62–1.78) or South Asian (combined OR = 1.72, 95% CI = 0.66–1.79) are reported to be more likely to be living with obesity than white women.[11] It is of course well recognised that rates of obesity have been rising quickly over the last two to three decades and, increasingly, pregnancy is being complicated by extreme or morbid obesity. In the UK, it has been estimated that approximately 1 in 1000 births are to women with a body mass index (BMI) > 50 kg m^{-2}, whereas, in Australia, a super-obesity prevalence of 2.1 per 1000 births was noted.[12,13]

This review discusses the implications of overweight and obesity for pregnancy complications and outcomes. As shown in Figure 1, overweight is a significant risk factor for infertility, loss of pregnancy, pregnancy complications, complications in labour, and fetal and maternal death. All of these risks are also associated with excessive weight gain during pregnancy. Because women can often struggle to lose weight gained in pregnancy,[14] excessive pregnancy weight gain can also put future pregnancies at risk of poor outcomes.[15] Greater inter-pregnancy weight gain is also a factor in establishing greater risk for future pregnancies.[16]

Gestational weight gain (GWG) comprises both fetal and maternal components. On the fetal side, there is mass laid down to develop the placenta, amniotic fluid and fetal tissues. Women also gain weight because of an increase in body water and blood volume to support perfusion of the placenta, deposition of tissue in the breasts in readiness for feeding post-partum, expansion of the uterus and deposition of fat in stores.[17] Achieving a satisfactory GWG is extremely important for successful completion of gestation. In some parts of the world, pregnant women are given some guidance on what would be appropriate GWG, whereas, in the UK, such advice is not available. In terms of evaluating GWG for research purposes, it is generally accepted that the United States Institute of Medicine guidelines are appropriate (Table 1).[18] Appropriate ranges of weight gain are dependent upon women's BMI going into pregnancy.[18]

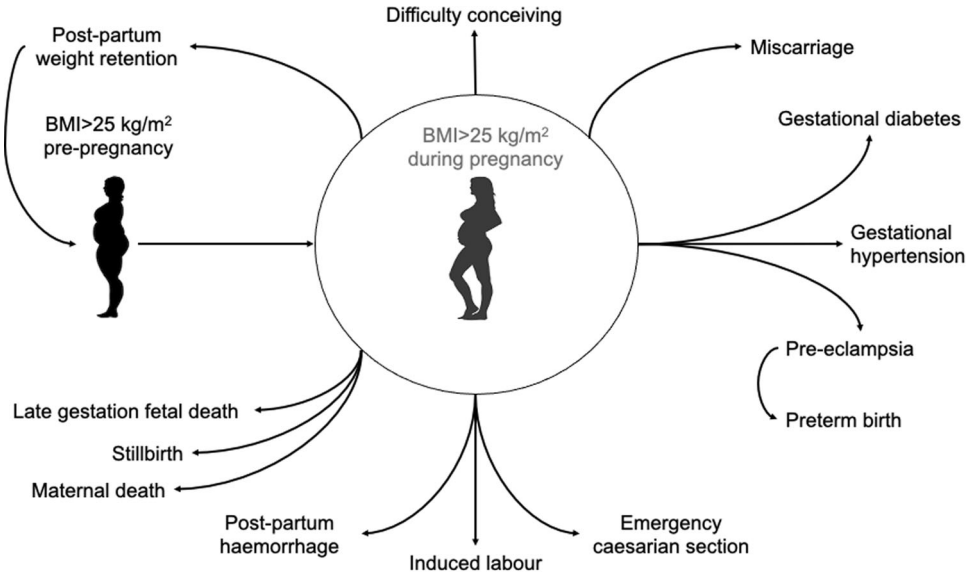

FIGURE 1 Obesity in pregnancy is a risk factor for adverse outcomes. BMI, body mass index. Adapted from Langley-Evans[16]

Women who are underweight prior to pregnancy need to gain more weight to avoid complications associated with inadequate GWG, whereas obese women should control weight gain to avoid excessive GWG. Greater weight gain is expected for women who are carrying twins, reflecting the greater amount of fetal and placental tissue to be laid down (Table 1).[19]

Although overweight and obesity greatly increase the risk of adverse outcomes in pregnancy, it is important to appreciate that the majority of women with BMI > 25 kg m^{-2} will have normal, uncomplicated pregnancies. Among 387 British women with a BMI in excess of 35 kg m^{-2} at booking, 75% went through a full gestation without developing any of the major complications of pregnancy (Figure 2). Similarly Relph *et al.*[20] found that among more than 115,000 Canadian women with BMI > 30 kg m^{-2}, with no underlying morbidities, nearly 60% had a normal pregnancy. The major complications of pregnancy are relatively uncommon events. One in 23 women in the UK develop gestational diabetes (GDM); one in 18 develop pre-eclampsia (PE) (of which only a one-third will have severe PE) and one in 13 give birth preterm (before 37 weeks; of which 10% do so as a result of PE). Although there are significant numbers of women affected each year and there are major concerns about health at the population level, the risk faced by individual women living with obesity remains small.[21]

TABLE 1 Recommendations for weight gain in pregnancy are related to pre-pregnancy body mass index

Body mass index at conception (kg m^{-2})	Optimal weight gain (kg) for singleton pregnancy	Optimal weight gain (kg) for twin pregnancy
Underweight < 18.5	13–18	23–28
Normal weight 18.5–24.9	11–16	18–25
Overweight 25–29.9	7–11	17–21
Obese > 30	5–9	13–17

Data Sources: Luke[19]; Institute of Medicine[18]. Optimal weight gain ranges are those associated with favourable pregnancy outcomes for mother and fetus and which lead to a birth weight between 3.1 and 3.6 kg.

OBESITY AND INFERTILITY

Body fatness is the main lifestyle-related factor that has effects on reproductive health in women. Both underweight and obesity are associated with menstrual cycle disorders including anovulation, amenorrhea and oligorrhea. This relationship is mediated through leptin, which is produced by adipose tissue. Leptin concentrations are directly proportional to the amount of adipose tissue in the body and this hormone has a permissive effect on the secretion of gonadotrophin-releasing hormone from the hypothalamus and luteinising hormone and follicle-stimulating hormone from the pituitary. In obesity, women develop leptin resistance leading to cycle disorders.[22] Rich-Edwards *et al.*[23] found that there was a U-shaped relationship between BMI and ovulatory disorders, with 25% of such disorders in the US population being explained by obesity. Among women with no obvious menstrual cycle disorders, overweight and obesity delay conception in women who are trying to become pregnant.[24] In women who have bariatric surgery to treat obesity, fertility improves and pregnancy outcomes are favourable.[25] In women who are undergoing assisted reproductive therapies (ART), obesity reduces the efficacy of those treatments. Comparing women with BMI > 30 kg m^{-2} with those at 18.5–24.9 kg m^{-2}, the odds of a

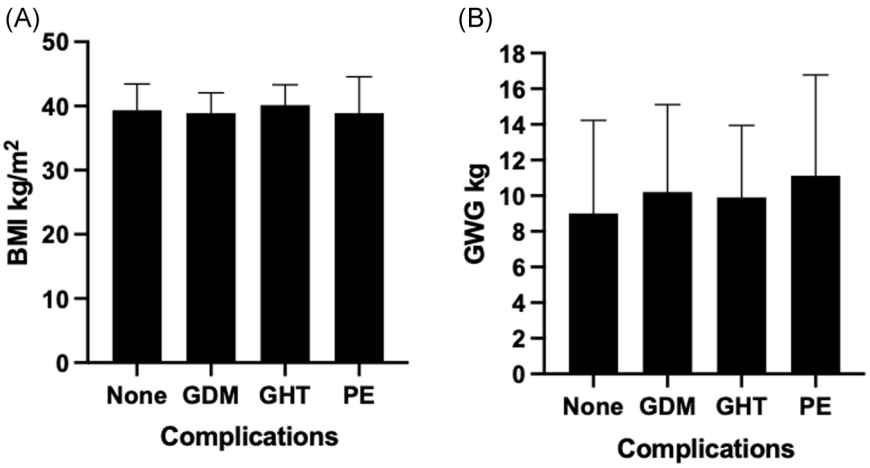

FIGURE 2 Early pregnancy body mass index (BMI) and gestational weight gain in relation to pregnancy complications (a) Distribution of BMI among severely obese pregnant women. (b) Distribution of gestational weight gain among severely obese pregnant women. All women were of BMI ≥ 35 kg m^{-2} at antenatal booking ($n = 387$). Gestational weight gain (GWG) was determined as weight gain between booking and 36 weeks of gestation. Data are shown as median and interquartile ranges. No complications $n = 291$ (75% of cohort); gestational diabetes (GDM), $n = 45$ (11.5%); gestational hypertension (GHT), $n = 37$ (9.5%); pre-eclampsia (PE), $n = 16$ (4%)

live birth were significantly reduced (relative risk [RR] = 0.85, 95% CI = 0.82–0.87).[26] Weight loss is generally advised for obese women prior to commencing ART.

Alongside infertility that is driven by leptin resistance, obesity is the major risk factor for polycystic ovary syndrome (PCOS). PCOS is one of the more common fertility issues in women and is associated with anovulation and irregular menstrual cycles. The cause of fertility problems in PCOS is elevated androgen concentrations, although this is secondary to insulin resistance.[27] Although more common in women living with obesity,[28,29] PCOS also occurs in women who are not obese and risk is related to visceral fat mass.[30,31] PCOS and its associated menstrual cycle disorders are readily treated with metformin to improve insulin sensitivity, or through weight loss of approximately 5% body weight.[32] Although a number of studies have evaluated whether low carbohydrate diets or similarly restricted approaches have particular efficacy in restoring fertility in PCOS, standard weight loss strategies (exercise and energy restriction) appear to be the most effective and straightforward approach.[33]

MISCARRIAGE, STILLBIRTH AND MATERNAL DEATH

Maternal BMI is a known determinant of the risk of spontaneous miscarriage in the first trimester of pregnancy. Both extremes of the BMI range are considered to increase risk, although the evidence of an adverse effect of underweight may be more robustly supported by the literature than overweight.[34,35] Both low concentrations of leptin and leptin resistance may play a role in miscarriage because this hormone has a role in embryonic implantation and the establishment of the placenta.[36] Although Bracken and Langhe[37] found no relationship between obesity and miscarriage, large studies of Asian populations indicate a modest but significant risk. Pan *et al.*[38] investigated more than half a million pregnancies in China and found that, although overweight was not a risk factor for miscarriage, BMI > 28 kg m^{-2} (cut-off for obesity in Asians) was associated with a 16% greater risk. Similar findings were reported by Haque *et al.*,[39] who noted 8% greater risk with overweight and 26% greater risk with obesity. Among women undergoing ART there are greater rates of miscarriage with obesity.[40,41]

The relationship between maternal BMI, GWG and risk of stillbirth is more complex than is often reported. Inadequate weight gain or weight loss in pregnancy have been demonstrated to increase stillbirth risk but, in women who were morbidly obese going into pregnancy, some weight loss in the second trimester reduced risk by 14%.[42] Johansson *et al.*[43] reported a greater risk of stillbirth with excessive GWG but only in women of normal weight pre-pregnancy. Other studies suggest that stillbirth is more likely in pregnancies complicated by overweight[44] or obesity.[39,42,45] In South Asian women, obesity increased risk substantially (OR = 1.46, 95% CI = 1.27–1.67).[39] Excessive GWG doubled stillbirth risk in women living with obesity in the study of Yao *et al.*[42,45] and the risk associated with obesity increased substantially in women whose pregnancies exceed 39 weeks in duration. A systematic review and meta-analysis including over 16,000 stillbirths across 38 studies concluded that for every increase in BMI of 5 kg m^{-2} above the ideal range, the odds of stillbirth increased by 24% (OR = 1.24, 95% CI = 1.18–1.30).[46]

Severe obesity is a risk factor for maternal death in the perinatal period. An analysis of the outcomes of 571,000 pregnancies in New York City (2008–2012) found that death was significantly more likely in women with BMI > 35 kg m^{-2} than in women of ideal weight.[47] The level of risk increased with severity of obesity (BMI = 35–39.9 kg m^{-2}, RR = 1.14, 95% CI = 1.05–1.23; BMI = 40–49.9 kg m^{-2}, RR = 1.34, 95% CI = 1.21–1.49; BMI > 50 kg m^{-2}, RR = 1.99, 95% CI = 1.57–2.54).[47] Knight *et al.*[48] considered all 209 UK women who died during pregnancy and up to 6 weeks post-partum between 2015 and 2017. Cardiovascular complications were the biggest cause of maternal death and 55% of such deaths occurred in women who were overweight or obese. A similar evaluation of maternal mortality in France (2013–2015) concluded that overweight increased the risk of death by 60% and obesity more than tripled the risk, particularly for cardiovascular deaths.[49] Maternal obesity was also a factor in the deaths of women who were infected with COVID-19 during pregnancy, with more than double the risk of death in women with BMI > 30 kg m^{-2}.[50]

PREGNANCY COMPLICATIONS

Women who are overweight or obese are at generally higher risk of all complications of pregnancy. The major complications include GDM and PE, both of which present a significant risk of mortality for mother and baby. Less serious complications are experienced by a high proportion of pregnant women and include heartburn and symphysis pubis dysfunction (SPD). Although neither are a threat to successful delivery of a live baby, both are debilitating and chronic conditions in pregnancy. Obesity is a modifiable risk factor for SPD and possibly a pelvic girdle syndrome that persists beyond delivery of the baby.[51] Denison *et al.*[52] reported that, when comparing women with BMI >30 kg m^{-2} with women with BMI under 25 kg m^{-2}, the risk of SPD was almost four-fold higher and risk of heartburn was increased by 2.65-fold.

Hypertensive disorders of pregnancy

Rising blood pressure is a normal feature of pregnancy and is generally not considered to be problematic. Blood pressure increases because renal function in pregnant women changes in order to handle a greater volume of blood and to perfuse the placenta. When the blood pressure increases to beyond the usual cut-offs for hypertension (systolic 140 mmHg/diastolic 90 mmHg) in the last trimester of pregnancy, this is termed gestational hypertension (GHT), if there is no pre-existing hypertension before conception and the condition arises no earlier than 20 weeks of gestation.[53] In most cases, GHT is not a major problem but the condition needs to be closely monitored to detect progression to PE (regular proteinuria screening and additional antenatal appointments). If blood pressure increases to more than 160/110 mmHg, this is regarded as an obstetric emergency putting the lives of both mother and baby at risk.[54,55] Women with GHT are also at greater risk of all complications that may arise post-partum, including haemorrhage.[56] In addition to anti-hypertensive medication, GHT is managed through lifestyle modification, including weight management and dietary sodium reduction.[55] In a population of predominantly overweight and obese women, higher compliance with the DASH dietary pattern was associated with lower diastolic blood pressure and mean arterial blood pressure.[57] GHT normally resolves within 3 months of giving birth but follow-up monitoring is advised in case chronic hypertension develops.[53,55]

Overweight and obesity are established risk factors for GHT.[52,58] In their very large cohort, Relph *et al.*[20] observed that, although only 2.6% of normal weight women developed the condition, the prevalence was 4.7% in overweight women, 7.8% in obese and greater than 10% in severely obese women. A study that modelled the risk factors for GHT concluded that BMI > 25 kg m^{-2} was the biggest single predictor of developing GHT.[59] Sormunen-Harju *et al.*[60] estimated that, compared to women with BMI 20 kg m^{-2}, the risk of GHT was 2.3-fold higher (95% CI 1.4–3.8) in women with BMI >25 kg/m^2. This risk rose markedly (42-fold) in women who had previously had a pregnancy complicated by GHT. Excessive GWG is also a risk factor for GHT across all maternal BMI categories.[61] GWG below guidelines was associated with a significant reduction in GHT risk in an analysis that drew on data from 18 cohort studies.[62]

PE is an extremely dangerous condition that threatens the lives of both mother and fetus. It is characterised by the development of hypertension after 20 weeks of gestation and urinary protein excretion in excess of 300 mg/24 h.[63] PE is caused by the development of arterial dysfunction in the placenta, which involves oxidative injury and an inflammatory response spreading beyond the placenta to impact upon all major organs in the mother.[64] PE is a progressive condition that cannot be reversed or controlled and, without intervention, women are at risk of developing eclampsia. Eclampsia is the end stage of the PE disorder and is characterised by maternal seizures and coma as a result of oedema of the brain. Eclampsia can result in multiple organ failure, renal collapse, abruption of the placenta and death of both mother and baby (Figure 3). PE is the major cause of preterm delivery because the only viable treatment is to deliver the baby early by caesarean section.

There is a strong genetic component to PE and women who have had a pregnancy complicated by PE are likely to do so again in future pregnancies.[65–67] Overweight and obesity are the next most important risk

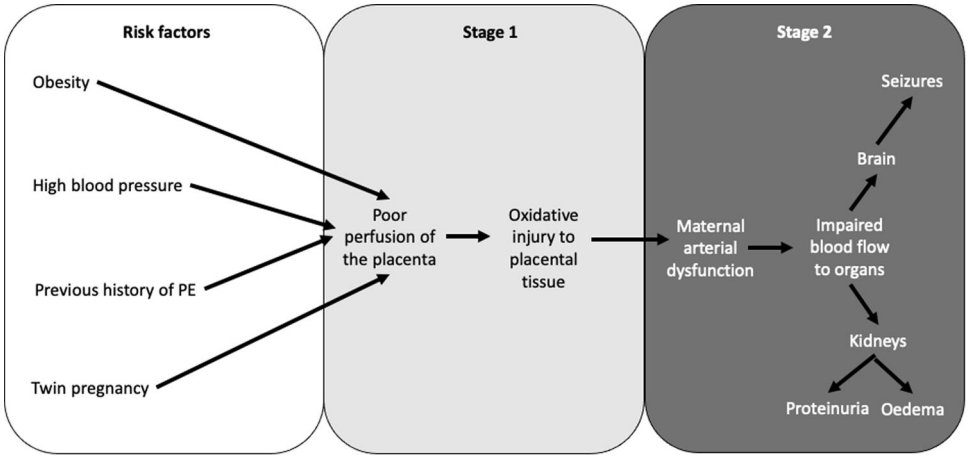

FIGURE 3 Factors that contribute to risk of pre-eclampsia (PE) and disease progression

factors for PE and make the development of PE more likely because of insulin resistance (a promoter of vascular endothelial dysfunction) and a systemic pro-inflammatory state as a result of the production of cytokines from adipose tissue. An analysis of a quarter of a million births in the Finnish Birth Registry showed that, among women under the age of 35 years, overweight increased risk of PE by 49%, whereas obesity increased risk by 2.45-fold. In older women, the risk was significantly greater.[68] A systematic review by He *et al.*[69] found that overweight (OR = 1.71, 95% CI = 152–1.91) and obesity (OR = 2.48, 95% CI = 2.05–2.69) were major risk factors for PE. Among women who had bariatric surgery prior to pregnancy, risk of PE declined compared to obese women who did not have the surgery and compared to the same women in their pregnancies prior to surgery.[70]

GDM

Pregnancy is an insulin resistant state in which changes to insulin signalling pathways suppress the uptake of glucose by the maternal muscle and liver.[71] In the fed state, this serves to drive glucose into the fetal compartment as a substrate for growth. In the fasted state, it means that women mobilise more triglycerides, free fatty acids and ketones for fetal metabolism.[72] Against this metabolic background, some women develop GDM, which has a number of potential adverse outcomes for babies when in utero and in the longer term. The most common outcome is macrosomia. Macrosomic infants weigh in excess of 4.5 kg at birth and this generally results in more caesarean sections because passage through the birth canal in a normal labour increases the risk of shoulder dystocia, bone fractures and subconjunctival haemorrhage.[73] GDM increases the risk of congenital heart defects.[74] and infants born to women with GDM are at greater risk of childhood obesity.[75]

The association between obesity and GDM is well documented. Systematic reviews and meta-analyses of case–control and cohort studies indicate that obesity increases risk by more than threefold,[76] and that excess visceral and central adiposity are greater risk factors than general obesity.[77,78] Both pre-pregnancy and early pregnancy BMI is associated with risk of GDM, as is excessive GWG.[79] Relph *et al.*[20] found that, among over 700,000 Canadian women, risk of GDM increased with increasing BMI across the whole range. Although 2.8% of women with BMI under 18.5 kg m^{-2} developed the condition, 12.4% did so among women with BMI ≥ 50 kg m^{-2}. A retrospective analysis of 4512 deliveries in Lagos found that excessive GWG was associated with greater risk of GDM (OR = 4.8, 95% CI = 1.93–12.62).[80] Among food insecure Malaysian women, excessive GWG in the second trimester of pregnancy increased risk of GDM by almost 10-fold.[81]

LABOUR COMPLICATIONS

Obesity and excessive GWG increase risk of complications leading into, during and after labour, both directly and indirectly. In indirect terms, weight related conditions such as PE and GDM increase the likelihood of preterm delivery and delivery by caesarean section.[58] In direct terms, labour is complicated by uterine dysfunction and caution on the part of medical professionals as they manage the labours of obese women.

Women who are overweight or obese are less likely than normal weight women to initiate and sustain spontaneous labour. Animal studies suggest that this is a consequence of reduced expression of uterine contractile proteins and production of labour-inducing prostaglandins.[82] As a result, these women are more likely to require labour induction.[83] However, induction is less likely to succeed and obese women are more than

three-fold more likely than normal weight women to require an emergency caesarean after induction.[83–85]

Intervention in labour is overall more likely in obese women, who are less likely than women of ideal weight to have a spontaneous vaginal delivery.[86] The most likely intervention is caesarean section (both elective and emergency).[58,87] and, to some extent, this is driven by medical staff seeking to minimise risk to the baby and mother. Women who are obese and whose labour is not progressing are less likely than women of ideal weight to be allowed to attempt a vaginal delivery with assistance from forceps or a vacuum cap (ventouse), but, when they are allowed to do so, appear to have better outcomes than ideal weight women.[88,89] With caesarean section recovery is slower and surgical complications are more likely with obesity.[90,91]

Post-partum haemorrhage occurs in up to 5% of women and is characterised by either heavy blood loss during delivery (in excess of 500 ml following vaginal delivery or 1 litre following a caesarean) or in the following days (as a result of placental retention, uterine atony or rupture). Obesity is one of the key risk factors for post-partum haemorrhage. Thies-Lagergren *et al.*[92] reviewed data on more than 400,000 pregnancies in the Swedish Birth Registry. Greater risk of blood loss exceeding 1 litre in the 2 h after birth was seen in women with BMI > 25 kg m^{-2}.[92] Similarly, risk of post-partum haemorrhage was found to be more than doubled in obese women in a study by Dalbye *et al.*[86] Some of this risk is driven by a larger birthweight and head circumference in babies of obese women, leading to tearing.

LONG-TERM IMPLICATIONS FOR THE INFANT

Obesity during pregnancy is not restricted to carrying risk for the outcome of that pregnancy. A growing body of evidence suggests that maternal obesity is responsible for programming long-term health and wellbeing in the growing fetus. Individuals who are exposed to maternal obesity or GDM in utero are, as adults, more likely to be obese,[93] develop type-2 diabetes[94,95] and die as a result of cardiovascular disease.[96]

THE ANTENATAL PERIOD AS A TEACHABLE MOMENT

Health promotion activities that target older children and adults are generally hampered by a lack of engagement by the target population. Although certain key messages about diet and nutrition can become well-embedded in the awareness of children and adults, compliance with such messages can be very poor. For example, the 5-a-day message relating to fruit and vegetable intake is almost universally known, but it fails to change behaviour across all age groups.[97] Similarly, although most women of childbearing age in the UK are aware of the need to take folic acid supplements to prevent neural tube defects should they become pregnant, less than 40% do so according to guidelines.[98] Although some of this is explained by around 40% of pregnancies being unplanned, there is clearly a significant proportion of women who do not follow guidelines despite being aware that they exist. The lack of engagement of some women with guidelines on lifestyle, and in particular weight, in pregnancy may be explained by a number of factors. A lack of awareness and education plays a big role, especially because most lifestyle changes need to be made before rather than during pregnancy. Having knowledge is no guarantee of action as making lifestyle changes is intrinsically difficult, especially if those changes are required without the incentive of benefitting the growing fetus. In a qualitative study of why women drink alcohol in pregnancy, Meurk *et al.*[99] found that many women who did so had not appreciated the risk involved, or lived in circumstances where healthy behaviours were not the norm. The desirability of maintaining their usual social behaviours outweighed the desirability of making a lifestyle change.[99] It is likely that the same factors apply to other unhealthy decisions made prior to and during pregnancy.

Pregnancy possibly represents the stage of life when women are most receptive to messages about health and at their most prepared to introduce lifestyle changes and has been described as a 'teachable moment'.[100] The motivation to change arises because women become aware that certain behaviours may put themselves and, more critically, the health of their baby at risk. Pregnancy forces a reevaluation of their role in their family and in society.[101] Pregnancy also brings women into more contact with health professionals and literature about health and lifestyle, thereby providing routes through which the teachable moment can be capitalised upon. Unfortunately, the willingness to seek advice and information can result in women accessing sources which are not reliable. Internet sources not only have the advantage of being instantly available at all times of day and night, but also are contaminated by error and deliberate misinformation. Lynch and Nikolova[102] found that pregnant women had a preference for finding information about their pregnancy and health on the Internet, and that they trusted what they read, although they did not question the source of the information. Internet sources are a major influence on decision making by pregnant women who are often dissatisfied by the information that they receive from health professionals, with the latter often being inaccessible to women when they have questions.[103]

The physiological response to pregnancy may in itself influence dietary behaviour right from the point of conception and this may not be conducive to making

changes that control body weight gain. Nausea and vomiting are commonplace and are sometimes the first sign of conception, appearing at between 2 and 6 weeks of gestation.[104] The nausea experienced by between 60% and 80% of women can influence food choices and the majority of women report changes in preferences for certain foodstuffs and beverages. Caffeine-based drinks, eggs, fish, meat and fatty foods are commonly avoided, whereas intakes of carbohydrate-rich foods tend to increase in the first trimester. Sweets, biscuits, chocolate and cakes are widely favoured, along with fruit and fruit juices.[105,106] Psychological influences are also important and some women use food to manage anxiety about their pregnancy and other negative states.[107]

Although most women undertake some degree of lifestyle change in response to becoming pregnant, if not prior to conception, the availability of a teachable moment and the health professional access that can deliver it do not guarantee that women will make the right choices. For example, although the UK Department of Health set a target of reducing the prevalence of smoking in pregnancy to 6% or less by 2022, in 2020/21, around 10% of pregnant women are still smoking by the time they give birth.[108] Similarly, more than 40% of British women reported consuming alcohol during pregnancy, against guidelines.[109] Generally compliance with recommendations on lifestyle change in pregnancy is greatest in women having their first baby and better educated women. Compliance is lower in younger women and those from impoverished backgrounds.[98]

In terms of managing weight and avoiding excessive weight gain in pregnancy, the opportunity to communicate clearly with women may be missed. Although, in some countries, there are clear guidelines on weight gain for pregnancy and monitoring weight is part of normal antenatal care, in the UK, the approach taken to dealing with obesity wastes an opportunity for action. Advice to women on what to eat during pregnancy, as well as what level of physical activity should be maintained, is very generalised and often poorly understood.[110] Similarly, communication about body weight, dealing with overweight in pregnancy and what constitutes healthy weight gain is ineffectual. In the UK, the National Institute for Healthcare and Clinical Excellence (NICE) recommends that women suffering from overweight or obesity should be advised to lose weight prior to, or after, pregnancy[111] and therefore places an emphasis on just monitoring weight gain during pregnancy. However, current clinical pathways mean that height and weight are usually only measured at the first antenatal appointment, without any further follow up. For women with a booking BMI in excess of 30 kg m^{-2}, there may be a referral offered to a dietitian or other agencies so that women can receive personalised support to help manage their weight, although this is inconsistent and infrequent.[110]

Routine weighing of women at antenatal appointments has been largely abandoned in the UK, despite the importance of maintaining a healthy rate of weight gain. There are a number of reasons for this. First, NICE guidelines state that routine monitoring of women's weight without their consent and without sufficient explanation or feedback is unacceptable.[111] There have also been studies indicating that overweight or obese women feel stigmatised, anxious or guilty when routinely weighed in pregnancy. A systematic review by Johnson *et al.*[112] concluded that focusing on weight may be a barrier to optimising diet and physical activity in pregnant women. However, in a study of almost 200 women in the first trimester of pregnancy, Swift *et al.*[113] found that most women would be happy to receive advice and guidance from health professionals on their weight, although only 15% of women reported having had any feedback on weight after having been weighed by their midwife, despite 31% having been overweight or obese going into pregnancy. Self-monitoring of pregnancy weight gain was a common behaviour in this group of women, indicating that they were both interested in their weight and engaged with tracking across their pregnancy. A 2020 feasibility study in Ireland reported that women found being weighed throughout their pregnancy a positive experience and gave them reassurance with regard to the growth of their babies.[114]

Obesity is a sensitive subject, and there is evidence that, in primary care, both patients and healthcare professionals may be embarrassed and reluctant to raise the issue of body weight. Generally, it is midwives who bear the responsibility for delivering health education and promoting a healthier lifestyle in pregnancy. Although being in regular contact with women and carrying a high level of trust as a source of information, they are not well equipped for dealing with conversations about overweight.[115,116] Those conversations may be compromised by ingrained weight stigma among health professionals, which training programmes need to overcome.[117] Midwives may lack the confidence to raise the issue of obesity and fear a hostile response from women that they are trying to form a professional bond with[112,117] and, with high workloads and time pressure, it can be difficult to maintain an awareness of unconscious bias around obese women and implement personal strategies to overcome that bias.[117,118] They also suffer from a lack of clear clinical guidelines that could enable referral to suitable personalised interventions.[118] In the absence of regular weighing, it is also possible that the high levels of overweight in society may normalise the appearance of obesity, meaning that midwives fail to recognise women who may need intervention.[118] Women want honest and respectful communication that provides personalised information about risk and facilitates informed lifestyle choices without scaremongering, and without proportioning blame about the causes of overweight.[119]

STRATEGIES FOR MANAGEMENT OF WEIGHT GAIN IN PREGNANCY

Although the US Institute of Medicine recommendations on GWG[18] are generally accepted as a good guide for achieving healthy outcomes for a pregnancy complicated by overweight or obesity, there are no clear guidelines on when or how to intervene to manage weight gain in pregnancy. In the UK, the emphasis in NICE guidelines is on achievement of a healthy weight in the interpregnancy interval[111] and there are concerns that interventions in pregnancy could result in weight loss or inadequate weight gain, with unknown consequences for babies. Despite this, many UK NHS Trusts have implemented local services to prevent excessive weight gain, although these often work from a limited evidence base.

As shown in Figure 4, it is likely that there is a very narrow window of time for a weight gain intervention to be initiated effectively. Across all BMI classes, weight gain in the first 20 weeks of gestation is modest (approximately 2.5 kg) and thereafter proceeds at around three to four times the early rate.[120] The route to weight-related complications will be established in the early-mid gestation period because GHT can manifest from 20 weeks and GDM from 24 weeks. Because most women are not booked into antenatal services and in regular contact with health professionals until 11–12 weeks of gestation, there is a period of only a few weeks in which to introduce strategies to avoid excessive GWG before rapid gain may limit efficacy of steps taken (Figure 4).

In terms of how weight should be managed, there should be little difference in approach between pregnant and non-pregnant individuals, except the goal for pregnancy is to allow weight gain within healthy limits rather than achieve a weight loss. Physical activity is important and previously sedentary women are advised to sit less, incorporate walking into daily life and engage with continuous exercise for up to 15 min day^{-1} (e.g. brisk walking or swimming) three times a week.[121] Otherwise, 150 min of moderate intensity exercise per week is advised.[111,121] The unique feature of pregnancy is the availability of access to health professionals who, if appropriately trained, could advise women on weight (midwives, obstetricians, general practioners). In some cases, women may benefit from the input of specialist dietitians. Increasingly pregnant women are able to access eHealth resources that may be tailored to their weight status, such as smartphone applications.[122–124] The latter is a particularly innovative example of taking advantage of the teachable moment in pregnancy, aiming to empower women to take control of their own health and fitness without being subjected to professional judgement and stigma. It is important that these applications provide the correct information, and also that it is tailored to the weight status of the user.

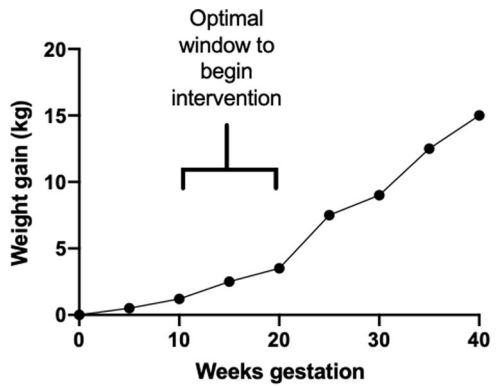

FIGURE 4 Weight gain profile for overweight women in pregnancy. The window of opportunity between antenatal booking and the rapid phase of weight gain is relatively short

There is an extensive literature that considers the efficacy of interventions designed to limit GWG in overweight and obese women, and these interventions have produced a diverse range of sometimes conflicting outcomes. For example, Mottola *et al.*[125] carried out an intervention based upon an individualised nutrition plan (2000 kcal day^{-1}) coupled with a programme of walking three to four times per week. This reduced the likelihood of women exceeding recommended rates of pregnancy weight gain, although average gain was still over 12 kg as a result of excessive gain in early pregnancy. The Mighty Mums intervention was delivered in primary care, exposing women to motivational talks from midwives, food advice from dietetic consultations and prescriptions of physical activity, with women able to access services to suit their preferences.[126] Participants had lower GWG than controls and lower post-partum weight retention. Exposure to dietetic consultation appeared to be the main driver of success. Liu *et al.*[127] implemented a very intensive programme amongst overweight and obese women in South Carolina. Participants attended an individual counselling session, 10 weeks of group sessions, had weekly phone calls from the intervention team and were given access to podcasts once a week.[127] These contacts focused on improving dietary quality, increasing physical activity and self-monitoring of weight. Overall, GWG was not impacted by the intervention. In African American obese women, GWG was increased in the intervention group, whereas overweight African American women had lower GWG than controls.[127] A lighter touch intervention using the Healthy Moms smartphone app for 6 months achieved lower GWG in women who were overweight or obese, but did not bring about changes in physical activity, glycaemia or insulin resistance.[123]

Alongside the many such small-scale interventions that focus on managing maternal weight gain in pregnancy, randomised controlled trials have sought to target either GWG or neonatal health as primary outcomes for diet or physical activity interventions in

pregnancy. Two large randomised controlled trials of approaches to limiting weight gain in pregnancy and the associated risks of poor pregnancy outcomes have been extensively reported. In the LIMIT trial conducted in Australia, Dodd *et al.*[128] found that a diet and lifestyle intervention reduced the risk of a birth weight above 4000 g by 18% (RR = 0.82, 95% CI = 0.68–0.99, $p = 0.04$), but the results for maternal weight gain showed no significance for the intervention group. The UPBEAT trial in the UK (2202 participants) had reduction of GDM as the primary outcome and although GWG was reduced by 0.55 kg by the intervention, GDM and other pregnancy complications were not impacted by the intervention.[129] A meta-analysis of 36 randomised controlled trials (RCTs) found that the success of interventions was highly dependent upon characteristics of the women recruited and whether the interventions were based upon diet, exercise or a combination of the two.[130] Physical activity-based interventions were generally ineffective. Interventions appear to be more effective if delivered by clinicians rather than non-clinical staff.[131] In women of low education, diet-based and mixed approaches reduced the risk of excessive GWG, whereas, in highly educated women, only diet-based interventions were successful. An earlier meta-analysis of 44 RCTs found that interventions that mixed physical activity goals with dietary change were ineffective, but diet-based interventions had the capacity to lower GWG and reduce risk of GHT, PE, GDM, preterm birth and labour induction.[132] The overall analysis found that GWG reductions could greatly exceed those attained by UPBEAT.[129] There is therefore little doubt that appropriately designed and targeted interventions can be effective tools in the management of pregnancies that are complicated by overweight and obesity. The devil lies in the detail, however, and the design, targeting and delivery of large scale, routine care to improve outcomes for overweight women is far from straightforward and is likely to be a major resource burden for local and national health services.

In 2015, we published an analysis of a pilot study for the Lincolnshire Bumps and Beyond intervention.[133] Bumps and Beyond was available to all pregnant women in Lincolnshire whose booking BMI was 35 kg m^{-2} or greater and comprised a programme of seven sessions, which covered healthy eating, physical activity, identification of triggers that lead to unhealthy lifestyle behaviours, and relapse to old behaviours around eating and physical activity. The programme was delivered by healthy lifestyle advisors with previous experience of delivering a smoking cessation programme. The pilot study showed that the intervention reduced GWG by approximately half and resulted in a reduced prevalence of GHT and PE.[133] Subsequent analysis (unpublished dataS.C. Langley-Evans and S. Ellis) with a bigger population confirmed that this intervention limited GWG in severely obese women and that the reduced GWG was associated with dramatically lower risk of PE (OR = 0.050 95% CI = 0.003–0.642).

There are some important lessons to be learned from the Bumps and Beyond intervention.[133] First, it was extraordinarily successful in achieving the goal of reducing GWG by half in severely obese women and in reducing pregnancy complications, which is something that the big randomised controlled trials have failed to do. RCTs are considered to be the pinnacle of the epidemiological hierarchy, but, in the field of nutrition, where the nature of the intervention may be rather different to an RCT utilising a pharmacological agent, they do have limitations. RCTs in nutrition are often less effective than similar studies where the treatments are drugs, because the subjects may become disaffected, fail to see any clear and immediate benefit of taking part, or are disturbed by minor side-effects and drop out, and also because the nature of the intervention may become apparent to the control group, prompting them to change their diet and behaviour in a way that detracts from the analysis. Both of these tendencies were seen with Bumps and Beyond where one-third of participating women failed to complete the full programme and, among some women who did not take part, good control over GWG was still observed, indicating that they had chosen to make beneficial lifestyle changes without the intervention of the delivery team. Rigid RCT protocols, although most useful for researching a specific question, are likely to be less effective than a more flexible, adaptive and multimodal approach in primary care practice and this may be why Bumps and Beyond[133] achieved results that were much greater than LIMIT[128] or UPBEAT.[129]

Another important lesson from Bumps and Beyond was the low uptake of the programme. Only 37.5% of women invited to take part did so, and those that declined were more likely to be living in deprivation or to be experienced mothers (one or more previous pregnancies). Effective intervention strategies need to find a way of including hard to reach social groups because these are the women at greatest risk. The final lesson to be learned from Bumps and Beyond comes from the experience of rolling out the same programme in a different setting. When the programme was operated in the neighbouring county of Nottinghamshire, there was no effect on either GWG or pregnancy outcomes, in contrast to the success in Lincolnshire. This may be explained by greater ethnic diversity in the population that was targeted, a more open recruitment strategy (women with BMI > 30 kg m^{-2}), or the new delivery team having a different skillset and approach to delivering the sessions. It is likely that successful intervention will require pregnant women to be given a more bespoke and culturally sensitive experience founded on a close partnership with a health professional trained in behaviour change techniques. There is evidence that training midwives in healthy conversation skills and extending appointment times

enables the use of those skills and allows them to be effective in raising issues around diet and physical activity with pregnant women.[134] Downs *et al.*[135] explored the feasibility of such an approach in a pilot study. Women in their trial were given 60 min with a dietitian per week between 8 and 20 weeks of gestation and this was either maintained in those who kept GWG within set limits, or was increased in those who exceeded limits. In effect, this was an intervention delivered at adaptive doses to suit the needs of each woman involved. Compliance was 87%, indicating that this intensive input was largely acceptable to the women. Although the intervention reduced GWG by 21%, the trial was too small to determine a statistically significant effect.[135]

CONCLUSIONS

As the global prevalence of overweight and obesity continues to increase year on year, the associated threat to the health and wellbeing of pregnant women and their infants, as well as the cost of managing adverse pregnancy outcomes, is becoming increasingly significant. It is clear that there are approaches that can be taken to reduce the risk of poor outcomes, although, for these to be successful in primary care, investment will be needed for both the training of health professionals and the delivery of interventions suited to the needs of individual women. For the greatest effect, conversations about weight management need to occur in the first trimester, which, although challenging, is likely to be the best time to capitalise on the teachable moment that early pregnancy offers. For greatest impact, the future needs of antenatal weight management in primary care may be best delivered through eHealth approaches.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the writing of this review.

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How to cite this article: Langley-Evans SC, Pearce J Ellis S. Overweight, obesity and excessive weight gain in pregnancy as risk factors for adverse pregnancy outcomes: A narrative review. J Hum Nutr Diet. 2022;35:250–264.
https://doi.org/10.1111/jhn.12999

Received: 14 September 2021 | Accepted: 5 January 2022

DOI: 10.1111/jhn.12990

INVITED REVIEW

JHND Journal of Human Nutrition and Dietetics | BDA The Association of UK Dietitians

Biology of human milk oligosaccharides: From basic science to clinical evidence

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Abstract

Human milk oligosaccharides (HMOs) have been researched by scientists for over 100 years, driven by the substantial evidence for the nutritional and health benefits of mother's milk. Yet research has truly bloomed during the last decade, thanks to progress in biotechnology, which has allowed the production of large amounts of bona fide HMOs. The availability of HMOs has been particularly crucial for the renewed interest in HMO research because of the low abundance or even absence of HMOs in farmed animal milk. This interest is reflected in the increasing number of original research publications and reviews on HMOs. Here, we provide an overview and critical discussion on structure–function relations of HMOs that highlight why they are such interesting and important components of human milk. Clinical observations in breastfed infants backed by basic research from animal models provide guidance as to what physiological roles for HMOs are to be expected. From an evidence-based nutrition viewpoint, we discuss the current data supporting the clinical relevance of specific HMOs based on randomised placebo-controlled clinical intervention trials in formula-fed infants.

KEYWORDS

animal milk, development, growth, human milk, immunity, infections, microbiota

Key points

- This review discusses different aspects of human milk oligosaccharides (HMOs): from their chemistry to biology.
- It provides a comprehensive overview of our current understanding of the clinical relevance of HMOs for infant development and health.
- Cumulative evidence from clinical observations and interventions backed by mechanistic basic research data indicates that HMOs are a meaningful and important component of human milk.
- Increasing evidence suggests that specific HMOs help establish immune competence, both local and systemically, partly through their effect on the metabolite activity of specific microbes mainly *Bifidobacterium* species.
- HMOs may also participate in a gut–brain connection, thereby modulating brain and cognitive development.
- HMOs likely act in concert with other bioactive components and act via different mechanisms that converge to specific functions.

INTRODUCTION

Human milk is the natural, adapted and sole recommended nutrition for infants. It provides not only nutrition for the growth of an infant, but also numerous bioactive components supporting age-appropriate development and immune protection. Consequently, pediatric societies and the World Health Organization (WHO) recommend exclusive breastfeeding to 6 months of age and breast-feeding to be continued to at least 2 years of age.[1,2] Breast milk is mostly composed of water and various solid components with nutritive values and bioactive functions.

Among the breast milk bioactive components, human milk oligosaccharides (HMOs) are a highly represented category, both in terms of amounts and structural diversity. Together with their resemblance to mucous and mucosal surface glycans, and the fact that they are largely undigestible, this has triggered extensive basic and applied research. To date, more than 160 different HMO structures have been described[3,4] with an estimated total concentration ranging between 5 and 15 g L^{-1}.[5–7]

Historically, HMOs were mainly recognised as a fraction in breast milk related to the presence of beneficial bacteria such as bifidobacteria and lactobacilli in infant feces.[8] However, there is now increasing evidence that HMOs could also contribute to broader health benefits of human milk. For over a century, breast milk has been recognised as protecting infants from morbidity and mortality. Indeed, breastfed infants generally experience less gastrointestinal and respiratory infectious illnesses, and show higher cognitive development and lower risk for being overweight or obese.[9] The potential effect of breastfeeding on development of allergies later in life is less clear. Notably, in infants born preterm, breast milk reduces the risk of life-threatening necrotising enterocolitis (NEC), as well as the risk of late onset sepsis, and supports medical treatments to prevent bronchopulmonary dysplasia.[10–12] These benefits of human milk for the infant are key inspirations in the quest to understand the physiological roles of breast milk components such as HMOs.

As Ajit Varki wrote; 'Nothing in Glycobiology Makes Sense, except in the Light of Evolution'.[13] This can serve as a guiding principle when trying to understand the variety and abundance of HMOs found in breast milk and their variability between mothers. In this work, we discuss different aspects of HMOs: from their chemistry to biology, aiming to provide an overview of our current understanding on their clinical relevance for infant development and health.

WHAT ARE HMOS?

HMOs are non-lactose oligosaccharides found in human milk. A stricter definition may be that HMOs are not only present, but also produced directly by the lactating mother's mammary glands. From a physiological angle, HMOs are not digested in the infant gut and hence are not part of the nutritive breast milk components, but, as a result of their numerous roles, are considered bioactive constituents.

From a chemical perspective, all known HMOs are elongations of the milk sugar lactose with one or several of the following monosaccharides: galactose (Gal), *N*-acetyl-glucosamine (GlcNAc), *N*-acetyl-galactosamine (GalNAc), fucose (Fuc) and sialic acid (*N*-acetyl-neuraminic acid [NeuAc]). Lactose is formed solely in the lactating mammary glands by the lactose synthase complex, starting from uridine diphosphate galactose (UDP-Gal) and glucose (Glc). Lactose synthase is a heterodimer composed of the milk protein alpha-lactalbumin and the enzyme beta-1,4-galactosyltransferase 1, which is encoded by the *B4GALT1* gene. The *B4GALT1* gene codes for two enzymatic forms that result from two distinct transcription initiation sites and subsequent post-translational processing. The ubiquitously present first form, a type II membrane-bound, trans-Golgi resident protein, is involved in glycoconjugate biosynthesis adding Gal from UDP-Gal to GlcNAc. The second transcription product results in the soluble lactose synthase, producing lactose by adding Gal from UDP-Gal to free Glc.

Lactose synthase is not reported to further elongate lactose with additional Gal. Rather, the next step of lactose elongation is brought about by a series of other glycosyltransferases (GTs) with different specificities as recently reviewed.[14] Lactose can be elongated by the disaccharides lacto-*N*-biose (LNB; β-Gal-(1→3)-β-GlcNAc) and *N*-acetyllactosamine (LacNAc; β-Gal (1→4)-β-GlcNAc) resulting in the tetrasaccharides lacto-*N*-tetraose (LNT) and lacto-*N*-*neo*tetraose (LNnT), respectively. The exact pathway leading towards the production of the latter two HMOs is yet to be discovered. Both LNT and LNnT are also further elongated by additional LNB and LacNAc units. Although the involved enzymes are not described, it is likely by tight sequential addition of GlcNAc and Gal units. The intermediate lacto-*N*-triose with only a GlcNAc added to lactose is only rarely reported in human milk.[15,16] Both LNT and LNnT as well as their further LNB- and LacNAc-elongated descendants can be decorated with Fuc and NeuAc. Because many GTs are involved in the formation of several linkage types, a large variety of structures differing in composition, conformation and chain length is found in human milk.[3,14,17] Trace amounts of galactosyl-lactoses (GLs), mainly 6'-GL, are also found in human milk[7,18,19] and, although the enzymes involved in their formation are not known, interestingly, no further elongation with Fuc or NeuAc has been reported. This could indicate that the GL synthesis is not localised in the endomembrane system together with the GTs that are involved in the major HMO synthesis pathways. We speculate that different microbes

present in milk, albeit in low concentration, could be responsible for synthesising these GLs in breast milk, similar to the GL formation during milk fermentation with starter cultures.[20]

Elongation of core HMOs with Fuc is catalysed by fucosyltransferases (FUT), which add Fuc from guanosine diphosphate fucose (GDP-Fuc) to lactose (or other acceptor oligosaccharides) through different linkages. These are encoded by *FUT2* and *FUT3*, respectively called the secretor and Lewis gene, as well as further *FUT* genes (e.g., FUT5,6). This leads for example to the formation of 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL) and lacto-difucosyltetraose (LDFT; 2',3-difucosyllactose) (Figure 1). Similarly, several sialyltransferases such as St3Gal4 and St6Gal1 add NeuAc (*N*-acetylneuraminic acid) to lactose (or other acceptor oligosaccharides) in different linkages, resulting in for example 3'-sialyllactose (3'-SL) and 6'-sialyllactose (6'-SL). The formation of the main HMOs that are generally analysed in most studies is depicted in Figure 1.

Based on their chemical composition HMOs may be classified into different categories. The most apparent subclassification is the separation of acidic HMOs harboring one or more sialic acids from neutral HMOs. The latter can be further grouped into those containing one or more fucose units vs. those without a fucose moiety. The acidic sialylated HMOs may be further split to discern acidic non-fucosylated and acidic fucosylated HMOs.

Biology is not only directed by chemical composition, but, importantly, also by the three-dimensional structure. Hence, it is essential to consider structural features when classifying HMOs. The same sugar monomer can be attached in different ways by different enzymes to another saccharide unit. For example, adding a Fuc to Lac can result both in 2'FL or in 3-FL depending on the catalysing GT. Investigating the structures of the major reported HMOs, eight primary differentiating structural features can be distinguished (Figure 1b). Generally, and as recently reviewed, each of these structural features is formed by a specific GT, although some can be formed by several GTs.[14] Although FUT2 specifically adds fucose in α1,2 to an acceptor galactose, FUT3 forms both α1,4 and α1,3 linkages between fucose and GlcNAc, as well as Glc. However, an additional FUT can also form the α1,3 linkages between Fuc and Glc (Figure 1).[21]

The presence or absence of the FUT2 and FUT3 enzymes also allows further stratification of human milk. The FUT2 genotype is linked to secretor status (Se), with women of a $FUT2^{-/-}$ genotype being unable to produce α1,2 fucosylated HMOs, such as 2'-FL and LNFP-I. The FUT3 enzyme determines the Lewis type (Le), resulting in strong reduction of α1,3 and absence of α1,4

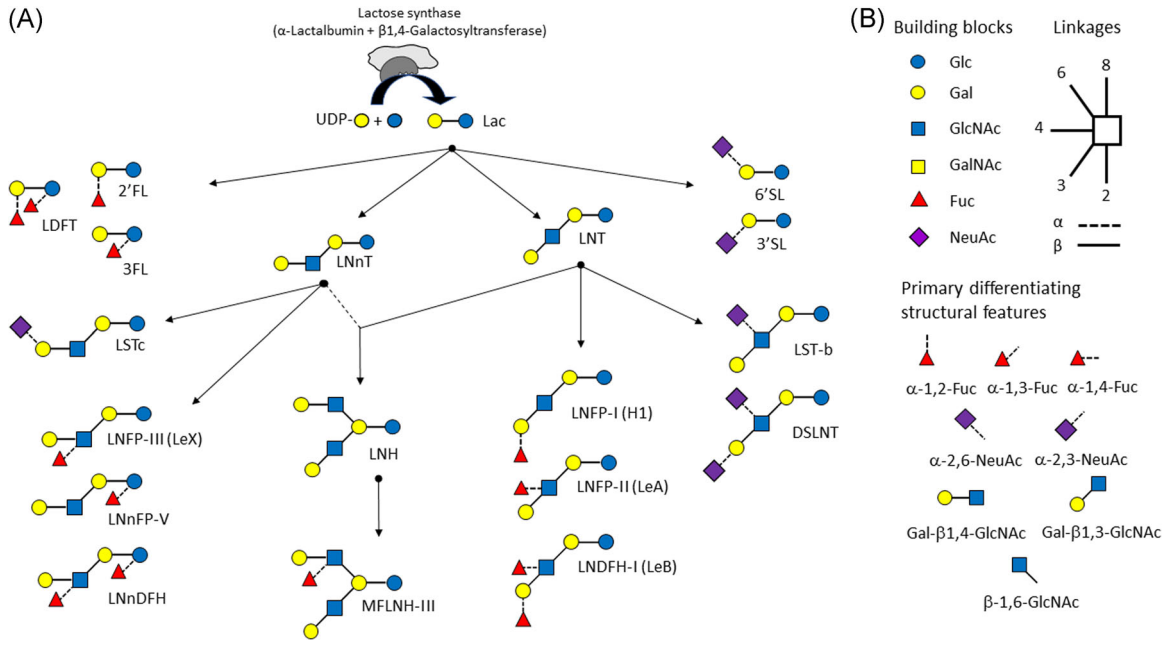

FIGURE 1 Main human milk oligosaccharides (HMOs) found in human milk. (a) Illustration of main HMOs and their synthetic route starting from lactose that is formed by lactose synthase complex from UDP-Gal and Glc in the mammary glands. (b) Key to the monosaccharide building blocks and linkages of HMOs and illustration of the eight primary differentiating structural features represented in HMOs. 2'FL, 2'Fucosyllactose; 3FL, 3Fucosyllactose; 3'SL, 3'Sialyllactose; 6'SL, 6'Sialylactose; DSLNT, DisialyllactoNtetraose; Fuc, fucose; Gal, galactose; GalNAc, *N*-acetyl-galactosamine; GlcNAc, *N*-acetyl-glucosamine; Glc, glucose; NeuAc, *N*-acetylneuraminic acid (sialic acid); LDFT, Lactodifucosyltetraose; Le (A,B,X), Lewis (A,B,X); LNnT, LactoNneotetraose; LNT, LactoNtetraose; LNFP (I,II,III), LactoNfucopentaose (I,II,III); LNnFP V, LactoNneofucopentaose V; LNnDFH, LactoNneodifucosylhexaose; LNDFH I, LactoNdifucosylhexaose I; LST (b,c), SialyllactoNtetraose (b,c); MFLNH III, MonofucosyllactoNhexaose III

fucosylated HMOs in milk. Lewis and Secretor type thus allow grouping of human milk in four main milk groups, also sometimes named 'lactotypes', which will be discussed later. Milk groups show significant differences in HMO profiles even beyond those HMOs directly affected by the *FUT2* or *FUT3* genotype.[7] Possible explanations are alterations in acceptor (e.g., LNT) or altered donor availabilities (GDP-Fuc) if one or the other enzyme is not active: for example, absence of a downstream fucosylation enzyme results in a higher proportion of sialylated HMO structures. HMO profiles are thus not solely dependent on the direct control by specific enzymes and their expression pattern. This means that the HMO structural diversity and composition are closely tied to their biosynthesis and any maternal factors, such as genetic background, health condition, environmental factors and diet, modulating the expression and function of maternal GTs, as well as substrate availabilities. Whether or not reported differences have a physiological significance for the suckling newborns is a topic of intensive research.

Of note, GTs involved in HMO biosynthesis in the mammary glands, such as FUT2 for example, are also expressed in other body parts, where they are involved in cell and mucous glycosylation. Therefore, HMOs may be considered as soluble lactose-bound analogues of typical mammalian cell glycocalyx and mucous glycans, which represent a dense glycan matrix at the interface with other cells and the environment, including the microbiome.

WHERE ARE HMOS FOUND?

Although human milk is particularly rich in both amount and number of oligosaccharides with a high diversity of structural features not generally seen in other mammals, all mammals produce the milk sugar lactose in their milk and all mammals are equipped with a series of GTs such as those involved in HMO synthesis (see earlier). Milk of different mammalian species greatly varies in amount, number and diversity of structural features of their milk oligosaccharides.[22] Some are common to those found in human milk, whereas others are not. Among the HMOs more universally found in mammals, including monotremes (e.g., platypus), marsupials and eutherians (i.e., placental mammals), are the sialyllactoses, primarily 3'-SL.[22,23] The milk of the egg-laying platypus contains 3'-SL and larger sialylated structures together with predominantly fucosylated lactose, such as LDFT, but also larger fucosylated oligosaccharides built on LNnT and LNT.[23] Similar oligosaccharides are also reported in milk of *Echidna*, another egg-laying mammalian species.[24] These observations suggest that specific oligosaccharides are ancestral features of milk. As a result of the immaturity of monotremes at birth, these oligosaccharides were also suggested to be of particular importance for development and immune protection. Noteworthy, 3'-SL and 6'-SL are found in mouse and rat milk as highly predominant milk oligosaccharides, rendering these animals, who are born relatively immature, relevant models to study their role for growth and development.[25–28]

Farmed animal milks also contain oligosaccharides, but at relatively low concentrations. Among them are 3'-SL, 6'-SL and primarily other neutral non-fucosylated oligosaccharides such as galactosyllactoses.[29] Generally, their concentration decreases very rapidly from colostrum to mature milk.[29] In bovine milk, 3'-SL is the most prominent oligosaccharide with a concentration reported to range between 50 and 100 mg L^{-1} compared to an approximately two-fold higher amount observed in mother's milk. Notably, 3'-SL was reported to increase in bovine milk around 2 weeks before parturition from around 100 to 700 mg L^{-1} and to steeply drop from around 800 mg L^{-1} in colostrum to around 100 mg L^{-1} by 3 days postpartum.[30,31]

Several structural features characterise human as opposed to animal milk oligosaccharides. Human milk shows a predominance of type 1 structures (LNB), built on LNT cores, whereas animal milks that have larger oligosaccharides mainly show type two structures (LacNAc), built on LNnT cores.[32,33] Because humans cannot synthesise the *N*-glycolyneuraminic acid (NeuGc) form of sialic acid, mother's milk only contains oligosaccharides with NeuAc, whereas animals do show also NeuGc on milk oligosaccharides in different proportions compared to NeuAc.[34,35] For example, goat milk contains approximately 70% NeuGc and 30% NeuAc, whereas, in bovine milk, over 95% of sialic acid is NeuAc.[36]

ARE ALL HMOS THE SAME?

As outlined in the previous section, each HMO is structurally distinct from another. However, some commonalities exist in composition and the primary differentiating structural features. Interestingly, it should be noted that, of all theoretically possible structures, only a limited and a finite number of structures are made and present in human milk. Although HMO diversity and richness suggest many structure-specific functions, the many different structures may also indicate a certain functional redundancy. The more universally present milk oligosaccharides, such as the sialyllactoses, are likely to contribute to similar more universal physiological needs of different mammals during the early postnatal life. On the other hand, different mammals have different postnatal nutritional and functional requirements that strongly depend on their maturity at birth, their speed of postnatal development and their environment. Hence, general milk composition differs among mammalian species and probably represents adaptation to their newborn's requirements. The question is: are milk oligosaccharides part of such an adaptation of milk?

In humans, HMO profiles strongly change by maternal polymorphisms in the Lewis blood group system (i.e. FUT2 and FUT3 polymorphisms) and by duration of lactation.[7,37,38] The distribution of the Lewis blood group polymorphisms indicates that this trait strongly depends on evolutionary pressure and selection with approximately 10%–35% FUT2 (secretor) negative genotypes in different geographies.[39–41] Population genetic studies confirm the presence of balancing selection acting upon FUT2, an indication of advantages linked to maintaining genetic variation.[39,42] The prevalence of FUT2 negative genotypes varies across different geographies, thus contributing to reported geographic differences in HMO profiles.[43] Additional environmental and maternal factors may also contribute to HMO variability, although their effect size may be rather modest.[7,43] Comparison of HMO profiles from mothers who gave birth to preterm vs. term infants indicated that sialyllactose is slightly higher and FUT2 dependent HMOs such as 2'-FL and LNFP I slightly lower in early milk of mothers who gave birth to a preterm infant.[18] Whether such observations represent an adaptation of the milk for the physiological needs of the infant or rather reflect the physiological state of the mother needs further investigation. Interestingly, birth appears to trigger a program that determines how the HMO profile changes over the period of lactation.[18] The stage of lactation is a key parameter affecting HMO profiles, with most HMOs decreasing and a few increasing in concentration over the first few months and even beyond the first year of lactation.[7,38] 3-FL is found among the HMOs generally increasing in concentration and, although the reason for this is unknown to date, we speculate that 3-FL may have a particular role beyond the exclusive breastfeeding period. When estimating the daily average intake, most HMOs show a relatively constant intake, whereas 3-FL intake increases with time of lactation.[44]

In basic research models, both redundancy and specificity with selected HMOs is reported. In animal models coupled with cell-based models and modelling, both 2'-FL and 6'-SL were shown to affect NEC via Toll like receptor 4 (TLR-4) mediated route.[45] Similarly, 2'-FL and 3-FL were shown to interact with dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), whereas LNT did not bind.[46] In yet another study, 6'-SL and LNT both activated the G-protein coupled receptor GPR35, with both together activating stronger, whereas 2'-FL, LNnT and 3-'SL did not activate this receptor.[47] When it comes to stimulation of specific *Bifidobacterium* species, many more examples of redundancy and specificity exist. Such redundancies indicate their physiological importance and are likely to come into play to compensate FUT2 and FUT3 polymorphisms. Detailed observational studies coupled with mechanistic insight are warranted to understand such initial observations and hypothesis.

MANUFACTURED HMOS ADDED TO INFANT FORMULA: ARE THEY SAFE AND WHAT ARE THEIR BENEFITS?

The relatively large amounts of diverse HMOs found in human milk contrasts with milk from farmed animals used for human nutrition, which contain only very small amounts of oligosaccharides. To close this gap for animal milk-derived breast milk substitutes in child nutrition, different technological strategies are possible. For example, the animal milk oligosaccharide-rich fraction can be concentrated, an approach that can provide some oligosaccharides such as the sialyllactoses but, as a result of the low initial amounts, this is technically challenging.[48] Alternatively, individual HMOs may be produced by chemical, enzymatic or biotechnological means. Recent progress in these fields has enabled industrial production of individual HMOs that are not typically found in farmed animal milks.

Today different biotechnological processes using bacterial fermentation are the most industrially viable technologies. A handful of individual HMOs are available at industrial scale, representing some of the most abundant HMOs found in breast milk. Although they have the primary structural features of HMOs (Figure 1), they are still relatively simple compared to the some of the larger HMOs found in human milk.

These technologies involve novel processes, as defined by the regulatory authorities such as the European Food Safety Agency (EFSA) or the Federal Drug Administration (FDA). Today, a select number of HMOs (2'-FL, 3-FL, LDFT, LNnT, LNT, 3'-SL, 6'-SL) have obtained Generally Recognized As Safe (GRAS) and Novel Food status. These HMOs, produced by biotechnology, are identical in structure to those naturally present in breast milk and are therefore dubbed 'human identical milk oligosaccharides' abbreviated as HiMOs. These ingredients are diligently analysed, characterised and subjected to a series of safety/toxicity tests according to guidelines established by the Organisation for Economic Cooperation and Development (OECD). Generally, to best match the target application in early life nutrition, the *in vivo* OECD testing protocol with animals was adapted to include a juvenile period.[49,50]

WHAT ARE THE PHYSIOLOGICAL ROLES OF HMOS?

The physical and physiological development of infants is intricately linked with their environment, including key influences such as nutrition and the developing gut microbiome. In terms of early life nutrition, breast milk feeding with its high abundance and variable amounts of different HMOs is the optimal nutrition for the developing infant and its microbiome. Yet some HMOs may

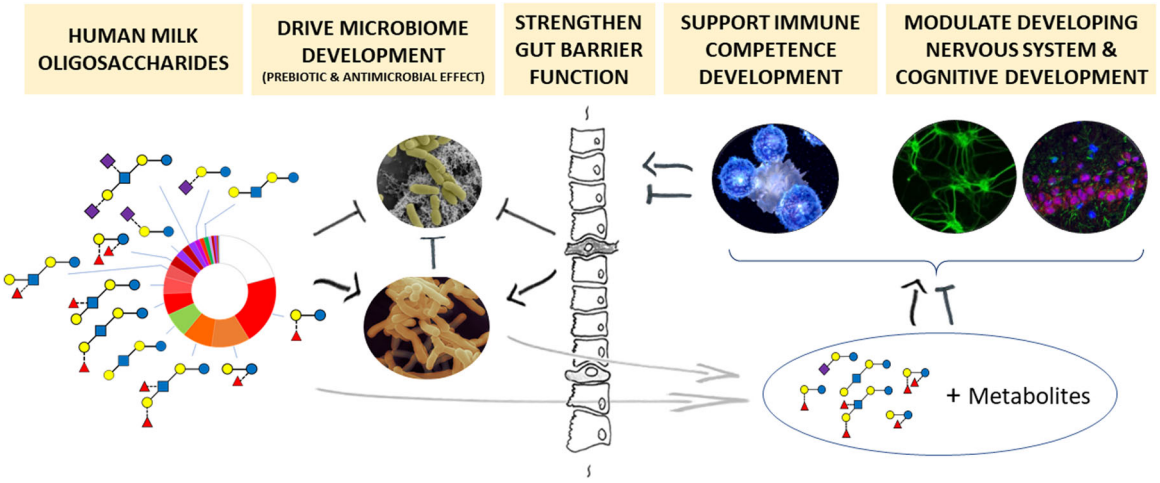

FIGURE 2 Summary illustration of main expected human milk oligosaccharide (HMO) functions reported in literature

have redundant roles and some may have synergistic functional effects, and hence simple associations between particular HMOs and their health effects may be difficult to establish. Ultimately, hypotheses need to be tested and causality established through mode-of-action research. In the following, we provide an overview of observations from different studies with breastfed infants and intervention trials with HMO formula-fed infants. Where possible, we discuss basic research data relevant to support clinical observations. Figure 2 illustrates the main proposed functions of HMOs.

HMOs and the development of the early life gut microbiome

A bona fide gut colonisation with microbes starts with maternal and environmental microbiota seeding, followed in part by appropriate selection through undigested dietary components. To this end, breast milk provides a multitude of diverse oligosaccharides (HMOs), together with numerous other key components such as immunoglobulins, lactoferrin and beyond. Primarily during the recommended exclusive breastfeeding period through 4–6 months of age, infants who are exclusively or partially breastfed show a different maturation trajectory of their gut microbiome compared to their non-breastfed peers. This is first reflected in a lower microbiota diversity index and secondly in a higher microbiota age or maturity compared to the infant's chronological age in formula fed infants.[51–53] These measures indicate that low or no breast milk intake accelerates maturation of the gut microbiome towards that observed in adults.[52]

At the microbiota taxonomic level, breastfed infants show primarily higher relative abundance of Bifidobacteriaceae family members during the early exclusive breastfeeding period. Notably, primarily strains from the classes Actinobacteria, including Bifidobacteriaceae, and Bacteroidia were found to be vertically transferred from the mother to her infant.[54] Additionally, breast milk was identified as the most important covariate explaining microbiome variance at genus and species taxonomy levels, as well as the microbiome functional capacity.[53] The importance of bifidobacteria especially during early life for gut ecology and development is well established[55–57] and highlights the importance of maternal seeding and feeding.

Generally, HMOs are not digested by the infant's digestive enzymes, although intestinal neuraminidase cleaving sialylated HMOs may be an exception.[58] Different studies with breastfed infant–mother dyads have investigated HMO profiles in breast milk and infant stools. Although some studies report only relatively small changes, others found that HMO profiles in some infant stools changed more dramatically.[44,59–61] In addition to important inter-individual variability, HMO profiles are strongly associated with specific microbiota taxa, primarily *Bifidobacterium*, *Bacteroides* and *Lactobacillus*.[61]

*Bifidobacterium*species are genetically particularly well equipped to take advantage of the HMO diversity and abundance in breast milk.[62–64] Their dominance in early life is largely attributed to this unique genetic glycan-foraging capacity, which is likely a result of coevolution with the host.[65–67] Several studies have investigated the complex glycan degradation capabilities of early life colonisers and all showed the unique capacity of bifidobacteria to utilise HMOs, whereas other strains favor less complex oligosaccharides and sugar monomers as carbon sources for growth.[68–70] In general, two large strategies are deployed by bifidobacteria to utilise HMOs: (1) the secretion of glycosidases that externally degrade HMOs followed by the uptake of sugar monomers by the bifidobacteria (e.g., *Bifidobacterium bifidum* and some *Bifidobacterium longum* strains) and (2) the expression of dedicated HMO transporters that can internalise HMOs for further internal degradation.[63,71] The

latter strategy is found for example in *Bifidobacterium breve*, *Bifidobacterium longum* subsp. *infantis* and most other *B. longum* strains.[63,71] Interestingly, strain-specific differences in HMO utilisation capacity were reported within the same *B. breve* and *B. longum* subsp. *infantis* species.[63,67,72,73] To what extent these strain specificities reflect a personalised mother–infant exchange of microbes and nutrition, or indicate a gradual gene pool loss of key microbes, needs to be established in detail.

Another group of microbes able to utilise HMOs for growth are *Bacteroides* species.[74] However, in a gnotobiotic mouse model associated with a *Bacteroides* and a *Bifidobacterium* species, the HMO LNnT boosted the abundance of the *Bifidobacterium* species over the *Bacteroides*, although both species were able to utilise LNnT.[75] Although the *Bacteroides* species can equally use mucous glycans and HMOs, the *Bifidobacterium* species can only use HMOs as growth substrate and HMOs appear to provide a selective advantage to *Bifidobacterium* species. Other early gut colonisers such as the Enterobacteriaceae, which includes several pathogens, are generally not able to grow on HMOs.[76] For *Streptococcus* species, 2'-FL contrary to lactose or galactooligosaccharides (GOS) was shown not to allow for *Streptococcus mutans* growth and LNT was identified to interfere with a Group B *Streptococcus* (GBS) cell wall synthesis, which leads to cell death and higher sensitivity to antibiotics.[77–80] The former may have a clinical relevance for oral health, whereas the latter may be of relevance to reduce sepsis risks and antibiotic dosing especially in infants born preterm. A second reason behind the dominance of *Bifidobacterium* species in the gut in early life, is its social behavior. Collaboration and cross-feeding may occur between different *Bifidobacterium* strains and between different species.[66,81–83] The first phenomenon relies on the presence of different HMO utilisation loci in different bifidobacteria, resulting in crossfeeding chains, further enhancing their dominance.[66] Crossfeeding of other strains relies on several HMO metabolites produced by bifidobacteria, such as the short chain fatty acid acetate supporting *Anaeostipes caccae* a butyrogenic species[71,84] or sugar oligo- and monomers supporting growth of other species unable to degrade HMOs, such as lactobacilli.[69] An elegant study showed that an early life gut microbiome community with a very high Bifidobacteriaceae dominance is established in presence of *Bifidobacterium* strains, in this case specific strains of *B. breve*, which have the genetic make-up to utilise specific HMOs such as 2'-FL.[67] Concomitantly, stool from infants with a microbiome harboring this 2'-FL utilising capacity was also shown to have lower pH, higher acetate and lower remaining 2'-FL from breast milk, indicating higher metabolic activity. Such gut ecology changes with higher acetate, likely combined with other metabolites, was shown in animal models to lead to improved protection against gastrointestinal and respiratory tract infections.[85,86] Infant gut microbiome HMO utilisation capacity was recently shown to relate to less inflammatory markers and specifically *B. longum* subsp. *infantis* derived metabolites such as indolelactate were shown to drive such pathways.[87,88] As expected, such bifidobacteria activity driven processes are very relevant for appropriate immune competence development.

HMOs and infant anthropometry

Along with environmental, genetic, epigenetic and metabolic factors, the developing gut microbiome is considered as a key factor affecting infant growth.[89] Considerable evidence suggests that the disruption of an age-appropriate gut microbiota assembly and succession could lead to growth faltering. For example, in infants born preterm, delayed microbiota succession or maturation was related to lower weight-for-age *z*-score (WAZ), leading to the hypothesis that the microbiome, influenced by nutrition, may play a causal role in promoting growth.[90] Similarly, studies focusing on undernourished infants found that an altered gut microbiota could be causally related to growth, with causality being established via studies in gnotobiotic animal models.[91–94] Similar to observations in preterm infants, the microbiota in stunted or undernourished infants is immature, as concluded from a modelling approach that used the microbiota composition to predict an infant's chronological age.[91,93] Based on their effect on the establishing gut microbiota, HMO are also investigated in relation to infant growth as summarised in Table 1.

Interestingly, in two Malawian mother-infant dyad cohorts with a total of 303 infants, lower fucosylated and sialylated HMOs (for the latter primarily LST-b) were observed in breast milk of non-secretor mothers whose infants were stunted compared to those showing normal growth.[92] No significant difference was seen in secretor mothers whose child was stunted or growing normally. Using gnotobiotic mice with a microbiota from stunted infants and sialylated oligosaccharides derived from bovine milk, primarily 3'-SL, a link between the HMOs, the microbiota and infant growth was reproduced.[92] This indicates that specific milk oligosaccharides may act via the microbiota to modulate infant growth. Importantly, a mechanistic link between the sialylated bovine milk oligosaccharides, essentially sialyllactose and bone formation was shown using the gnotobiotic mouse model.[95] A recent observational study in rural Malawi ($n = 647$) reported a significant association of HMO absolute abundance at 6 months with length-for-age change from 6 to 12 months, but no relationship between sialylated HMOs and growth.[96]

Several observational studies have investigated a possible link between breast milk oligosaccharides and infant growth in well-nourished breastfed infants born at term. Although some associations were found, only a few are consistent across different studies.[96–105] For example,

TABLE 1 Summary of reported associations between human milk oligosaccharides (HMOs) and anthropometric measures

Anthropometry measure	Infant age (months)	Associated HMOs		Study type	Feeding mode	Reference
		Positive	Negative			
Stunted growth			Fucosyl- HMOs sialyl- HMOs	Obs	BF	92
Height/length-for-age *z*-score change	6–12	HMOs abundance[a]		Obs	BF	96
Height/length-for-age *z*-score	5	LNFP I + III, DFLNHa		Obs	BF	107
Height/length *z*-score	3–12	2'-FL	LNnT, LST-b	Obs	BF	100
Height/length-for-age *z*-score[b]	5	3'-SL, LDFT	LNnT, DFLNH	Obs	BF	101
Weight	6		LNFP I	Obs	BF	97
Weight gain	1–6		LNFP II	Obs	BF	104
Weight *z*-score	3–12	2'-FL, 3-FL	LNnT	Obs	BF	100
Weight velocity[b]	0–5	2'-FL	LNnT	Obs	BF	101
Weight-for-age *z*-score	2–6	3'-SL, 6'-SL		Obs	BF	106
Weight-for-age *z*-score	5	3'-SL	LST-c	Obs	BF	107
Weight-for-length gain	0–4	3'-SL		Obs	BF	103
Head circumference SDS	3–12	Non-secretor milk		Obs	BF	105
BMI-for-age *z*-score[b]	5		6'SL	Obs	BF	101
BMI SDS	3–6	Non-secretor milk		Obs	BF	105
Lean mass	6		LNFP I	Obs	BF	97
Fat mass	6	LNFP II, DSLNT	LNFP I	Obs	BF	97
Fat mass	2–6	3'-SL, 6'-SL, DSLNT		Obs	BF	106
Fat mass index[b]	5	2'-FL, LDFT,	LNnT, DFLNH	Obs	BF	101
Percent fat	6		LNnT	Obs	BF	97
Weight, length, head circumference and their *z*-scores	0–4	No association seen in secretor positive vs. secretor negative milk		Obs	BF	99,103
Weight, length, head circumference and their *z*-scores	0–4	No differences observed with 2'-FL alone or combined with LNnT or LNT, 3'-SL, 6'-SL, LDFT or 3-FL		RCT	FF	109–111,113–116

Abbreviations: BF, breastfed; BMI, body mass index; DFLNH, DifucosyllactoNhe; FF, formula fed; HMO, human milk oligosac charides; Obs, observational; RCT, randomised placebo-controlled trial; SDS, standard deviation score.

[a]Abundance assessed by integration of collected ion signals.

[b]Association seen in secretor positive milk fed infants only.

in Hispanic mother-infant pairs ($n = 157$), a higher LNFP II concentration in breast milk at 1 month of lactation was associated with lower weight gain from 1 to 6 months of age,[104] whereas, in a previous analysis of a small cohort ($n = 25$), LNFP II at 6 months of lactation was associated with higher fat mass at 6 months of age.[97] However, major differences exist among the different studies with respect to design, geography, sampling during lactation, number of time points at which growth parameters are assessed, the specific HMOs that were analysed and the statistical methods applied to model the associations. Disialyllacto-*N*-tetraose (DSLNT) concentration at 6 months of lactation was associated with higher fat mass of infants at 6 months of age.[97] In another US cohort, DSLNT intake at 2 months was also related to subsequent fat mass through 6 months.[106] Additionally, in the same study, 3'-SL and 6'-SL at 2 months of lactation were associated with higher fat mass and WAZ from 2 to 6 months of age.[106] In a small cohort of mothers and infants from The Gambia, 3'-SL was found to be associated with an increase in WAZ, whereas other sialylated HMOs such as LST-c showed the opposite association.[107] In a recent European multicenter study of 370 mother–infant dyads, 3'-SL was the only HMO associated with higher weight for length gain during the first 4 months of lactation.[103] Similarly, associations between fucosylated HMOs (e.g., 2'-FL, LNFP I, LNFP II) or neutral non-fucosylated HMOs

(e.g., LNnT) and growth, show conflicting results in different observational studies. In two studies, 2'-FL was associated with higher growth velocity and fat mass index from birth to 5 months and length and weight *z*-scores at 3 months of age.[100,101] In the same studies, LNnT was inversely correlated with 2'-FL, and it was proposed that the 2'-FL/LNnT ratio at 3 months is associated with higher length and weight *z*-scores.[100,101] To date, no other study has confirmed these observations.

Biological plausibility for some of these observations may be built on the following hypothesis. As previously mentioned, HMO intake may increase food efficiency through microbiome related processes, explaining how specific HMO–microbiota pairs could affect infant anthropometry. Another hypothesis, proposed recently, is that HMOs affect food-responsiveness and appetite through a microbiome driven process that affects the entero-endocrine system or central nervous system. Indeed, specific HMOs were recently shown to be both positively and negatively associated with food-responsiveness.[108] However, a consistent picture explaining associations between HMOs and anthropometric findings has not yet emerged.

As a result of insufficient data, it is not possible to explain the above inconsistent and often contradictory associations between HMO and infant growth. However, we can speculate that factors such as gut microbiota differences (e.g., epigenetic and genetic differences) and maternal nutritional status during pregnancy may be important confounding variables. It should also be noted that, generally, the observed effect sizes of associations between breast milk HMOs and growth in healthy well-nourished infants are modest and within normal growth trajectories. Moreover, because associations do not imply a causal relationship, causality needs to be established using randomised placebo-controlled interventional trials (RCTs) and supporting mechanistic studies.

Under controlled and randomised conditions, growth of infants fed formula containing individual HMOs in different combinations, 2'-FL either alone or in combination with LNnT or in combination with LNT, 3'-SL, 6'-SL, LDFT or 3-FL, was similar to control formula-fed infants, without HMOs and whenever assessed, equivalent to infants exclusively breastfed for at least 4 months.[109–116] These RCTs assessed infant growth in healthy infants born at term with growth as the primary study end point. All trials concluded that addition of specific HMO or HMO blends to infant formula are well tolerated and allow for age-appropriate growth. However, whether the addition of specific HMOs could help to improve growth in specific conditions of malnutrition and growth faltering, or in infants born preterm, is unknown and needs to be established in RCTs coupled with mechanistic studies. Clinical findings on the link between HMOs and immunity and infections are summarised in Table 2.

TABLE 2 Summary of observed associations between human milk oligosaccharides (HMOs) and reduced risks for infant health related outcome measures

Measure	Infant age (months)	HMOs	study type	feeding mode	References
Necrotising enterocolitis	Preterm	DSLNT	Obs	HM	127,128
Necrotising enterocolitis	Preterm	HMO diversity	Obs	HM	129
Immunoglobulin E-associated eczema[a]	48	2'-FL, secretor positive milk	Obs	BF	136
Cow milk protein allergy	18	LNFP III, LNFP I, 6'-SL, DSLNT	Obs	BF	141
Sensitisation	12	HMO profile[b]	Obs	BF	145
Plasma cytokine profile[c,d]	1.5	2'-FL	RCT	FF	147
Diarrhea	9	2'-Fucosyl-HMOs	Obs	BF	153
*Campylobacter*diarrhea	9	2'-FL	Obs	BF	153
Morbidity	4	2'-Fucosyl-HMOs	Obs	BF	107
Diarrhea	ca 11	2'-Fucosyl-HMOs	Obs	BF	159
Morbidity	3	LNFP II	Obs	BF	160
Prescribed antibiotic use[d]	12	2'-FL + LNnT	RCT	FF	109
Lower respiratory tract infections[d]	12	2'-FL + LNnT	RCT	FF	109
Overall infections[d]	1.5	2'-FL	RCT	FF	111

Abbreviations: BF, breastfed; FF, formula fed; HM, human milk fed; HMO, human milk oligosaccharides; Obs, observational; RCT, randomised placebo-controlled trial.

[a]In C-section born only.

[b]Relative higher concentrations of FDSLNH, LNFPII, LNnT, LNFPI, LSTc, FLNH and lower concentarions of LNH, LNT, 2'-FL and DSLNH.

[c]Interleukine receptor antagonist (IL-1ra), IL-1a, IL-1b, IL-6 and tumour necrosis factor α (TNF-αa).

[d]Secondary exploratory outcome measures.

HMOs and immune competence development

Development of the immune system starts *in utero* and continues with exposure to new stimuli during postnatal development. Several stimuli important for immune development stem initially from the maternal microbiome passing through the placenta to the fetus.[117] Postnatally, although maternal microbial metabolites continue to affect the newborn (e.g., through breast milk), exposure to metabolites and components from the infant's developing intestinal microbiome is much greater and more important. HMOs are considered to affect immune system development by their major influence on the establishment of the infant gut microbiome and its metabolic activity.

Development of the immune system is particularly important in infants born preterm, who are at increased risk of numerous health problems such as NEC, sepsis and cerebral palsy, which are all at least partly linked to an inappropriate immune reaction. Human milk feeding strongly reduces the risk of developing these diseases, probably by its immune modulating effects through components with direct action and others that involve the development of the gut microbiome.[11,118–121] Several blends and individual HMOs have been investigated for their protective effects in preclinical NEC models. In rodent models, not only DSLNT,[122] but also 6'-SL and 2'-FL,[45,123,124] showed some protection against the severity of NEC. Although a mechanism of action has yet to be established for DSLNT, 2'-FL appears to ameliorate NEC symptoms through the modulation of endothelial nitric oxide synthase (eNOS) leading to increased gut perfusion.[123] Interestingly, 2'-FL was previously shown to improve vascularisation in another model system.[125] Additionally, in mouse and piglet models of NEC, both 2'-FL and 6'-SL reduce clinical measures of NEC and inflammation, partly through the inhibition of TLR-4 signalling, which is implicated in the onset of NEC.[45] From *in silico* modelling, both 2'-FL and 6'-SL were predicted to dock to TLR-4, thus inhibiting signalling. In other preterm pig models for NEC, 2'-FL alone or in combination with other HMOs including 6'-SL did not lead to significant reduction in NEC symptoms.[126] Of note, in one study a blend of > 20 HMOs including DSLNT was tested, but did not reduce NEC symptoms in a preterm pig model.[126] Although clinical observations have not shown associations between 2'-FL or 6'-SL with NEC, higher DSLNT concentrations in breast milk were found to be associated with, and to be a good predictor for, a lower risk of NEC in two independent preterm infant cohorts,[127,128] although this association was not confirmed in another smaller study.[129] This latter study also reported a link between low HMO diversity in breast milk and NEC.[129] Although possible explanations for the association between DSLNT and NEC remain elusive, a recent study[128] suggests that DSLNT intake may be associated with a more age-appropriate microbiome progression.[128] Clearly, further research using an interventional design is needed to establish a causal link between DSLNT and NEC risk. It would also be worthwhile to investigate whether the same benefits of DSLNT on reduced NEC risk are observed with donor breast milk as with mothers' own milk reported in the current studies. This will allow us to better understand whether additional maternal factors need to be considered in combination with the HMOs to understand their possible physiological role.

The role of breastfeeding in relation to risks of developing allergic diseases is ambiguous[9,130–132] possibly partly because of the large variability in breast milk composition. As iterated before, HMO composition in breast milk is highly variable and has profound effects on the neonatal microbiome development, which itself is related to sensitisation and development of allergic manifestations.[133–135] Hence, several studies have investigated possible associations between the HMO composition of human milk and allergic manifestations in breastfed infants.

In a Finnish cohort ($n = 266$), infants with a hereditary risk of developing allergies and born by Caesarian section had an earlier onset of immunogloulin E-associated eczema when breastfed by secretor-negative mothers compared to those fed by secretor-positive mothers.[136] The infants fed secretor-negative breastmilk also showed a more pronounced delay in establishing a bifidobacterial-dominated microbiome at 3 months of age compared to fed with secretor-positive breast milk.[137] Among the affected bifidobacteria, specifically *B. breve* has been associated in independent studies to reduce the risk of pediatric eczema.[138,139] As summarised in a recent systematic review,[139] observational data indicate that lower Bifidobacteriaceae abundance in infancy is associated with a higher risk of eczema, especially in infants with family history of atopy. However, the underlying mechanisms and the individual *Bifidobacterium* species involved remain unknown.

A small case–control study of 20 mother-infant pairs from a larger birth cohort in Sweden found no association between the concentrations of nine neutral HMOs and risk of developing allergic disease up to 18 months of age.[140] In another case–control study ($n = 39$ and 41), several individual HMOs (LNFP III, LNFP I, 6'-SL, DSLNT) were observed to be lower in breast milk fed to infants with cow milk protein allergy, with LNFP III showing the strongest signal[141] compared to non-allergic infants. Of these, not only 6'-SL, but also 2'-FL provided some protection against development of a food allergy compared to lactose in an ovalbumin food allergy animal model.[142] The mechanism for this effect was probably partly related to mast cell stabilisation leading to less histamine release. For LNFP III, further mechanistic insight may be gained based on its immune modulatory functions.[143,144]

Another large clinical observation study ($n = 421$ mother–infant dyads) did not observe any association between individual HMOs and food sensitisation.[145]

Instead, out of the 19 measured HMOs, a specific profile classified using projection on latent structures-discriminant analysis was found to be related to lower risk for food sensitisation. This profile could be characterised by relative higher concentrations of FDSLNH, LNFPII, LNnT, LNFPI, LSTc and FLNH, and relatively lower concentrations of LNH, LNT, 2'-FL and DSLNH. Similarly, in another birth cohort ($n = 285$) of infants at risk of allergies, specific HMO profiles classified by latent class analysis (LCA) were reported to be associated with allergies up to 18 years of age.[146] Although the approach to classify HMOs in profiles is promising and deserves to be extended to other breast milk components, its interpretability can be challenging. For example, Lodge et al.[146] used LCA, a method that works with binary 'yes/no' data. To transform the HMO concentrations into a 'yes/no' signal, HMO was considered as 'yes' when above the median and as 'no' when below it.

To understand if and how HMOs modulate sensitisation and allergy risk in breastfed infants, large well-controlled cohort studies with nested case-control analysis are needed. To help interpretability, it may be useful to include HMO classifications with large differences between HMO concentrations. For example, profiles determined by maternal FUT2 and FUT3 genotypes or classifications considering highest and lowest quartile comparison. In addition to HMOs, other known immune active breast milk components such as transforming growth factor β should also be considered. The developing gut microbiome may also strongly affect expected functions of HMOs and should therefore be part of such investigations. Information may be gained by machine learning approaches to better understand whether symptoms or sensitisation can be explained by a combination of input features such as infant and maternal parameters, environmental factors, HMOs and infant microbiome data.

To date, no randomised controlled intervention trial has investigated the role of HMOs in the prevention of sensitisation and allergic diseases. Only one intervention trial assessed plasma cytokine profiles as a proxy for immune maturation in a subgroup analysis of infants fed formula supplemented with 2'-FL at two concentrations and in combination with GOS, against only GOS in the control formula.[147] Infants who received 2'-FL in the formula showed similar basal and stimulated plasma cytokine profiles compared to the profiles in breastfed infants, but not those who received formula with GOS alone.

HMOs and infectious illnesses

HMOs are largely undigested by the infant digestive enzymes. This observation, together with the recognition that the HMOs resemble mucous and cell surface glycans, triggered the hypothesis and concept that HMOs may serve as soluble ligands for pathogens and their toxins, as these often first attach via glycan ligands to epithelial cells.[148,149] Today, many different gastrointestinal and respiratory tract viral and bacterial pathogens have been shown to either bind to specific HMOs, or specific HMOs were shown to block adhesion of specific pathogens.[150] Interestingly, specific HMOs, including LNT for example, were reported to have antibacterial activity by interfering with biofilm formation, cell wall synthesis and function of opportunistic pathogens such as *Streptococcus agalactiae* (Group B *Streptococcus*, GBS), *Staphylococcus aureus* and *Acinetobacter baumannii*.[77,151] Such HMO fragilised bacteria were shown to be more sensitive to antibiotic treatment.[77,80,152] Interestingly, Chambers et al.[80] reported increased 12,13-DiHOME production in GBS treated with HMOs. Possibly, *in vivo* this may trigger an increased effector immune response for pathogen clearance.

In clinical observation studies, primarily the alpha 1,2-linked fucosylated-HMOs were associated with protection from infectious illnesses. In a pioneering study with 93 breastfed infants and their mothers from Mexico, Morrow et al.[153] observed fewer cases of enteropathogenic induced diarrhea in infants of mothers expressing higher amounts of alpha 1,2-linked fucosylated-HMOs. Notably, this was observed for diarrhea caused by calicivirus, including norovirus, and for *Campylobacter*, against which 2'-FL was specifically suggested to be protective. In two different mouse models, 2'-FL was shown to reduce *Campylobacter jejuni* load and clinical symptoms. Moreover, *in vitro* adhesion to model cells was strongly reduced by 2'-FL.[154,155] For specific norovirus strains, binding of 2'-FL and also 3-FL was shown to lead to reduced adhesion to their blood group antigen ligands *in vitro*.[156,157] Earlier work showed inhibition of norovirus particles by secretor-positive milk, but not secretor-negative milk indicating alpha 1,2-linked fucosylated-HMOs might be involved.[158] However, HMOs seem not to have been involved. Rather, the alpha 1,2-linked fucosylated-glycans on milk mucins and lipase were found to inhibit norovirus adhesion.[158] Alpha 1,2-linked fucosylated-HMOs were associated with reduced diarrhea and morbidity in independent cohorts in Africa.[107,159] Additionally, in another small study, the HMO LNFP II that is FUT3 dependent was associated with reduced gastrointestinal and respiratory illnesses in early infancy.[160]

From a molecular point of view, fragilising, blocking and deviating pathogens from adhering to their cognate cell surface ligands comprise plausible mechanisms of action for HMOs. These add a line of innate protective functions to the colonisation resistance brought about by an appropriately developing gut microbiome. In relation to respiratory pathogens, an additional effect is expected through gut microbial metabolites as elegantly demonstrated in basic research models that show protection

from respiratory viral infections through immune active microbial metabolites.[86,161] Together, an intricate interplay between different breast milk glycan structures, including free HMOs, the gut microbiome and specific pathogens, is expected.

Not only infants, but also adults, who are genetic non-secretors, are often at a lower risk of diarrheal and respiratory infections caused by specific pathogens.[162,163] This is an important confounding factor especially when investigating associations between HMOs that strongly depend on maternal secretor status and infectious illnesses in breastfed infants. Although breastfeeding reduces the risk of diarrhea, the exact HMO composition of breast milk as determined by maternal secretor status might not have a large impact on this protective effect. This indicates that a certain functional redundancy may exist within the diversity of HMOs in mother's milk. Mechanistically, this can be exemplified by the similar effects of 2'-FL and 3-FL on *B. longum* subsp *infantis*,[164] and of 2'-FL and 6'-SL on models of NEC and allergic disease.[45,142]

To date, only few individual manufactured HMOs, 2'-FL alone or in combination with LNnT, have been tested in randomised controlled intervention trials in formula fed infants[109–114] and children.[164] All trials in infants investigated growth as the primary safety objective and also investigated infectious morbidity, not only as part of the reporting of adverse events, but also with an *a priori* hypothesis to investigate whether HMOs reduce infectious illnesses. For 2'-FL alone at 0.25 g L^{-1}, Storm et al.[111] reported a trend for a lower number of the overall infection related adverse events compared to controls. On the other hand, Marriage et al.[110,147] observed a higher incidence rate for reported adverse events related to overall infections in the control group of infants (GOS alone) and infants fed formula with 2'-FL at 1 g L^{-1} with GOS compared to infants fed the lower dose of 2'-FL at 0.2 g L^{-1} with GOS. In a cohort of healthy children aged 1–2.5 years, 2'-FL at 3 g L^{-1} provided in two portions of 200 mL per day over a 6-month period did not change the incidence of upper respiratory tract, nor gastrointestinal tract infections.[165] Rather, and somewhat surprisingly, a slight increase in duration of upper respiratory tract infections was observed. Infants fed formula with the two HMOs 2'-FL and LNnT experienced significantly fewer reported lower respiratory tract infections up to 1 year of age.[109] In the same trial, infants fed the formula with 2'-FL and LNnT also required significantly less antipyretics and prescribed antibiotics compared to control formula fed infants. Although these observations were based on an a priori hypothesis, they were part of the analysis of exploratory outcome measures in the trial. The observation of a lower risk of respiratory infections and lower need for antibiotics with 2'-FL and LNnT supplementation is further supported by recent studies linking a microbiome community structure highly dominated by *Bifidobacterium* species at 3 months of age with a reduced requirement for antibiotics.[47] Additionally, metabolites such as acetate, derived from HMO stimulated *Bifidobacterium* metabolic activity, could also contribute to a lower risk of respiratory tract infections.[166] For example, in basic research models, acetate was shown to be protective against gastrointestinal pathogenic *Escherichia coli*[85] and respiratory syncytial virus through a type of interferon mediated pathway.[86] Similarly, in cow milk protein allergic infants fed extensively hydrolysed formula feeding with the same 2'-FL and LNnT, there was a trend for lower respiratory tract infections and antibiotic use in supplemented vs. control fed infants although this difference did not reach statistical significance because of the limited sample size.[113]

HMOs and cognitive development

The brain is highly sialylated and many developmental and functional processes in the brain depend on sialic acid bound to proteins and glycolipids (i.e., gangliosides). As a result of the high sialic acid demand during early development and the high sialic acid content in breast milk, primarily in the form of HMOs, sialic acid is considered an important conditional nutrient in early life.[167–169] Studies in animal models suggest that most dietary sialic acid is largely catabolised to pyruvate and GlcNAc and is not used directly as sialic acid,[26,170,171] whereas some is reused directly through a salvage pathway as shown by the uptake and incorporation of the non-human sialic acid NeuGc.[172] Although the mechanisms are not fully established, these studies have led to the hypothesis that sialylated HMOs play a role in brain and cognitive development.

Today, numerous basic research models indeed support that sialyllactoses affect brain and cognitive development. In preterm pigs, a bovine milk preparation with sialyllactoses improved cognitive performance and upregulated hippocampal genes of sialic acid metabolism, ganglioside biosynthesis and myelination, whereas the concentration of hippocampal sialic acid was not affected.[173] In neonatal pig studies, sialyllactose supplementation increased ganglioside bound sialic acid in the corpus callosum and cerebellum,[174] affected sialic acid profiles in additional brain regions such as the prefrontal cortex as well as the hippocampus,[175] and affected metabolic signatures including neurotransmitters.[176] However, such changes did not translate into improved recognition memory or sleeping patterns.[177] In different rodent models, 3'-SL and 6'-SL were found to improve learning and memory using different testing paradigms and models.[28,178,179] Using a cross-feeding model with dams genetically unable to synthesise 6'-SL in their milks, wild-type animals fed 6'-SL deficient milk showed long lasting deficits in prefrontal cortex mediated executive functions.[28] Analysis of early life brain, plasma and gut microbiota hinted to affected serotoninergic pathways, linking the gut and brain, as well as neurochemical and

neuroanatomical adjustments in the brain. In another rodent experiment, using social disruption as a stressor, both 3'-SL and 6'-SL feeding prevented stress-induced dysbiosis and anxiety such as behavior indicating that, at least in part, these HMOs may act via the microbiome involving the gut–brain axis pathways.[180]

Several studies have investigated the role of sialyllactoses in breastfed infants. In a cohort of 99 infant–mother dyads, a higher breast milk 3'-SL concentration was associated with higher scores for expressive and receptive language development. However, this association was seen only in infants who were fed breast milk that contained the HMO A-tetrasaccharide (only produced by mothers with blood group A) but not in infants fed breast milk without this HMO.[181] In Malawian breastfed infants receiving FUT2 positive milk ($n = 485$), total sialylated HMOs, especially the concentration of total fucosylated HMOs, was positively associated with language development, whereas the non-sialylated and non-fucosylated HMOs structures showed an inverse relation.[96] In a pilot study, breast milk 6'-SL amounts at 1 month of age correlated positively with the composite cognitive score at 18 months of age ($n = 76$).[182]

Similar to sialyllactoses, 2'-FL is also reported in numerous basic research models to help improve cognitive development. The first studies found that brain exposure to 2'-FL, but not fucose or 3-FL improved hippocampal long-term potentiation.[183,184] Interestingly, direct effects of 2'-FL and 3-FL on enteric neuronal functions were also postulated from findings with an *ex vivo* model on gut contractility. Both 2'-FL and 3-FL, but not sialyllactoses, LNnT or GOS, had an immediate effect on colonic motor contractions, indicating that this effect is probably not driven by the gut microbiome.[185] Furthermore, additional *ex vivo* tests with animals that were subjected to restraint stress showed that 2'-FL alleviated stress-induced gut dysmotility.[186] Several recent studies tested 2'-FL feeding in rodent models to assess cognitive abilities and its possible mode of action.[187,188] The long lasting and improved learning and memory outcomes with 2'-FL feeding were related to effects on hippocampal memory related gene expression and long-term potentiation. In additional studies, the effect of 2'-FL was shown to be mediated via the vagus nerve[188] and not by direct uptake of 2'-FL or derived fucose into the brain.[189,190] Rather, for any uptake, microbial cleavage of 2'-FL is necessary. There are relatively few data in humans but, in one study of breastfed infants, greater 2'-FL intake at 1 month of age predicted better infant cognitive development at 24 months of age.[191]

Although infant cognitive development is affected by multiple environmental and nutrition factors, emerging data raise the possibility that HMOs make an important contribution and could partly help to explain some of the cognitive advantages of breastfeeding compared to formula feeding. Although the exact nature and mechanisms are not fully established, gut–brain communication processes involving gut microbiome metabolites are likely to be important. Well controlled and designed intervention trials with specific HMOs will be required to establish a causal link in this emerging field.

CONCLUSIONS AND OUTLOOK

From a structural perspective, HMOs represent numerous structural features that are generally present on mucosal and cell surface glycans and play important modulatory roles in cell–cell and host–microbe interactions. From a physiological perspective, HMOs show many structure function-specific activities, only observed with specific HMO species and not generally seen with unrelated glycans that are often used as prebiotics. However, there is redundancy of some functions between different HMO species, possibly acting as safeguard mechanisms for some of their important roles.

Although human milk is particularly rich in amounts and structural diversity of HMOs, some oligosaccharides are common across the animal milks. For example, 3'-SL appears to be quite universally present in animal and human milks, suggesting universal and important functions across mammals.

Recent progress in manufacturing of individual HMOs has triggered a revival of research and great interest in the application of HMOs as seen by the exponential increase in published studies. Together, the cumulative evidence indicates that HMOs are a meaningful and important component of human milk, the optimal nutrition for early life. Increasing evidence also suggests that specific HMOs help establish immune competence, both local and systemically, partly through their effect on the metabolite activity of specific microbes such as specific *Bifidobacterium* species. HMOs may also participate in a gut–brain connection, thereby modulating brain and cognitive development. As expected from many biological processes, HMOs work in concert with other bioactive components and additionally act via different mechanisms that converge to specific functions.

Although we have started to accumulate clear evidence on the benefits of specific individual HMOs and blends thereof from randomised controlled trials, observational studies in breastfed infants have added to our knowledge and evidence supporting the importance of HMOs in early life. However, why human milk contains such diverse HMOs and what are the key drivers besides genetic polymorphism and time of lactation that explain the high variability in amounts of some HMOs remain key questions. To what extent the microbes and milk composition provided by mothers to their infants is personalised also remains an intriguing question for future research.

ACKNOWLEDGEMENTS

We thank Dr Elizabeth Forbes Blom and Dr Eline van der Beek for their critical review and support.

CONFLICT OF INTERESTS

Norbert Sprenger, Hanne L. P. Tytgat, Aristea Binia and Sean Austin are employees of Société des Produits Nestlé, Switzerland. Atul Singhal has no conflict of interst regarding this work, but previously received research funding from Nestlé and Abbott Plc, as well as honoraria to give lectures and attend advisory boards for Nestlé Nutrition Institute, Danone, Wyeth Nutrition, Reckitt, Phillips, Abbott Nutrition and several academic institutions.

ETHICS STATEMENT

Not applicable.

AUTHOR CONTRIBUTIONS

Norbert Sprenger conceptualised and drafted the manuscript. Atul Singhal, Sean Austin and Atul Singhal completed the writing of the manuscript. Norbert Sprenger and Hanne L. P. Tytgat prepared the figures. Norbert Sprenger and Sean Austin prepared the tables. All authors reviewed and approved the final version of the manuscript submitted for publication.

TRANSPARENCY DECLARATION

The peer review history for this article is available at https://publons.com/publon/10.1111/jhn.12990.

DATA AVAILABILITY

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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How to cite this article: Sprenger N, Tytgat HLP, Binia A, Austin S, Singhal A. Biology of human milk oligosaccharides: From basic science to clinical evidence. J Hum Nutr Diet. 2022;35: 280–299. https://doi.org/10.1111/jhn.12990