The Influence of Early Infant-Feeding Practices on the Intestinal Microbiome and Body Composition in Infants

Supplementary Issue: Parental Nutritional Metabolism and Health and Disease of Offspring

Aifric O'Sullivan[1], Marie Farver[2] and Jennifer T. Smilowitz[3,4]

[1]UCD Institute of Food and Health, University College Dublin, Belfield, Dublin, Ireland. [2]Sutter Davis Hospital Birthing Center, Davis, CA, USA. [3]Department of Food Science and Technology, University of California Davis, Davis, CA, USA, [4]Foods for Health Institute, University of California Davis, Davis, CA, USA.

ABSTRACT: Despite many years of widespread international recommendations to support exclusive breastfeeding for the first six months of life, common hospital feeding and birthing practices do not coincide with the necessary steps to support exclusive breastfeeding. These common hospital practices can lead to the infant receiving formula in the first weeks of life despite mothers' dedication to exclusively breastfeed. Consequently, these practices play a role in the alarmingly high rate of formula-feeding worldwide. Formula-feeding has been shown to alter the infant gut microbiome in favor of proinflammatory taxa and increase gut permeability and bacterial load. Furthermore, several studies have found that formula-feeding increases the risk of obesity in later childhood. While research has demonstrated differences in the intestinal microbiome and body growth between exclusively breast versus formula-fed infants, very little is known about the effects of introducing formula to breastfed infants either briefly or long term on these outcomes. Understanding the relationships between mixed-feeding practices and infant health outcomes is complicated by the lack of clarity in the definition of mixed-feeding as well as the terminology used to describe this type of feeding in the literature. In this commentary, we highlight the need for hospitals to embrace the 10 steps of the Baby Friendly Hospital Initiative developed by UNICEF and the WHO for successful breastfeeding. We present a paucity of studies that have focused on the effects of introducing formula to breastfed infants on the gut microbiome, gut health, growth, and body composition. We make the case for the need to conduct well-designed studies on mixed-feeding before we can truly answer the question: how does brief or long-term use of formula influence the health benefits of exclusive breastfeeding?

KEYWORDS: Baby Friendly Hospital Initiative, *Bifidobacterium*, breastfeeding, formula-feeding, human milk oligosaccharides, lactation, microbiome, body composition

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CORRESPONDENCE: jensm@ucdavis.edu

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Introduction

Breastfeeding is the biological norm for the human species, and milk alternatives have the potential to negatively impact infant and maternal health.[1] Breast milk meets the infant's needs by providing nutrients appropriate to the infant's developmental stage, as well as growth factors, antimicrobial peptides, and proteins to support their developing immune system. Humans are born neurologically and physiologically immature in comparison to other nonhuman primates.[2] Indeed, a recent investigation revealed that human milk contains higher concentrations of proteins that support gastrointestinal system, immune system, and neurological development compared to rhesus macaque milk.[3] Furthermore, anthropological evidence shows that human neonates are born with the least-developed brains of any primate.[4] Therefore, human neonates are considered *secondary altricial* and would require a gestation period of 18–21 months instead of nine months to reach the same neurological and cognitive developmental stage as other nonhuman primates at birth.[4] This highlights the importance of the initial period after birth and emphasizes the need for strategies to ensure normal infant growth and development.[5]

Mother–infant contact, latching and suckling, drive human milk production and support successful breastfeeding. Consequently, hospital practices that separate mothers and their babies[6] and birth interventions that hinder infant latching and suckling[7,8] impact milk production[9] and lengthen the time to onset of lactogenesis II (copious milk production).[10] Insufficient mother–infant contact and reduced milk production are barriers to successful breastfeeding[11] that can result in weight loss and/or jaundice and ultimately compromise infant health.[10,12] All these factors can lead to the introduction of formula-feeds in the first weeks of life despite mothers' dedication to exclusively breastfeed.[13] Maternal concerns over infant fussiness as well as weight loss also drive formula supplementation rates.[14] Health care providers can address mothers' concerns and improve exclusive breastfeeding rates by communicating evidence-based information.[15] Exclusive and partial formula-feeding have been shown to alter the gut microbiome toward adult patterns,[16,17] increase proinflammatory bacterial taxa,[16,18–20] increase gut permeability,[21] and result in lower concentrations of fecal short-chain fatty acids[22,23] compared with exclusive breastfeeding. Exposure to proinflammatory bacteria and antigens during the neonatal period may profoundly influence oral tolerance and have long-term consequences on immune health.[24] While research has demonstrated differences in the intestinal microbiome and body growth between exclusively breastfed and formula-fed infants, very little is known about the effects of introducing formula-feeds to breastfed infants either briefly or over longer periods. Indeed, mixed breast and formula-feeding is a common practice; however, the impact of *mixed-feeding* on infant health outcomes is unclear. This missing information is crucial as the majority of infants in most developed nations are mixed-feeders by the time they are three months of age. In this commentary, we will highlight the factors that facilitate successful exclusive breastfeeding and the methods that lead to the introduction of infant formula. Herein, we make a case for more research to investigate how the common practice of mixed-feeding impacts the intestinal microbiome, infant growth, and body composition.

Global Recommendations for Breastfeeding

According to the American Academy of Pediatrics, the World Health Organization, United Nations International Children's Emergency Fund, and the Center for Disease Control (CDC), exclusive breastfeeding is recommended for the first six months of life, followed by breastfeeding in combination with the introduction of complementary foods until at least 12 months of age, and continuation of breastfeeding for up to two years and beyond or as long as mutually desired by mother and baby.[1,15,25] In a systematic review of over 400 individual studies, breastfeeding was associated with a range of short- and long-term health outcomes including a reduction in the risk of acute ear infections, asthma (in young children), atopic dermatitis, gastrointestinal infections, respiratory tract diseases, obesity, type 1 and 2 diabetes, childhood leukemia, sudden infant death syndrome in term infants, and necrotizing enterocolitis in preterm infants.[26] Unfortunately, breastfeeding initiation and continuance rates in the United States are alarmingly low. According to the 2014 report card of the CDC, 79% of US babies were ever breastfed and only 41% of babies were exclusively breastfed at three months of age (http://www.cdc.gov/breastfeeding/pdf/2014breastfeedingreportcard.pdf). The picture is similar across other regions of the world. While some European countries, including Portugal, Latvia, Slovenia, Switzerland, and the Czech Republic, report breastfeeding initiation rates above 95%, others perform very poorly on this indicator. The most recent European Perinatal Health Report lists Ireland as the country with the lowest breastfeeding initiation rates (~54%).[27] In addition, there is a steep drop in exclusive breastfeeding in the first few months with ~19% of mothers reporting exclusive breastfeeding at three months and a further 15% practicing mixed-feeding.[28] By six months, only 13% of Irish mothers are still exclusively breastfeeding.[28]

Most Important Factors that Support Successful Breastfeeding

Breastfeeding is fundamental to lifelong human health.[15] Health care organizations have a public obligation to promote health. Yet, it is well established that many traditional hospital maternity care practices undermine exclusive breastfeeding.[29] More than 85% of US mothers entering the hospital intend to exclusively breastfeed, but only 32% of these mothers meet this goal after hospital discharge.[13] Seventy-eight percent of US hospitals inappropriately supplement infant formula to breastfed infants.[30] Hospital supplementation of breastfed infants is associated with delayed onset of copious milk production and shorter duration of exclusive breastfeeding.[10,31] Separation of mothers and infants by using a centralized nursery not only inhibits establishment of breastfeeding but also promotes inappropriate use of formula and artificial nipples as hospital staff deal with fussy, hungry babies in the absence of their mothers.[6] Lack of prenatal breastfeeding education leaves mothers with the impression that formula-feeding and breastfeeding are equivalent options for their infant. Without guidance and support from hospital staff during breastfeeding, mothers often give up or come to the misguided conclusion that they or their infants cannot breastfeed. Recognizing the impact these and other hospital practices have on breastfeeding, UNICEF and the WHO developed the 10 steps to successful breastfeeding.[32] By reforming maternity care practices through the implementation of these 10 steps, breastfeeding rates have more than doubled in participating facilities.[29] Having a written breastfeeding policy, training the staff to assist patients with breastfeeding,[33] keeping mothers and babies together continuously from birth,[34] eliminating inappropriate use of formula and artificial nipples,[13] encouraging cue-based unrestricted breastfeeding, and ensuring continued support for the breastfeeding mother after discharge[35] transform maternity care to allow the establishment of successful breastfeeding.[36]

Factors that Support Formula-Feeding

The United States' "Surgeon General's Call to Action to Support Breastfeeding" lists five obstacles that prevent mothers from successful breastfeeding initiation and continuance: (1) the lack of experience or understanding among family members of how best to support mothers and babies; (2) not enough opportunities to communicate with other breastfeeding mothers; (3) lack of up-to-date instruction and information from health care professionals; (4) hospital practices that make it difficult to successfully breastfeed; and (5) lack of accommodation to breastfeed or express milk at the workplace.[37] In addition, other cultural, demographic, and phenotypic factors such as socioeconomic status, age, education, and body mass index (BMI) are reported in the scientific literature as factors associated with duration of exclusive breastfeeding and early introduction of formula-feeds.[38–40]

Hospital practices that lead to in-hospital formula supplementation increase the risk of breastfeeding cessation by two months of life (threefold) despite adjustments for the strength in breastfeeding intentions.[41] Hospitals that do not embrace the 10 steps to successful breastfeeding typically employ the use of infant formula to offset excess weight loss, which, in most cases, is a result of hospital birthing practices. For example, excess weight loss in infants is positively associated with intrapartum fluid balance.[42,43] IV fluid administration during labor often leads to elevated fluid levels in these infants, thus artificially increasing what is measured as birth weight. Rapid weight loss due to the elimination of this excess fluid typically leads to in-hospital supplementation of formula for these babies.[44] Additionally, infants exposed to pain medications or epidural anesthesia during labor demonstrate fewer breast-seeking reflexes and reduced rooting and suckling scores and consume less milk at initial feedings.[7,8,45] Epidural anesthesia during labor can also reduce milk supply[46] and delay the onset of lactogenesis II.[10,47]

In an attempt to improve breastfeeding rates, a recent randomized controlled pilot trial of 40 mother–infant dyads, the Early Limited Formula (ELF) study, hypothesized that breastfeeding rates would improve if infants were fed small amounts of formula for a limited time.[48] The ELF study reported that breastfeeding rates were improved because infants lost less weight and were more hydrated during the period of time before the onset of copious milk production. They also reported that mothers had less concern regarding their milk supply related to infant fussiness. While significantly fewer infants in the ELF group had consumed formula by one week of life and more infants in the ELF group were breastfeeding exclusively by three months compared with the control group, the study design was limited and the rationale for improving breastfeeding rates was flawed and misleading. First, infants enrolled in this study were defined as at-risk for weight loss, even though 5%–10% weight loss is normal. Second, mothers randomly assigned to the ELF group were instructed to feed their infants 10 mL of formula after each breastfeeding session using a feeding syringe until copious milk production began. However, to control for the amount of time the study personnel spent with mothers in the ELF group, the mothers randomized to the control group had 15 minutes of instruction in infant–soothing techniques. It is unclear what types of soothing techniques were used and whether they interfered with breastfeeding. Third, 10 mL of formula after each breastfeeding session may not seem like much, yet for a mother whose milk comes in late, it can be. For example, a 48-hour-old infant who nurses 10 times each day could consume 100 mL of formula for several days especially if the onset of copious milk production is delayed. Fourth, although not statistically significant due to a lack of power, there was a higher percentage of multiparous mothers in the ELF group (70%) versus the control group (40%), which may partly explain the improved breastfeeding outcomes of ELF. Parity is the strongest predictor of delayed onset of copious milk production with a prevalence of delayed onset to be 33% among primiparous versus 5% among multiparous mothers.[10] Fifth, attempting to ameliorate mothers' concerns about milk supply using infant formula is a temporary stopgap and sends an erroneous message to the mother, family, and health care providers that the biological norm—breastfeeding—is insufficient to nourish the infant. Finally, promoting the use of infant formula to mothers who express a desire to exclusively breastfeed their at-risk infants without the use of artificial milk is misleading. It is imperative that mothers receive lactation support and education by a trained health care professional for successful breastfeeding continuance.[25,37] Access to support from health care professionals who can provide education, encouragement, and support for breastfeeding during the early postpartum period is extremely critical to successful breastfeeding.[25] Sufficient milk production is heavily reliant on effective, frequent, and early removal of colostrum.[49,50] Breast milk production is time sensitive and begins immediately after delivery. When expression ensues within the first hour postpartum (vs one to six hours postpartum), the time for the onset of lactogenesis II is decreased and the production of milk is increased by 130% by three weeks postpartum.[50] Additionally, hand expression versus pumping in the first 48 hours increases the volume of colostrum removed.[51] These health care methods are employed in settings that prioritize exclusive breastfeeding without the use of formula. Finally, providing breastfeeding support and education at specific periods in the postpartum period that coincide with developmental and physiological changes in the infant and the mammary gland is imperative. For example, breastfeeding concerns expressed by mothers such as *feeding difficulty* peaked on day 7 and concerns regarding *milk quantity* peaked on day 14. These breastfeeding concerns were associated with breastfeeding cessation.[35] Thus, it is critical for women to receive lactation support during the first two weeks of their infants' lives to ensure successful breastfeeding continuance rates.

Many hospitals focus on infant weight loss from birth and use formula to facilitate regaining birth weight.[52] This practice is common despite the fact that weight loss up to 10% of birth weight is normal, and it is expected that the infant will regain birth weight by two weeks of life.[53] It is unknown how early introduction of formula impacts the immature gastrointestinal tract, gut microbiome, and naive immune system. A recent systematic review by the Cochrane Collaboration on the use of formula in breastfed infants only reported outcomes on breastfeeding duration, weight loss, blood glucose, body temperature, and serum bilirubin but did not include data on any immunological or gastrointestinal outcomes.[54] Most studies have compared the effects of exclusive breastfeeding with exclusive formula-feeding on gut health. For example, exclusive formula-feeding within the first few weeks of life has been shown to increase intestinal permeability and bacterial translocation compared with exclusive breastfeeding (reviewed in Ref.[21]). Based on the most recent report card of the CDC, 59% of babies are mixed-fed by the time they are three months of age. Thus, understanding the consequences of mixed-feeding, whether brief or for longer periods, is critical as it represents the most common feeding practice. The question we need to ask is: how does brief or long-term use of formula influence the health benefits of exclusive breastfeeding?

Impact of Early Formula-Feeding on Infant Health

Infant gut microbiome and health. Infants are exposed to their first inoculum of human microbes in utero.[55,56] The microbial colonization pattern of the infant is complicated and relies heavily on the mode of delivery and diet. Infants born vaginally are colonized by resident bacteria of the vagina and perineal area with a dominance of *Lactobacillus* and *Prevotella* spp., and infants born by C-section acquire species endemic to maternal skin from *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*.[57] Additionally, the fecal microbiota of infants born vaginally resembles maternal fecal microbiota by day 3 and day 7 of age, which is not observed in infants delivered by cesarean section.[58] Thus, the vertical transmission of beneficial microbiota from mother to infant relies on the vaginal mode of delivery. In addition to the mode of delivery, infant diet plays a substantial role in shaping the infant gut microbiome.[16,17,59] It is well documented that exclusively breastfed infants have higher taxa from the protective bacterial class Actinobacteria and formula-fed infants have higher levels of the proinflammatory bacterial class γ-Proteobacteria.[16,18–20] Using metagenomic sequencing, Bäckhed et al recently discovered that the complexity of the infant gut microbiome increased over time and resembled more adult-like patterns, but the adult-like patterns were more prominent in formula-fed than exclusively breastfed infants. Furthermore, the intestinal microbiota of breastfed infants was less diverse than that of formula-fed infants, but this lower alpha diversity in breastfed infants was consistent with the enrichment of genes required for the degradation of human milk oligosaccharides (HMOs) from breast milk.[16] The differences in the microbiome between breastfed and formula-fed infants result in differences in gene transcription in the human host.[17,59] Using metagenomics and host gene transcriptomic analysis, Praveen et al[17] found that the intestinal microbiota diversity was significantly lower in breastfed infants, but their microbial genes interacted twofold more with host genes associated with immunological, metabolic, and biosynthetic activities compared with formula-fed infants. Schwartz et al[59] discovered that the expression of microbial virulence factors was higher in breastfed infants, but this finding was concomitant with the downregulation of inflammatory genes in host epithelial cells. Thus, compared with formula-fed infants, the gut microbiome of exclusively breastfed infants is dominant of protective gut bacteria that utilize the complex sugars in human milk and interact more with host cells.

Breast milk provides a wide spectrum of biologically active factors that aid in the development and maturation of the gut, systemic metabolism, and the innate and acquired immune systems. It also develops a supportive and protective microbiota. Glycosylated proteins such as lactoferrin, lysozyme, and immunoglobulins are important nonnutritive factors that protect infants from infection and, as a result, affect the development of the intestinal microbiome. These bioactive proteins are the first line of defense against potential pathogens within the infant gut and exert their protective effects via multiple and often overlapping mechanisms. These may have either direct or indirect effects on the intestinal microbiome by interacting with bacteria, engaging in pathogen destruction/deflection including immune exclusion, interacting with the infant mucosal immune system, and stimulating epithelial barrier function.[60–63]

Recent advances in mass spectrometry-based tools have revealed the detailed chemical structures of the complex and diverse-free and conjugated glycans in human milk.[64–66] HMOs, the third most abundant component in human milk (~10–20 g/L), is a constellation of complex sugars that are nondigestible by the infant but support the competitive growth of protective *Bifidobacterium* strains within the intestine of the breastfed infant. Specifically, two main bifidobacterial species populate the breastfed infant colon, *Bifidobacterium longum*[67–72] and *B. breve*.[73] The *B. longum* clade contains two subspecies found in humans, *B. longum* subsp. *longum* (herein termed *B. longum*) and *B. longum* subsp. *infantis* (*B. infantis*). Breakthroughs in microbiology have led to a detailed description of the natural colonization of protective *Bifidobacterium* in breastfed infants and identified HMO in breast milk as natural prebiotics that selectively enrich the growth and function of these beneficial bacteria.[67–70,72,74–80] Specifically, HMO consumption is most efficient among *B. infantis* strains but is absent in *B. longum* strains.[79] When grown in the presence of HMOs, *B. infantis* upregulates its expression of two groups of bacterial genes-specific binding proteins that import HMOs[72] and 16 glycosyl hydrolases with specificity for every linkage in HMOs.[67,69,70,77,81] This suggests that *B. infantis* is able to efficiently transport intact HMOs into its cytoplasm and digest HMOs within the bacterial cytoplasm. These HMO-associated genes are not upregulated when *B. infantis* is grown on more simple prebiotics added to formula, such as fructo-oligosaccharides, galacto-oligosaccharides and inulin,[72,82] even though other strains of *Bifidobacterium* efficiently utilize them.[83] Levels of intestinal *B. infantis* are positively associated with immune protection through several proposed mechanisms (reviewed in Ref. 84). These mechanisms include: (1) through enhanced adherence to intestinal cells and inhibition of gut invasion by undesirable bacterial strains;[85] (2) through direct interaction with intestinal cells and dendritic cells resulting in the production of anti-inflammatory cytokines and reduction of proinflammatory cytokines and enhanced gut barrier function;[86,87] (3) through the secretion of bioactive factors that directly reduce expression of toll-like receptors 2 and 4, reduce the production of proinflammatory cytokines, enhance the release of anti-inflammatory cytokines, and enhance gut barrier function;[88,89] (4) through increased activation of T-regulatory cells with concomitant inhibition of chemokine secretion within the mucosa during pathogen infection;[90] and (5) through inhibiting the translocation of gram-negative bacterial toxins via the increased production of short-chain fatty acids as by-products of carbohydrate fermentation.[91] Microbial production of short-chain fatty acids was also found to directly reduce gut inflammation by increasing the number of colonic T-regulatory cells and their production of interleukin-10.[92] Clinically, Bangladeshi infants colonized by high levels of gut *B. infantis* were shown to have larger thymus glands and better peripheral T-cell responses following vaccinations against the oral polio virus and tetanus toxoid.[93] In premature infants, *B. infantis* but not *Bifidobacterium animalis* subspecies *lactis*, given in combination with human milk increased *Bifidobacterium* and decreased γ-Proteobacteria in feces.[94] Additionally, probiotics containing *B. infantis* was found to decrease the risk of necrotizing enterocolitis in premature infants.[95] Thus, mammalian lactation has evolved to selectively nourish targeted strains of *Bifidobacterium* that in turn protect and guide the intestinal health and immune system of the developing infant.

Most of what is known about how diet influences the infant gut microbiome is through the comparison of exclusively breastfed versus formula-fed infants. However, it is not well understood how mixed-feeding influences the gut microbiome. It is also unknown whether there is a difference in mixed-feeding early in life when the gut is naïve versus in later life after the infant's gut microbiome is established and the gut is more mature. On the first day of age, Enterobacteriaceae, *Streptococcus*, and *Staphylococcus* are present in infant feces. *Bifidobacterium*, the protective obligate anaerobic genus, does not appear until day 2 of age, and its level is not stable until day 4 of age.[96] It has been proposed that the gradual consumption of oxygen in the intestine by aerobic microorganisms decreases the oxidation–reduction potential and thus provides the conditions for the settlement of a dominance of anaerobic bacteria.[97] The intestinal level of the genus *Bifidobacterium* has been reported to be similar between breast and formula-fed infants at one month of age;[19,20] yet, few studies examined the fecal microbiology to the level of species and subspecies belonging to this genus. Among *Bifidobacterium*, *B. infantis*, *B. breve*, *B. adolescentis*, *B. longum*, and *B. bifidum* are typical of breastfed infants.[73,98–100] On the other hand, *B. longum*, the adult strain of *Bifidobacterium*,[100] is higher in formula-fed infants.[17] It has been reported that relatively small amounts of formula supplemented to breastfed infants shifted the gastrointestinal environment and microbiota from a breastfed to a formula-fed pattern. Bullen et al reported that the infant fecal microbiology was similar in the first week of life regardless of the type of milk they were receiving: (1) exclusively breastfed, (2) exclusively formula-fed, or (3) mixed-fed (breastfed + fed formula one bottle every 24 hours). However, the class Clostridia was not present or present in very low levels in breastfed infants (10^3) over the first six weeks of life but rose from 10^5 to 10^7 over the six–week study period in the formula-fed and mixed-fed infants. Proteus bacteria, belonging to the proinflammatory class γ-Proteobacteria, was not detected in breastfed infants, yet was found in 30% of formula-fed infants and 20% of mixed-fed infants.[23] These data suggest that in breastfed infants, consumption of formula for only one week resulted in unfavorable consequences on the gut microbial ecology throughout the six–week study duration. Well–designed studies are needed to understand the long-term effects of brief formula-feeding in breastfed infants (a common practice) on the infant gut microbiome and gut health.

The nature of mucosal gut microbial ecology ecology acquired in early infancy has been proven to be critical in the determination of mucosal immune response and tolerance, so that alterations of the gut environment are directly responsible for mucosal inflammation and disease, autoimmunity, and allergic disorders in childhood and adulthood.[24,101] Breastfed infants have a much lower colonic pH, and more breastfed infants have higher concentrations of fecal acetate than formula-fed or mixed-fed infants.[22,23,102] Acetate production by protective bifidobacteria has been shown to act in vivo to promote defense functions of the host epithelial cells and protect the cell from enteropathogenic infection.[91] Additionally, levels of colonic acetate was found to be anti-inflammatory through the regulation of colonic T-regulatory cells.[92] These data suggest that even in a breastfed infant, consumption of one bottle of formula per day for the first week of life is enough to shift the gastrointestinal microbiology toward proinflammatory taxa with concomitant lower concentrations of colonic acetate several weeks postnatally. More research is needed with long-term studies on breastfed infants who are also formula-fed early in life, a common practice used by hospitals that do not adhere to the Baby Friendly guidelines established by the WHO and UNICEF.

Infant growth and body composition. Infancy is a period of rapid growth; from birth to two years of life, body length increases by ~75% and weight increases 3.5-fold (http://www.who.int/childgrowth/standards/weight_for_age/en/). At birth, body fat accounts for ~15% of weight, and this increases to ~25% at six months, peaking at ~30% at 12 months. Deviations in growth in early life are associated with increased risk of disease in the short and long term;

hence, growth is often used as a proxy measure of infant health. Some of the most referenced infant growth studies date back to the 1980s and 1990s when it was apparent that the growth patterns of exclusively breastfed infants deviated significantly from WHO standard growth charts, which at the time were based on predominantly formula-fed populations.[103] Most of these earlier publications investigating infant growth reported weight and length gains, with the majority identifying a more rapid growth trajectory for formula-fed infants and a higher absolute body weight.[103] Although some of the early studies reported measures of adiposity suggesting that breastfed infants were generally leaner than formula-fed infants, these investigations were limited by the measurement techniques used.[103] In contrast, a recent systematic review and meta-analysis reported higher fat-free mass in formula-fed infants over the first year of life. Fat mass was higher in breastfed infants at three to four months and at six months; however, by 12 months, this effect was no longer significant and there was a trend toward a higher fat mass in formula-fed infants.[104] Recognizing the differences among breast and formula-fed infants, other investigations have targeted potential consequences of different feeding practices/growth trajectories and reported a protective effect of breastfeeding against obesity risk[105] and a positive association between rapid weight gain and obesity risk.[106] Since exclusive breastfeeding is associated with the highest standard of health, the growth pattern of breastfed infants is regarded as the standard for all infants, and so international growth reference charts have been revised accordingly. The mechanisms that explain how breast milk consumption and breastfeeding can impact infant growth and protect against obesity can be broadly classified under two major categories: (1) components/composition of breast milk and (2) behaviors related to infant feeding. The sources, quantities, and qualities of macronutrients and micronutrients are different in breast milk and formula. Taking protein as an example, despite recent modifications, formula still contains more protein and different types of proteins than breast milk. The higher protein intake reported in formula-fed infants results in higher concentrations of plasma amino acids, particularly branched-chain, insulinogenic amino acids (valine, leucine, isoleucine). The formula-fed infants' amino acid profile is associated with higher concentrations of insulin that is thought to impact growth and potentially obesity risk.[107] In addition to nutritive components, breast milk also provides a range of nonnutritive bioactives such as immune cells, growth factors, and hormones that must also be considered in relation to growth and body composition (bioactives reviewed in Refs 108,109). For example, studies show that breast milk concentrations of leptin and adiponectin are associated with infant weight gain.[110,111] Leptin regulates appetite and therefore provides a link to improved self-regulation of milk intake in breastfed infants compared to formula-fed infants. Indeed, regulating milk intake is one aspect of feeding behavior that is different between breast and formula-fed infants. However, behaviors of the caregiver also play an important role in infant feeding. Formula-fed infants are often encouraged to empty the bottle, which may override infants' internal satiety cues and result in poor self-regulation of intake.[112] This highlights another key point that bottle-feeding, regardless of the type of milk, is distinct from breastfeeding in its effect on the infant's ability to self-regulate intake.[112]

Studies examining the relationship between infant feeding and growth typically compare exclusive breastfeeding and full formula-feeding, despite the fact that mixed-feeding is a common practice. Consequently, there are a limited number of studies that include a group of infants that consume breast milk and formula. One recently published paper from a large Canadian cohort classified infant–feeding practices as (1) only breastfed, (2) mixed-fed, or (3) only formula-fed.[113] This study reported that a higher proportion of children who were introduced to formula in early infancy were overweight or obese compared to children who received formula in later infancy or who never received formula. When classified by feeding type, there was no difference in the proportion of obese children in the *only breastfed* or *mixed-fed* groups; however, the proportion of obese children in the *only formula-fed* group was higher.[113] In slight contrast, NHANES data showed similar rates of childhood obesity among mixed-fed and only formula-fed infants, which were higher than the only breastfed group.[114] Based on the Canadian data, one might expect that the consumption of any breast milk will be protective against obesity, while the NHANES data suggest that any breast milk will not ameliorate the effects of formula on body weight. The conflicting findings are potentially due to the age at which BMI/obesity was measured and/or due to variations in the definition of mixed-fed infants. Indeed, one final study that included mixed-feeding as an independent group reported that the duration of partial breastfeeding that was necessary to impart a protective effect against obesity was longer than when breastfeeding was exclusive.[115] In a population of low-income children, Bogen et al[115] found that breastfeeding for at least 16 weeks without formula or at least 26 weeks with formula was associated with a reduced risk of obesity at age four years. Similarly, studies investigating obesity risk and duration of exclusive breastfeeding consistently report a protective effect when formula is delayed.[116,117]

Conclusion

Despite many years of widespread international recommendations to support exclusive breastfeeding for six months, common hospital feeding and birthing practices do not appear to coincide with the necessary steps to support exclusive breastfeeding. In fact, by the age of three months, the majority of infants are mixed- or formula-fed. Hospital practices that do not embrace the 10 steps to support breastfeeding created by UNICEF and the WHO often use formula as a stopgap to treat infant morbidity (ie, infant weight loss, dehydration, jaundice). These common hospital practices can lead to infant formula-feeding in the first weeks of life despite mothers' dedication to exclusively breastfeed. Effective communication of evidence-based information from health care providers addressing maternal concerns over infant fussiness and weight loss could increase exclusive breastfeeding rates. While research has demonstrated differences in the intestinal microbiome and body growth between exclusively breast versus formula-fed infants, very little is known about the effects of introducing formula to breastfed infants either briefly or long term on these health outcomes. Understanding the relationship between mixed-feeding practices and infant outcomes is complicated by the lack of clarity in the definition of mixed-feeding as well as the terminology used to describe this type of feeding in the literature. Based on the paucity of published findings, it is extremely difficult to determine whether studies are actually reporting mixed-feeding or merely a switch from exclusive breastfeeding to formula only. We need studies that classify infants into strict groups based on breast milk and formula consumption before we can truly answer the question: how does brief or long-term use of formula influence the health benefits of exclusive breastfeeding?

Author Contributions

Conceived the concepts: AOS, JTS. Wrote the first draft: JTS. Contributed to the writing of the manuscript: AOS, MF, JTS. Jointly developed the structure and arguments for the paper: AOS, JTS. Made critical revisions and approved final version: AOS, MF, JTS. Agree with manuscript results and conclusions: AOS, MF, JTS. All authors reviewed and approved of the final manuscript.

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