Abstract
Breastfeeding confers the infant short- and long-term health benefits and significantly modulates the developing infant gut microbiome. A specific human milk microbiome has relatively recently been discovered, but its origin remains poorly understood. Data from experimental and clinical studies suggest that the bacteria in milk may originate in the maternal gut and be transported via a specific enteromammary pathway, the details of which have not been elucidated yet. The milk microbiome is affected by the maternal metabolic state, antibiotic use, as well as the mode of delivery. We are only in the initial stages of understanding the biological function of the milk microbiome and its potential contribution to infant gut colonization. Several clinical studies indicate, however, that despite considerable differences in the overall composition of the milk and infant gut microbiomes, specific bacteria are detectable both in human milk and infant feces, and that the bacteria in milk are a source of microbes colonizing the neonatal gut. If the microbes in human milk are discovered to contribute to the beneficial effects of breastfeeding, modulating or mimicking the milk microbiome may provide a novel means of improving child health.

Introduction
Breastfeeding not only provides the neonate and infant with optimal nutrition, it also confers protection against acute and chronic diseases [reviewed in 1]. It has been estimated that, in the developing world, the risk of death of exclusively
Breastfed infants is only 12% of that of formula-fed infants [1]. Breastfeeding could potentially prevent half of all diarrheal diseases and one third of respiratory tract infections in infancy, which would correspond to an estimated 823,000 lives saved annually [1]. These protective effects are thought to be mediated by a variety of antimicrobial compounds present in human milk, albeit the fact that exclusively breastfed infants are less likely to be exposed to potentially contaminated food and water probably also plays a role. Interestingly, in addition to protection against infectious disease in infancy, breastfeeding has been associated with long-term health benefits, including a reduced risk of developing chronic diseases in later life [1].

**Breastfeeding and the Risk of Noncommunicable Diseases in Later Life**

It is important to recognize the problems inherent in studies assessing the long-term health impact of breastfeeding. Performing randomized controlled studies comparing breastfeeding of different durations with formula feeding on the individual level is not possible for practical and ethical reasons. The results of epidemiological studies must be interpreted with caution because of potential confounding by factors including maternal obesity and mode of delivery, which are known or suspected to affect both breastfeeding rates [2, 3] and chronic disease risk in the child [4, 5]. Furthermore, the increased risk of infectious disease in infancy related to formula feeding may result in more frequent antibiotic use, which in turn has been suggested to increase the risk of childhood overweight [6] and asthma [7]. Many of the available epidemiological studies rely on parental reports of breastfeeding duration and childhood diseases, which together with potential recall bias in retrospective studies further decreases the reliability of the data.

Despite the methodological difficulties discussed above, there are convincing data to suggest that breastfeeding confers health benefits, which extend beyond the period of breastfeeding and even into adulthood. It has been estimated that breastfeeding reduces the risk of overweight and obesity by 13–26% and that of type II diabetes mellitus by 35%, but no effects on the other components of the metabolic syndrome, including hypertension and hypercholesterolemia, have been reliably documented [1]. The data regarding the associations between breastfeeding and the risk of allergic diseases, asthma, and type I diabetes are somewhat inconsistent.

The mechanisms between breast milk exposure and reduced risk of noncommunicable immune-mediated or inflammatory diseases are currently not well understood. Human milk contains a large variety of immunoactive molecules,
such as hormones, growth factors, cytokines, and chemokines, as well as maternal lymphocytes, which may and, to some extent, have been shown to modulate mucosal and systemic immune responses in the infant and induce immune maturation [reviewed in 8]. The individual variation in immune molecule concentrations in the milk associated with maternal health or the metabolic state may explain the somewhat discrepant data regarding breastfeeding and chronic disease risk. Future research will hopefully shed more light on these interesting associations and the underlying mechanisms.

Breastfeeding and the Infant Gut Microbiome

The developing intestinal microbiome is a potential mediator of the long-term health effects of breastfeeding. Early gut colonization and the establishment and development of the gut microbiome during the first year of life are a dynamic process which is profoundly affected by breastfeeding and exposure to formula and solid foods [reviewed in 9]. Disturbances in the intestinal microbiome in early life have been linked with later development of chronic noncommunicable diseases, including overweight and obesity [10] as well as allergic disease and asthma [11, 12]. Since breastfeeding has also been suggested to modulate the risk of these disorders, it is warranted to speculate whether causal connections exist between breast milk, the gut microbiome, and later disease.

The gut microbiome of exclusively breastfed infants is drastically distinct from that of formula-fed infants of the same age. The initial neonatal gut microbiome in healthy term newborns is characterized by *Escherichia coli*, enterococci, streptococci, and clostridia, which are soon followed by anaerobes and particularly members of the genera *Bifidobacterium* and *Bacteroides* [13, 14]. Several studies indicate that bifidobacteria dominate the gut microbiome of breastfed infants [15–17], whereas that of formula-fed infants exhibits greater diversity [18]. Indeed, both the diversity and composition of the infant gut microbiome are affected by the intake of breast milk in a dose-dependent manner [19]. This has been observed also after the introduction of solid foods, and it appears that the cessation of breastfeeding modulates the infant gut microbiome more profoundly than complementary feeding [17].

Human milk contains a vast variety of molecules, which may influence the developing infant gut microbiome. The protection against infectious diseases alluded to above is mediated at least partially by IgA antibodies, Toll-like receptors, complement, and other antimicrobial proteins and peptides present in human milk [reviewed in 9], and it is likely that they elicit their effects on the potentially colonizing and indigenous as well as pathogenic bacteria. In addition
to antibacterial components, human milk contains substances which selectively promote the growth of specific bacteria. A considerable body of scientific evidence suggests that human milk oligosaccharides (HMOs) are the most significant modulators of the developing infant gut microbiome present in human milk [reviewed in 20, 21]. HMOs are a large group of molecularly diverse non-digestable carbohydrates, the structure and function of which are discussed in detail elsewhere in this volume. One of the most comprehensively characterized biological effects of HMOs in breastfed infants is providing substrates and hence a survival benefit to specific members of the intestinal microbiome and particularly bifidobacteria. Whether individual differences in milk HMO composition resulting from maternal genetics, metabolic state, or disease are reflected in the effect of breastfeeding on gut microbiota or child health is the focus of future research.

The Human Milk Microbiome

Breastfeeding may influence the developing infant gut microbiome by directly inhibiting or promoting the growth of bacteria or by modulating intestinal immune function. In addition, breast milk harbors live bacteria, which are thought to provide a colonizing inoculum for the infant gut. We are currently only in the initial stages of understanding the composition, origin, and significance of the human milk microbiome, which are discussed in detail elsewhere in this volume. It is not always clear to what extent the microbes detected in human milk samples reflect the bacterial population in the mammary epithelium and whether the microbes detected in milk originate from the maternal skin or even the infant mouth. Nonetheless, bacterial DNA has been detected in surgically obtained samples from nonlactating mammary gland specimens [22], which has been interpreted to suggest that the human mammary epithelium harbors a distinct microbiome. As of present, more than 200 bacterial species have been detected in human milk samples [21]. Interestingly, the bacterial taxa in human milk are distinct from those encountered on the areolar skin, and gut-associated obligate anaerobes such as bifidobacteria are often detected [21, 23]. This may result from differences pertaining to the exposure to oxygen and other environmental factors, but also raises the question of the origin of the mammary gland and human milk microbiome. Relatively few studies have systematically approached this question. Several species of obligate anaerobic bacteria, including members of the genera Bifidobacterium, Bacteroides, Parabacteroides, and the clostridial family, usually thought to be characteristic of the human gut microbiome, were detected in both milk and fecal samples in a study of 7 lactating women [23].
Interestingly, in clinical trials, specific probiotic lactobacilli have been recovered in human milk after oral consumption by the mother [24, 25]. While these data do not provide insight into the mechanisms of microbial transfer from the gut to the breast milk, and contact contamination is possible, it is intriguing to speculate that a mechanism exists by which enteral bacteria are transported to the mammary gland and then milk. This notion is consistent with data from an elegant series of studies by Perez et al. [26], according to which increased bacterial translocation from the gut to mesenteric lymph nodes was detected in the perinatal period in mice. Furthermore, *B. longum* DNA was detected in the maternal gut microbiome, circulating immune cells, and milk, and finally also in infant feces [26]. These data indeed suggest that enteromammary transfer of microbes takes place at least during lactation. It may be hypothesized that this process is triggered by labor, since the human milk microbiome is reportedly different in mothers who have given birth by elective cesarean section delivery [27, 28].

**The Human Milk Microbiome and Infant Gut Colonization**

Dissecting the contribution of the human milk microbiome to infant gut colonization is challenging. Human gut colonization is a stepwise process, which is influenced by a series of exposures during early life [reviewed in 9]. In recent years, it has been suggested that microbes may be present already in the intrauterine environment, and that gut colonization may begin during fetal life [29, 30]. Whether an intrauterine microbiome exists during healthy pregnancy and whether fetal microbial colonization takes place remain open questions and active areas of debate and research. It is well established, however, that the neonate receives an important inoculum of maternal vaginal and intestinal bacteria during delivery. The importance of this early massive bacterial exposure is highlighted by studies showing differences in gut microbiome composition in children born by cesarean section and thus not exposed to the maternal microbiome days, weeks, months, and even years after the fact [reviewed in 9]. Whether the differences in the milk microbiome between mothers who have delivered by cesarean section or vaginal delivery play a role in the aberrant gut colonization in infants born by cesarean section is currently not known.

As discussed above, breastfeeding is the single most important determinant of gut colonization patterns after delivery and during infancy. The role of breast milk bacteria as a source of colonizing bacteria is a new and largely uncharted area of research (Table 1). In a study of 15 infants born by elective cesarean section and their mothers [30], the colostrum microbiome was observed to be...
dominated by members of Enterobacteriaceae and streptococci. The dominant bacterial family detected in the meconium of these cesarean section-delivered neonates was staphylococci. However, in the newborn fecal samples obtained later in the first week of life, the relative abundance of staphylococci decreased and that of streptococci and Veillonellaceae increased. Moreover, using an unweighted UniFrac distance matrix, the neonatal gut microbiome began to resemble the maternal milk microbiome during the first week of life. Even though not direct proof, these data may be interpreted to support the notion that microbes in milk might colonize the newborn gut. This notion is corroborated by a recent study according to which several bacterial taxa, particularly those belonging to the genera *Streptococcus* and *Staphylococcus*, are detected in precolostral samples collected before delivery and oral samples from the neonates. Importantly, the design of the study with milk collection before the child is born excludes the possibility of the milk bacteria originating from the infant mouth [31].

Table 1. Selected studies investigating the association between the human milk microbiome and infant gut colonization

<table>
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<tr>
<th>Study</th>
<th>n</th>
<th>Method</th>
<th>Results</th>
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<tbody>
<tr>
<td>Collado et al. [30]</td>
<td>15</td>
<td>16S sequencing</td>
<td>The neonatal gut microbiome shifted during the first week of life to resemble more closely that in colostrum</td>
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<tr>
<td>Grönlund et al. [32]</td>
<td>61</td>
<td>qPCR</td>
<td>Maternal <em>Bifidobacterium</em> frequencies and counts in feces but not in milk correlated with those detected in the infant gut at 1 month of age</td>
</tr>
<tr>
<td>Jost et al. [23]</td>
<td>7</td>
<td>Several, e.g., culture and 16S sequencing</td>
<td>Members of <em>Bifidobacterium</em>, <em>Bacteroides</em>, <em>Parabacteroides</em>, <em>Blautia</em>, <em>Clostridium</em>, <em>Collinsella</em>, and <em>Veillonella</em> were detected in maternal feces, milk, and neonatal feces. Viable <em>Bifidobacterium breve</em> was detected in maternal feces and milk as well as in infant feces.</td>
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<tr>
<td>Pannaraj et al. [19]</td>
<td>107</td>
<td>16S sequencing</td>
<td>Infant fecal microbiome at the median age of 40 days resembled more closely the microbiome in their own mother’s milk as compared to nonrelated mothers. As per source tracking analysis, 15% of the fecal microbiome was derived from the bacteria in milk.</td>
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<tr>
<td>Williams et al. [33]</td>
<td>21</td>
<td>16S sequencing</td>
<td>The milk and infant fecal microbiomes have some similarity in early life but become increasingly different over time. Source tracking analyses indicated that on day 2 of life, milk microbes contribute 4.9% to the infant gut microbiome, but this diminishes to 0.3% at 6 months of age.</td>
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The contribution of human milk bacteria to infant gut colonization may indirectly be assessed based on reports on the similarities between the maternal milk and the infant gut microbiome (Table 1). In a study of 61 mother-infant pairs, bifidobacteria were analyzed by PCR from milk and infant fecal samples obtained at the age of 1 month [32]. In addition, the same analyses were performed on maternal fecal samples collected during the last trimester of pregnancy. Maternal fecal *Bifidobacterium adolescentis* and *B. bifidum* frequencies and counts correlated significantly with those detected in their infants’ gut. *B. longum* was the most frequently detected *Bifidobacterium* species in human milk, but no correlation was observed between human milk and infant gut bifidobacteria in total or at species level. According to these data, at least in the case of bifidobacteria, the mother is an important source of gut bacteria to the infant, but other routes of transfer may exceed human milk in importance.

Further insight into the associations between the milk microbiome and infant gut colonization was provided by Jost et al. [23], who systematically studied the microbial composition of maternal feces and milk and serial infant fecal samples in 7 mother-infant pairs using both culture-independent and -dependent techniques. Bacteria belonging to the genera *Bifidobacterium*, *Bacteroides*, *Parabacteroides*, *Blautia*, *Clostridium*, *Collinsella*, and *Veillonella* were observed to be detectable in all 3 sample types (maternal feces, milk, and neonatal feces). Furthermore, viable *Bifidobacterium breve* was detected in maternal feces and milk as well as infant feces. These data lead the authors to conclude that their results support the hypothesis of enteromammary transfer of bacteria influencing infant gut colonization. Even more compelling yet still indirect evidence for the role of human milk microbes in infant gut colonization was provided by a recent study by Pannaraj et al. [19], who collected and analyzed areolar, milk, and infant fecal samples from 107 mother-infant pairs. The infant gut microbiome assessed by fecal samples obtained at the median age of 40 days resembled more closely the microbial profiles detected in their own mother’s milk as compared to nonrelated mothers. It is important to note, however, that the milk and infant fecal microbiomes were clearly distinct from each other. Based on source-tracking analyses, approximately 15% of the fecal microbiome in predominantly breastfed infants originated from the bacteria in milk and 10% from the areolar skin during the first 30 days of life. Recently published data from serial fecal and milk sampling from 21 mother-infant pairs suggest that the human milk microbiome may contribute to infant oral colonization but less to gut colonization [33]. The milk and infant gut microbiomes displayed some similarities in the neonatal period with source-tracking analyses suggesting an approximately 5% contribution from milk bacteria to the neonatal gut microbiome during the first days of life. Later in infancy, the role of milk microbes in infant gut colonization patterns was found to be negligible.
The clinical significance of the human milk microbiome is currently not well understood. Recent advances in research indicate, however, that maternal characteristics and exposures affect the bacterial population in human milk (Table 2). Maternal overweight or excessive weight gain have been reported to be associated with an altered milk microbiome [27, 34]. Interestingly, the HMO composition of human milk has been reported to be associated with the microbial profile in milk [35].

As alluded to above, the milk microbiome of mothers who have given birth by cesarean section is significantly different from those who deliver vaginally [27, 28]. Furthermore, maternal intrapartum antibiotic administration has recently been reported to affect the bacterial composition of human milk 1 month after delivery [28]. Interestingly, intrapartum antibiotics are also associated with increased abundance of antibiotic resistance genes in the neonatal gut microbiome, but this potentially detrimental consequence of antibiotic exposure appears to be ameliorated by breastfeeding, possibly at least in part via modulation of the taxonomic composition of the infant gut microbiome in favor of bifidobacteria [36].

Given the established significance of early gut colonization patterns to later health and the potential contribution of the bacteria in milk to the developing infant gut microbiome, future research should focus on establishing whether the milk microbiome is associated with health outcomes. Experimental and clinical

### Table 2. Factors affecting the human milk microbiome

<table>
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<tr>
<th>Exposure/factor</th>
<th>Impact on the human milk microbiome</th>
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| Excessive weight gain during pregnancy [34]         | ↑ staphylococci  
|                                                     | ↓ bifidobacteria  
|                                                     | ↑ Akkermansia muciniphila-type bacteria                                                              |
| Cesarean section delivery [27]                      | ↓ diversity  
|                                                     | ↓ richness  
|                                                     | ↓ bifidobacteria  
|                                                     | ↑ staphylococci                                                                                 |
| Intrapartum antibiotics [28]                         | ↑ diversity  
|                                                     | ↑ richness  
|                                                     | ↓ bifidobacteria                                                                                 |
| Human milk oligosaccharide profile                   | ↑ bifidobacteria with increased HMO total concentration and sialylated HMOs  
|                                                     | ↑ Akkermansia muciniphila with increased fucosylated human milk oligosaccharides                   |

### Clinical Significance of the Human Milk Microbiome

The clinical significance of human milk bacteria and their contribution to the developing infant gut microbiome is currently not well understood. Recent advances in research indicate, however, that maternal characteristics and exposures affect the bacterial population in human milk (Table 2). Maternal overweight or excessive weight gain have been reported to be associated with an altered milk microbiome [27, 34]. Interestingly, the HMO composition of human milk has been reported to be associated with the microbial profile in milk [35].

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Given the established significance of early gut colonization patterns to later health and the potential contribution of the bacteria in milk to the developing infant gut microbiome, future research should focus on establishing whether the milk microbiome is associated with health outcomes. Experimental and clinical
studies are needed to elucidate the contribution of the bacteria in milk to the beneficial effects of breastfeeding. In the future, modulating or mimicking the human milk microbiome may offer a means to influence early gut colonization and improve child health.

**Disclosure Statement**

The author declares no conflict of interest.

**References**


