The Human Milk Microbiome – Possible Implications for Infant Health

The human body contains and carries a large number of microorganisms and, surprisingly, the mammary gland and mother’s milk also contain their own microbiota. Very little is known, however, about the function of the human milk microbiome or the factors responsible for the marked variation observed within and among populations all over the world. It is hypothesized that the human milk microbiome plays an important role in the development of an infant’s gastrointestinal microbiota and thus for its health in the future. Scientific exploration of the human milk microbiome and possible strategies for modification has just began but appears to be very promising.

The first studies investigating the bacterial content of human milk were undertaken in the 1970s and 1980s because of concerns that bacteria might affect pre-term babies fed with human milk. At that time, the only bacteria that had been detected in human milk were Staphylococci and Streptococci, reported Dr. Esther Jiménez, Madrid (Spain). It was not until 2003 that lactic acid bacteria were isolated from human milk [1]; these were regarded as beneficial because they were able to suppress the growth of Staphylococcus aureus in the laboratory [2].

Culture-dependent and culture-independent techniques

According to Jiménez, about 50 genera and 200 species have been detected in human milk using culture techniques. The normal concentration of bacteria in milk from healthy women was shown to be about $10^3$ colony-forming units (cfu) per milliliter. Culture techniques, however, have limitations. They are semi-quantitative, and only 30% of bacteria can be cultured. On the other hand, culture-independent methods such as 16S rRNA gene sequencing are expensive but may provide in-depth information about the microbial composition in human milk. 16S rRNA sequencing data indicated that the bacterial communities of human milk are complex and often stable over time within an individual [3]. In addition, substantial inter-individual variability was observed. “Each woman’s milk has its own bacterial profile,” concluded Jiménez.

In particular, 16S rRNA sequencing has been very useful in identifying bacteria that cannot be cultured, such as those that cannot survive in the presence of oxygen (anaerobes), in different body fluids. For instance, a Swiss group detected such obligate anaerobic genera like Bifidobacterium, Veillonella, and Bacteroides in the feces of healthy breastfed neonates using a comprehensive approach with culture and molecular methods [4].

Entero-mammary pathway

The presence of obligate anaerobic bacteria was not compatible with the traditional view that human milk is sterile by nature. Bacteria detected in milk were thought to stem exclusively from the breast skin or from the baby’s mouth colonizing the mammary gland, said Jiménez.

In another study the same lactic acid bacteria were found in locally fermented food, maternal milk, and fecal samples of breastfeeding mothers and their babies [5]. This observation supports the hypothesis that bacteria are transferred from the mother’s gastrointestinal tract to her milk and through the milk to the infant’s gastrointestinal tract.

As Jiménez reported, evidence has increased that a mother’s gastrointestinal bacteria are able to reach the mammary gland via an endogenous route. For this bacterial...
translocation, dendritic cells appear to play an important role. These specialized cells were shown to open the tight junctions between epithelial cells in the digestive tract. By sending projections into the intestine, dendritic cells were able to sample bacteria residing there and take them to the lamina propria [6]. From there, bacteria likely travel with immune cells through the body; indeed, DNA signatures of intestinal bacteria were found in immune cells in both human milk and blood of lactating mothers [7].

**Human milk: what is normal?**

Little is known about what is normal in terms of human milk composition, including its microbiome. Therefore, the INSPIRE project was started to determine (using standardized data collection methods) how human milk composition and microbiome vary in different parts of the world. As Dr. Michelle McGuire, Pullman (USA), reported, 413 healthy breastfeeding women with healthy babies belonging to populations from Africa, Europe, South America, and the USA were included in this study.

Substantial population differences were found regarding the content of milk protein, immune factors, human milk oligosaccharides (HMOs) such as 2'-fucosyllactose (2'FL), and even lactose, which is regarded as being the least variable component of human milk [8–10]. HMO profiles in milk produced by mothers categorized as secretors (2'FL producers) differed substantially from those of non-secretor mothers [10]. Factors influencing these variations are widely unknown. In McGuire’s opinion, “we need to rethink the idea of there being a normal range of milk compounds.”

**Marked variations**

Furthermore, the INSPIRE project revealed clear population differences and variation within populations in the maternal milk microbiome [11]. For instance, in a Hispanic population from California (USA) more Staphylococcus and Escherichia/Shigella were found as compared with a population from Washington/Idaho (USA), in which Lactobacillus didn’t even belong to the 30 most frequently encountered genera. In populations from Peru or Spain, Streptococcus was predominant; in Sweden, however, Staphylococcus was most frequently encountered. While milk microbiomes in urban and rural populations in Gambia were similar, a remarkable dissimilarity was observed between an urban and a rural population from Ethiopia.

McGuire reported that Staphylococcus and Streptococcus (present in 99% and 98% of the samples) were universal core bacteria [11]. The next-frequent genera were Propionibacterium (in 78% of the samples) and Corynebacterium (in 74% of the samples). The data showed, however, that there are different core bacteria in different regions.

“At the time being we don’t understand what is a normal or healthy human milk microbiome,” said McGuire. What is regarded as normal in one population may be suboptimal for another. In McGuire’s opinion, however, “understanding this is a must before we can understand what is unhealthy or intervene.” And “normal” or “healthy,” she said, is clearly not a one-size-fits-all construct.

**Modulation by diet**

In another 21 healthy breastfeeding women, the McGuire research group performed detailed dietary assessment over a period of 6 months postpartum to investigate the influence of diet on the human milk microbiome [12]. As McGuire reported, multiple relationships were found. For instance, high-calorie diets were associated with a higher abundance of Firmicutes in milk, whereas milk of mothers consuming less-energy-den-
bacteria as Veillonella, Leptotrichia and Prevotella was observed.

Maternal health may also be an important factor. For instance, milk Bifidobacterium levels were significantly lower in allergic as compared with non-allergic mothers (p=0.003) [14]. Collado and co-workers detected lower Bifidobacterium and higher Staphylococcus levels in milk produced by obese mothers than in normal-weight mothers [15]. In milk of normal-weight mothers a higher bacterial diversity was observed [13]. The milk microbiomes from mothers with vaginal delivery or Cesarean section differed in bacterial composition and diversity [13, 16]. Interestingly, differences were observed in milk microbiotas of mothers with emergency or programmed Cesarean section [13], reported Collado. In her opinion, these differences may be due to differential stress levels impacting intestinal permeability. In a recent study, lower levels of Staphylococcus and Pseudomonas spp. were found in the colostrum of mothers after vaginal delivery than after Cesarean section [17]. In addition, the mode of delivery had a strong influence on the microbiota network.

HMOs – the role of the secretor status

In non-secretor mothers (as opposed to the whole study population) McGuire and co-workers found a strong relationship between HMO profiles in milk and the milk microbiome and similarly (in contrast to secretor mothers) between immune factors in maternal milk and the milk microbiome.

Maternal and perinatal factors appear to affect not only the human milk microbiota, but also biologically active milk compounds (such as HMOs, fatty acids, cytokines, polymamines, proteins), concluded Collado, and “we need to understand the interactions between the human milk microbial communities/milk-born compounds and the infant gut development.”

The relationship between milk and the infant fecal microbiomes seemed to be modulated by the maternal secretor status as well. In the whole study population, McGuire found no association between milk microbiomes and the infant fecal microbiomes, but in non-secretor mothers a clear relationship was evident. According to McGuire, there is a clear pattern “indicating that the secretor status is somehow interacting with many relationships of interest.”

The association between maternal secretor status and the infant fecal microbiota composition has been confirmed in other studies. For example, infants fed by non-secretor mothers were delayed in the establishment of Bifidobacterium-laden microbiota (Fig. 1) [18]. Another study suggested an influence of maternal and infant secretor status on the infant microbiota composition at the age of 2 or 3 years [19].

Shaping the infant gut microbiome

Maternal microbiomes and diet (e.g., human milk versus formula feeding, introduction of solid food) have been shown to be associated with variation in the infant’s gastrointestinal microbiome composition [20].

“Human milk is a constant source of microbes,” said Collado. The importance of the milk microbiome for the establishment of the infant gastrointestinal microbiome has been confirmed in a study in which the infant gut microbial communities were shown to be more closely related to the mother’s milk and skin as compared with the milk and skin of a random mother [21]. During the first month of life, breastfed infants obtained 27.7% of their intestinal bacteria from human milk and 10.4% from areolar skin. Changes in the infant fecal microbiome were associated with the proportion of breastfeeding in a dose-dependent manner.

The infant intestinal microbiome undergoes a dynamic process. Initially the diversity of the microbiome is low, but it increases over the first 2 years of life (Fig. 2) [22]. For specific bacteria specific patterns of relative abundance are observed. For instance, levels of Bifidobacterium and Enterobacteriaceae steadily decrease during maturation of the gastrointestinal microbiome, while the abundance of Bacteroidaceae increases [23].

As Collado pointed out: “Any disturbance of the microbiota development during this critical phase of life would affect the microbial composition.” For example, antibiotic exposure or Cesarean section may disrupt the establishment of the infant’s intestinal microbiome [22].

Infant gastrointestinal microbiota and health

A mother’s microbiome is now recognized as one of the essential factors determining neonatal health outcomes. Early exposure to mother’s milk microbiota is thought to impact the immune system development and

**Figure 1**

| Relative levels of gut microbiota in infants fed by secretor mothers (S) as compared with non-secretor mothers (N) |
|---------------------------------------------------------------|
| **Secretor Status:**         | **N** | **S** |
| **Bacteroides**                      | [3]  | [3]  |
| **Bifidobacterium**                  | [3]  | [3]  |
| **Clostridium**                       | [3]  | [3]  |
| **Enterobacteriaceae (Other)**       | [3]  | [3]  |
| **Escherichia/Shigella**              | [3]  | [3]  |
| **Streptococcus**                    | [3]  | [3]  |

modified after [18]
metabolism, said Collado. This may be one reason why breastfeeding has been shown to protect infants from infections and to decrease the risk of developing such diseases as atopic dermatitis, asthma, obesity, diabetes, necrotizing enterocolitis, gastroenteritis or sudden infant death syndrome in epidemiological studies.

According to Collado, alterations in the milk microbiome may be transferred to the infants via breastfeeding, potentially providing at times also a dysbiotic microbiota, “making them adults with a higher risk of disease.”

Mother’s milk for preterm infants
Preterm infants often are delivered by Cesarean section and receive antibiotics; they differ from term babies in nutrition, have delayed hospital stays and often an impaired gastric barrier and an aberrant gastrointestinal microbiota, characterized mainly by a low number of Bifidobacterium and Lactobacillus and a high prevalence of Enterobacteria. Therefore, feeding human milk may be even more important for preterm infants than for term infants. According to Jiménez, the best option is the infant’s own mother’s milk, followed by donor human milk and formula milk.

Jiménez pointed out that distortions in the development of intestinal microbiota may have a strong impact on preterm infants, for instance causing an increased risk of developing necrotizing enterocolitis or sepsis.

Breast cancer linked with bacterial dysbiosis
A mother’s microbiome may be important not only for the health of the infant but also for the mother’s health.

A link between breast cancer and dysbiosis in breast tissue was suggested by Xuan et al., who analyzed microbiota in tumor tissue and paired adjacent breast tissue from the same patients [24]. As Jiménez pointed out, bacteria of the Methylobacteriaceae family occurred in much higher abundance in tumor tissue (p=0.0237), whereas bacteria of the Sphingomonadaceae family were significantly enriched in adjacent breast tissue (p=0.0079) (Fig. 3). Among these families, M. radiotolerans and S. yanoikuyae were the most abundant species. The relative abundances of M. radiotolerans and S. yanoikuyae correlated inversely in normal breast tissue but not in tumor tissue. In addition, the bacterial load in tumor tissue was reduced compared with that in adjacent normal breast tissue or breast tissue of healthy women.

In a lactating woman diagnosed with Hodgkin’s lymphoma in the 2nd trimester of pregnancy, chemotherapy affected both bacterial diversity and profiles of her milk [25]. According to Jiménez, bacterial composition changed completely between baseline and weeks 4–16 of therapy. In particular, chemotherapy was accompanied by a striking increase in the abundance of Acinetobacter and Xanthomonadaceae and a marked decrease in bacteria believed to confer beneficial effects for infants (Bifidobacterium and Eubacterium, but not Lactobacillus).

Mastitis – response to milk lactobacilli
Mastitis is a painful condition and regarded as the principal reason to stop breastfeeding. According to Jiménez, mastitis is characterized by bacterial dysbiosis and high counts of one or two predominating species. In a study by Mediano et al., the num-
bacillus salivarius, or antibiotics as prescribed.

were randomized into three groups to be
treated with Lactobacillus fermentum, Lacto-
...were not detectable in the milk of the control
group but were found in substantial numbers
at the end of treatment, lactic acid bacteria
biotic group as compared with controls. At
...in milk samples of both groups were simi-
lar within each group. Moreover, mean staphy-
occal counts ranged between 1 and 7 [26]. The milk
samples were tested for Staphylococcus epi-
dermidis, often in combination with Streptoco-
coccus (mitis or sali-

...samples contained a mixture of mainly different Strep-
tococcus species (especially mitis) together
with Rothia spp., Staphylococcus aureus, or S.
epidermidis.

In clinical studies performed by Jimé-
nez’ group, lactating women with staph-
lococcal mastitis derived benefits from oral
treatment with lactic acid bacteria isolated
from milk of healthy mothers [27, 28].

In a pilot study, women with mastitis
who had previously failed to respond to an-
tibiotics randomly received Lactobacillus sa-
livarius and Lactobacillus gasseri or placebo
for 4 weeks [27]. Mean staphylococcal counts
in milk samples of both groups were simi-
lar at baseline. Following treatment, how-
ever, they were substantially lower in the pro-
biotic group as compared with controls.

by their healthcare providers. After 3 weeks,
probiotic treatment was associated with a
clear reduction of bacterial counts and less
breast pain [28].

Where science and regulation meet
Knowledge regarding the influence of mater-
nal diet on human milk, specifically the hu-
man milk microbiome, and the possible im-
 pact of nutrition on the infant’s outcome is
steadily increasing. Consequently, new ques-
tions are arising, said Prof. Dr. Seppo Salmi-
nen, Turku (Finland). For instance:
— Do we need to introduce “protective fac-
tors originating from human milk” in addi-
tion to breastfeeding?
— Would it be beneficial to enrich the ma-
ternal diet or infant formula diet with
prebiotic supplements?
— Should it be an aim to influence the ma-
ternal microbiota and thereby possibly
the long-term outcome of the child?
— And finally: “Do we have sufficient sci-
etific data to convince the regulator and
get approval?”

Convincing the regulator, however, has pro-
ven difficult in many cases. One reason is that
“science is always ahead of regulation, which
only very gradually adapts,” reported Salmi-
nen. For instance, in microbiology the use of
culture techniques has been standard. How-
ever, new methods have been introduced,
such as PCR (polymerase chain reaction) or
16S rRNA DNA sequencing, and the regula-
tor asks: “What is the generally accepted sci-
cence?” Furthermore, the regulator often re-
gards the relation between cause and conse-
quence as not being clear enough to support
a health claim for a specific pre- or probiotic.

In Salminen’s opinion, the decision pro-
cess is especially complicated in the Euro-
pean Union (EU). The European Food Safe-
ty Authority (EFSA), responsible for assessing
submitted health claims for scientific sub-
stantiation, is an independent authority ad-
vising the European commission and parlia-
ment. The decision about approval, however,
is always made by the European commis-
sion, often in consultation with the European
parliament or the EU member states. In spe-

Human milk has always been considered the natural model for the production
of infant milk formula, as human milk optimally supports infants’ natural development.
Therefore, the “human milk research group” initiated by HiPP – has been intensively
exploring the composition of human milk and its positive effects on human health for
years.

The research group has met for thematic workshops on a regular basis. Representa-
tives of HiPP’s nutritional science and product development divisions discussed the
issue of “The microbiome of human milk” together with Professor Michelle McGuire,
School of Biological Sciences at Washington State University in Pullman (WA, USA),
Dr. Esther Jiménez Quintana, Departamento de Nutrición, Bromatología y Tecnología
de los Alimentos, Universidad Complutense, Madrid (Spain), Dr. Maria Carmen Collado,
Instituto de Agroquimica y Tecnologia de Alimentos – Consejo Superior Investigaci-
ones Cientificas (IATA-CSIC), Valencia (Spain), and Professor Seppo Salminen, Func-
tional Foods Forum, Faculty of Medicine, University of Turku (Finland).
cific cases the assessment of health claims, especially if they are related to vulnerable groups, such as children or pregnant women, has to be done according to the highest standards. Most important for the decision, in Salminen’s opinion, is the relation between causes, risk factors and consequences. But with regard to microbiota, neither the risk factors potentially leading to bacterial dysbiosis nor the consequences are generally accepted. Therefore, at the present time, “it would be very difficult to get a positive evaluation or a positive decision on health claims related to microbiota composition and activity,” concluded Salminen.

Summary
There is good evidence that mothers’ and infants’ microbiomes are related. The functional significance of this relationship, however, has yet to be determined. Up to now the focus has been on understanding what is a “normal” or “healthy” milk microbiome. A substantial variation has been found around the globe which appeared to be driven by such factors as environmental exposure, genetics, diet, and maternal health. The relationship between the human milk microbiome and the infant fecal microbiome varies by population and by the maternal secretor status. The next step will be to conduct intervention studies to see if it is possible to influence the human milk microbiome, for instance by diet. In addition, it is necessary to relate the variation in maternal milk microbiomes to infant health. However, it is not all about babies: variation in the human milk microbiome apparently influences maternal health, as indicated by links with diseases such as breast cancer or mastitis.

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