The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery[1–4]

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ABSTRACT

Background: Breast milk is recognized as the most important postpartum element in metabolic and immunologic programming of health of neonates. The factors influencing the milk microbiome and the potential impact of microbes on infant health have not yet been uncovered.

Objective: Our objective was to identify pre- and postnatal factors that can potentially influence the bacterial communities inhabiting human milk.

Design: We characterized the milk microbial community at 3 different time points by pyrosequencing and quantitative polymerase chain reaction in mothers (n = 18) who varied in BMI, weight gain, and mode of delivery.

Results: We found that the human milk microbiome changes over lactation. *Weisella, Leuconostoc*, *Staphylococcus*, *Streptococcus*, and *Lactococcus w*ere predominant in colostrum samples, whereas in 1- and 6-mo milk samples the typical inhabitants of the oral cavity (eg, *Veillonella*, *Leptotrichia*, and *Prevotella*) increased significantly. Milk from obese mothers tended to contain a different and less diverse bacterial community compared with milk from normal-weight mothers. Milk samples from elective but not from nonelective mothers who underwent cesarean delivery contained a different bacterial community than did milk samples from individuals giving birth by vaginal delivery, suggesting that it is not the operation per se but rather the absence of physiological stress or hormonal signals that could influence the microbial transmission process to milk.

Conclusions: Our results indicate that milk bacteria are not contaminants and suggest that the milk microbiome is influenced by several factors that significantly skew its composition. Because bacteria present in breast milk are among the very first microbes entering the human body, our data emphasize the necessity to understand the biological role that the milk microbiome could potentially play for human health. *Am J Clin Nutr* 2012;96:544–51.

INTRODUCTION

Breast milk gained its nutritional superiority after the divergence of mammals millions of years ago (1). Breast milk contains several functional and protective nutrients, which help to create the right microenvironment for gut development and maturation (2, 3). Protection is provided by components including regulatory cytokines and growth factors. In addition, breast milk contains several other factors such as lysozyme, lactoferrin, and oligosaccharides, which assist in preventing infections and supporting the growth of beneficial bacteria. Furthermore, breast milk is also a continuous source of microbes, their growth factors, and components that regulate host-microbe interactions. These factors emphasize the key position of breastfeeding in conferring protection during a critical period in life, when breast milk is the sole source of nutrition for the infant and when the neonate's own immune defenses, including the integrity of the gut barrier, are immature (4–7).

Breastfeeding has been reported to modify infant gut microbiota development (8–10), and breast milk is recognized as one of the most important postpartum elements modulating metabolic and immunologic programming related to the child's health (11). In overweight and obese pregnant women, a vicious circle of unfavorable metabolic development may be generated if the aberrant gut microbiota associated with overweight and obese or excessive weight gain during pregnancy is transferred to the infant (12–15). Differences in microbiota composition in infants delivered by cesarean delivery compared with vaginally born infants (15, 16) suggest that mother-infant transmission of microbiota occurs during vaginal delivery. Recent work has opened up new angles in the uncovering of a previously unknown source of microbiota, that in breast milk (17), which suggests that maternal factors can influence the composition and activity of the microbiota. The purpose of the present study was thus to identify pre- and postnatal factors that can potentially influence the bacterial communities inhabiting human milk and to compare the bacterial composition of breast milk with that

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from other sites in the human body. For this purpose, we analyzed breast-milk composition through the recommended period of breastfeeding in women who varied in BMI, weight gain during pregnancy, and mode of delivery.

SUBJECTS AND METHODS

Subjects and design

Mothers were selected from an ongoing prospective, randomized follow-up study that started in April 2002 (NCT00167700, section 3; registered through clinicaltrials.gov) (18). Pregnant women were recruited at their first visit to a maternal welfare clinic if they had no metabolic or chronic diseases other than allergy. For the present report women were selected according to their prepregnancy BMI, early exclusive breastfeeding practices, and breastfeeding follow-up during 6 mo as well as breast-milk sample availability. A total of 18 women were included: 10 obese women with a BMI (in kg/m^2) >30 and 8 normal-weight women with a BMI ≤25 who served as controls. Details of delivery and early infant feeding were collected after birth. Data on duration of breastfeeding as well as infant feeding practices were obtained by interview and recorded at each visit. Maternal dietary guidance was provided by a nutritionist during each study visit to conform to the current dietary recommendations. Written informed consent was obtained from the participants, and the Ethics Committee of the Hospital District of South-West Finland approved the study protocol. "Normal" weight gain was defined according to the Institute of Medicine's recommendations: 11.5–16.0 kg and 7.0–11.5 kg for normal-weight and obese women, respectively. Clinical characteristics of mother-infant pairs are shown in **Table 1**.

TABLE 1
Clinical characteristics of the infants and their mothers (n = 18 mother-infant pairs) included in the study

	Values
Maternal age (y)	32.01 ± 5.12[1]
Weight before pregnancy (kg)	76.11 ± 13.87
Normal weight	62.88 ± 4.58
Obese	86.70 ± 8.17
BMI before pregnancy (kg/m^2)	27.71 ± 4.77
Normal weight	22.90 ± 1.11
Obese	31.86 ± 2.22
Maternal weight gain over pregnancy (kg)	12.45 ± 5.82
Normal weight	14.70 ± 5.60
Obese	10.49 ± 4.60
Duration of pregnancy (wk)	40.40 ± 1.11
Method of delivery (% vaginal)	50.0 (9/18)[2]
Medical induction of labor by using Cytotec (Pfizer; oxitocin) (%)	11.1 (2/18)
Problems during pregnancy (%)	
Normal, no problems	88.8 (16/18)
Gestational diabetes	11.1 (2/18)
Streptococcus B positive	0 (0/18)
Other infections	0 (0/18)
Parity (%)	
1	33.3 (6/18)
2	33.3 (6/18)
>2	33.3 (6/18)
Sex (% female)	55.5 (10/18)
Infant head circumference at birth (cm)	35.28 ± 1.11
Infant weight (kg)	
At birth	3.69 ± 0.32
Age 6 mo	8.13 ± 0.90
Age 12 mo	9.87 ± 1.40
Age 24 mo	12.41 ± 1.03
Infant length (cm)	
At birth	51.25 ± 1.84
Age 6 mo	68.80 ± 2.82
Age 12 mo	76.50 ± 3.65
Age 24 mo	87.67 ± 2.80
Maternal antibiotic treatment (%)	
Before delivery	0 (0/18)
During delivery	16.6 (3/18)
After delivery	5.5 (1/18)
Infant antibiotic treatment (%)	
At birth	0 (0/18)
First months	0 (0/18)
Total time exclusively breastfeeding (mo)	4.10 ± 1.10
Breastfeeding at 1 mo (%)	100 (18/18)
Breastfeeding at 6 mo (%)	100 (18/18)
Infant diet at age 6 mo (%)	
Exclusively breast milk	0 (0/18)
Breast milk + formula	0 (0/18)
Breast milk + solid foods	66.7 (12/18)
Breast milk + formula + solid foods	33.3 (6/18)
Formula	0 (0/18)

[1] Mean ± SD (all such values).
[2] Percentage and prevalence reflect the percentage of the number of positive out of total samples included in the study.

Breast-milk samples

Breast-milk samples from mothers were collected within 2 d after mothers gave birth in the maternity hospital (colostrum) and at 1 and 6 mo after delivery at home. Mothers were given written instructions for standardized collection of samples each morning, and the samples were frozen and stored at −20°C for later analysis. Before sample collection, the breast was cleaned with an iodine swab to reduce bacteria residing on the skin, and breast milk was collected manually, discarding the first drops, with a sterile milk collection unit.

DNA extraction and quantitative real-time polymerase chain reaction analysis

Before analysis, the milk samples corresponding to colostrum and 1 and 6 mo of infant age were thawed and centrifuged at 10,000 × g for 10 min to separate cells and fat from whey. Thereafter, total DNA was isolated from the pellets by using the QIAamp DNA Stool Mini Kit (Qiagen). Quantitative polymerase chain reaction (qPCR)[5] and specific bacterial group analyses were conducted as previously described (12, 13). qPCR amplification and detection were performed with an ABI PRISM 7300-PCR sequence detection system (Applied Biosystems). Each reaction mixture of 25 μL was composed of SYBR Green PCR Master Mix (Applied Biosystems), 0.5 μL of each of the specific primers at a concentration of 0.25 μmol/L, and 1 μL template DNA. The fluorescent products were detected in the last step of each cycle. A melting curve analysis was performed

[5] Abbreviations used: OTU, operational taxonomic unit; PCA, principal components analysis; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

after amplification to distinguish the targeted from the nontargeted polymerase chain reaction (PCR) product. The bacterial concentration in each sample was calculated by comparing the Ct values obtained from standard curves. These were created by using serial 10-fold dilution of pure culture-specific DNA fragments corresponding to 10–10^9 gene copies/mL. Statistical analyses were performed by using SAS version 9.2 software for Windows (SAS Institute Inc). For the BMI and weight gain groups, microbial data were expressed as medians with IQRs, and the Mann-Whitney test was used for comparisons. Differences in the prevalence of bacterial groups were established applying the chi-square test.

PCR amplification and pyrosequencing

The first 500 base pairs (bp) of the 16S rRNA genes were amplified with the universal eubacterial primers 27F and 533R by using high-fidelity AB-Gene DNA polymerase (Thermo Scientific) with an annealing temperature of 52°C and 20 cycles to minimize PCR biases (19). A secondary amplification was performed by using the purified PCR product as a template, in which the universal primers were modified to contain the pyrosequencing adaptors A and B and an 8-bp "barcode" specific to each sample, following the method used in Cole et al (20). Barcodes differed from each other in ≥3 nucleotides to avoid errors in sample assignments. Three secondary PCRs were performed per sample; the PCR products were pooled before purification by using an Ultrapure PCR purification kit (Roche). The final DNA per sample was measured by picogreen fluorescence in a Modulus 9200 fluorimeter from Turner Biosystems. PCR products were pyrosequenced from the forward primer end only by using a GS-FLX sequencer with Titanium chemistry (Roche) at the Center for Public Health Research in Valencia, Spain. One-eighth of a plate was used for each pool of 20 samples.

Sequence analysis

Sequences with an average quality value of <20 and/or with >4 ambiguities in homopolymeric regions in the first 360 flows were excluded from the analysis. Only sequences longer than 200 bp were considered. Sequences were assigned to each sample by the 8-bp barcode and passed through the Ribosomal Database Project classifier software (21), where each sequence was assigned a phylum, class, family, and genus, as long as the taxonomic assignment was unambiguous within an 80% confidence threshold. To estimate total diversity, sequences were clustered at 97%, 95%, and 90% nucleotide identity over a 90% sequence alignment length by using the Ribosomal Database Project pyrosequencing pipeline. For this analysis, sequences >97% identical were considered to correspond to the same operational taxonomic unit (OTU), representing a group of sequences that presumably belong to the same species (22). Principal components analysis (PCA) was performed with UNIFRAC (23) by using clustering at 97% sequence identity. The DNA sequences were deposited in the MG-RAST server database (http://metagenomics.anl.gov/) with access numbers 4475188.3–4475213.3, 4475215.3, and 4475220.3–4475235.3 under the project name "Milk Microbiome."

RESULTS

Milk bacterial diversity across lactation time

After quality filtering and length trimming, 119,652 16S rRNA sequences were analyzed, with an average number of taxonomically assigned, high-quality sequences of 2623 per sample. The taxonomic assignment of the sequences showed that the composition of human breast milk is dominated by bacilli, which account for >76% of the total number of sequences obtained (**Figure 1**A). In colostrum, the most common genera were *Weisella* and *Leuconostoc* (both lactic acid bacteria from the order Lactobacillales) followed by *Staphylococcus*, *Streptococcus*, and *Lactococcus* (Figure 1B). Although lactic acid bacteria genera were still among the most abundant in 1- and 6-mo milk samples, typical inhabitants of the oral cavity such as *Veillonella*, *Leptotrichia*, and *Prevotella* or members of the TM7 phylum (24) increased significantly.

Milk from colostrum compared with 6-mo milk samples showed different patterns of bacterial diversity. The rarefaction curves indicated >1000 OTUs when sequences were clustered at 97% sequence identity (the consensus value for determining species boundaries), >800 OTUs when clustered at 95%, and >400 OTUs when clustered at 90% (**Figure 2**). With the sequence length (400-bp average length) and potential pyrosequencing errors taken into consideration (25), the high number of OTUs obtained, even at conservative clustering values, points to several hundred species in human breast milk, with colostrum having higher diversity than transition and mature milk.

Milk microbiome and maternal obesity

Maternal BMI influenced the milk microbiome composition. A PCA was performed for milk samples from mothers who had delivered their infants vaginally to eliminate the effect of mode of delivery. Clustering of samples in obese women indicated a more homogenous bacterial composition in comparison with the milk samples of normal-weight women, which were more scattered (**Figure 3**A), which suggests that bacterial composition may have been influenced by body weight. Further PCAs clearly separated samples from mothers who gained excessive weight during pregnancy from those of mothers with normal weight increase (Figure 3B).

The qPCR data corroborated the differences in the proportion of specific taxonomic groups between obese and normal-weight mothers (*see* Table S1a under "Supplemental data" in the online issue) and those with excessive and normal weight gain over pregnancy (*see* Table S1b under "Supplemental data" in the online issue). Higher maternal BMI was related to higher numbers of *Lactobacillus* in colostrum ($r = 0.600$, $P = 0.026$). Similarly, higher numbers of *Staphylococcus* ($r = 0.560$, $P = 0.038$) and lower numbers of *Bifidobacterium* in breast milk 6 mo postpartum ($r = -0.651$, $P = 0.012$) were related to higher maternal BMI. With the use of mixed models to analyze the effect of BMI on breast-milk microbiota composition during lactation, we detected higher total bacteria counts (ratio: 0.34; 95% CI: 0.08, 0.60; $P = 0.011$), *Staphylococcus* (ratio: 0.62; 95% CI: 0.30, 0.93; $P = 0.0001$), and *Lactobacillus* (ratio: 0.52; 95% CI: 0.02, 2.02; $P = 0.038$) and lower *Bifidobacterium* numbers (ratio: −0.48; 95% CI: −0.78, 0.18; $P = 0.002$) in obese compared with normal-weight women over the first 6 mo of breastfeeding. Excessive weight gain during pregnancy was also associated with higher amounts of the genus *Staphylococcus* and *Staphylococcus aureus* ($P = 0.09$ and 0.03

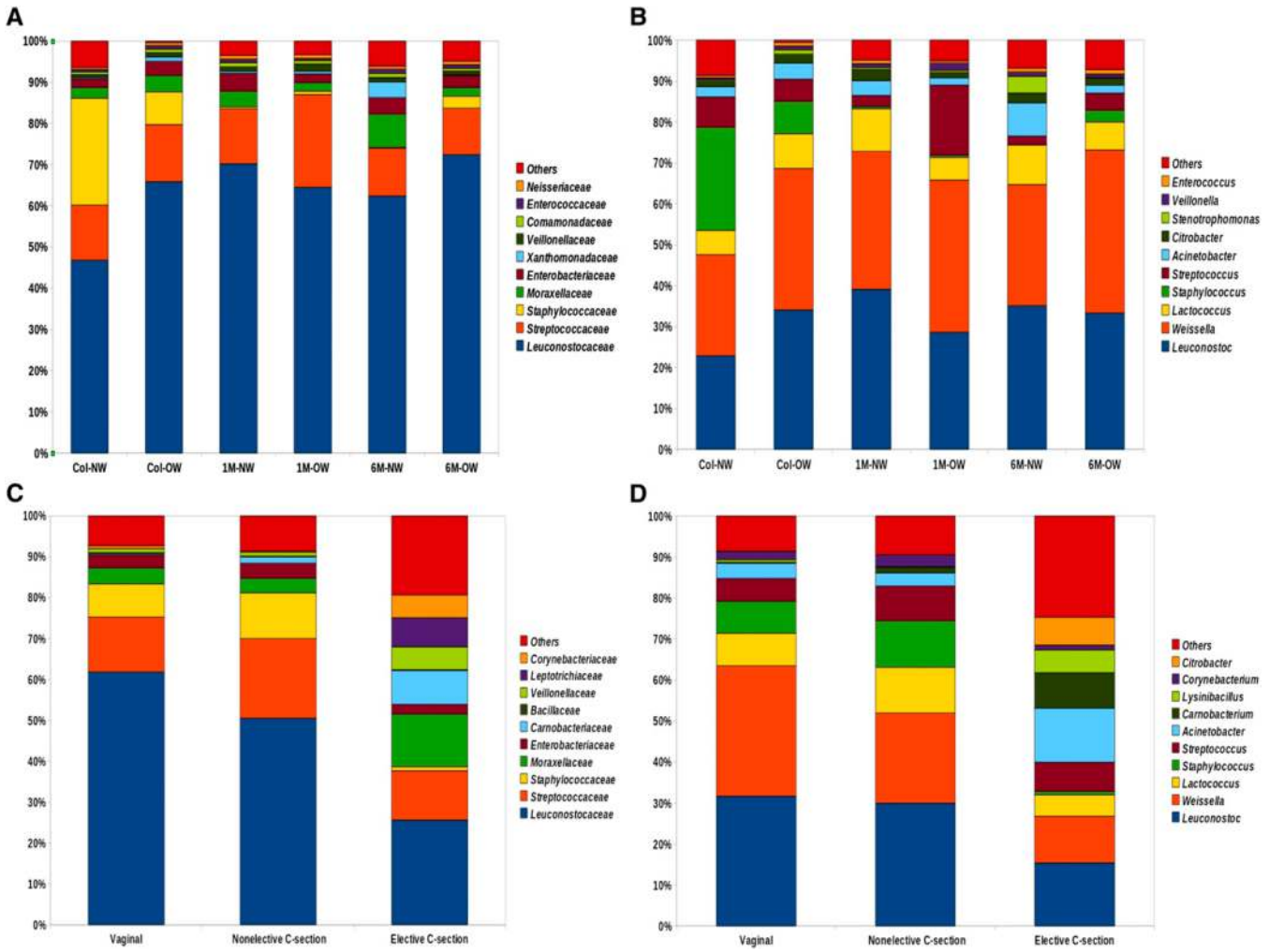

FIGURE 1. Bacterial taxonomic composition of human breast milk. The graphs show the proportion of bacterial families (left) and genera (right) as inferred by polymerase chain reaction amplification and pyrosequencing of the 16S rRNA. A and B: bacterial composition of the milk microbiome through time (colostrum, 1 and 6 mo after delivery) in normal-weight (n = 8) and obese (n = 10) mothers. C (colostrum samples) and D (milk samples during breastfeeding period): bacterial composition of milk from mothers who delivered vaginally (n = 9) and by nonelective (n = 3) and elective (n = 6) cesarean section. Col-NW, colostrum samples from normal-weight mothers; Col-OW, colostrum samples from obese mothers; C-section, cesarean section; 1M-NW, milk samples at 1 mo of breastfeeding in normal-weight mothers; 1M-OW, milk samples at 1 mo of breastfeeding in obese mothers; 6M-NW, milk samples at 6 mo of breastfeeding in normal-weight mothers; 6M-OW, milk samples at 6 mo of breastfeeding in obese mothers.

respectively) in 1-mo samples, as well as higher amounts of *Lactobacillus* (P = 0.03) and lower amounts of *Bifidobacterium* (P = 0.03) in 6-mo samples (*see* Table S1b under "Supplemental data" in the online issue).

Milk microbiome and mode of delivery

A striking difference in bacterial taxonomic composition was found between mothers who delivered their infants vaginally and those who delivered by cesarean section. Mothers who gave birth by elective cesarean delivery showed a significant compositional shift, with decreased amounts of *Leuconostocaceae* and increased amounts of *Carnobacteriaceae*, among others, compared with those who delivered vaginally (Figure 1, C and D). This difference was already present in the colostrum and was maintained in breast milk at 1 and 6 mo. qPCR analysis of the samples with the use of universal primers indicated that the total bacterial load in the colostrum was not different between elective and nonelective cesarean-section deliveries (*see* Table S1c under "Supplemental data" in the online issue). Interestingly, milk samples from mothers who gave birth by nonelective cesarean delivery displayed a composition more similar to milk from mothers who gave birth by vaginal delivery than to milk from mothers who gave birth by elective cesarean delivery (Figure 1, C and D).

Clustering of the different samples according to their composition showed that the milk microbiota was not associated with any mucosal, fecal, or skin samples. PCA showed that milk microbiota with a skewed bacterial composition as a result of the influence of mode of delivery clustered with microbes from the oral and vaginal mucosa (**Figure 4**), which suggests that the bacterial transfer mechanism could be modified.

DISCUSSION

The results of the current study provide new insight into the composition of microbiota in breast milk. In addition to nutritional support, breast milk provides bioactive constituents that both

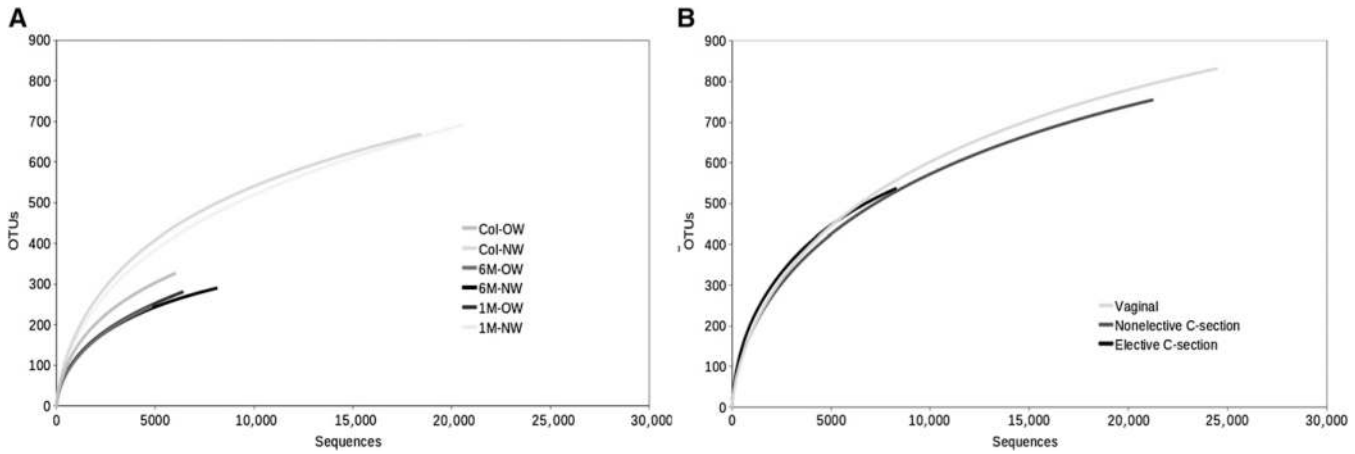

FIGURE 2. Diversity of the human milk microbiome. Graphs show rarefaction curves relating the sequencing effect compared with an estimate of the number of bacterial species, as inferred by the number of OTUs. An OTU was a cluster of 16S rRNA sequences that were >95% identical, a conservative estimate for the boundary between species, established at 97% for full-length 16S gene sequences. Panel A shows rarefaction curves for milk samples through time (colostrum, 1 and 6 mo after delivery) from normal-weight and obese mothers. Panel B shows rarefaction curves for the 3 delivery modes studied. Col-NW, colostrum samples from normal-weight mothers; Col-OW, colostrum samples from obese mothers; C-section, cesarean section; OTUs, operational taxonomic units; 1M-NW, milk samples at 1 mo of breastfeeding in normal-weight mothers; 1M-OW, milk samples at 1 mo of breastfeeding in obese mothers; 6M-NW, milk samples at 6 mo of breastfeeding in normal-weight mothers; 6M-OW, milk samples at 6 mo of breastfeeding in obese mothers.

directly and indirectly enhance mucosal barrier function and shape immune development (5, 6). Among these components, our work shows an extraordinarily diverse array of microorganisms whose potential functional role should be evaluated. The data also indicate significant changes in milk microbiome composition and diversity through lactation in a manner similar to what has been reported for the protein and fat content of breast milk (7). The higher amount of diversity and the different bacterial composition found in our data sets when compared with the other available data (*see* Figure S1 under "Supplemental data" in the online issue) could be a result of geographic and environmental differences in the mothers and secondly of differences in the experimental protocol [eg, the use of nondegenerate primers, a high annealing temperature, and a large number of cycles in the previous study (17)], which taken together may reduce the identified diversity.

In our samples, the first and second components in PCA clearly separated the colostrum samples from the 1- and 6-mo breast-milk samples (Figure 3, A and B, respectively), indicating that the initial colostrum is compositionally distinct from later milk samples. Although pyrosequencing of the 16S rRNA gene allows an extraordinary degree of detail in the analysis of microbial diversity, this approach has limitations such as PCR amplification biases (19). This is well known in the case of some bacterial groups, which are not easily amplified by universal primers, including bifidobacteria, whose presence is a hallmark of the gut microbiota in healthy breastfed infants (9). Hence, qPCR experiments with the use of primers to amplify specific taxonomic groups were also performed, including taxa such as *Bifidobacterium* spp., whose high G+C content disfavors amplification under standard conditions.

Our results show that maternal BMI and weight gain during pregnancy have an impact on breast-milk microbiome taxonomic composition and diversity. Colostrum and 1-mo milk samples from obese mothers showed a lower diversity than did those from normal-weight mothers, although the difference disappeared in milk samples taken at 6 mo (Figure 2A). The interlink between the gut microbiota and obesity has been extended to high prepregnancy body weight and excessive weight gain during pregnancy, which act as an inoculum for the gut microbiota in infants (11, 12). The qPCR data also showed significant differences in the presence of specific bacterial genera depending on BMI and weight gain during pregnancy, corroborating the conception that obesity influences the milk microbiome. Nevertheless, it must be emphasized that the sample size (18 mothers were sampled in the present study) limits the interpretation of the results, and the suggested trends would need to be confirmed by larger-population studies. Given that milk bacteria are among the first bacterial cells transferred to the infant's orogastrointestinal tract, a skewed composition in the milk could be a factor contributing to acquire shifts in bacterial composition from mothers to infants. Thus, our data may indicate an additional mechanism explaining the heightened obesity risk in infants of obese and overweight mothers.

Industrialized countries worldwide are experiencing a progressive increase in immunologic and metabolic diseases, and the velocity of this increase is particularly conspicuous in children. The mode of delivery is known to influence the neonatal gut microbiota composition (15, 16). Our data further show that breast milk from mothers who had given birth by elective cesarean delivery showed a significant compositional shift compared with those who had a nonelective cesarean section and those who delivered vaginally (Figure 1, C and D; Figure 4). The difference was already present in the colostrum and was maintained in breast milk at 1 and 6 mo, indicating that the shift in bacterial composition had a long-term effect. The fact that the milk microbiome of mothers who gave birth by nonelective cesarean section had a normal microbial composition that was comparable to that of breast milk from mothers who delivered vaginally suggests that physiologic (eg, hormonal) changes produced in the mother during the labor process may influence the composition of the bacterial community. Given the increased risk of diseases such as allergic rhinitis, asthma, and celiac disease reported in children born by cesarean

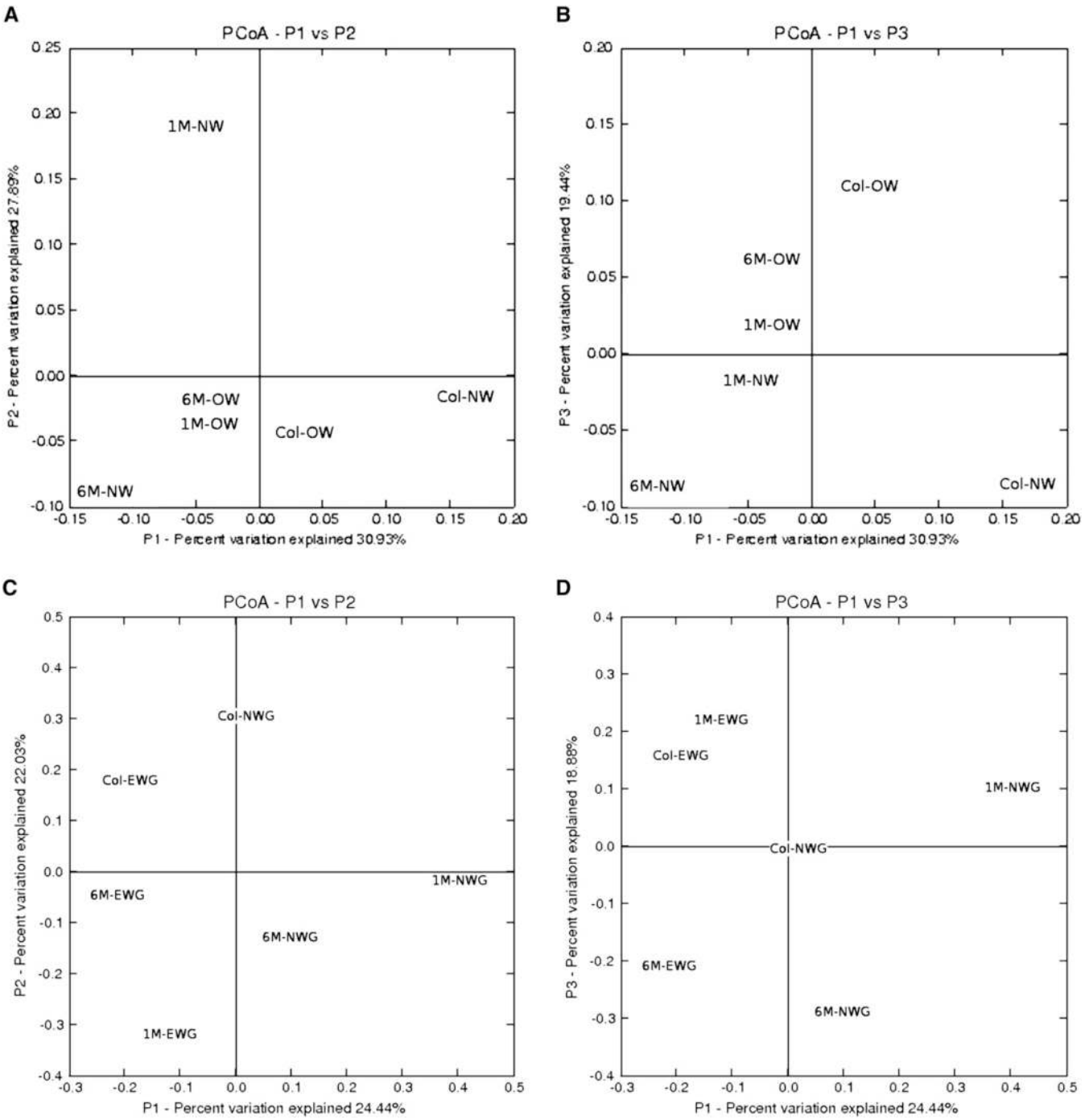

FIGURE 3. PCoA on the basis of the bacterial composition of human breast milk. A and B: Distribution of colostrum and 1- and 6-mo samples from obese and normal-weight mothers. C and D: Distribution of colostrum and 1- and 6-mo samples from mothers with normal weight gain and excessive weight gain during pregnancy. All samples were collected from mothers who gave birth by vaginal delivery (n = 9). Col-EWG, colostrum samples from excessive-weight-gain mothers; Col-NW, colostrum samples from normal-weight mothers; Col-NWG, colostrum samples from normal-weight-gain mothers; Col-OW, colostrum samples from obese mothers; P, component of the principal coordinates analysis; PCoA, principal coordinates analysis; 1M-EWG, milk samples at 1 mo of breastfeeding in excessive-weight-gain mothers; 1M-NW, milk samples at 1 mo of breastfeeding in normal-weight mothers; 1M-NWG, milk samples at 1 mo of breastfeeding in normal-weight-gain mothers; 1M-OW, milk samples at 1 mo of breastfeeding in obese mothers; 6M-EWG, milk samples at 6 mo of breastfeeding in excessive-weight-gain mothers; 6M-NW, milk samples at 6 mo of breastfeeding in normal-weight mothers; 6M-NWG, milk samples at 6 mo of breastfeeding in normal-weight-gain mothers; 6M-OW, milk samples at 6 mo of breastfeeding in obese mothers.

delivery (28, 29), further evaluation of the possible impact of the skewed bacterial composition of breast milk needs to be performed.

It is a widely held belief that sources of colonization of the bacteria in breast milk include the skin and oral cavity of newborns exposed to the mother's vaginal and intestinal microbiota during childbirth (15). Therefore, to shed light on the potential origin of the milk microbiome, its microbiota was compared with the available data for female skin, vaginal, oral cavity, fecal, and gut mucosal microbiota, including a total of 500,000 16S rRNA sequences. Our results suggest that the milk microbiome is

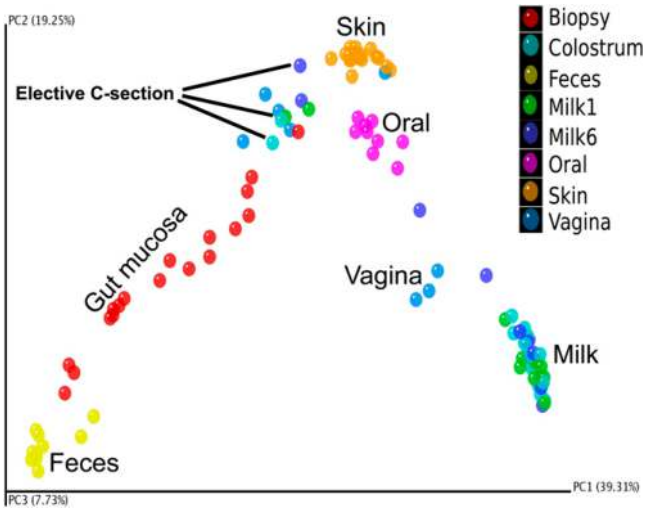

FIGURE 4. Relatedness between milk bacteria and the rest of the human microbiome. The graph shows a principal components analysis of the bacterial composition of milk from colostrum and at 1 and 6 mo compared with available adult female sequences from the same 16S gene region (hypervariable regions V1 and V2) from skin, vagina, feces, gut mucosa, and oral epithelium. Milk samples from the 3 time points clustered together and separately from the rest, except for samples collected from mothers who gave birth by elective and nonelective cesarean section, which overlap with oral samples. Data from the human microbiome are from fecal samples (26); from skin, vagina, and oral samples (15); and from Durban et al (27). C-section, cesarean section; Milk1, milk at 1 mo; Milk6, milk at 6 mo; PC, principal components; PC1, first component of the principal components analysis; PC2, second component of the principal components analysis; PC3, third component of the principal components analysis.

compositionally distinct from any other human niche and that it is not a simple contaminant from the skin (Figure 4), although part of the differences could be a result of the different sampling procedures. Clustering of the different samples according to their composition showed that the milk microbiota was not associated with any mucosal or fecal samples (data not shown). Thus, the milk microbiome does not appear to be a subset of any specific human niche. In addition, the suggested transfer of bacteria present in the human gut to reach the mammary gland through an endogenous route (the so-called entero-mammary pathway) via the mucosal associated lymphoid system (30, 31) remains unclear. It has been reported that a bacterial peptidoglycan originating from gut microbes could translocate into the circulation and serve as a molecular mediator responsible for the remote systemic priming of neutrophils in the bone marrow (32). Another theory suggested is endocytosis, which could be supported by our observed differences between elective and nonelective cesarean sections and the stress of labor and the delivery process affecting gut permeability. There should be a selective process by which only specific taxa are transferred or able to survive to form the characteristic, lactic acid–dominated microbiota of the milk. This theory is further supported by reports that the enteric nerve system affects gut sampling of bacteria and their transfer to Peyer patches (33, 34). Another source of colonization of these bacteria could be the skin and oral cavity of newborns exposed to the mother's vaginal and intestinal microbiota during childbirth (15). We found an increase in typical oral inhabitants in 1- and 6-mo-old milk, suggesting that during breastfeeding and nursing, bacteria from the infant's mouth could colonize the milk ducts and areola. However, recent molecular studies have shown that milk secreted by the mammary glands during the weeks previous to labor (and thus not submitted to any kind of infant contact) contains bacterial species similar to those isolated from fresh milk obtained after labor (35). Dendritic cells have been described to penetrate the intestinal epithelium to take up commensal bacteria from the gut lumen (36), to reach the systemic circulation, and to retain even live bacteria for several days (37); recently, the transfer of intestinal bacteria to the mammary glands within dendritic cells has been proposed (28, 38).

Prenatal and postnatal microbial exposures have profound effects on the microbial colonization of the intestine and maturation of the naive immune system. Given that the bacteria present in breast milk are among the very first microbes that enter the human body and given the vital role of bacteria in the infant's physiology and development of the immune system, our data emphasize the necessity to understand the biological role that the breast milk microbiome could potentially play for human health. If the bacterial composition of human breast milk has coevolved to maximize the infant's metabolic efficiency and to optimally stimulate the immune system, a skewed microbial milk composition might have important consequences for the infant's health, and those potential consequences would need to be evaluated for future recommendations in child nutrition.

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