Dear Sir:

Reply to Z Weizman

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Dear Sir:

We thank Dr Weizman for his valuable comments and the Editor for giving us the opportunity to clarify our results on milk microbial diversity obtained by 16S rDNA analysis. Although we agree with Weizman on the potential sharing of specific bacteria between milk and other body parts, this is clearly not the case for the whole bacterial community, as our article shows. The knowledge of bacterial diversity in breast milk is limited and almost exclusively based on the use of culture methods. However, the application of culture-independent molecular techniques has allowed a more complete assessment of milk’s biodiversity. In addition, recent advances in genomics and sequencing technologies based on 16S rDNA analyses have provided the possibility to study bacterial composition as a whole as opposed to evaluating the contribution of individual bacterial species. Our study focused on showing the large microbial biodiversity in milk samples by use of pyrosequencing, and we showed that this community was distinct from those found in skin, gut, vagina, or mouth. This does not imply that specific bacteria are not shared among human environments; several bacterial species and strains of Bifidobacterium, Lactobacillus, and Staphylococcus have been found in breast milk and infant fecal samples (1). But when considering the whole milk microbiome as detected by massive DNA sequencing, the bacterial composition is so different from other sites that the degree of contamination during sampling must be minimal. Our comparison of bacterial diversity, however, does not identify the origin of milk bacteria, because the milk microbiome composition appears to be different from the studied body sites. We also want to emphasize that breast-milk bacteria are not the only source of bacteria from the mother to the infant because transmission of specific intestinal Bifidobacterium strains from mothers to infants has been observed (2), supporting the hypothesis of maternal microbial transfer and suggesting that each mother–infant pair might have unique family-specific strains. We believe that the use of new sequencing technologies in multiple body sites of mother–infant pairs such as breast milk, fecal samples, and oral cavity through time could reveal the relative contribution of breast milk to the developing gut microbiome of the newborn, and this clearly needs to be further clarified.

A second issue raised by Weizman is geographic variability. Although the initial colonization process is heavily influenced by infant diet (breast milk compared with infant formula), mode of birth, and antibiotics, geographic location seems to have a great impact as well (3). In fact, the supplementary information with our article included a comparison of our data (Europe) with previous milk microbiome data from the United States (4) and showed important differences in bacterial diversity and composition. Future studies in samples from other geographic locations will show whether genetic or dietary factors are behind those differences, and we encourage other research groups to collect breast-milk samples from different geographic and cultural areas. However, for rigorous comparisons between data sets, the protocols for DNA extraction, PCR amplification, and sequence analysis should be standardized.

The milk microbiome should be partly influenced by the glycomicome, but this was not the aim of our study. It has been hypothesized that human milk oligosaccharides (HMOs) are able to interact with milk microbes (5). A positive correlation was observed between total HMO content and the relative abundance of Staphylococcus (5). Furthermore, HMOs are able to promote the in vitro growth of specific microbes isolated from human milk (5) and of gut-related microbes such as specific bifidobacterial species and Bacteroides (6).

It is important to remember that milk bioactive factors are related to the host genotype. Most HMOs are fucosylated, their production depends on enzymes determined by the Lewis blood group, and the mother’s secretor status determines the presence of FUT2+1–2-, 1–3-, and/or 1–4-fucosylated core structures of HMOs (6). However, the role of the host genotype in controlling the microbiota and the influence of gene-environment interactions on the microbiota composition and function is largely unexplored. Recently, intestinal bifidobacterial diversity and composition were shown to be strongly associated with the secretor status of the host (8). The FUT2 gene defines the secretor status and thus the expression of the ABO and Lewis
hista-blood group antigens. Recent studies (9) showed the contribution of host genetic factors (the \textit{FUT2} genotype in particular) on differences in the gut microbiota between patients with Crohn disease and healthy individuals. Thus, an intimate relation between host genetics and bioactive components is expected and should be linked to both infant microbiota colonization and immune system development.

The process of microbial colonization of the infant’s gut plays a pivotal role in the metabolic and immunologic development of the child. Deviations from the characteristic microbiota of the healthy breastfed infant are associated with an increased risk of allergic and inflammatory conditions as well as obesity. The transfer of microbiota via breast milk from mothers to infants is influenced by maternal allergic and obesity status (10, 11). Therefore, the different species of bifidobacteria and lactic acid bacteria present in the breast milk of allergic and obese mothers are expected to influence infant gut colonization through a constant daily inoculum during breastfeeding. Thus, modulation of maternal gut microbiota during pregnancy and lactation could have a direct effect on infant health, and microbes present in milk should be characterized to evaluate if this microbiota could be a source for future and specific probiotic strains.

We hope that our article stimulates further work to determine the role of host factors in modulating the composition of the human microbiota, enabling new applications in the field of personalized nutrition and medicine.

The authors did not declare any conflicts of interest.

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**Nutrient biomarkers are not always simple markers of nutrient intake**

Dear Sir:

In a recent meta-analysis (1), blood measurements of carotenoids were more strongly associated with lower breast cancer risk than were dietary measurements. When results from studies based on nutrient biomarkers provide a stronger association than those based on dietary assessments, the frequently accepted explanation for the discrepancy is that the true relative risk is attenuated from measurement error by using dietary questionnaires. Assuming that a causal association exists, the blood measurement may more directly assess the relevant exposure. However, potential limitations of nutrient biomarkers as representing a causal association need to be considered. A causal association here assumes that altering the concentration of the biomarker directly through intake of the nutrient will directly affect disease risk. I will briefly describe 5 examples where results from nutrient biomarkers are confounded on the basis of nondietary determinants of the concentration of the