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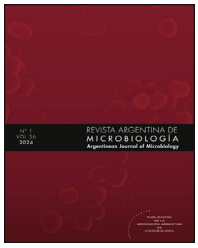

ORIGINAL ARTICLE

Impact of gestational diabetes mellitus in gut and human breast milk microbiome in Colombian women and their infants

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KEYWORDS
Bacteriome;
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Abstract Human breast milk (HBM) is a vital source of macronutrients and micronutrients that are crucial for an infant's development. Recent studies have shown that HBM contains diverse microorganisms, including bacteria, viruses, protozoa, and anaerobic fungi. Additionally, novel research has revealed that individuals with metabolic disorders, such as diabetes mellitus, are prone to dysbiosis in their gut microbiome. Our study aimed to investigate the impact of gestational diabetes mellitus (GDM) on HBM and the pair mother–infant gut microbiota. We conducted a comprehensive analysis of two groups from Pereira, Colombia: a GDM group and a non-GDM group. Each group consisted of five infants and their mothers. HBM and stool samples were collected from GDM and non-GDM mother–infant pairs. DNA was purified, and the 16S V3–V4 region was amplified and sequenced. Reads obtained were quality filtered and classified by homology according to the Ribosomal Small Subunit SILVA database. We found significant differences in the relative abundances of gut bacteria between GDM and non-GDM groups. Notably, *Bifidobacterium*, *Serratia* and *Sutterella* were negatively associated in women's gut with GDM. In HBM, *Sutterella*, *Serratia* and *Lactococcus* were found in low RA in the GDM group. Moreover, in the infants, *Bifidobacterium*, *Lactobacillus*, *Sutterella*, *Serratia*, *Streptococcus*, and *Veillonella* had a low presence in GDM. Our findings indicate that there are variations in gut bacteriome profiles between healthy women and those with GDM. These variations may impact the bacterial diversity in HBM, potentially leading to gut bacterial dysbiosis in their infants.

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PALABRAS CLAVE
Bacterioma;
Diabetes mellitus gestacional;
Leche materna;
Lactancia

Impacto de la diabetes mellitus gestacional en el microbioma de la leche materna y el intestino en una cohorte de mujeres colombianas y sus lactantes

Resumen La leche materna es una fuente vital de macro- y micronutrientes, cruciales para el desarrollo del recién nacido. Estudios recientes han demostrado que la leche materna contiene un amplio rango de microorganismos que incluyen bacterias, virus, protozoarios y hongos anaerobios. Asimismo, se ha encontrado que individuos con trastornos metabólicos como diabetes mellitus tienen tendencia a presentar un microbioma intestinal en disbiosis. El objetivo del presente estudio fue investigar el impacto de la diabetes mellitus gestacional (GDM) en la microbiota de la leche humana y el intestino del binomio madre-hijo. Se analizaron 2 grupos de madres e hijos del municipio de Pereira (Colombia), uno con GDM y otro sin GDM (non-GDM). Cada grupo incluyó 5 pares de madres y sus hijos. Se colectaron muestras fecales y de microbiota de la leche humana. Se purificó el ADN y se amplificó y secuenció la región V3-V4 del 16S ARNr. Las lecturas de ADN se filtraron por calidad y se clasificaron por homología empleando la base de datos ribosomal SILVA. Se encontraron diferencias significativas en las abundancias relativas bacterianas entre los grupos GDM y non-GDM. *Bifidobacterium*, *Serratia* y *Sutterella* estuvieron negativamente asociadas con el bacterioma intestinal de mujeres con GDM. En la leche materna de mujeres con GDM, *Sutterella*, *Serratia* y *Lactococcus* se encontraron en baja abundancia relativa. En los infantes del grupo GDM, *Bifidobacterium*, *Lactobacillus*, *Sutterella*, *Serratia*, *Streptococcus* y *Veillonella* tuvieron baja proporción. Nuestros resultados indican que los perfiles del bacterioma intestinal difieren entre mujeres sin diabetes gestacional y mujeres con dicha afección, lo cual podría ocasionar disbiosis en el bacterioma intestinal de sus hijos.

Introduction

Human breast milk (HBM) is a complex biofluid produced by the mammary glands that nourishes and acts as the first source of infant nutrition. The composition of human breast milk can differ both qualitatively and quantitatively due to genetic and physiological factors in women, but it also changes over time to fit the infant's nutritional requirements according to their age[2,16]. In addition to the high content of macro and micronutrients present, recent evidence has shown that the healthy HBM is also a rich source of microorganisms, mainly belonging to four phyla, Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria[25]. Several studies using culture-independent techniques have encountered at genus level that the main genera in HBM are *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Propionibacterium*, *Serratia*, *Corynebacterium*, and *Ruminococcus*, among others[20,22,23,32,49,53]. However, the definition of the ''core'' HBM bacteriome is still controversial since most studies worldwide exhibit significant differences in the bacterial composition of healthy individuals[34].

The HBM-associated bacteriome is crucial as it provides beneficial bacteria that can colonize the infant's GI tract and perform important metabolic and protective functions. It is important to note that a newborn's gastrointestinal tract is sterile, and bacterial colonization begins during passage through the birth canal in vaginal delivery[19]. However, the gut colonization process may be disrupted if delivery occurs via C-section; there is evidence that infants born through vaginally delivery exhibit higher abundances of various species, such as *Bifidobacterium* and *Lactobacillus*, compared to those delivered via C-section[8]. Recent evidence suggests that pathogenic and gut bacteria transmission could also occur vertically through the placenta[30]. Additionally, it is known that the initial bacterial community in newborns corresponds to the mother's vaginal and fecal microbiota and is enriched during the first weeks through feeding and environment exposure[30,48,50]. HBM is a key factor in shaping the GI microbiota, and this early gut colonization will have a significant impact on the composition of the individual's future gut microbiota[17,41]. Gut bacterial colonization during breastfeeding is critical for developing its function as immunomodulatory and neuromodulatory factors as well as for performing its metabolic functions. It is known that several factors, such as diet, age, gender, and health status, shape bacterial populations in the gut and other human environments. However, how hyperglycemia during GDM in the mother could affect the structure of bacterial communities in the affected women and their breast-fed children is still unclear[12,28,51].

Materials and methods

Subjects

This is an exploratory, descriptive study registered and approved on July 2019 by the UAMRA-Universidad Autónoma de Tamaulipas Ethics in Research Committee (1RB00010860) under registration number 001/2019/CEI. To participate in this study, the GDM group consisted of five Colombian women

and their infants, who were contacted through the clinical records provided by the Obstetrics Department at Risaralda Comfamiliar Hospital. Every woman participating in this study had a singleton pregnancy; twin pregnancies were excluded. Selected women were recruited during their third trimester of pregnancy after they were clinically diagnosed as hyperglycemic. The socioeconomic level of every woman participating in this study was similar, all of them belonging to the Colombian middle class. Women who reported consumption of special diets such as ketogenic, vegan, or vegetarian were excluded from this study. Participating women were interviewed and did not report any other known additional comorbidities, including obesity, diabetes mellitus type II (DM), HIV or cancer.

GDM was diagnosed by the medical staff at the Risaralda Hospital. A fasting glucose level lower than 7.8 mmol/l (140 mg/dl) after 1 h of glucose solution consumption was considered normal; blood glucose levels above these values were considered suspicious. If this happened, three-hour tests were performed. The three-hour test was considered positive if blood glucose values were higher than 10 mmol/l (180 mg/dl) after 1 h, higher than 8.5 mmol/l (153 mg/dl) after 2 h, or higher than 7.8 mmol/(140 mg/d) after 3 h of glucose solution consumption with diabetes symptoms[21]. The infants met the age criteria established between one and three months old; every infant included in the study was fed exclusively with HBM through skin contact at the time of sampling. Infant and mother stool samples were collected during the first month of lactation by the mother and placed in a sterile plastic container. HBM samples were collected manually by the donors and placed directly into sterile plastic containers. All the samples were transported on ice to the laboratory at the Universidad Libre, Seccional Pereira in Colombia, and stored at −20 °C until their processing.

The non-GDM group comprised five women and their infants selected from women with singleton pregnancies, who claimed to be in good health and have no previously known comorbidities. In both groups, if the mother had received antibiotic treatment during pregnancy and/or if the newborn was given other nourishment besides HMB, they were excluded from the analysis. In every case, if the mother agreed to participate in this study, an informed consent was signed.

DNA purification and 16 rRNA sequencing

For the HBM and stool samples, genomic DNA was purified using the Invisrob Spin Universal Kit (Stratec. Berlin, Germany). DNA libraries were constructed and subjected to Illumina MiSeq pair-ended high throughput sequencing of the 16S V3-V4 region (2 × 300) according to the Illumina Multiplexing Sample Preparation Guide (Illumina, San Diego, CA). Sequencing was performed in two locations, first at the Laboratory of Genomic Services of the National Laboratory of Genomics for Biodiversity (Irapuato, Mexico) using the primers 367F (5′-CTCCTACGGGAGGCAGCAG-3′) and CDR (5′-CTTGTGCGGGCCCCCGTCAATTC-3′) and at the Colombian Center of Bioinformatics and Computational Biology (Manizales, Colombia) amplifying with the primers 5′-CCTAYGGGRBGCCASCAG-3′ and 5′-GGACTACNNGGGTATCTAAT-3′.

Sequencing data analyses

Raw reads analyses were performed in the hpc-cbg bioinformatics cluster (Instituto Politécnico Nacional, México), quality control was carried out, and then reads were assembled in contigs using the Mothur pipeline[40]. The contigs assembled were quality filtered and classified by homology according to the Ribosomal Small Subunit SILVA database v. 132[36]. Observed and estimated alpha and beta indexes for phylum and genus were calculated. Alpha diversity was reported as observed and estimated, accounting for less frequent species through the nonparametric Chaol method under ANOVA statistical analysis[5]. Additionally, beta diversity among the individuals was calculated through the Bray-Curtis index distance method and tested using a permutational multivariate analysis of variance (PERMANOVA). Differential abundance was estimated through edgeR[38] and LEfSe[42]. Abundance graphs were visualized through the Microbiome Analyst Portal (https://www.microbiomeanalyst.ca)[6].

Results

Subjects

In this study, we enrolled 20 subjects from Pereira, Colombia, distributed in two groups of five non-GDM and five GDM mother–infant pairs; the mother's age range in both groups was 19–41 years old. All of them did not report addictions to drugs, tobacco, or alcohol. GDM women were reportedly treated with metformin (800 mg/day) during their pregnancy. Body mass index (BMI) ranged from 24.7 to 31.1 in the GDM group and between 20.5 and 27.3 in the non-GDM group (supplementary Table 1).

Nucleic acid purification and illumina sequencing

Raw reads were deposited in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA728120; after quality control (QC) and filtering of non-bacterial reads, we analyzed between 4259 and 114 731 informative reads per sample (supplementary Table 2). According to the sequenced region, we identified distinct bacterial communities in HBM, the mother's gut, and the infant's gut. In HBM from both non-GDM and GDM mothers, the gut bacterial composition was dominated by Proteobacteria, Firmicutes and Bacteroidetes in terms of relative abundance. However, the gut microbiome of the GDM mothers showed lower diversity and predominantly higher abundance of Firmicutes (75.1%) compared to non-GDM women's fecal samples (28.9%), which exhibited large amounts of Bacteroidetes (45.7%) and higher presence of Proteobacteria (25.3%) compared to GDM women (19 and 5.5% respectively). Finally, we found that the infant's gut from non-GDM mothers showed higher abundance of Proteobacteria (63.6%) in contrast to GDM-fed infants that showed a large relative abundance of Firmicutes (79.4%) (Fig. 1).

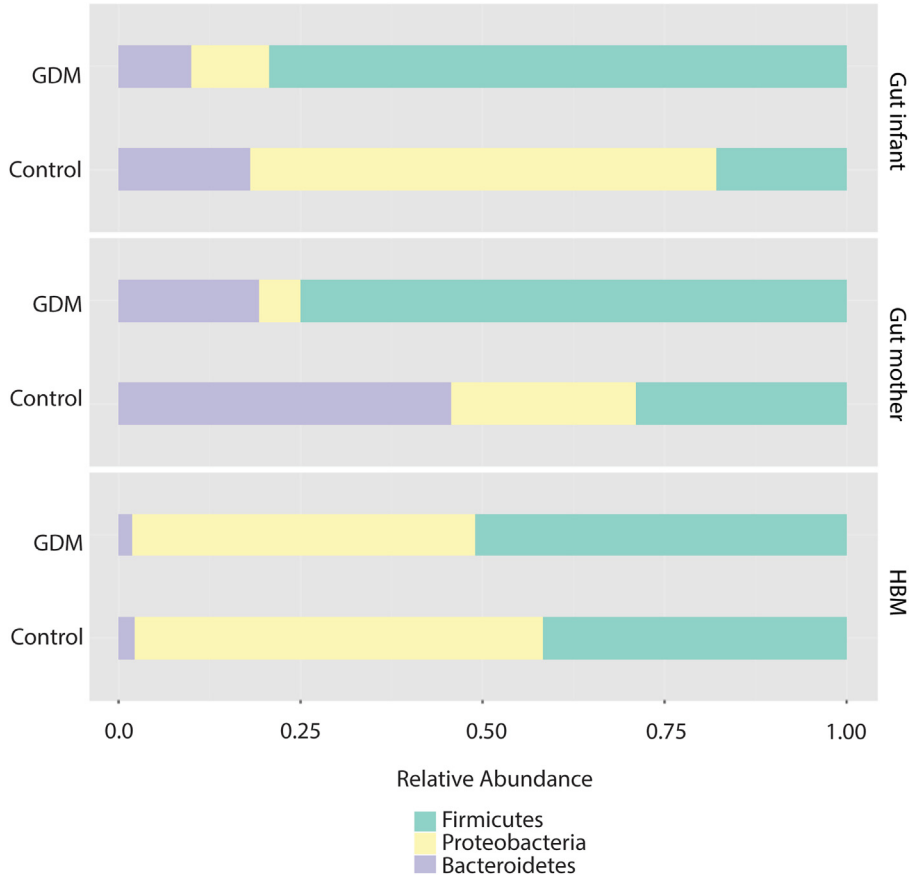

Figure 1 Relative abundance of phyla in the analyzed samples, merged by health condition. GDM samples were obtained from women with gestational diabetes and their children, while non-GDM samples refer to those obtained from healthy women without gestational diabetes and their children. GutL: infants' gut; GutM: mothers' gut; HBM: human breast milk; GDM: gestational diabetes mellitus.

Bacteriome profile of infants' gut

We identified ~451 operational taxonomic units (OTUs) among the analyzed samples. The total phyla identified within the infants' bacteriome included mainly Firmicutes, Proteobacteria, and Bacteroidetes. However, Actinobacteria, Chloroflexi, Synergistetes, and Verrucomicrobia were found in very low abundance ($p < 0.001$). Relative abundance of Firmicutes predominated in GDM-fed infants ($p < 0.001$). On the other hand, infants from the non-GDM mother's group generally showed higher species diversity and a greater abundance of Actinobacteria, Proteobacteria, and Bacteroidetes, although the differences were not statistically significant.

At genus level, we identified ~103 total genera among the analyzed samples, and we found significant differences in the abundance of several genera in the guts of GDM and non-GDM mothers and their infants (Fig. 2 and supplementary Table 3). However, each genus was tested independently and, according to the differential abundance analysis method (edgeR) and the LEfSe analysis, *Flavonifractor* ($p = 1.06E-5$), *Clostridium* senso stricto ($p = 0.0012$), *Clostridiales* ($p = 0.0022$), *Paenibacillus* ($p = 0.0028$), *Oscillibacter* ($p = 0031$), *Ruminococcus* ($p = 0.0035$), *Gemmiger* ($p = 0.0042$) and unclassified Clostridiaceae species ($p = 0.0048$) were agents positively associated to the gut samples from GDM HBM (Fig. 3A).

Moreover, *Escherichia/Shigella* ($p = 0.047$), *Streptococcus* ($p = 0.028$), *Serratia* ($p = 0.009$), *Megasphaera* ($p = 0.047$), *Clostridium* XVIII ($p = 0.0758$), *Lactobacillus* ($p = 0.016$), *Veillonella* ($p = 0.0162$), *Staphylococcus* ($p = 0.028$), unclassified Burkholderiales ($p = 0.026$), unclassified Pasteurellaceae ($p = 0.044$), *Sutterella* ($p = 0.047$), *Psychromonas* ($p = 0.007$), *Azoarcus* ($p = 0.007$), and *Aeromonas* ($p = 0.016$) were found in relatively low abundance in infants fed on non-GDM HBM, the lactic acid bacteria *Bifidobacterium* sp. was consistently found in low abundance in non-GDM HBM-fed infants, but not to a statistically significant extent ($p = 0.07$) (Fig. 3A).

Alpha diversity in the gut bacteriome at OTUs level from infants fed on GDM HBM was significantly higher compared with those fed with non-GDM HBM ($p = 0.0025$) and higher at phylum level ($p = 0.07$). Beta diversity among non-GDM and GDM infants was significant at the OTU level ($p < 0.01$) and barely significant at the phylum level ($p < 0.033$) and genus level ($p < 0.028$). The Firmicutes/Bacteroidetes ratio was significantly higher in the GDM group compared to the non-GDM.

In HBM from GDM mothers, we found that the analyzed samples were composed of approximately 189 OTUs from 47

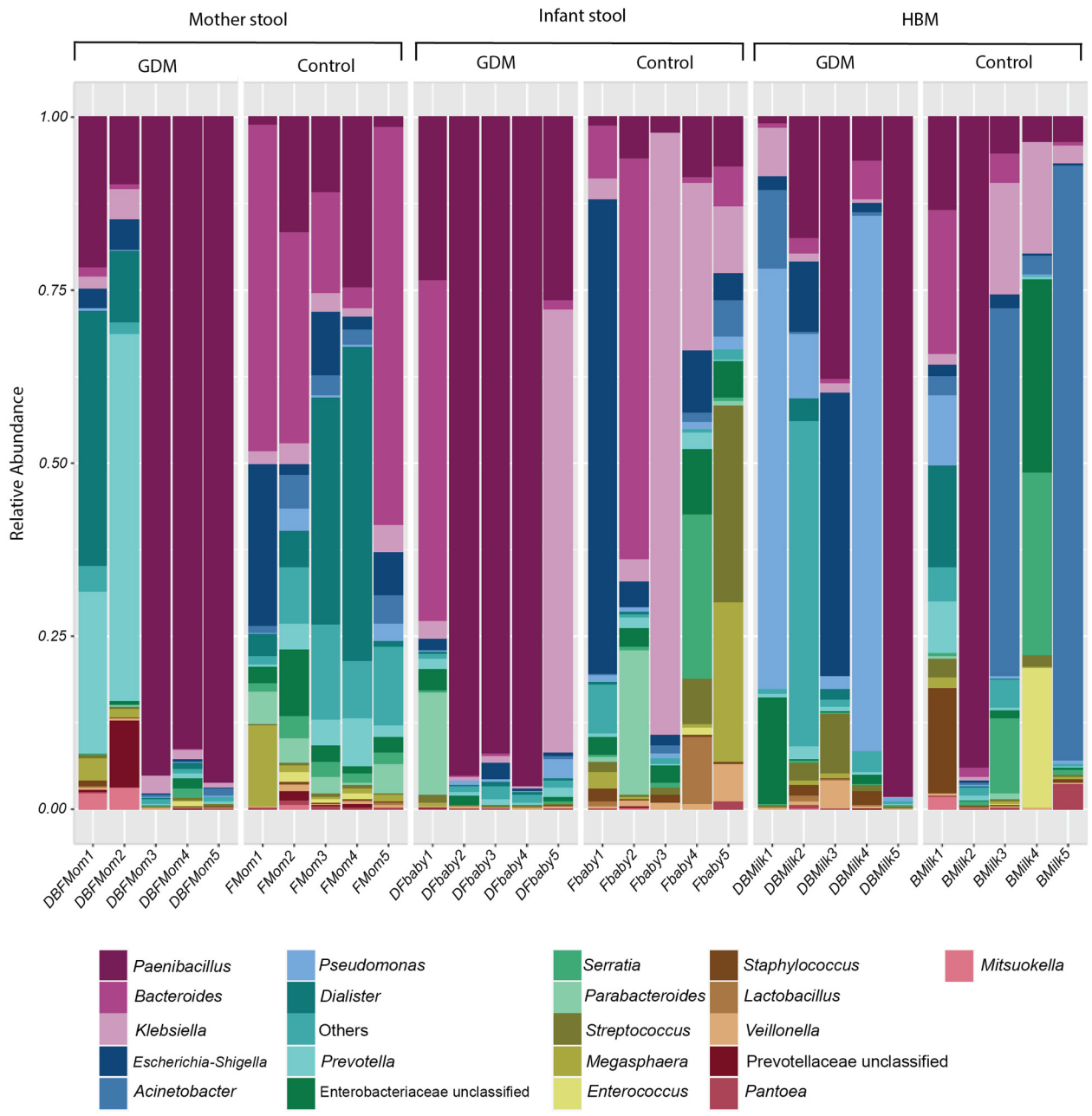

Figure 2 Bar chart of relative abundance (RA) at the genus level for each sample. DBFMom1-5: fecal sample from GDM mothers' gut; FMom1-5: fecal sample from non-GDM mothers' gut; DFbaby1-5: fecal sample from GDM infants; Fbaby1-5: fecal sample from non-G DM infants; DBMilk1-5: GDM HBM; BMilk1-5: non-GDM HBM; GDM: gestational diabetes mellitus; HBM; human breast milk.

identified bacterial genera, which belonged to three phyla: Proteobacteria, Firmicutes, and Bacteroidetes. However, we found no significant differences in the relative abundances of any phylum among the samples. At genus level, unclassified *Bacteroidetes* ($p = 2.82E\text{-}4$), *Veillonella* ($p = 0.001$), *Syntrophorhabdus* ($p = 0.001$), *Bacillales* ($p = 0.0021$) were found in higher relative abundance within the GDM samples, whereas *Enterococcus* ($p = 0.005$) and *Serratia* ($p = 0.005$) were found with significant higher relative abundance in non-GDM samples (Fig. 3B). We found no significant differences in alpha diversity among the non-GDM and GDM groups. However, a slightly higher alpha diversity index was observed at the genus level in the non-GDM group. No significant beta diversity among the tested groups was registered ($p < 0.422$).

The gut bacteriome of GDM and non-GDM mothers showed ~136 OTUs distributed across 62 identified genera. The bacteria found mainly belong to three phyla, Firmicutes, Bacteroidetes, and Actinobacteria. Comparing the two groups, we found that Firmicutes were significantly associated with the GDM group ($p = 0.0015$). At the genus level, unclassified Paenibacillaceae ($p > 0.001$), *Paenibacillus* ($p > 0.001$), *Staphylococcus* ($p = 0.006$), *Brevibacillus* ($p = 0.01$), *Bacillus* ($p = 0.01$) were differentially associated to GDM women whereas *Alistipes* ($p > 0.001$), *Phascolartobacterium* ($p > 0.001$), *Bacteroides* ($p > 0.001$),

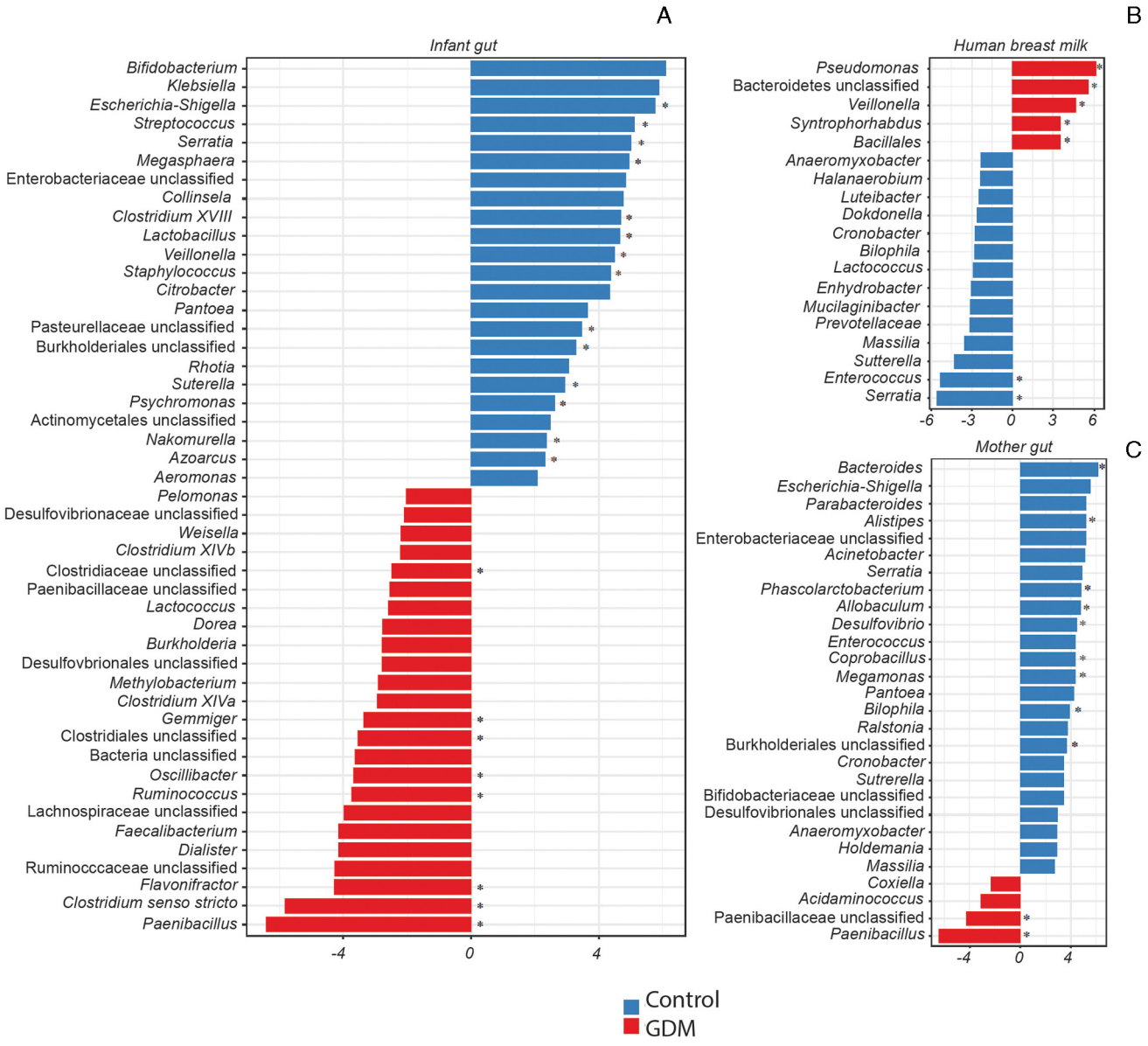

Figure 3 Linear discriminant analysis (LDA) with effect size (LefSe) showing bacterial genera with significant differential abundance among GDM and non-GDM groups. (A) Bacterial genera with significant differential abundance in the gut of non-GDM (blue) and GDM (red) infants. (B) Bacterial genera with significant differential abundance in the gut comparing non-GDM (blue) and GDM (red) in HBM. (C) Bacterial genera with significant differential abundance in the gut of non-GDM (blue) and GDM (red) women. *Denotes statistically significant values determined using edgeR as an additional tool. GDM: gestational diabetes mellitus.

Coprobacillus ($p > 0.001$), *Allobaculum* ($p = 0.001$), *Megamonas* ($p = 0.005$), *Bilophila* ($p = 0.009$), *Parabacteroides* ($p = 0.01$) showed higher relative abundance within the non-GDM group (Fig. 3C). Alpha diversity showed no significant differences at the taxonomic levels tested, while beta diversity between the groups was significant only at the genus level ($p < 0.018$).

Discussion

GDM increasing prevalence represents a major public health challenge for Latin American countries; it is known that metabolic diseases such as diabetes mellitus type 2 have a strong relationship with the gut microbiome structure. Considering recent evidence, it has been suggested that the gut microbiome may play a role in gestational metabolism, potentially leading to the development of GDM[27]. Recent observational studies have focused on the microbiome of HBM in GDM. These studies found that it remains unclear whether there is an association between HBM microbiota and GDM conditions, at least during the first weeks postpartum. However, evidence suggests that *Streptococcus* and *Staphylococcus* are the dominant species in HBM microbiota with and without GDM[39].

Our study aims were to compare the gut and HBM microbiota between GDM and healthy non-GDM women as well as

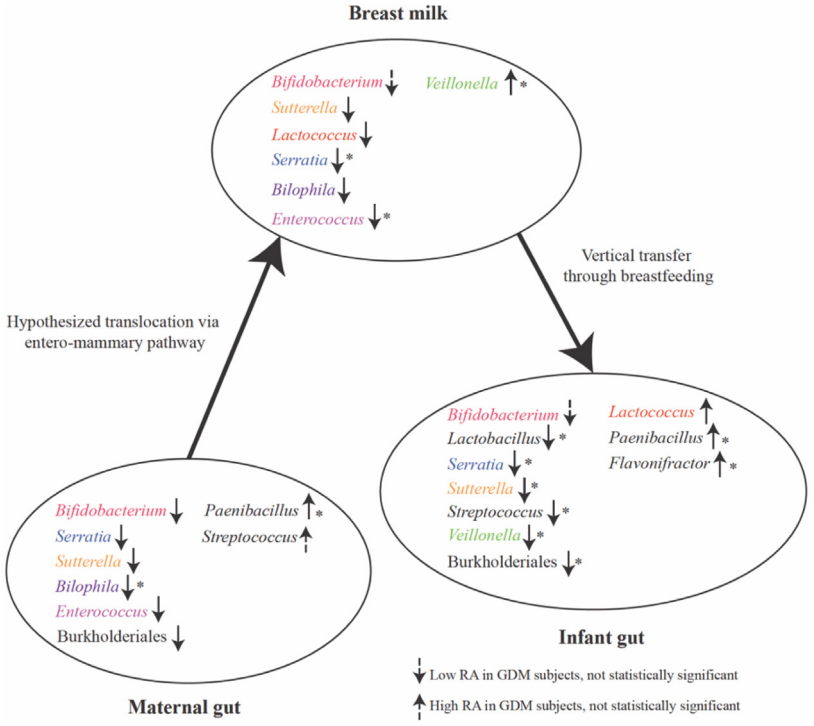

Figure 4 Bacterial differential abundance in GDM subjects. Statistical significance was calculated using a linear discriminant analysis (LDA) with effect size (LEfSe). Arrows show the significance of LEfSe (p-value cutoff = 0.05), and asterisks indicate additional significance in edgeR (p-value cutoff = 0.05). GDM: gestational diabetes mellitus.

the gut microbiota of their offspring. The overall findings of this study suggest that gut bacteriome profiles vary between healthy and GDM women, leading to gut bacterial dysbiosis in their infants (Fig. 4). In this study, GDM women were reportedly prescribed metformin during their pregnancy. While this could potentially affect the gut bacteriome, there are conflicting results in various studies worldwide that aim to describe the gut bacteriome in patients with type 2 diabetes[35]. Consistently, we observed low abundance of several bacterial genera in the infant's gut, such as *Bifidobacterium*, *Sutterella*, and *Serratia*, which can be traced to the maternal gut, and HBM from GDM affected women. These results support the hypothesis of entero-mammary translocation of internal bacteria, originally proposed by Fernández[11].

Our results showed that the abundance of *Lactobacillus* ($p = 0.016$) and *Bifidobacterium* tends to decrease in the gut of infants fed HBM from GDM. Additionally, we found that *Lactobacillus* abundance is decreased in mother gut and HBM with GDM. The species associated with human fecal samples are *L. acidophilus*, *L. casei*, *L. crispatus*, *L. gasseri*, *L. paracasei*, *L. plantarum*, *L. reuteri*, and *L. ruminis*, and it is believed that this genus plays a role in maintaining microbiota homeostasis[37,48].

In this study, we found the gram positive *Flavonifractor* sp. to be highly associated with the gut bacteriome of GDM-fed infants. The resolution obtained with our analysis had not allowed us to identify it to species level; interestingly, we have found no evidence of *Flavonifractor* in the mothers' group. However, it is known that *Flavonifractor plautii* is a human gut microbiome species that is able to degrade naturally produced flavonoids present in commonly consumed fruits and vegetables commonly, specifically quercetin, which is the most abundant one. Previously, *Flavonifractor* was considered a possible biomarker since it is reported as one of the microorganisms heavily associated with colorectal cancer in two recent studies[18,54]. *Flavonifractor* sp. was also found in high presence in diabetes mellitus affected mice[15]. In contrast, it appears to be associated with lower GDM risk when the diet is rich in polyphenols and flavonoids[14]. The high abundance and strong association of *Flavonifractor* in GDM infants could be effectively linked and serve as a potential marker for gestational hyperglycemia.

It is noteworthy that *Paenibacillus* spp. reads are present in very high counts across the analyzed samples. *Paenibacillus* is a facultative anaerobic, rod-shaped, gram positive bacterium. This species has been barely described in previous studies. To date, only two species, *P. faecis* and *P. phocaensis*, have been reported as isolated from the human gut[7,46]. Our results showed a high presence of *Paenibacillus* in GDM maternal gut and their children. However, to determine the species found in this study, further analyses are required.

It is known that gut bacteria have an essential role in human metabolism, including the production of essential nutrients such as vitamin K_2, complex B vitamins, and short chain fatty acids (SCFA). Vitamin K_2 is a menaquinone (MK) (2-methyl-3-multipreny-1,4-naphtoquinone), a fat-soluble vitamin produced by the human gut microbiota synthesized during bacterial anaerobic respiration. Major MKs in the human gut are produced by members of the genera *Bac-*

teroides (MK-10, MK-11), *Enterobacteria* (MK-8), *Serratia* (MK-4), *Lactococcus* (MK-7, MK-8, MK-9) and *Lactobacillus* (MK-4)[9,10,24,31,43]. We found a low presence of Enterobacteriaceae, *Serratia* sp., and *Lactobacillus* sp. in the gut samples of GDM-fed infants. Remarkably, *Lactococcus* had increased its presence in non-GDM children. Within the HBM samples, GDM HBM had a significantly low abundance of *Serratia* ($p = 0.009$). Remarkably, *Lactococcus* showed low counts in GDM HBM samples in contrast to the observed results in the GDM infants' gut. Here, we hypothesize that the high levels of glucose occurring under GDM could affect the abundance of *Lactococcus* in HBM. However, their number increases again once they are established in the infant colon. Our findings suggest that GDM infants could have a diminished supplementation of MKs at least during the first months of life. However, further research measuring MK concentration under hyperglycemic conditions is needed to elucidate the impact of GDM in women and their infants.

Thiamine (vitamin B_1) functions as a cofactor for several important enzymatic reactions, including the citric acid cycle reactions catalyzed by pyruvate dehydrogenase and α-ketoglutarate dehydrogenase[55]. Thiamine is produced by several gut and probiotic bacterial species such as *Bacteroides fragilis*, *Ruminococcus lactaris*, *Prevotella copri* and some *Lactobacillus* spp., *Bifidobacterium* spp., and *Fusobacterium* spp. According to our findings, at least *Lactobacillus* spp. and *Bifidobacterium* spp., are in low abundance in GDM women and infants, and this could lead to a reduced amount of bacterial synthesized thiamine, which, in consequence, could increase the competition for thiamine between gut bacteria and the host.

Rivoflavin (vitamin B_2) is a precursor of the redox coenzymes, flavin mononucleotide and flavin adenine dinucleotide, which are essential in TCA and fatty oxidation pathways. Although the diet is an important source of rivoflavin, it is also synthesized by several members of the human gut microbiota, including *Lactobacillus* spp., *Lactococcus* spp., and *Weisella* spp.[45,55]. In this study, the relative abundance of *Lactobacillus* sp. is reduced in GDM HBM-fed infants while it increases in *Lactococcus* sp. and *Weissella* sp.

Folates (vitamin B_9) are anionic hydrophilic carrier molecules that are not naturally synthesized by human cells; thus, they must be acquired through dietary components or produced by the gut microbiota. Bacterial folate biosynthesis in the form of tetrahydrofolate (THF) is carried out mainly by members of the phyla Bacteroidetes (*Bacteroides* spp. and *Prevotella* spp.), Firmicutes (*Lactobacillus* spp. and *Streptococcus* spp.) and Actinobacteria (*Bifidobacterium* spp.)[44,55]. Additionally, there is a low count of *Lactobacillus* and *Streptococcus* in GDM individuals, which could lead to stress levels of THF in affected infants since HBM is the only folate source at that stage, and folate levels in HBM are maintained by the HBM microbiota[1,26].

Cobalamin (Vitamin B_{12}) is produced exclusively by microorganisms, animals, plants, and fungi that do not have the ability to biosynthesize it. In the human gut, *Lactobacillus reuteri* is the indigenous bacterium that supplies the daily requirements[29]. A study by Boran et al.[4] compared the gut microbiota between healthy infants with sufficient and insufficient levels of vitamin B_{12} and found no difference between the two groups. However, vitamin B_{12} insufficiency could be related to several factors, including GDM. Our results suggest that low abundance of *Lactobacillus* spp. could be linked to a low presence of *L. reuteri*[52].

In the early stages of life, the gut bacteriome is mainly made up of Enterobacteriaceae species. However, as the breastfeeding stage comes to an end, the healthy bacteriome is dominated by members of the phylum Firmicutes, including species of Lactobacillaceae, Ruminococcaceae, and Lachnospiraceae[33,47]. These bacteria are responsible for producing butyrate and other SCFAs, which have a key role in the anti-inflammatory effect of acetate and propionate, and the proinflammatory effect of butyrate on innate immune system cells through the free fatty acid receptors 2 and 3[3,13].

The analysis of the bacteriome structure in the infants indicates that those with GDM show lower levels of *Lactobacillus* and *Streptococcus* bacteria, and potentially reduced levels of *Bifidobacterium*, compared to non-GDM samples. This could result in a diminished production of various complex B vitamins.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version available at https://doi.org/10.1016/j.ram.2024.10.006.

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