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Analysis of human colostrum reveals differential co-occurrence networks of metabolites, microbiota and cytokines in maternal obesity†

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Breastmilk is essential for neonatal development, particularly in seeding the gut microbiota and modulating the immune system. This proof-of-concept study explores the systemic nature of colostrum and the influence of maternal obesity on co-occurrences of colostrum bioactives. Using 16S-rRNA sequencing, untargeted metabolomics, and cytokine quantification, we analyzed co-occurring elements in the colostrum of mothers with normal weight (18.5 < BMI < 25) or obesity (BMI > 30). We identified 5 different co-occurrence networks, characterized by positive correlations of taxonomically related bacteria. Our integrative analysis reveals that *Aeromonadaceae*, *Xanthomonadaceae* and *Staphylococcaceae* negatively correlate with pro-inflammatory cytokines TNF-α, IL-6, and IL-12p70 in the colostrum of mothers with obesity (WO). Additionally, lipid mediators, including 15-HEDE and LysoPC (16:00), were associated with cytokines IL-10 and IL-8 and microbiota taxa *Burkholderiaceae*, *Beijerinckiaceae* and *Planococcaceae* – first reported in the colostrum of mothers WO. Our findings suggest a pervasive regulation of bioactives in the colostrum of mothers WO. This may have implications for distinctive neonatal intestine development.

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Introduction

Breastmilk is a complex and dynamic fluid that supplies hydration, nutrients, and bioactive molecules essential for the optimal growth and development of neonates.[1] The bioactive components of breastmilk regulate vital functions, including passive immunity to pathogens, tolerance to the colonizing microbiota, and other processes necessary to mature the intestinal system.[2,3] Multiple maternal factors have been linked to alterations in the breastmilk composition including BMI, age, ethnicity and diet.[4–6]

Most of the current studies draw direct correlations between maternal physiological states and alterations in individual milk components.[7] While informative, this approach fails to capture a more comprehensive breadth of how maternal condition correlates to breastmilk bioactives. This becomes evident when considering breastmilk as a complex and dynamic ecological system. As an example, during pregnancy, a clear biological network emerges within the maternal gut, wherein bacterial fermentation produces short-chain fatty acids (SCFAs), triggering the proliferation of regulatory T (Treg) cells and modulating the balance between pro-inflammatory and anti-inflammatory cytokines, thereby promoting an immunotolerant environment that allows the establishment of commensal bacteria.[8] Understanding breastmilk as a biological system and how it is influenced by maternal health will provide valuable insights into its potential impacts on neonatal health.[9,10]

One in three pregnant women worldwide is affected by obesity. This metabolic disorder has been correlated with systemic alterations, including chronic inflammation. The breastmilk bioactive components of mothers with obesity are also altered.[5,11,12] For instance, the breastmilk microbiota of mothers with obesity has increased proportions of

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Staphylococcus and *Corynebacterium*.[13,14] Yet, little is known about the possible co-dependences of these alterations on other breastmilk bioactive components. The breastmilk microbiota is a living component that produces metabolites and other molecules while utilizing maternal factors. Therefore, it is crucial to examine the interactions among soluble bioactives together with the microbiota composition.[15–17] Approaching breastmilk as an ecosystem, and more importantly, colostrum – the initial biological message that a lactating mother has formulated for the neonate – will open avenues to explore the complexity and dynamism of its interacting components.

Using co-occurrence networks to analyze the patterns of interactions among bioactives represents a promising approach for exploring the structure of complex biological systems and their interactions with the host.[18] This approach provides valuable insights into potential interactions that are difficult to detect by characterizing bioactives independently or by using general ecological metrics such as alpha/beta diversity.[19] Here, we integrate colostrum cytokine concentrations, microbiota composition and metabolomics in the context of maternal obesity as an approach for identifying distinctive co-occurring networks. By exploring these interconnections, we provide a proof of concept of the systemic nature of colostrum and begin to dimension the complex biological networks at play within this microenvironment.

Materials and methods

Experimental model and study participant details

This is an observational study conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The protocol was approved by the IRB at Escuela de Medicina y Ciencias de la Salud, Tecnologico de Monterrey, with the ID: P000487; it has been registered at Clinical Trials with the ID: NCT04812847. Every participant was provided with details regarding the study, and written consent was acquired from each of them, ensuring the confidentiality of the personal information.

Healthy breastfeeding mothers who had given birth to full-term infants in the Hospital Regional Materno Infantil de Alta Especialidad were recruited between November 2020 and July 2022. The inclusion criteria for participants encompassed mothers between 18 and 35 years, with confirmed residency within the metropolitan area of Monterrey. The exclusion criteria included a history of antibiotic usage in the 3 months prior delivery, a prolonged antibiotic exposure exceeding 3 weeks at any stage of pregnancy, antibiotic requirement for more than 24 hours post-delivery, immunosuppressive, or immunomodulatory corticosteroid therapy, bariatric surgery, experienced delayed lactogenesis or issues with milk supply, pre-term delivery or if required intensive care post-delivery. Women with pregnancy complications such as gestational diabetes mellitus (GDM), hypertensive disorders, thyroid dysfunction, or history of feeding disorders were also excluded, as well as cases involving multiple birth or neonates with congenital anomalies.

Pre-pregnancy BMI was determined by the clinician and reported in the corresponding clinical records. Participants were divided into two groups according to the World Health Organization (WHO) classification of the body mass index (BMI):[20] women with obesity ("WO"; BMI $\geq$ 30 kg m^{-2}; n = 28) and mothers with normal weight ("NW"; BMI $\leq$ 25 kg m^{-2}; n = 20).

Sample collection and processing

Colostrum samples were collected within the first 48 hours post-partum from a total of 48 mothers by a trained medical team, utilizing sterile gloves for the process. Following a gentle cleansing of the areola with sterile water, when possible, 3 mL of colostrum was obtained by manual expression into a sterile 15 mL polypropylene tube. To ensure the purity of the sample, the initial drops were discarded. After collection, the samples were promptly transported to the laboratory and stored at −20 °C until subsequent processing.

Cytokine measurement

Due to limited sample volume availability, cytokine quantification was performed in a subset of 47 colostrum samples (NW = 20 and WO = 27). Samples were centrifuged (3000*g*, 15 minutes) to remove any cellular structure. Cytokines were quantified in the supernatant using a LEGENDplex® kit (inflammation cytokine markers thirteen, Bio Legend Cat #740 808) which includes IL-1β, TNFα, IL-6, IL-8, IL-10 and IL-12p70, according to the manufacturer's instructions with some modifications. Briefly, standard curves with known concentrations of each cytokine were generated through double serial dilutions, resulting in an eight-point curve performed in duplicate. Five microliters of the supernatant were combined with a fluorescent bead's reagent at a 1 : 1 volume ratio and incubated for two hours at room temperature. Following incubation, the beads were washed by resuspending them in wash buffer and then centrifuged (250*g*, 5 minutes). The supernatant was carefully discarded, and a mix of secondary antibodies was added and incubated for one hour. Subsequently, streptavidin–phycoerythrin (SA–PE) conjugates were introduced into the same reaction and incubated for 30 minutes at room temperature. After an additional washing step, the samples were analysed using a BD® FACSCelesta flow cytometer fitted with 405 nm, 488 nm, and 633 nm lasers and operated through BD® FACSDiva software v.8. The detection limits for cytokines were IL-1β, 1.5 ± 0.6 pg mL^{-1}; TNF-α, 0.9 ± 0.8 pg mL^{-1}; IL-6, 1.5 ± 0.7 pg mL^{-1}; IL-8, 2.0 ± 0.5 pg mL^{-1}; IL-10, 2.0 ± 0.5 pg mL^{-1}; IL-12p70, 2.0 ± 0.2 pg mL^{-1}. Data analysis was performed using BD software, which is accessible online at the website of LEGENDplex™ cloud-based data analysis software (**https://legendplex.qognit.com**).

Genomic DNA extraction and 16S sequencing

Genomic DNA extraction from 48 colostrum samples (NW = 20 and WO = 28) was performed following an optimized phenol–chloroform protocol, as previously described.[13] Briefly, 1 mL of colostrum was utilized for DNA extraction whenever feasible.

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The procedure involved the removal of the fat layer using a sterile hyssop and pelleting bacterial cells with a high-speed centrifugation followed by a sterile PBS wash. The resulting pellet was then treated with a lysis buffer and mechanically lysed using a FastPrep system (MP Biomedicals, Santa Ana, CA). Enzymatic lysis with proteinase K, lysozyme, and RNAse was subsequently performed. DNA extraction was carried out using a phenol : chloroform : isoamyl-alcohol (25 : 24 : 1) mixture, followed by precipitation of DNA with isopropanol. The DNA pellet was washed with 70% ethanol and resuspended in nuclease-free water. As controls, an in-house mock community with two bacterial isolates (*Escherichia coli* and *Pseudomonas putida* in a 1 : 1 ratio) and a DNA extraction of sterile PBS were included as positive and negative controls, respectively. DNA integrity was assessed by agarose gel electrophoresis, and concentration was determined using a NanoDrop ND-1000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Unless stated otherwise, all reagents were obtained from Sigma-Aldrich.

For sequencing, the prepared DNA samples were subjected to a Zymo Research's Quick-16S kit, utilizing phased primers 341F (5′-CCTACGGGDGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) targeting the V3–V4 regions of the 16S rDNA. The sequencing was performed on a V3 MiSeq 622cyc flowcell, generating 2 × 301 bp paired-end reads.

Colostrum taxonomic and differential profiles

The sequencing reads were imported into QIIME2 v.2022.8 and demultiplexed for subsequent analysis.[21] DADA2 plugin was used for denoising and quality control with optimized parameters to remove low quality regions of the sequences (--p-trim-left-f 12 --p-trim-left-r 16 --p-trunc-len-f 285 --p-trunc-len-r 250).[22] Taxonomic species profiling was accomplished by aligning amplicon sequence variants (ASVs) against the Silva 138.1 database with 99% of identity threshold,[23] using the q2-feature-classifier classify-sklearn naive Bayes taxonomy classifier.[24] To ensure data quality, potential contaminants identified through batch effect analysis or the positive control (archaea, mitochondria/chloroplast, *Cyanobacteria*, *Streptomyces*, *Stenotrophomonas*, and *Pseudomonas*) were removed.[25] Additionally, rare taxa were identified as ASVs with a total read count of ≤18 in at least three samples and subsequently excluded from the analysis.[26]

Rarefaction curves were generated to assess the sequencing depth and ensure adequate coverage of microbial diversity in colostrum. The rarefaction was conducted at a depth of 7712 sequences. Diversity analyses were performed in R using vegan v2.6-4[27] and phyloseq v1.44.0 libraries.[28] Alpha diversity analyses included the calculation of the Shannon diversity index, observed ASVs, evenness, and phylogenetic distance. Comparisons between groups were conducted using the Mann–Whitney's *U* test. Beta diversity was assessed through ANOSIM (performed with 999 permutations) using the UniFrac and robust Aitchison distance matrices. A Principal Coordinate Analysis (PCoA) and a Robust Principal Component Analysis (RPCA) were created and visualized using EMPEROR.[29] Differential abundance analysis at the taxonomic family level was conducted using DESeq2 v1.40.2 with batch correction applied[30] and ANCOMBC v2.6.0.[31] Only those families that were significantly different in both tools were graphed.

Colostrum metabolite extraction

Due to limited sample volume availability, 25 colostrum samples (NW = 5 and WO = 20) were used to extract metabolites. 100 μL of colostrum was combined with 600 μL of acetonitrile (ACN). The mixture was vortexed for 1 minute and subjected to 30 minutes of sonication in ice-cold water to enhance the extraction of metabolites. Subsequently, the samples were centrifuged using optimized centrifuge parameters (20 817*g*, 4 °C for 10 minutes). From the resulting supernatant, 400 μL were carefully collected for vacuum evaporation of ACN. The dried metabolome was then resuspended in 100 μL of an injection solution composed of a water : acetonitrile mixture in an 80 : 20 ratio containing 0.1% formic acid (v/v). To ensure effective resuspension, the samples were vortexed for 1 minute and sonicated in ice-cold water for 30 minutes. To obtain a particle-free solution suitable for injection, the resuspended samples underwent centrifugation at the optimized parameters before being transferred to the injection vials. Quality control (QC) samples were generated by pooling all resuspended samples in equal volumes.

LC-MS2 data acquisition

We employed the instrumentation and followed the equipment's parameters previously described,[32] with minor modifications. Four μL of resuspended metabolomes were injected into an LC Agilent 1260 system (Agilent Technologies, Inc., Santa Clara, CA, USA). The separation of metabolites was done using a ProtID-Chip-43 II column (C18, 43 mm, 300 Å, 5 μm particle size, equipped with a 40 nL enrichment column). Mobile phase consisted of water with 0.1% formic acid (solution A) and acetonitrile (ACN) with 0.1% formic acid (solution B). The chromatographic method separation started with a mobile phase composition of 5% B, which was then linearly increased to 40% B over 20 minutes. Subsequently, the gradient was elevated to 100% B within 5 minutes and maintained at this composition for an additional 5 minutes. Next, the system was returned to its initial condition of 5% B within 1 minute and held for 9 minutes to ensure complete column re-equilibration before the next sample analysis. The total process time was 40 min, utilizing a flow rate of 300 nL min^{-1}. To mitigate potential carryover effects, two blank samples of 6 μL each – one comprising the injection solution and the other comprising ACN – were run between every sample injection. The separated metabolites were then introduced into an Agilent 6530A Q-TOF mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA) through a Chip Cube–LC interface using nanospray ionization in positive mode. Data-dependent acquisition was used. For MS1, mass range was 110–2000 *m*/*z* with 4 spectra per s velocity. The top 5 most intense precursor ions per cycle reaching 150 cps were selected for fragmentation (MS2 acquisition) in a mass range of 50–2000 *m*/*z*, at 3 spectra per s rate.

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The active exclusion option was on, set to 2 spectra, and released after 0.25 min. Ramped collision energy was used with a slope of 6 and an offset of 4. Calibration was done before sample acquisition and every 24 hours with an ESI-L low mix concentration tuning mix solution (Agilent Technologies, Inc., Santa Clara, CA, USA) to ensure a mass accuracy <5 ppm for MS1 and MS2 data. The samples were randomly allocated in the autosampler for data acquisition.

LC-MS2 data processing

Processing of LC-MS2 datasets was performed to reach two main goals: feature or peak (signals with m/z and retention time) extraction (peak-picking) and metabolite annotation at the structure (metabolomics standard initiative (MSI) levels 2 and 3), substructure, and chemical class (MSI, level 3) levels.[33] Raw datasets were transformed from commercial .d format to open source .mzXML format using an MSconvert tool within ProteoWizard v3.[34] Transformed datasets were processed in MZmine v2.53 for peak-picking using the parameters described in Table S1.†[35] For feature annotation by spectral matching (MSI, level 2), open-source raw and processed data were uploaded and analyzed in the Global Natural Products Social Molecular Networking (GNPS) platform[36] for classical molecular networking[37] and feature-based molecular networking (FBMN),[38] respectively. To expand the annotations not achieved by spectral matching, we employed MolDiscovery[39] and Dereplicator+[40] within the GNPS environment. Additionally, we employed SIRIUS v5.8.1[41] for database-independent chemical formula annotation and correction by ZODIAC,[42] in-silico structural metabolite annotation by CSI: FingerID,[43] and chemical class assignment by CANOPUS algorithm (MSI, level 3). The chemical landscape representation was created using the molecular network provided by the FBMN analysis in GNPS, enriched with the chemical superclass annotations of CANOPUS. Substructure (motifs; MSI, level 3) annotation was done using the MS2LDA webpage.[44] Molecular structure assignments were filtered by a mass error less than 10 ppm to keep annotations with high-mass accuracy. Additionally, peaks and annotations derived from blank samples were filtered out from the analysis.

Quantification and statistical analysis

Unless other specified, all statistical analyses were performed and plotted using GraphPad Prism version 8.0.1, GraphPad Software, San Diego, California, USA (**https://www.graphpad.com**). The clinical data (maternal age, gestation weeks, delivery mode and neonatal sex) were compared between groups using independent-sample median test or Fisher's exact test to evaluate their influence as confounding factors. The Kruskal–Wallis test and GLM were used to assess the influence of maternal and neonatal demographic and clinical data on the microbiota composition, metabolomic profile, and cytokine quantification. Mann–Whitney's U test was used for evaluating the difference in cytokine concentrations between the NW and WO groups. A p-value ≤ 0.05 was considered statistically significant.

For metabolomic data analysis, feature abundance normalization was conducted using the quantile method, and subsequent differential abundance analysis was carried out within the NormalyzerDE online platform.[45] Metabolites exhibiting a log 2-fold change (Log 2FC) of ±0.58 and a p-value <0.05 (calculated with the limma package[46]) were considered differentially abundant between WO and NW groups. Volcano plots were created to facilitate data visualization using EnhancedVolcano R package v1.16.0.[47] To assess differences among groups at the chemical class level, the summed abundance (area under the curve) of all the quantified features belonging to each respective class was calculated, followed by a two-group comparison using the Wilcoxon test, assuming unequal variance, implemented with the wilcox.test() function in R. For comparing colostrum chemical diversity between groups, the Shannon and Simpson indexes were calculated as community ecology metrics[48] using the vegan package v2.6-4. Statistical significance was determined through a two-group comparison using the Wilcoxon test, as described above.

Bacterial interactive network was constructed by linking taxonomic families that exhibited a Spearman rank correlation exceeding an absolute value of 0.5 and a p-value < 0.05. Network analysis was performed using MicrobiomeAnalyst.[49] Correlations between clinical data, cytokine concentrations, bacterial families, and metabolite abundances were calculated with Spearman's correlation (ρ) using R. Only correlations with a $p < 0.10$ were kept for visualization in the heatmaps.

Results

Demographic and clinical characteristics of the study cohort

This study cohort consisted of 48 women between 18 and 35 years old. As per the study design, the body mass index (BMI) was significantly higher in the group "with obesity" (WO) compared to the "Normal Weight" (NW) group (medians: 22.45 *vs.* 32.05, in the NW and WO groups, respectively, $p < 0.0001$, Table 1). There was no difference in maternal age, duration of gestation, delivery mode or biological sex of the offspring.

There were no evident alterations in colostrum pro-inflammatory cytokines in maternal obesity

We quantified colostrum cytokines relevant in inflammatory processes. All cytokines were detected in all samples. However, no significant differences were observed between the groups and correlations with the maternal BMI (Table 1). Although we explored the relationships between the maternal clinical characteristics (maternal age, gestational age, and way of delivery) and colostrum cytokine concentrations, no clear correlation patterns were observed across the groups.

Changes in the microbial composition in the colostrum of mothers with obesity

We analysed the colostrum microbial community using 16S ribosomal RNA amplicon sequencing. We obtained 2 280 755 high-quality reads, corresponding to 45 615 reads

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Table 1 Study population characteristics, colostrum cytokine concentrations, and metabolite data, grouped in accordance with maternal weight classification

Variables	Mothers with normal weight (NW)	Mothers with obesity (WO)	*P*-Value
Number of participants	20	28	
Maternal age (years)[a]	23.5 (23.0 to 28.8)	26.0 (21.0 to 29.0)	0.60
BMI[a]	22.5 (22.0 to 23.7)	32.1 (31.0 to 36.8)	**<0.0001**
Gestational age (weeks)[a]	39.0 (38.0 to 39.0)	38.4 (38.0 to 40.0)	0.28
Way of delivery[b]			0.16
Vaginal	18 (90)	20 (71.4)	
Caesarean	2 (10)	8 (28.6)	
Neonatal characteristics			
Sex assigned at birth[b]			0.14
Female	12 (60)	10 (35.7)	
Male	8 (40)	18 (64.3)	
Cytokines[c]			
IL-12p70	0.76 (0.01 to 1.77)	1.15 (0.10 to 2.33)	0.30
IL-10	4.33 (1.91 to 9.49)	2.72 (1.23 to 5.75)	0.34
IL-1β	16.53 (3.86 to 76.56)	8.16 (2.96 to 31.34)	0.31
TNF-α	7.66 (3.43 to 28.22)	11.49 (4.62 to 18.50)	0.94
IL-6	46.54 (26.64 to 236.9)	53.05 (26.74 to 85.60)	0.40
IL-8	12 498 (3221 to 31 737)	22 294 (10 881 to 36 852)	0.19
Metabolites[d]			
(12*Z*)-9,10-Dihydroxyoctadec-12-enoic acid	2706 (2011 to 4156)	2406 (1576 to 15 898)	0.72
12(13)Ep-9-KODE	3164 (2746 to 20 827)	3091 (2046 to 14 327)	0.41
15-HEDE	1070 (741 to 3694)	244.5 (141.60 to 749.90)	**0.02**
1-*O*-Elenoloyl-2-*O*-(9*Z*-octadecenoyl) glycerol	4432 (2313 to 10 431)	9572 (5217 to 16 373)	0.11
3-Acetyl kabiramide D	180.2 (30.04 to 2515)	210.9 (11.00 to 1650)	0.77
4-Hydroxynonenal	2290 (974.70 to 4708)	1361 (912.60 to 3593)	0.67
7,26-Epoxy-2,7-dihydro-cycloirid-16-enal	192.10 (88.44 to 1046)	366.70 (316.80 to 1169)	0.37
8-Hydroxy-9,10-epoxystearic acid	8762 (7063 to 11 353)	6994 (5829 to 24 920)	0.49
8*S*-Hydroxy-9*E*,11*Z*,14*Z*-eicosatrienoic acid	8441 (1712 to 27 819)	1937 (998.60 to 5005)	0.15
9(*S*)-HOTrE	3370 (1432 to 5267)	2938 (2053 to 3423)	0.92
Aminopentol	11 710 (7385 to 26 755)	29 520 (12 087 to 45 859)	0.13
Carveol	12 052 (6423 to 19 590)	7979 (4180 to 12 588)	0.37
Conodutarine A 19′-ketone	81.16 (2.79 to 8660)	451.70 (28.10 to 2134)	0.87
Decanoylcarnitine	2622 (2023 to 11 551)	1563 (639.30 to 2862)	**0.04**
M531	1483 (977 to 2144)	2771 (1946 to 8447)	**0.02**
Ervahainamidine B	3150 (27.33 to 6736)	1138 (47 to 4016)	0.76
Isomotuporin D	102.10 (11.63 to 1986)	295.70 (25.36 to 967.90)	0.87
Kaimonolide A	1683 (1100 to 1786)	2625 (1532 to 6364)	0.15
LysoPC(16:0)	668 (362.50 to 1594)	671.90 (547.30 to 1152)	0.72
Linoleic acid	23 852 (1272 to 41 630)	4997 (1412 to 63 942)	0.92
Linolenic acid	4284 (1943 to 33 102)	2387 (1329 to 9357)	0.30
Monoelaidin	1041 (214.50 to 23 263)	896.80 (319.4 to 5932)	0.72
1-Monolinolenoyl-*rac*-glycerol	5879 (3722 to 10 378)	6145 (2921 to 21 338)	0.87
Myxochromide S3	3208 (2865 to 37 604)	3883 (2274 to 9778)	0.53
PA (18:4(6*Z*,9*Z*,12*Z*,15*Z*)/22:2(13*Z*,16*Z*))	499.20 (294.90 to 873.60)	819.70 (528.80 to 1420)	0.19
Palmitic acid	1839 (1548 to 3776)	1462 (963 to 2440)	0.22
Pargamicin B	400 (218.70 to 7937)	406.20 (71.86 to 1409)	0.41
PC (18:1(9*Z*)/16:0)	1040 (93.18 to 10 347)	530 (125 to 2864)	0.57
PE (18:4(6*Z*,9*Z*,12*Z*,15*Z*)/20:1(11*Z*))	1183 (769.40 to 1521)	1839 (1001 to 4197)	0.27
PE (18:4(6*Z*,9*Z*,12*Z*,15*Z*)/P-18:1(11*Z*))	1736 (1250 to 2028)	2559 (1583 to 6457)	0.11
Progesterone	10 698 (3653 to 11 922)	7071 (3148 to 19 685)	0.77
Sphingosine	1547 (942.10 to 2905)	2675 (1420 to 3388)	0.37
TG (16:0/18:1(9*Z*)/20:4(5*Z*,8*Z*,11*Z*,14*Z*))	1603 (625.50 to 13 417)	3493 (667 to 10 753)	0.92
TG (16:1(9*Z*)/18:2(9*Z*,12*Z*)/18:3(6*Z*,9*Z*,12*Z*))	393.30 (176.8 to 6832)	533.60 (186.70 to 2450)	0.92
Vitamin E	559.80 (160.60 to 690.60)	271.80 (105.50 to 1005)	0.67

BMI = body mass index [kg m^{-2}]. [a] Values expressed as median (interquartile range); statistical analysis by an independent-samples Mann–Whitney *U* test. [b] Values expressed as frequency (intra-group percentage); statistical analysis by Fisher's exact test. [c] Cytokine concentrations are reported as median [pg ml^{-1}] (interquartile range); statistical analysis by the Mann–Whitney *U* test. NW = 20 and WO = 27. [d] Metabolite abundances are reported as median (interquartile range); statistical analysis by the Mann–Whitney *U* test. NW = 5 and WO = 20.

per sample. We identified 1255 amplicon sequence variants (ASVs) annotated within 4 major phyla: Firmicutes (66.5%, NW; 77.5%, WO), Proteobacteria (21.9%, NW; 12.3%, WO), Actinobacteriota (4.5%, NW; 4.8%, WO), and Bacteroidota (2.3%, NW; 1.6%, WO) (Fig. 1A). Overall, the taxonomic families *Staphylococcaceae* (37.8%, rank 0.17%–97.8%) and *Streptococcaceae* (28.8%, rank 0.02%–92.7%) exhibited the highest relative abundance. Lower abundances were observed for *Gemellaceae* (3.7%), *Burkholderiaceae* (2.2%), *Enterobacteriaceae* (2%), and *Sphingomonadaceae* (1.3%) taxo-

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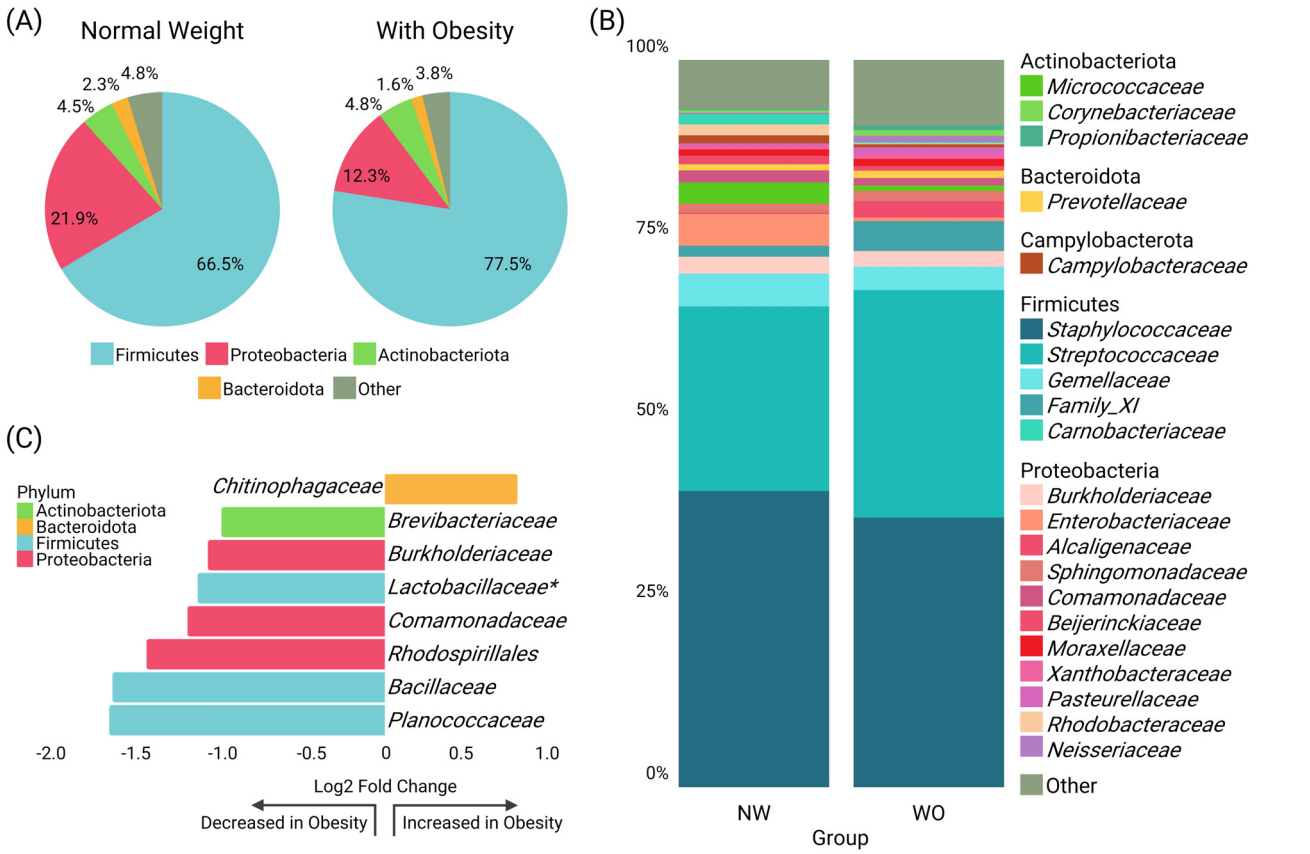

Fig. 1 Composition of the microbiota of colostrum collected from women with and without obesity. (A) Relative abundance of the most abundant taxa at phylum and (B) family levels. "Other" classification represents bacterial families with less than 1% of the total relative abundance. (C) Differential abundance analysis performed at the taxonomic family level using ANCOMBC. The graph provides information on the phylum, family, and corresponding log 2 fold change values ($p < 0.05$), which indicate the magnitude and direction of the differential abundance. NW, normal weight group ($n = 20$); WO, with obesity group ($n = 28$); *$p < 0.10$.

nomic families. Additional families (102), including *Bacillaceae*, *Lactobacillaceae* and *Bifidobacteriaceae*, contributed to <1% of the total relative abundance (Fig. 1B and raw data). There was no difference between the groups looking at the main ecological diversity indices (Fig. S1†). The Firmicutes to Bacteroidota (F/B) ratio and the Firmicutes to Proteobacteria (F/P) ratio were higher in samples from women with obesity, although these differences were not statistically significant (mean ratio: 28.7 *vs.* 47.7, $p = 0.30$, and 3.0 *vs.* 6.3, $p = 0.14$, respectively). In contrast, the Proteobacteria to Bacteroidota (P/B) ratio was significantly decreased in WO (9.4 *vs.* 7.6, $p = 0.046$), which agrees with a relative reduction in the abundance of the Proteobacteria phylum, although not significant (21.9% ± 23.5% *vs.* 12.3% ± 17.2%, $p = 0.14$; Fig. S2†).

To our knowledge, this is the first report identifying an increase abundance of the *Chitinophagaceae* family in the colostrum of women with obesity, with a 0.78 Log2FC relative to samples from the NW group (Fig. 1C). In contrast, 7 taxonomic families were less abundant, including *Brevibacteraceae* (Log 2FC: −0.96), *Burkholderiaceae* (Log 2FC: −1.04), *Lactobacillaceae* (Log 2FC: −1.10), *Comamonadaceae* (Log 2FC: −1.16), *Rhodospirillales* (Log 2FC: −1.40), *Bacillaceae* (Log 2FC: −1.60), and *Planococcaceae* (Log 2FC: −1.62).

Maternal obesity correlates with changes in the colostrum metabolite environment

We used untargeted metabolomics to explore discrepancies in the colostrum of women with obesity compared to normal weight subjects. Our pipeline led to the quantification of 2808 features across the samples. We annotated 1027 MS2-containing features across 12 superclasses, leaving 473 features unassigned (Fig. 2A and Table S2†). Metabolite diversity at the class level was assessed using the Shannon and Simpson indexes, revealing a reduction in the colostrum samples from the WO group compared to the NW group ($p = 0.05$; Fig. S3†). Subsequently, we observed 73 features with decreased abundance and 49 features with increased abundance in the WO group (log 2FC ± 0.58, limma test, $p < 0.05$; Fig. 2B). Decreased features were mostly linked to carboxylic acids, fatty acyls and prenol lipids, whereas the distribution of the increased features was primarily carboxylic acids, organooxygen compounds, azoles, and organic phosphines (Fig. 2C). To gain

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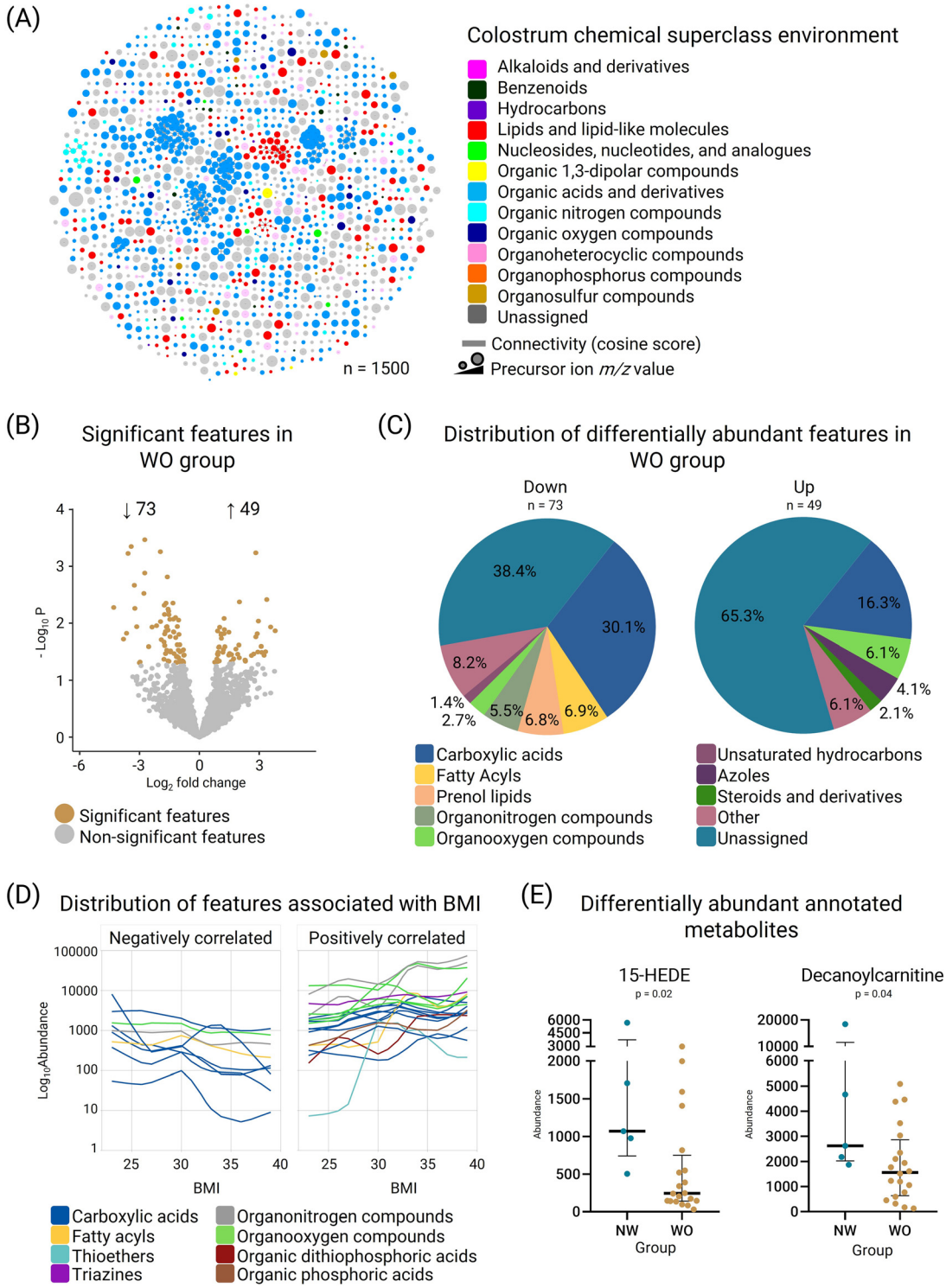

Fig. 2 Metabolite environment in the colostrum of women with obesity (WO). (A) Molecular network of the identified MS2-containing features in colostrum samples. Each node represents a feature, and the color denotes its assigned chemical superclass. Node size highlights the *m/z* value. Edge thickness indicates the MS2 similarity (cosine score) among the features and (B) volcano plot of all quantified features highlighting the differentially abundant features between the WO and NW groups. Brown dots represent significantly increased or decreased features in the WO group (log 2FC ± 0.58, limma $p < 0.05$), while gray dots indicate features without significant differences; (C) pie charts of the chemical class distribution of the differentially increased ($n = 49$) or decreased ($n = 73$) features in the colostrum samples from the WO group. "Other" classification corresponds to classes that only contained one feature; (D) LOESS curves of each feature significantly associated with maternal BMI classified by their Spearman's correlation value (ρ). Curves are color-coded based on the feature chemical class assigned as described in the color key. Only features with a correlation value higher than absolute 0.45 were plotted; (E) boxplots of the relative abundance of the dysregulated (limma test, $p < 0.05$) lipid related identified metabolites, 15-HEDE and decanoylcarnitine; NW, normal weight group ($n = 5$); WO, with obesity group ($n = 20$). See also Tables S2 and S3.†

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insights into the metabolic markers of obesity, we performed correlation analyses between metabolites (identified at the chemical class level) and maternal BMI levels. We identified 57 features distributed across 11 chemical classes that were significantly associated with the maternal BMI (Fig. 2D and Table S3†), mainly comprising carboxylic acids and derivatives (n = 31), followed by fatty acyls (n = 7), and organooxygen compounds (n = 6). Through high-confidence putative structural annotation (mass error ≤ 10 ppm) and differential abundance analysis (limma test), we found that decanoylcarnitine (alongside an unidentified carnitine, as revealed through MS2LDA analysis) and 15-HEDE (fatty acyl) presented reduced abundance in the colostrum of the WO group compared to the NW group (Fig. 2E and Table 1). This is the first time that 15-HEDE is detected in differential abundance in the colostrum of mothers with obesity.

Bacterial co-occurrence network in the colostrum microbiota

Expanding upon the abundance analysis of individual elements, we used co-occurrence networks to define distinctive bacterial interaction modules within the samples. This approach provides a comprehensive view of the coexistence of bacterial taxa and their potential influence with each other. Using Spearman's rank coefficients, we defined five distinct modules of taxonomic families according to their degree of interaction. A positive correlation in this context denotes a potential coordinated and mutualistic interaction, either through direct or indirect mechanisms, which implies that the presence of specific taxa positively influences the presence of others within the microbial community. Conversely, a negative correlation indicates a direct or indirect antagonistic interaction, wherein the coexistence of certain taxa is perturbed by the presence of others (Fig. 3 and raw data).

In the first module, composed of Firmicutes members, *Streptococcaceae* appears as a central taxon, negatively correlated to *Staphylococcaceae* and positively correlated to *Gemellaceae*. These associations suggest potential competition between dominant breastmilk bacterial families and highlights the role of *Streptococcaceae* in the microbial structure. The second module includes mostly members of Proteobacteria, including *Yersiniaceae*, *Erwiniaceae*, *Shewanellaceae*, *Reyranellaceae* and *Rhodanobacteraceae*. Strong positive correlations were observed between *Candidatus Kaiserbacteria* and both *Reyranellaceae* (ρ = 0.81) and *Rhodanobacteraceae* (ρ = 0.83), suggesting cooperation or shared environmental preferences. Notably, their similar median relative abundance in both groups indicate that the interactions are potentially resilient to the maternal BMI. In the third module, we observed moderate positive correlations ($\rho \approx 0.51$–0.53) among *Saccharimonadales*, *Fusobacteriaceae*, *Neisseriaceae* and *Carnobacteriaceae*. *Neisseriaceae* and *Carnobacteriaceae* showed higher median relative abundance in the colostrum of the NW group, indicating potential obesity-mediated disruptions on the mutualistic interactions of this module. The fourth module presented positive correlations between *Prevotellaceae*, *Campylobacteraceae*, *Veillonellaceae* and *Peptostreptococcaceae*. *Veillonellaceae* was the most interconnected family within this module, suggesting a key structural role in the bacterial network. Conversely, *Prevotellaceae* showed a decreased median relative abundance in the WO group, which may imply shifts in the dynamics of the colostrum microbiota. The fifth module was the largest and most diverse, comprising 19 taxonomic families with positive correlations. Potential key taxa in the network included *Alcaligenaceae* and was identified as a potential key player, participating in 7 interactions, followed by *Caulobacteraceae* and *Nocardiopsaceae*, each involved in 6 interactions. We identified families with reductions in relative abundance in the colostrum of women with obesity, including *Comamonadaceae*, *Burkholderiaceae*, *Xanthobacteraceae*, *Beijerinckiaceae*, *Sphingomonadaceae*, *Caulobacteraceae*, *Bifidobacteriaceae*, *Nocardiaceae*, *Deinococcaceae*, and *Moraxellaceae*, which suggest that maternal obesity may compromise a core mutualistic module essential to colostrum's ecological and functional integrity. Further investigation is required to unravel the complex network of interactions that may hold biological significance in the context of maternal obesity.

Lipid mediators are correlated with pro-inflammatory cytokines and Proteobacteria members in the colostrum

We performed a co-occurrence analysis to explore potential interactions between putatively annotated metabolites and cytokines in the colostrum (Fig. 4A and B). We found that 15-HEDE negatively correlated with IL-12p70 and IL-10, suggesting a potential role of this regulatory lipid in inflammation. Lysophosphatidylcholine (16:00; LysoPC [16:00]), on the other hand, correlated positively with IL-1 and IL-8, suggesting a role in pro-inflammatory signalling pathways (Fig. 4B and Table S4†).

In addition, we analysed the interrelationship between metabolites and bacterial groups. For instance, the regulatory lipid 15-HEDE, found to be decreased in the colostrum of women with obesity, exhibited positive correlations with taxonomic families such as *Beijerinckiaceae*, *Burkholderiaceae*, and *Erwiniaceae*, which were also less abundant in the colostrum of women with obesity (Fig. 1C and Fig. 4C). Moreover, despite the absence of a significant difference between the study groups, 12(13) Ep-9-KODE showed a positive correlation with *Micrococcaceae*, *Aeromonadaceae*, and *Beijerinckiaceae*. On the other hand, LysoPC (16:00) showed negative correlations with *Xanthobacteraceae*, *Reyranellaceae*, *Xanthomonadaceae*, *Caulobacteraceae*, *Cellulomonadaceae*, *Pasteurellaceae*, *Rhodospirillales*, *Streptococcaceae*, *Weeksellaceae* and *Bacteroidaceae*, but exhibited positive correlations with *Planococcaceae*, *Alphaproteobacteria* and *Burkholderiaceae*.

We also identified that the steroid hormone progesterone displayed negative correlations with *Caulobacteraceae*, *Comamonadaceae*, *Devosiaceae*, *Nocardioidaceae* and *Staphylococcaceae*, which may imply instances where negatively affects abundance of the bacterial community. Furthermore, fat-soluble vitamin E was negatively correlated with *Comamonadaceae*, *Neisseriaceae*, *Fusobacteriaceae*,

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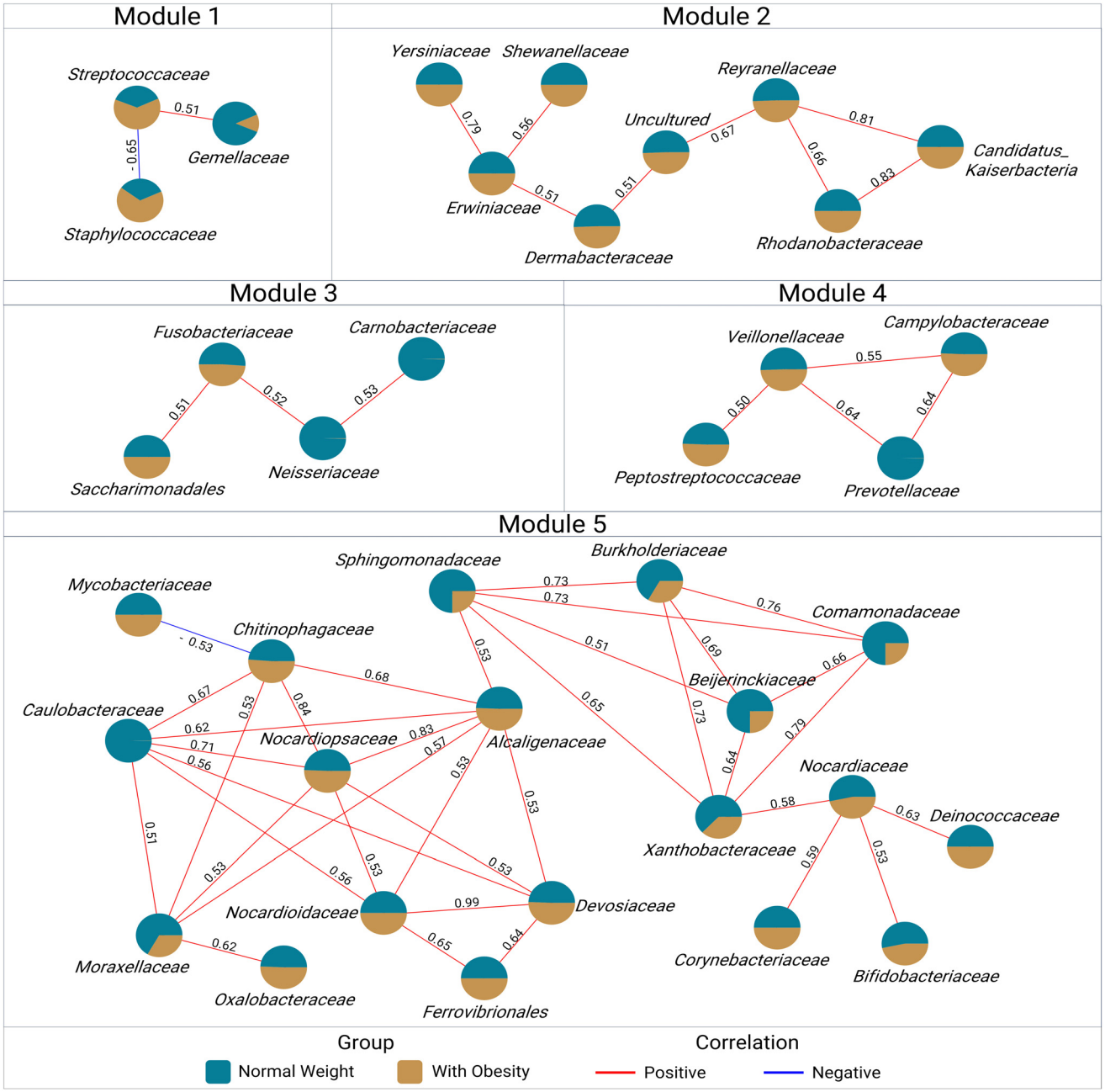

Fig. 3 Colostrum bacterial co-occurrence network. Visualization of the interactions between taxonomic families' abundances within the normal weight (blue; n = 20) and with obesity (brown; n = 28) groups. Five distinct modules, each comprising at least 3 members, were obtained based on Spearman's rank correlation of coexistence. Each node is represented by a pie chart displaying the median relative abundance of a taxonomic family within each group. Edges connect two families if their Spearman's rank correlation exceeds an absolute value of ρ = 0.5 with a p-value < 0.05. The edges are annotated with Spearman's correlation values. Positive correlations between differentially abundant taxonomic families are represented by red edges, while negative correlations are indicated by blue edges as described in the color key.

Staphylococcaceae, *Erwiniaceae* and *Carnobacteriaceae* (Fig. 4C and Table S4†). Further research into the specific metabolic pathways and ecological roles associated with these metabolites could support their significance in the colostrum system and their implications in obesity.

Pro-inflammatory cytokines are negatively correlated to Proteobacteria members in the colostrum of mothers with obesity.

We also delve into the interplay between microbial communities and immune-modulating factors (Table S5†). In samples obtained from normal weight participants, members of the Proteobacteria phylum such as *Enterobacteriaceae* and *Burkholderiaceae* displayed negative correlations with IL-10, while *Prevotellaceae* showed a positive correlation with this anti-inflammatory cytokine. Other members of the Proteobacteria phylum, including *Neisseriaceae* and

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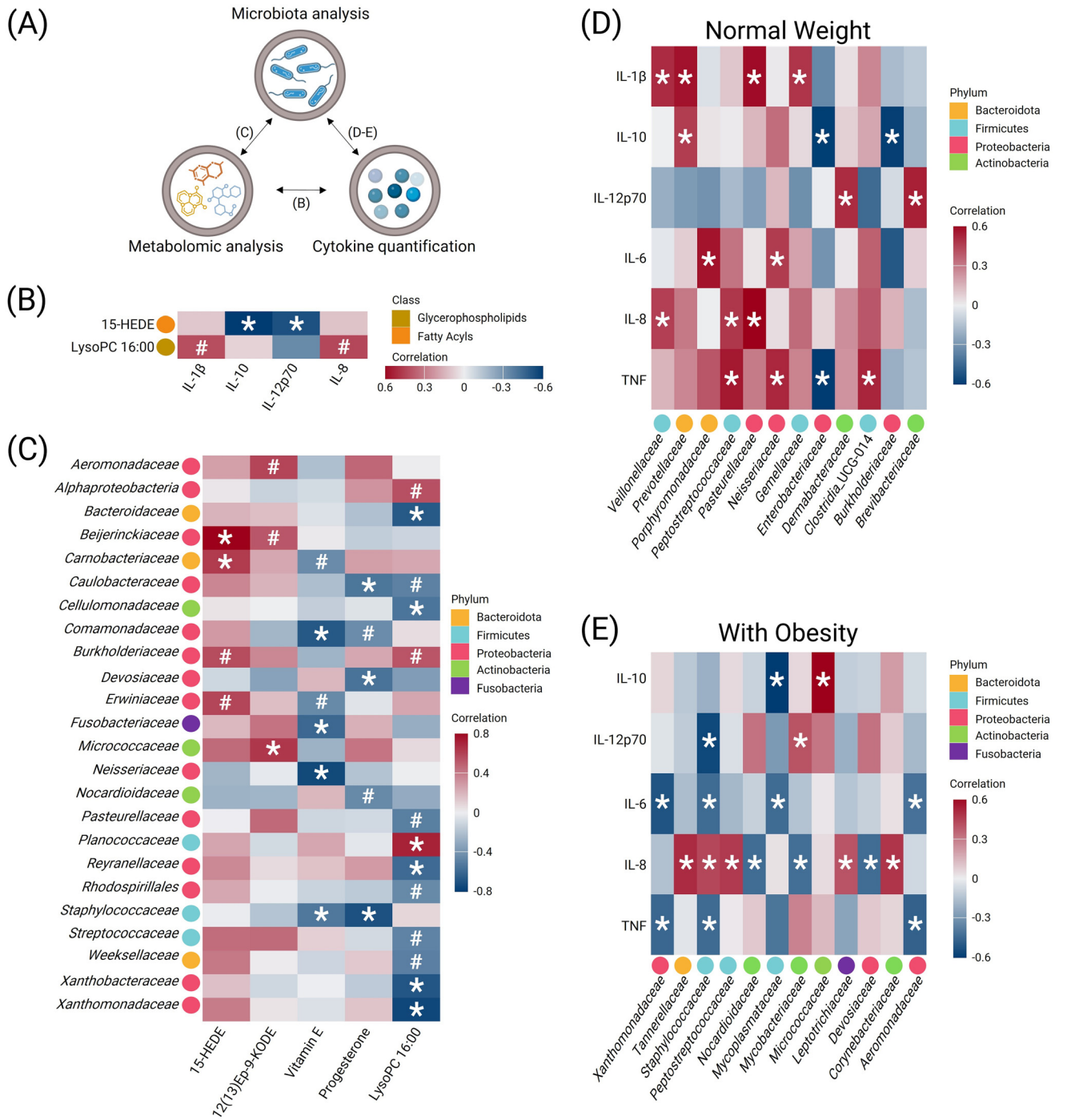

Fig. 4 Correlation analysis between colostrum bioactive elements. (A) Scheme of the different correlations performed between the bioactive elements. (B) Heatmap of Spearman's correlation between metabolites and cytokines in colostrum; (C) Heatmap of Spearman's correlation analysis of taxonomic families and metabolites in colostrum; (D and E) Heatmap of Spearman's correlation analysis of taxonomic families and cytokines in the colostrum of women with (D) normal weight and (E) obesity. Red represents a positive correlation, white represents a low correlation, and blue represents a negative correlation, as shown in the color key. *p-value < 0.05; #p-value < 0.10. See also Tables S4 and S5.†

Pasteurellaceae, exhibited positive correlations with IL-6, TNF-α, IL-1β, and IL-8, suggesting a potential pro-inflammatory modulation (Fig. 4D). Meanwhile *Veillonellaceae*, *Prevotellaceae*, and *Gemellaceae* presented a positive correlation with IL-1β, and *Dermabacteraceae* and *Brevibacteraceae* were positively correlated with IL-12p70. Additionally, *Porphyromonadaceae* was positively correlated with IL-6.

In contrast, samples from women with obesity showed negative correlations between the taxonomic families *Aeromonadaceae*, *Xanthomonadaceae*, and *Staphylococcaceae*,

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and the immune-modulating factors IL-6, TNF-α, and IL-12p70. *Devosiaceae*, *Mycobacteriaceae*, and *Nocardioidaceae* were negatively correlated with IL-8, while *Corynebacteriaceae*, *Staphylococcaceae*, *Leptotrichiaceae* and *Tannerellaceae* showed positive correlations (Fig. 4E). Notably, *Peptostreptococcaceae* exhibited a positive correlation with IL-8 across the entire cohort independently of the study group.

Discussion

Colostrum is a complex and dynamic fluid that seeds neonatal intestines within the initial hours of life. The impact of colostrum depends on the composition and interactions among its bioactive components.[10,14] Integrative research initiatives, such as the BEGIN project, are exploring breastmilk as a biological system, emphasizing the interactions and coordinated functionality between its bioactive components.[10,50] In this study, we profiled the colostrum microbiota, cytokines, and metabolites and established networks of co-occurring elements to provide insights into the effect of maternal obesity on interacting bioactive components.

Despite extensive research on the associations between breastmilk components and maternal obesity, the findings remain heterogeneous.[4,11,13,51–53] We identified subtle differences while analysing individual bioactive components, *i.e.* cytokines, microbiota, and metabolites. While variability can be attributed to technical and methodological factors,[54–56] studying colostrum as a dynamic biological system with interconnected components could provide valuable insights into the relationships within microbial communities and their interactions with bioactive molecules.

In our study, we identified a negative correlation between *Staphylococcaceae* and *Streptococcaceae*, indicating a potential competitive relationship. This association could be influenced by ecological factors within the mammary gland microenvironment, such as niche competition, resource availability, or immune responses, which could benefit the dominance of one bacterial group over the other.[57,58] Moreover, co-occurrence network analysis revealed bacterial relationships among Proteobacteria members exhibiting reduced relative abundance in the colostrum of women with obesity. Within the same co-occurrence module, these taxa demonstrated positive correlations, indicating mutualistic interactions and functional similarities.[57]

The observed variations in the colostrum microbiota of the WO group may impact the microbiota development of neonates. Offspring born to mothers with obesity presented a decrease in the abundance of Gammaproteobacteria two weeks after birth.[59] This is critical for the establishment of a healthy gut microbiome functioning, since Proteobacteria members are the primary colonizers during the first week of life and play a crucial role in preparing anaerobic conditions in the gut for successive colonization by strict anaerobes.[60–62] Additionally, their presence in the postnatal period is essential for promoting a healthy immune system, facilitating host–microbe interactions. A reduction in Proteobacteria supply in the colostrum of mothers with obesity may compromise immune system training during the critical early days of life, potentially leading to impaired immunological tolerance and increased risk of immune-related diseases later in life.[63,64]

Alterations in specific microbial taxa within breastmilk may potentially be linked to cytokine levels, exerting an impact on breast-fed infants.[65] We report that the abundance of *Enterobacteriaceae* in the colostrum of women with normal weight was negatively correlated with TNF-α, while Proteobacteria such as *Neisseriaceae* was positively correlated with TNF-α and IL-6. This pattern aligns with previous observations in mice, where the presence of the early colonizer *Enterobacteria* triggers TNF-α production during the first week of life. This interaction prevents excessive inflammatory and autoimmune gastrointestinal disorders in the future.[64] Furthermore, the production of TNF-α by macrophages and monocytes within the first three days of life, stimulated by the microbiota, is a prerequisite for the maturation of pre-conventional dendritic cell 1 (pre-cDC1) into conventional dendritic cell 1 (cDC1).[66]

The increased levels of Gram-negative bacteria in the gut are known to stimulate the local production of pro-inflammatory cytokines.[67] In mothers with obesity (WO group), we observed negative correlations between pro-inflammatory cytokines (TNF-α, IL-6, and IL-12p70) and certain bacterial families such as *Aeromonadaceae*, *Xanthomonadaceae* and *Staphylococcaceae* (Fig. 5). Our unexpected correlative findings align with the observations of lower TNF-α and IL-6 concentrations in the breastmilk of mothers with obesity compared to mothers with normal BMI.[68] This suggests a potential local regulatory system within the mammary gland that may modulate systemic inflammation during obesity.

Metabolites are key elements of microbial communities as they reflect interactions within the ecosystem. Different metabolites have been associated with interactions in the breastmilk of women with obesity.[5,69,70] We observed a reduction in fatty acyls and prenol lipids, which suggests potential alterations in the lipid profile of colostrum associated with maternal obesity. For instance, we observed that decanoylcarnitine exhibited a negative correlation with BMI and was found to be decreased in the WO group. Our results contrast previous observations where elevated levels of various acylcarnitines were detected in the serum and breastmilk of individuals with obesity.[5,71] Acylcarnitines play a crucial role in mediating the beta-oxidation of fatty acids within the mitochondria, a pivotal process in energy production.[72]

In addition, the breastmilk of women with overweight or obesity exhibits decreased levels of polyunsaturated fatty acids (PUFAs).[73] The metabolism of PUFAs processed through the enzymatic action of lipoxygenases and cyclooxygenases can lead to the formation of oxylipins.[74] Breastmilk has a diverse array of bioactive lipids, which play significant roles within the neonatal immune system.[75,76] The presence of oxylipins in breastmilk may be a marker of maternal health and could influence the health outcomes and inflammatory status of breast-fed infants.[74]

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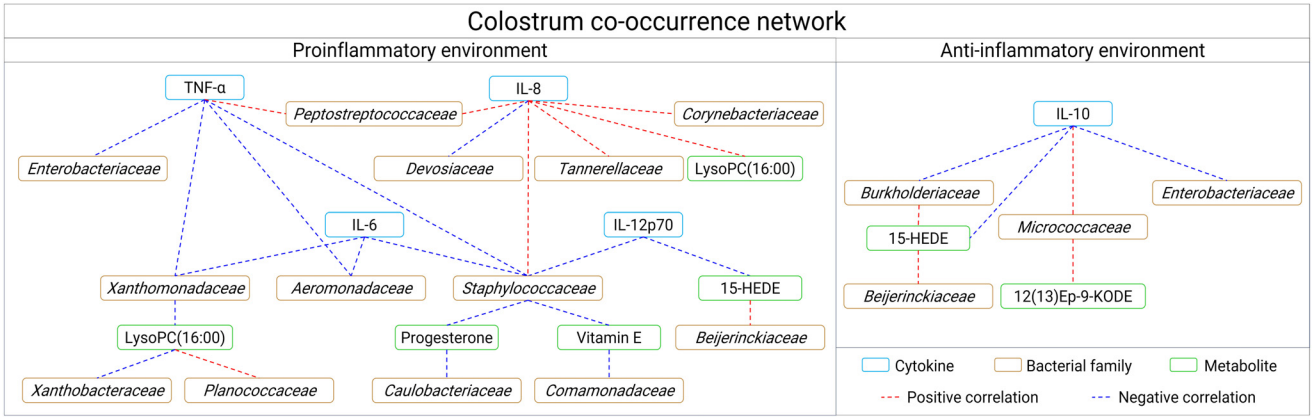

Fig. 5 Integrated biological network of the colostrum environment in the obesity context. Summary of Spearman's correlation analysis between colostrum inflammatory cytokine quantification, bacterial families, and metabolites in the maternal obesity context ($p < 0.05$). The left panel corresponds to the correlation network between pro-inflammatory cytokines and bioactives in the colostrum samples of women with obesity. The right panel corresponds to the colostrum anti-inflammatory cytokine correlation network of women with obesity. Rectangle colors represent bioactive elements: the blue rectangle corresponds to cytokines, brown to bacterial families, and green to metabolites. Red and blue edges represent the positive and negative correlations, respectively.

Some hydroxy fatty acids appear to be linked to inflammatory processes with robust functions, for example, with anti-inflammatory and pro-resolving properties, either directly or through their conversion into lipid mediators.[77,78] One notable example is 15-HEDE, a macrophage-oxidized product of the inflammatory modulator 6-PUFA eicosadienoic acid (EDA).[79,80] We found that 15-HEDE levels were decreased in the colostrum of women with obesity, showing a negative correlation with IL-10 levels and positive correlations with Proteobacteria members, such as *Beijerinckiaceae* and *Burkholderiaceae* (Fig. 5). Despite the fact that 15-HEDE has been described as an inhibitor of 5-lipoxygenase (5-LO),[81] it has also been reported to induce vasodilation and vascular hyperpermeability, acting as a pro-inflammatory lipid mediator that exacerbates allergic rhinitis.[82] Elevated levels of 15-HEDE have also been observed in allergic airway inflammation in mice after a house dust mite exposure and in psoriasis.[79,83] The negative correlation with IL-10 suggests that IL-10 may act as a modulator of inflammation resolution in colostrum. In the chronic inflammatory milieu of obesity, circulating levels of IL-10 are elevated compared to normal weight women.[84,85] Therefore, we hypothesized a potential compensatory mechanism to counterbalance chronic inflammatory processes and mitigate the impact of reduced but highly immunogenic bacteria. In normal-weight mothers, the higher abundance of 15-HEDE may enhance the vasodilatory capacity of the mammary vasculature, facilitating efficient nutrient transfer and immunological component delivery to the neonate.[86]

While progesterone showed no significant difference between the NW and WO groups, its potential role in shaping colostrum microbial ecology warrants further investigation. We observed negative correlations when compared to specific bacterial taxa, including *Staphylococcaceae*, *Ferrovibrionales*, *Caulobacteriaceae*, and *Devosiaceae* (Fig. 5). Progesterone has been shown to influence microbial communities *in vitro* by reducing the growth, adhesion, and virulence of certain *Staphylococcus* strains.[87] While progesterone is dominant during late pregnancy, pregnant women with obesity often exhibit reduced serum progesterone levels.[88,89] Moreover, progesterone levels in the breastmilk of women with a high fat and protein diet are diminished[90] and negatively correlated with infant weight at 6 months.[91]

Our study presents some limitations. For instance, detailed information regarding maternal diet and lifestyle was not collected, which could represent potential confounding factors. Although small cohorts are common in colostrum-related studies, we acknowledge that the implementation of larger sample sizes would enhance the statistical power and robustness of findings. Some correlations in our study fell within the moderate range ($|\rho| = 0.40–0.59$). These values, though statistically significant, should be interpreted with caution, as they may miss nonlinear relationships or interactions mediated by unmeasured variables. In addition, due to limited sample volumes, this work presents high variability in sample size across datasets (16S sequencing, cytokines, and metabolomics), which may negatively impact data integration and interpretation. Finally, while co-occurrence networks offer insights into the microbial community structure and their interactions with bioactive molecules that traditional methods might overlook, they do not establish directional interactions or causal mechanisms.

In summary, using co-occurrence networks, we integrated metataxonomic data, metabolomics, and cytokine profiles to provide novel insights and a proof of concept for colostrum as an interconnected system and demonstrate the influence of maternal obesity on bioactive interactions within this microenvironment. Our analysis identified a negative co-occurrence between the lipid mediator 15-HEDE and IL-10 while also showing a positive association with microbiota members such as *Beijerinckiaceae* and *Burkholderiaceae* – findings that have

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not been previously reported in the colostrum of mothers with obesity. Additionally, we identified that *Aeromonadaceae*, *Xanthomonadaceae*, and *Staphylococcaceae* were negatively correlated with pro-inflammatory cytokines (TNF-α, IL-6, and IL-12p70), suggesting a potential regulatory effect on inflammatory responses in colostrum associated with maternal obesity. Our findings reveal that maternal obesity influences alterations in the ecosystem structure. However, further research is needed to elucidate the neonatal health implications of the disrupted interactions among the bacterial community, metabolites and local inflammatory markers observed in the colostrum of women with obesity.

Author contributions

JSGV: data curation, formal analysis, investigation, methodology, writing – original draft, and writing – review & editing. KCC: data curation, formal analysis, investigation, supervision, and writing – review & editing. ESSS: investigation, methodology, and writing – review & editing. MRAG: investigation and methodology. CNLV: investigation and methodology. RACC: formal analysis, methodology, and writing – review & editing. AMU: supervision, formal analysis, methodology, and writing – review & editing. VJLD: investigation, methodology, supervision, and writing – review & editing. MEGB: conceptualization, investigation, methodology, supervision, funding acquisition, writing – original draft, and writing – review & editing. CLC: conceptualization, data curation, formal analysis, funding acquisition, project administration, supervision, writing – original draft, and writing – review & editing.

Data availability

All fastq-format reads analyzed have been deposited and are publicly available at the NCBI Sequence Read Archive (SRA). Metabolite raw datasets are available at the GNPS/MassIVE public repository and are available as of the date of publication. Accession numbers are listed in the ESI (Table S6†). This paper does not report the original code.

Conflicts of interest

All authors have read the manuscript and declare no conflicts of interest.

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