Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 144 (2025) 109967

Journal of Nutritional Biochemistry

RESEARCH PAPER

Differences in mature human milk metabolic profiles based on delivery mode and parity

Jiayue Tang[a], Cai Shen[b], Dan Yao[a], Jingwen Yu[a], Yanan Liu[a], Maolin Tu[a], Hong Zhang[c], Xuebing Xu[c], Oi-Ming Lai[d], Ling-Zhi Cheong[b,*]

[a] Zhejiang-Malaysia Joint Research Laboratory for Agricultural Product Processing and Nutrition, Key Laboratory of Animal Protein Food Deep Processing Technology of Zhejiang Province, College of Food Science and Engineering, Ningbo University, Ningbo, China
[b] School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, The University of Melbourne, Melbourne, Australia
[c] Research and Development Center, Wilmar (Shanghai) Biotechnology Research and Development Center Co Ltd., Shanghai, China
[d] Department of Bioprocess Technology, Faculty of Biotechnology and Bimolecular Science, University Putra Malaysia UPM, Serdang, Malaysia

Received 8 July 2024; received in revised form 20 March 2025; accepted 16 May 2025

Abstract

Human milk (HM) is regarded as the gold standard for infant nutrition. The metabolite profiles of mature human milk (MHM) have been reported to be affected by maternal physiological conditions, lactation stage, and maternal diets. We collected MHM (3–6 months postpartum) from 32 healthy mothers with different parities and delivery modes. Then, GC-MS and LC-MS were used to perform an untargeted metabolomic study. A clear distinction can be observed in terms of MHM metabolites of mothers with different delivery modes and parities with a 95% confidence interval. A total of 119 differentially expressed metabolites (DEMs) were identified in MHM of women with different delivery modes. Metabolic pathway analyses indicated that these DEMs were mainly associated with fatty acid biosynthesis. The higher abundances of these DEMs in MHM of cesarean women may be due to the differing levels of cortisol and oxytocin between mothers with different delivery modes. Meanwhile, 284 DEMs were identified in MHM of women with different parities. These DEMs were primarily related to ABC transporters, center carbon metabolism in cancer, and D-amino acid metabolism. These findings highlighted the impact of delivery mode and parity on HM metabolite composition. Further research is needed to explore the long-term health implications of these metabolic differences and optimize infant nutrition strategies.

Keywords: Human milk; Untargeted metabolomics; Delivery mode; GC-MS; LC-MS.

1. Introduction

Human milk (HM) is a dynamically changing fluid that provides essential nutrients and bioactive substrates for infants [1]. It contains a variety of small-molecule metabolites such as lipids, fatty acids, oligosaccharides, peptides, immunomodulators, vitamins, and minerals [2]. Bioactive lipids and fatty acids promote brain development [3], meanwhile oligosaccharides have been shown to increase beneficial microbes for infants [4]. Peptides reduce the risk of acute and chronic pediatric disorders [5].

There are many factors affecting the composition of HM metabolites including gestational age, lactation stages, maternal diet, geographic location, and health conditions. For example, lactose and oligosaccharide levels are significantly higher in mothers' milk with preterm infants than those of term infants [6]. Another research showed significantly higher caproic (C6:0) and alpha-linolenic acids (C18:3 n-3) in colostrum than in mature milk [7]. Increased maternal intake of fats and oils, particularly linoleic acid, may lead to the inhibition of omega-3 (n-3) long-chain polyunsaturated fatty acid (n-3 LCPUFA) metabolism, such as the biosynthesis of docosahexaenoic acid (DHA) in HM [8].

HM metabolites can be determined using nuclear magnetic resonance (NMR), capillary electrophoresis-mass spectrometry (CE-MS), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). Each of these analytical techniques has its own merits and drawbacks [9]. NMR has the advantages of easy sample preparation, simultaneous metabolite characterization and quantification, and high experimental reproducibility, but it has lower detection sensitivity [10]. Meanwhile, CE-MS can characterize only ionic, mildly ionic, and highly polar compounds [11]. Although compounds must be firstly derivatized, GC-MS is suitable for analyzing highly polar metabolites including

* Corresponding author at: The University of Melbourne, Grattan Street, Parkville VIC 3010, Melbourne, Australia.
E-mail address: lingzhi.cheong@unimelb.edu.au (L.-Z. Cheong).

https://doi.org/10.1016/j.jnutbio.2025.109967

organic alcohols, organic amines, monosaccharides and disaccharides [12,13]. LC-MS has been applied to identify the majority of semi-polar substances, such as peptides, nucleotides, nucleosides, sterpenoids and alkaloids [14]. Both GC-MS and LC-MS can analyse intermediate polar metabolites, like fatty acids, amino acids, organic acids, sugars, etc. [13].

Metabolomics have also been widely utilized in HM researches in recent years. Studies on milk metabolomics have principally concentrated on (1) comparing HM with other mammalian milk [15], (2) exploring the effects of maternal and infant factors on HM metabolites (such as health conditions of mothers [16], maternal diet [17] and gestational age of infants [6]), (3) finding the differences between HM and infant formula [18], (4) uncovering the physiological function of HM metabolites on infant health [19]. These studies have helped to deepen the understanding of HM and expand ideas for studying HM.

Although several studies have explored the influence of HM composition by some factors, few have examined the effects of delivery mode and parity on the metabolites of HM. Our previous proteomic study revealed that parity, delivery mode, and maternal age significantly influenced the proteomic profile of mature human milk (MHM) [20]. Building on these findings, the current study further investigates differences in MHM metabolic profiles based on delivery mode and parity. We employed multivariate statistical analysis to screen the differentially expressed metabolites (DEMs) between different groups of women. The results of this study will help to understand how maternal factors affect HM metabolites and early life nutrition.

2. Materials and methods

2.1. Materials

Fatty acid methyl esters standards [methyl octanoate (C8:0), methyl nonanoate (C9:0), methyl decanoate (C10:0), methyl laurate (C12:0), methyl myristate (C14:0), methyl palmitate (C16:0), methyl stearate (C18:0), arachidic acid (C20:0), methyl sanyuate (C22:0), methyl lignocarbonate (C24:0)] were purchased from NU-chek (Minnesota, USA) and Dr. Ehrenstorfeh GmbH (Augsburg, Germany). These compounds with varying carbon chain lengths were selected as internal standards for GC-MS to better cover the retention time range of metabolites. This approach facilitates quality control, retention time correction, and metabolite identification. HPLC-grade L-2-chlorophenylalanine (98.0%) was purchased from Hengchuang Biological Co., Ltd (Shanghai, China), which was used as internal standard for GC-MS and LC-MS. N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) was purchased from TCI (Shanghai) Development Co., Ltd. (Shanghai, China). HPLC-grade methanol (99.9%), acetonitrile (A998-4, 99.95%), and formic acid (A117-50, 99.0%) were obtained from Fisher Scientific (Hampton, NH, USA).

2.2. MHM collection

Participants were screened using questionnaires. All participants were healthy women aged 23-39 (mean: 29 years) with no history of diseases (mastitis, breast cancer, diabetes, hypertension) or lifestyle behaviors (smoking, alcohol, drug use) that could impact infant development during pregnancy or lactation. MHM samples were collected from 32 healthy lactating mothers in Ningbo, China, at 3-6 months postpartum to control for lactation stage and geographical variations. Participants were evenly allocated into four groups (n=8 per group) based on delivery mode [cesarean section (CS) vs. vaginal birth (VB)] and parity [multiparous (MP) vs. primiparous (PP)]. Key confounding factors, including maternal age, prepregnancy BMI, parity (all primiparous in CS and VB groups), and cesarean rates (balanced between MP and PP groups), were controlled, as detailed in Table S1, to ensure robust study design.

Full breast milk samples were collected from the same breast at Ningbo University Affiliated Hospital under dim light between 9:00 and 11:00 AM (fasting was not required) using an electric pump, aliquoted into storage bags, and immediately frozen at −80°C to preserve photosensitive metabolites. To reduce dietary and individual variability, 3 individual samples were pooled into 1 analytical sample (each individual sample used in ≤3 pools), resulting in 8 pooled samples per group for metabolomic analysis. All participants had informed consent and the study had approval from the human research ethics committee of Ningbo University (NBU-2021-142).

2.3. GC-MS analysis of MHM metabolome

2.3.1. Extraction of MHM metabolites

MHM metabolites were extracted using an established method with slight adaptations [21]. MHM was thawed at room temperature. About 150 μL of MHM was added to 450 μL of methanol-acetonitrile solution (2:1, v/v, to precipitate proteins) with 2 μg/mL L-2-chlorophenylalanine (internal standard). After vortexing for 1 min, the mixtures were sonicated (ice bath, 10 min) and then frozen (−40°C, 30 min), thawed at 4°C, followed by centrifuging (4°C, 10 min, 12,000 rpm). The supernatant was transferred for further GC-MS and LC-MS experiments.

2.3.2. Derivatization of MHM metabolites

For GC-MS analysis, the supernatant (150 μL) was transffered to a glass sampling vial and subsequently dried utlizing a freeze concentrate centrifugal dryer. Methyltyramine hydrochloride (80 μL, 15 mg/mL) in pyridine solution was then added and incubated (37°C, 60 min) for the oxime reaction. Following that, BSTFA derivatization reagent (50 μL, with 1% trimethylchlorosilane, introducing silyl groups), n-hexane (20 μL), and internal standards (10 μL, C8/C9/C10/C12/C14/C16/C18/C20/C22/C24) were added to the blend and derivatized at 70°C for 60 min. Mixtures were cooled (ambient temperature, 30 min) prior to analysis. Quality control was prepared by pooling aliquots of all samples.

2.3.3. GC-MS analysis

GC-MS analysis was performed using a reported method [22]. Separation of the MHM metabolites was conducted using Agilent 7890B gas chromatography and MSD system (Agilent Technologies Inc., CA, USA) equipped with a DB-5MS capillary column (30 m×0.25 mm×0.25 μm, Agilent J&W Scientific, Folsom, CA, USA). High-purity helium (purity>99.999%, flow rate=1 mL/min) was used as carrier gas. The injection condition was set as: injection temperature, 260°C; injection volume, 1 μL; solvent delay time, 4.8 min. The conditions of GC-MS heating procedure were applied as previously described [23]. The remaining GC-MS conditions were set as: mass spectrometer quadrupole temperature, 150°C; electron bombardment ion source temperature, 230°C; collision energy, 70 eV; mass spectral data, full scan mode (50–500 m/z).

2.3.4. GC-MS data processing

The GC-MS data were processed via MS-DIAL software as described previously [21], covering peak detection, peak recognition, MS2Dec deconvolution, metabolite characterization, peak alignment, wave filtering and missing value interpolation. Metabolites were identified using the LUG database.

2.4. LC-MS analysis of mature human milk metabolome

2.4.1. Extraction of mature human milk metabolites

The steps for metabolite extraction were consistent with those used for GC-MS. For LC-MS analysis, the supernatant (150 μL) was passed through a polytetrafluoroethylene membrane filter (0.22 μm, Millipore, Germany) into LC vials for subsequent analysis.

2.4.2. LC-MS analysis

The metabolic analysis was performed applying Dionex Ultimate 3000 RS UHPLC fitted with Q-Exactive quadrupole-Orbitrap mass spectrometer equipped with heated ESI source (Thermo Fisher Scientific, Waltham, MA, USA). The chromatographic conditions were as follows: an ACQUITY UPLC HSS T3 column (100 mm×2.1 mm, 1.8 μm, Waters, Milford, Massachusetts, USA) was used with a column temperature of 45°C, the mobile phase consisted of A-water (containing 0.1% formic acid) and B-acetonitrile, the flow rate was 0.35 mL/min, and the injection volume was 5 μL. Gradient separation was performed as previously reported: 0.01 min, 5% B; 2 min, 5% B; 4 min, 30% B; 8 min, 50% B; 10 min, 80% B; 14 min, 100% B; 15 min, 100% B; 15.1 min, 5% B; 18 min, 5% B [24]. The MS conditions used an ESI ion source with sample signals collected in positive and negative ion scan modes. Parameter settings were: spray voltage, 3800 (positive)/−3000 (negative) V; capillary temperature, 320°C; Aux gas heater temperature, 350°C; sheath gas flow rate, 35 Arb; Aux gas flow rate, 8 Arb; S-lens RF level, 50; mass range, 100-1200 m/z; Full ms resolution, 70000; MS/MS resolution, 17500; NCE/stepped NCE, 10, 20, 40 for both modes.

2.4.3. LC-MS data processing

The LC-MS data were analyzed using MS-DIAL and Progenesis QI V2.3 software (Nonlinear, Dynamics, Newcastle, UK) according to a previously described method, which included baseline filtering, peak detection, integration, retention time correction, peak alignment, and normalization [25]. Metabolites were identified by referencing the Human Metabolome Database (HMDB), EMDB, PMDB, Lipidmaps (V2.3), Metlin, and our proprietary in-house databases. Peaks (>50% missing values, ionic strength=0) were excluded, and compounds scoring (<36, out of 60) were also considered imprecise and deleted. The zero value was substituted by half of the minimum value and the compounds were selected according to their qualitative outcomes. The positive and negative ion data were coupled to build the data matrix.

2.5. Statistical and bioinformatic analysis

To investigate the overall distribution of samples and the stability of the overall analytical procedure, Principal Component Analysis (PCA) was employed. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was applied to identify the first principal component from PCA and to select metabolites that differed between cohorts. The overall contributions of all variables to the differences between groups were assessed using variable importance level (VIP) values derived from the OPLS-DA model. The OPLS-DA model was verified by sevenfold cross-validation and 200 response permutation tests. R2 is an index of fitness and robustness, and Q2 is usually an assessment of the predictive power of the model [26]. To identify significant differences in metabolites between groups, a two-tailed Student's t-test was conducted. The conditions for DEMs screening was established in the following manner: VIP>1.00 and *P*-value<.05. Venn diagrams and heatmaps were generated using OECloud tools at https://cloud.oebiotech.com. Volcano plots and pie charts were created using Origin 2021 software. Metabolic pathway analyses were carried out using Metaboanalyst 5.0 and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. The graphical abstract was created using Adobe Illustrator 2020. All untargeted metabolomic data used in this study have been deposited to the EMBL-EBI Metabolights database.

3. Results and discussion

3.1. Multivariate statistical analysis

Figure 1 shows the OPLS-DA score plots and corresponding validation plots of the OPLS-DA results derived from MHM metabolites of women with different delivery modes and parities determined using both GC-MS and LC-MS. Regardless of the analysis techniques, clear distinctions can be observed in terms of MHM metabolites of mothers with different delivery modes (Fig. 1A-B) and parities (Fig. 1C-D) with a 95% confidence interval. The R2Y values of the corresponding validation plots (Fig. 1E-H) are 0.944, 0.904, 0.973, and 0.734, respectively. The share of Y variables rises and Q2 progressively falls as the replacement retention rate falls indicating the model is predictive and not overfitted.

3.2. Screening and analysis of DEMs

3.2.1. DEMs in MHM of mothers with different delivery modes

A total of 119 DEMs were detected in MHM of mothers with different delivery modes using both GC-MS (53 DEMs) and LC-MS (54 DEMs in positive ion mode, 12 DEMs in negative ion mode) (Table 1, Fig. 2A). 89 of the DEMs were up-regulated and the remaining 30 were down-regulated in CS mothers as compared to VB mothers (Fig. 2B). Heat map analysis shows distinct differences of MHM metabolites of mothers with different delivery modes (Fig. 2D). A pie chart was used to illustrate the major classification of the DEMs (Fig. 2C). DEMs in MHM of mothers with different delivery modes can be categorized as lipids and lipid-like molecules (36 DEMs), organic oxygen compounds (26 DEMs), organic acids and derivatives (12 DEMs), organoheterocyclic compounds (7 DEMs), phenylpropanoids and polyketides (6 DEMs), and benzenoids (5 DEMs).

Among these DEMs, we found several HM oligosaccharides (HMOs) [27] and their metabolites, such as 3-fucosyllactose, lactodifucotetraose, lacto-N-difucopentaose II, lacto-N-tetraose, 3′-sialyllactose, lacto-N-biose I, which are of paramount importance for infant development. Comparative analysis revealed that 3-fucosyllactose [$\log_2$(FC)=−0.4126], lactodifucotetraose [$\log_2$(FC)=−1.1109] and lacto-N-difucopentaose II [$\log_2$(FC)=−1.0982] had lower abundance in CS vs. VB, while lacto-N-tetraose [$\log_2$(FC)=0.3953], 3′-sialyllactose [$\log_2$(FC)=0.6455], and lacto-N-biose I [$\log_2$(FC)=0.3939] exhibited higher abundance (Table 1). Previous studies demonstrated that the levels of 3′-sialyllactose, 2′-fucosyllactose, and 6′-sialyllactose in HM after CS were lower than those after VB [28]. The concentration of nonfucosylated HMOs in the HM of mothers who delivered via VB was lower than that in the HM of mothers who delivered via CS [29]. 3-Fucosyllactose significantly influenced gut microbiota growth, exhibited antimicrobial properties, modulated immune function, offered antiviral defense, and contributed to brain development [30]. Lactodifucotetraose, found in both HM and infant urine, has been implicated in alleviating platelet function and inflammatory cytokine release [31]. Lacto-N-tetraose was extensively demonstrated to have biological functions in modulating the composition and activity of the microbiota, preventing the adhesion of pathogens, and directly stimulating epithelial or immune

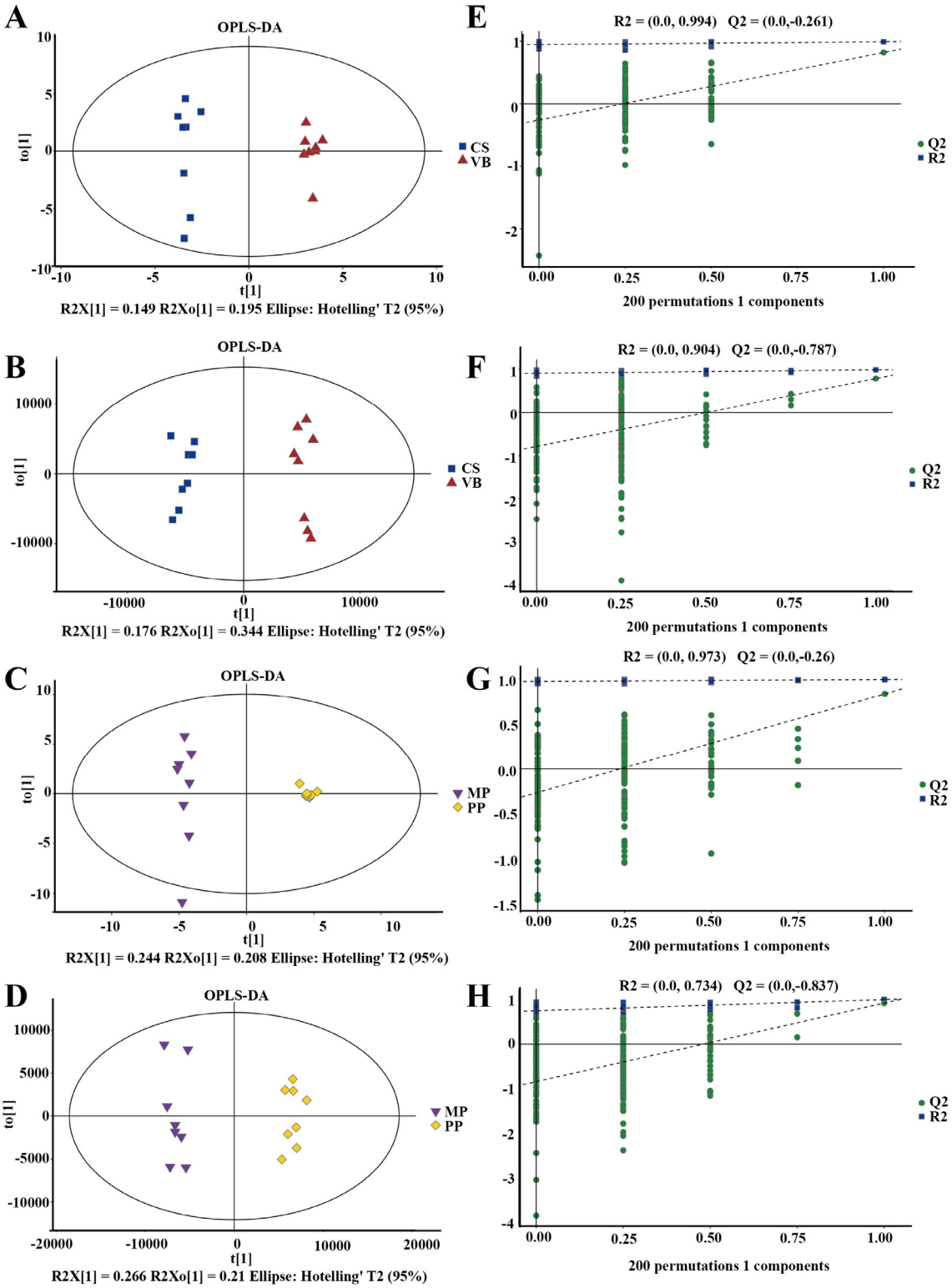

Fig. 1. Orthogonal projections to latent structures-discriminate analysis (OPLS-DA) score plot (A-D), corresponding validation plot of OPLS-DA (E-H) of metabolites in mature human milk. Blue dot, cesarean section; red dot, vaginal birth; purple dot, multiparous; yellow dot, primiparous. (A, C, E, G) represent the results of GC-MS; (B, D, F, H) represent the results of LC-MS.

Table 1
Identification of differentially expressed metabolites (DEMs) in mature human milk (MHM) from cesarean section (CS) and vaginal birth (VB) mothers.

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	$\log_2$(FC)
GC	23.804	-	Mg (16:0/0:0/0:0)	Unclassified	2.4806	.0035	2.2202
GC	19.338	-	Gluconic acid	Organic oxygen compounds	2.1189	.01318	2.2111
GC	19.721	-	Palmitelaidic acid	Lipids and lipid-like molecules	2.8274	.00022	1.9104
GC	23.769	-	Docosahexaenoic acid	Lipids and lipid-like molecules	2.2537	.00302	1.8521
GC	17.565	-	9-Tetradecenoic acid	Unclassified	2.8987	.00042	1.7507
GC	24.834	-	Mg (18:0/0:0/0:0)	Unclassified	2.3386	.00014	1.6461
GC	19.783	-	Cis-9-Palmitoleic acid	Unclassified	2.1190	.00034	1.4436
GC	23.618	-	Mg (0:0/16:0/0:0)	Lipids and lipid-like molecules	1.8096	.02175	1.3960
LC	0.669966667	pos	L-Aspoxicillin trihydrate	Organic acids and derivatives	5.9631	.00067	1.3600
LC	14.31251667	neg	S-Japonin	Lipids and lipid-like molecules	4.0889	.03697	1.3045
GC	22.606	-	Arachidonic acid	Lipids and lipid-like molecules	2.3578	.00876	1.2556
LC	7.557616667	pos	PE (20:3(5Z,8Z,11Z)/PGE1)	Unclassified	1.3001	.00479	1.2355
GC	24.48	-	Turanose	Lipids and lipid-like molecules	2.4177	.01311	1.2256
LC	5.128916667	pos	ascr#11	Lipids and lipid-like molecules	1.0011	.00082	1.2207
GC	18.901	-	Galactaric Acid	Organic oxygen compounds	1.7772	.012	1.0988
LC	0.7615	pos	N-(1-Deoxy-1-fructosyl) serine	Organic acids and derivatives	1.1916	.03694	1.0047
GC	8.461	-	Ketoleucine	Organic acids and derivatives	1.8757	.00231	0.9813
GC	21.474	-	Linoleic acid	Lipids and lipid-like molecules	1.5241	.01156	0.9750
GC	22.697	-	Glycerol 1-Myristate	Unclassified	1.5275	.00469	0.9735
LC	0.983466667	pos	Vidarabine	Nucleosides, nucleotides, and analogues	1.3364	.02728	0.9668
GC	17.687	-	Myristic acid	Lipids and lipid-like molecules	1.6539	.00091	0.9548
GC	13.226	-	L-Aspartic acid	Organic acids and derivatives	1.6607	.01124	0.9494
GC	21.502	-	Oleic acid	Lipids and lipid-like molecules	1.5348	.00463	0.9222
GC	15.12	-	Lauric acid	Lipids and lipid-like molecules	1.5268	.00238	0.8972
LC	6.551433333	pos	TG (16:1(9Z)/14:0/22:5(7Z, 10Z,13Z,16Z,19Z))	Lipids and lipid-like molecules	1.7669	.0003	0.8962
LC	0.88645	pos	9-Hydroxy-risperidone	Organoheterocyclic compounds	1.4695	5.1E-05	0.8942
LC	14.30571667	pos	Petroselinic acid	Lipids and lipid-like molecules	1.2043	.03819	0.8671
GC	10.639	-	Fumaric acid	Organic acids and derivatives	1.6589	3.5E-05	0.8401
LC	0.619683333	pos	2-Feruloyl-1,2′-disinapoylgentiobiose	Phenylpropanoids and polyketides	1.1662	.01454	0.8210
GC	12.748	-	Malic acid	Organic acids and derivatives	1.4835	.0017	0.8204
LC	12.74593333	pos	Octaethyleneglycol monododecyl ether	Organic oxygen compounds	1.0174	.02434	0.7836
GC	6.2	-	Caproic acid	Lipids and lipid-like molecules	1.1961	.01021	0.7652
GC	17.549	-	D-Fucitol	Unclassified	1.4790	.00299	0.7324
LC	0.636766667	pos	TG (22:5(4Z,7Z,10Z,13Z,16Z)/ 15:0/O-18:0)	Lipids and lipid-like molecules	2.3851	.00969	0.7302
GC	22.868		Cis-Gondoic acid	Lipids and lipid-like molecules	1.2189	.04023	0.7287
LC	0.686716667	pos	a-L-Arabinofuranosyl-(1->3)-b-D-xylopyranosyl-(1->4)-D-xylose	Organic oxygen compounds	11.0369	.00017	0.7193
GC	24.585	-	Lactobionic acid	Unclassified	1.3815	.02026	0.7123
LC	12.76373333	pos	Heptaethylene glycol monododecyl ether	Organic oxygen compounds	1.0477	.02358	0.6931
GC	20.637	-	D-Ribulose 5-Phosphate	Organic oxygen compounds	1.0868	.04509	0.6777
LC	14.06135	pos	PE (17:0/18:0)	Lipids and lipid-like molecules	4.2834	.03452	0.6755
LC	13.54106667	pos	Mangiferic acid	Lipids and lipid-like molecules	1.6708	.02641	0.6673
LC	0.686716667	pos	3′-Sialyllactose	Organic oxygen compounds	6.3040	.04995	0.6455

(*continued on next page*)

Table 1 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	$\log_2$(FC)
GC	19.129	-	Beta-D-Glucopyranuronic acid	Organic oxygen compounds	1.4068	.00151	0.6442
LC	13.52355	pos	6-Amino-4-(4-phenoxyphenylethylamino)quinazoline	Benzenoids	1.0752	.02866	0.6418
LC	12.4441	pos	LysoPC (18:0/0:0)	Lipids and lipid-like molecules	1.1764	.00196	0.6358
LC	0.619683333	pos	2-trans-7,10,13,16,19-all-cis-Docosahexaenoyl-CoA	Lipids and lipid-like molecules	1.6023	.01895	0.6330
LC	13.52355	pos	3-(4-(2-Dimethylamino-1-methylethoxy)phenyl)-1H-pyrazolo(3,4-b)pyridine-1-acetic acid	Organoheterocyclic compounds	1.6353	.02675	0.6316
LC	14.30571667	pos	Atevirdine	Organoheterocyclic compounds	1.3202	.01462	0.6305
LC	0.90185	pos	Imidazolepropionic acid	Organoheterocyclic compounds	2.0721	6.4E-05	0.5796
GC	19.68		Myo-Inositol	Organic oxygen compounds	1.3416	4.2E-05	0.5460
LC	1.2019	pos	n-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide	Organoheterocyclic compounds	1.8477	.02521	0.5439
LC	0.627433333	neg	Nuciferoic acid	Lipids and lipid-like molecules	1.8461	.01177	0.5382
LC	0.653416667	pos	Isovitexin 2''-(6'''-(E)-p-coumaroylglucoside)	Phenylpropanoids and polyketides	13.8232	.03832	0.5230
GC	25.114	-	Adenosine Monophosphate	Unclassified	1.0942	.01351	0.5160
GC	24.906	-	Coniferin	Organic oxygen compounds	1.2712	.00099	0.5138
GC	21.175	-	N-Acetylgalactosamine	Organic oxygen compounds	1.0426	.0178	0.5120
GC	15.358	-	L-Lyxulose		1.2505	.00016	0.5045
LC	5.696766667	pos	Hexanoylcarnitine	Lipids and lipid-like molecules	1.1026	.02617	0.5015
LC	0.73815	neg	Setipiprant	Benzenoids	3.3643	.00054	0.4785
GC	18.556		Sorbitol	Organic oxygen compounds	1.2514	.00024	0.4702
LC	0.636766667	pos	Quercetin 7-methyl ether 3-alpha-L-arabinopyranosyl-(1->3)-[galactosyl-(1->6)-galactoside]	Lipids and lipid-like molecules	1.4384	.03482	0.4628
LC	13.20436667	pos	Palmitic amide	Lipids and lipid-like molecules	1.9909	.03648	0.4489
LC	12.97471667	pos	9E,15E,19Z-docosatrienoic acid	Lipids and lipid-like molecules	1.0517	.04334	0.4423
GC	18.389	-	Allose	Organic oxygen compounds	1.1391	.00131	0.4302
GC	18.389	-	Glucose	Organic oxygen compounds	1.1389	.00132	0.4301
GC	17.833	-	D-Fructose	Organic oxygen compounds	1.1915	5.8E-06	0.4158
LC	13.46973333	pos	3E,13Z-Octadecadienal	Lipids and lipid-like molecules	6.3870	.03652	0.4037
LC	11.94818333	pos	12,13-EpOME	Lipids and lipid-like molecules	1.3377	.01554	0.3976
LC	3.1252	pos	Isobutyryl-L-carnitine	Lipids and lipid-like molecules	2.6783	.01874	0.3968
LC	4.386883333	pos	Pentaethylene glycol	Organic oxygen compounds	1.0048	.03722	0.3959
LC	0.669966667	pos	Lacto-N-tetraose	Organic oxygen compounds	10.4116	.02499	0.3953
LC	0.659866667	neg	(a-D-mannosyl)2-b-D-mannosyl-N-acetylglucosamine	Organic oxygen compounds	6.0310	.01701	0.3952
LC	0.669966667	pos	Lacto-N-biose I	Organic oxygen compounds	8.1164	.01108	0.3939
LC	14.25311667	pos	N-Stearoyl Valine	Organic acids and derivatives	1.4486	.03969	0.3757
LC	0.807316667	pos	9–hydroxy-7E-Nonene-3,5-diynoic acid	Lipids and lipid-like molecules	1.5538	.02182	0.3653
LC	0.78505	neg	Pseudouridine	Nucleosides, nucleotides, and analogues	2.5299	.03776	0.3591

(*continued on next page*)

Table 1 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log_2(FC)
LC	0.732066667	pos	Trigonelline	Alkaloids and derivatives	1.2635	.01883	0.3587
GC	20.715	-	Beta-N-Acetylglucosamine	Unclassified	1.0105	.00554	0.3546
LC	14.09605	pos	9-tridecynoic acid	Lipids and lipid-like molecules	1.2394	.02233	0.3514
LC	0.717216667	pos	2-Deoxy-2,3-dehydro-n-acetyl-neuraminic acid	Organic acids and derivatives	2.0378	.04114	0.3485
LC	1.970066667	pos	Cinnamic acid	Phenylpropanoids and polyketides	3.0707	.02339	0.3466
LC	0.999866667	pos	2-Hydroxycinnamic acid	Phenylpropanoids and polyketides	1.8323	.04803	0.3339
LC	10.71973333	pos	9-OxoODE	Lipids and lipid-like molecules	1.5730	.04458	0.3324
LC	0.653416667	pos	Galactotriose	Organic oxygen compounds	1.6874	.03451	0.3278
LC	0.7615	pos	Pyridoxal	Organoheterocyclic compounds	1.5216	.03746	0.3078
LC	0.732066667	pos	N-Acetylmannosamine	Organic oxygen compounds	3.6793	.03881	0.2981
LC	0.732066667	pos	Aminoadipic acid	Organic acids and derivatives	1.5209	.03735	0.2838
LC	0.732066667	pos	pyramid	Organoheterocyclic compounds	1.3803	.04977	0.2644
LC	0.659866667	neg	Hygromycin A	Organic oxygen compounds	2.5567	.03786	0.2518
GC	12.714	-	(E)-2-Methylglutaconic acid	Lipids and lipid-like molecules	1.0813	.00046	−0.3627
GC	7.234	-	3-Cyclohexene-1-Methanol	Unclassified	1.1879	.00018	−0.4037
LC	0.72345	neg	3-Fucosyllactose	Organic oxygen compounds	15.5819	.00154	−0.4126
GC	14.371	-	Hydroxyhydroquinone	Unclassified	1.1134	.02731	−0.4147
GC	11.561	-	2-Aminoethyl Methacrylate	Unclassified	1.0142	.03389	−0.4149
GC	10.881	-	Ethylmethylmalonic acid	Unclassified	1.1478	.00358	−0.4231
GC	8.321	-	Methylmalonic acid	Organic acids and derivatives	1.0171	.02968	−0.4272
GC	25.127	-	Inosinic acid	Unclassified	1.0474	.0208	−0.4345
GC	11.549	-	2-(Methylthio)Phenol	Organosulfur compounds	1.2355	.00216	−0.4623
GC	6.849	-	Sarcosine	Organic acids and derivatives	1.0927	.01261	−0.4708
GC	11.361	-	Erythrofuranose	Unclassified	1.2985	.04726	−0.5244
LC	0.708583333	neg	Phloretin 2′-O-(6′′-O-acetylglucoside)	Lipids and lipid-like molecules	14.7089	.00016	−0.5541
LC	4.989083333	pos	3 Hydroxycoumarin	Phenylpropanoids and polyketides	1.1235	.00363	−0.5716
LC	0.708583333	neg	Desaminosulfamethazine	Benzenoids	8.7229	5.9E-05	−0.5799
GC	10.021	-	4-Cyclohexene-1,2-Diol	Unclassified	1.4074	.00316	−0.5988
GC	5.546	-	1,4-Dioxan-2-Ol	Unclassified	1.2005	.01297	−0.6198
GC	7.69	-	2r-Amino-Butanoic acid	Unclassified	1.1920	.02442	−0.6803
LC	1.35185	pos	Tyrosylglycine	Organic acids and derivatives	1.1518	.0055	−0.6858
GC	9.176	-	Ethanolamine	Organic nitrogen compounds	1.4982	.00073	−0.7092
GC	26.481	-	Pulchelloside I	Unclassified	1.6567	.03512	−0.8175
LC	7.808366667	pos	2-oxoglutaryl-CoA	Lipids and lipid-like molecules	1.5977	.01945	−0.8255
LC	0.636766667	pos	Spinacetin 3-(2′'-apiosylgentiobioside)	Phenylpropanoids and polyketides	1.2114	.00538	−0.8290
LC	4.975116667	neg	Hippuric acid	Benzenoids	1.8197	.01753	−0.8900
LC	0.669966667	pos	Lewis y Tetrasaccharide	Organic oxygen compounds	8.9612	.00017	−0.9468
LC	0.644466667	neg	Lacto-N-difucopentaose II	Organic oxygen compounds	9.2801	1.6E-05	−1.0982
LC	0.73815	neg	Lactodifucotetraose	Lipids and lipid-like molecules	6.7433	.00029	−1.1109
LC	2.95715	pos	Xamoterol	Unclassified	1.7963	.00478	−1.1552
GC	11.453	-	Hydroquinone	Benzenoids	3.1027	.00159	−1.6427
LC	2.822783333	pos	3,5,7,9,11-dodecapentaenoic acid	Lipids and lipid-like molecules	1.1394	.042	−1.8890
GC	12.931	-	Erythritol	Organic oxygen compounds	2.3681	.02903	−3.6391

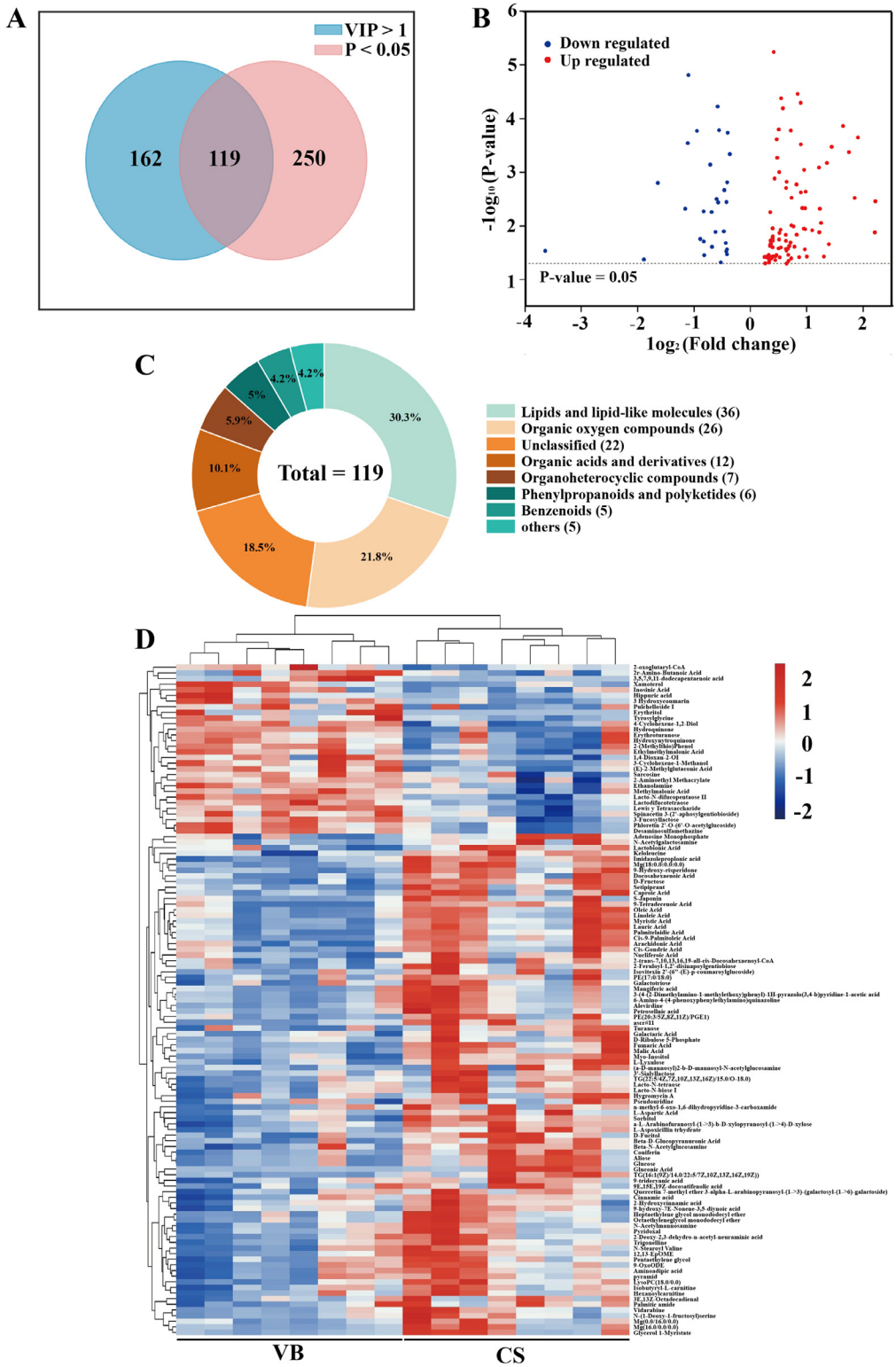

Fig. 2. Bioinformatics analysis of differential metabolites in different delivery mode groups. (A) Venn diagram of variable importance in the projection (VIP) and *P*-value results of the metabolites in mature human milk; (B) Volcano plot of DEMs screened by VIP and *P*-value results; (C) Heatmap analysis of DEMs; VB represents vaginal birth group; CS represent cesarean section group; (D) The superclass of differentially expressed metabolites (DEMs) between cesarean section and vaginal birth mothers.

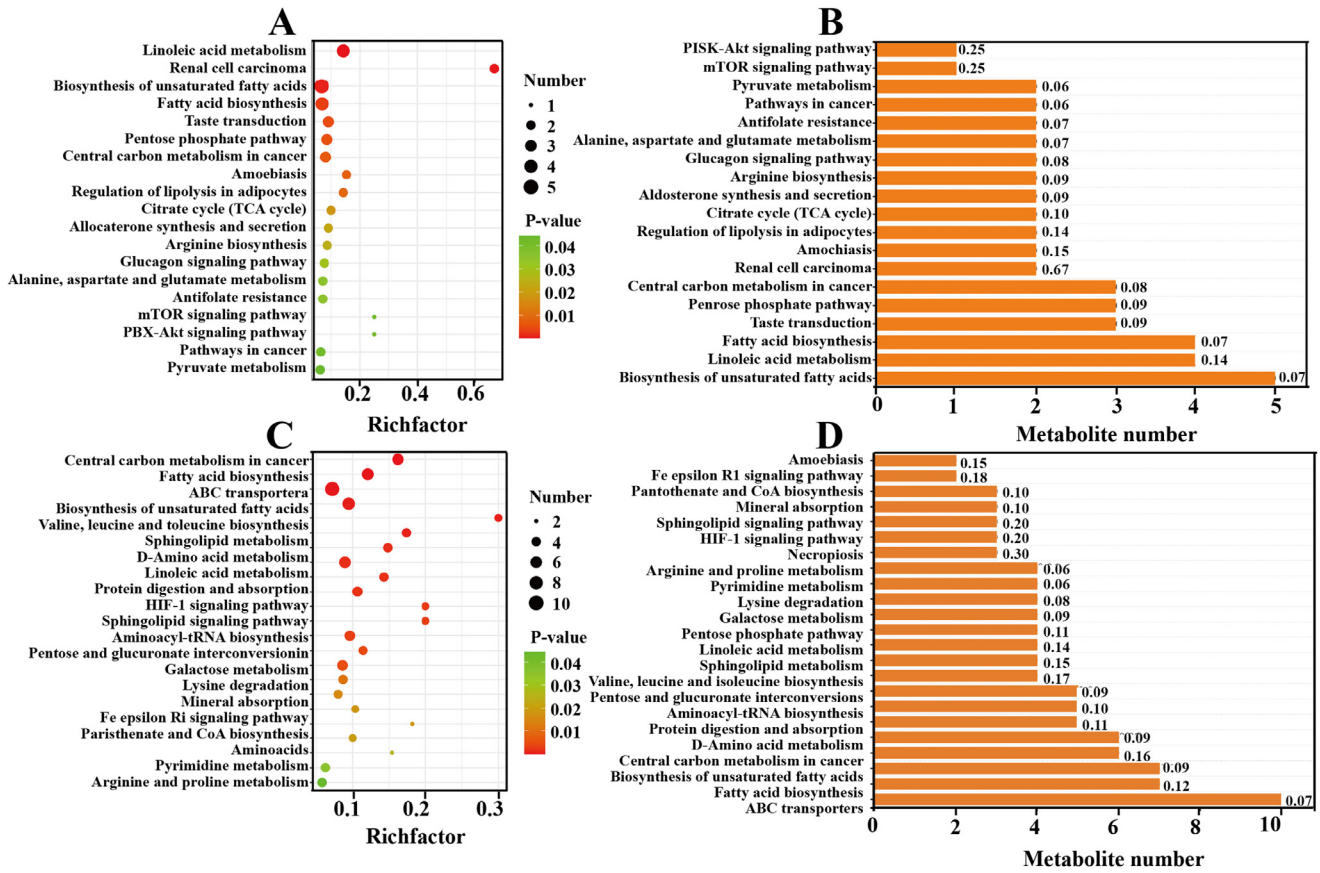

Fig. 3. (A) Metabolomic view map of the 119 significantly different metabolites of biosynthetic pathways between different delivery mode groups. The x-axis represents the pathway effect, and the y-axis represents the pathway name. Large sizes and dark colors represent the major pathway enrichment and high pathway effect values, respectively. (B) Histogram of KEGG pathway between different delivery mode groups. The x-axis represents the number of metabolites, the y-axis indicates the pathway names, and the numbers on the bars represent the enrichment factors. (C) Metabolomic view map of the 284 significantly different metabolites of biosynthetic pathways between different parity groups. (D) Histogram of KEGG pathway between different parity groups.

cells [32]. As previously mentioned, 3′-sialyllactose was able to improve learning as well as memory performance and to affect the gut microbiota-brain axis [33]. Lacto-N-biose I is beneficial for promoting the growth of bifidobacteria, modulating immune response and ameliorating allergies [34].

KEGG analysis was used to analyze the metabolic pathways of the DEMs. DEMs identified in MHM of mothers with different delivery modes were found to be significantly enriched in 19 differential metabolic pathways ($P<.05$) (Fig. 3A-B). Three of the most significant metabolic pathways are biosynthesis of unsaturated fatty acids [5 DEMs: arachidonic acid, oleic acid, linoleic acid, docosahexaenoic acid (DHA), and cis-gondoic acid]; fatty acid biosynthesis (4 DEMs: DHA, lauric acid, myristic acid, and palmitelaidic acid); and linoleic acid metabolism (4 DEMs: arachidonic acid, oleic acid, 9-OxoODE, and 12,13-EpOME) (Fig. 4). Biosynthesis of some of the fatty acids such as DHA, lauric acid, myristic acid, and palmitelaidic acid involves malonyl-(acyl-carrier protein), a key metabolite in the fatty acid pathway that provides two carbon units for the growing fatty acid chain during each step of biosynthesis (Fig. 4). In this study, higher abundances of palmitelaidic acid [$\log_2$(FC)=1.9104], DHA [$\log_2$(FC)=1.8521], arachidonic acid [$\log_2$(FC)=1.2556], linoleic acid [$\log_2$(FC)=0.9750], myristic acid [$\log_2$(FC)=0.9548], oleic acid [$\log_2$(FC)=0.9222], lauric acid [$\log_2$(FC)=0.8972], cis-gondoic acid [$\log_2$(FC)=0.7287], 12,13-EpOME [$\log_2$(FC)=0.3976], and 9-OxoODE [$\log_2$(FC)=0.3324] were observed in the HM of CS mothers. This finding aligns with a previous study reporting elevated levels of specific lipids, such as lauric acid, pentadecanoic acid, palmitic acid, margaric acid, stearic acid, elaidic acid, and erucic acid, in HM from CS mothers [35]. Previous studies have shown that the delivery mode affects the fatty acid composition of HM. CS mothers' milk exhibited higher omega-6 (n-6) PUFAs, arachidonic acid/DHA ratios, and n-6/n-3 ratios [36]. DHA levels are higher in HM from VB women compared to CS women [37]. This may be due to differing levels of cortisol and oxytocin between mothers with different delivery modes. Both corticol and oxytocin have been reported to have effects on biosynthesis of fatty acids [38]. As shown in Table S1, the CS group (male/female infants = 2/6) had more female infants than the VB group (male/female infants = 6/2). Besides delivery mode, HM fatty acid composition also varies by infant sex. Male infants' HM has higher fat content [39], while female infants' HM contains more eicosadienoic acid and a higher ARA/DHA ratio, potentially reducing food sensitization and atopic dermatitis risks [40,41].

Fatty acids including arachidonic acid, DHA, linoleic acid, and alpha-linolenic acid are important to meet the growth and metabolic needs of infants especially in their first six months of life [42]. Recommended levels of these acids have been established in many countries, such as China and Europe [43]. Infant formulas fortified with DHA and arachidonic acid have numerous benefits for infant growth, including cognitive performance, visual capacity, and immune function, bringing formula feeding closer to breastfeeding [44]. As precursors to DHA and arachidonic acid, alpha-linolenic acid and linoleic acid are classically regarded as essential fatty acids. They cannot be synthesized by mammalian body and

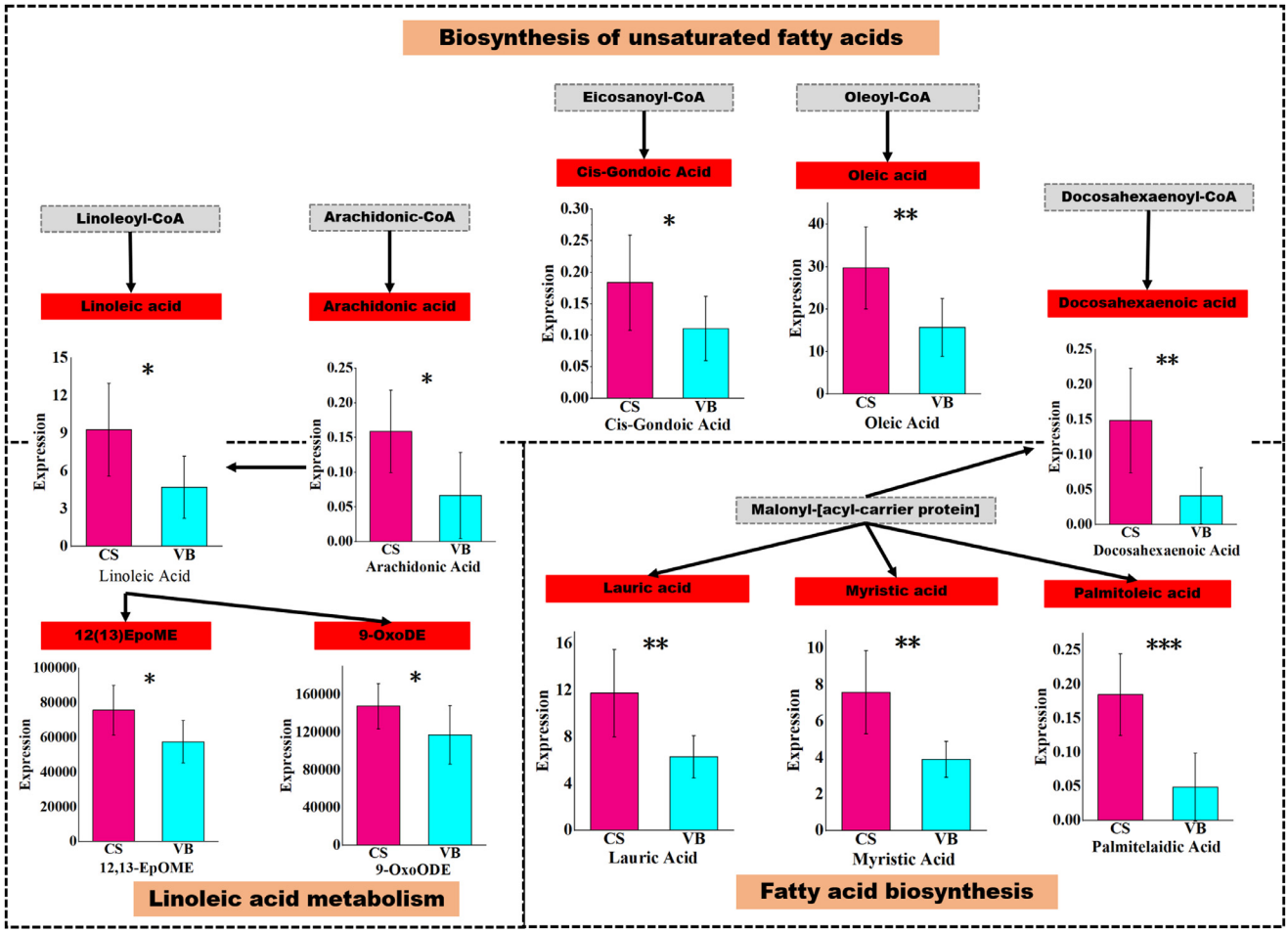

Fig. 4. Hypothesized scheme pathway most related to mature human milk metabolite changes between different delivery mode groups. The y-axis represents the relative abundance of metabolites. Error bars indicate SEM. *means $P<.05$, **means $P<.01$, ***means $P<.001$.

need to be obtained through food, etc. [45]. Both 9-OxoODE and 12,13-EpOME are oxygenated metabolites of linoleic acid which are important components of biomembrane and immunomodulatory compounds [46]. These fatty acids are modulated by dietary intake and their concentrations in MHM vary depending on dietary habits among mothers [42]. Supplementation with multiple micronutrients, lutein, and DHA during lactation increased maternal milk DHA by 30%, maternal blood DHA by 17%, and eicosapentaenoic acid (EPA) by 4% [47]. Dietary consumption of total PUFAs, EPA, and DHA showed a positive correlation with the levels of PUFAs, n-6 PUFAs, and linoleic acid in HM, while the intake of n-3 PUFAs and alpha-linolenic acid was inversely related to the levels of saturated fatty acids in HM [48]. The major fatty acids (palmitic acid, oleic acid, linoleic acid, and alpha-linolenic acid) and PUFA ratios (linoleic acid/alpha-linolenic acid and n-6/n-3) were significantly affected by maternal edible oils [49]. Rapeseed oil consumers showed the lowest linoleic acid (~19%) and highest alpha-linolenic acid (~1.9%) in HM [49]. Milk lipids originate from dietary intake, body storage, and de novo synthesis. Fatty acids are primarily stored in adipose tissue before being released into the bloodstream and secreted into HM [50]. Diet may influence metabolic outcomes by providing the necessary material foundation or affecting relevant metabolic pathways. Our results showed that delivery mode has a significant effect on lipids and lipid-like molecules which may lead to differences in physiological development in newborns. Maternal n-3 fatty acids deficiency from prepregnancy through lactation significantly affects gene expression related to offspring hippocampal neurogenesis [51]. Further research is necessary to explore the underlying mechanisms of how birth mode affects the constitutions of HM and the possible effects on infants' health.

3.2.2. *DEMs in MHM of mothers with different parity*

A total of 284 DEMs were detected in MHM of mothers with different parities using both GC-MS (83 DEMs) and LC-MS (177 DEMs in positive ion mode, 24 DEMs in negative ion mode) (Fig. 5A and Table 2). 103 DEMs were upregulated and the remaining 181 were downregulated in MHM of multiparous mothers as compared to those of primiparous mothers (Fig. 5B). Hierarchical clustering shows clear separation in terms of metabolites of mothers with different parities (Fig. 5D). These DEMs can be categorized as lipids and lipid-like molecules (86 DEMs), organic oxygen compounds (44 DEMs), organic acids and derivatives (37 DEMs), organoheterocyclic compounds (34 DEMs), benzenoids (11 DEMs), and nucleosides, nucleotides and their analogs (7 DEMs) (Fig. 5C).

Among these DEMs, we also identified several important HMOs, such as 3-fucosyllactose and 3′-sialyllactose, which exhibited lower abundances in MP mothers compared to PP mothers. Consistent with these findings, the Finnish birth cohort reported higher levels of 3-fucosyllactose and 3′-sialyllactose in PP mothers, while lacto-N-tetraose levels were lower [52]. Similarly, another study demonstrated that MP women had higher

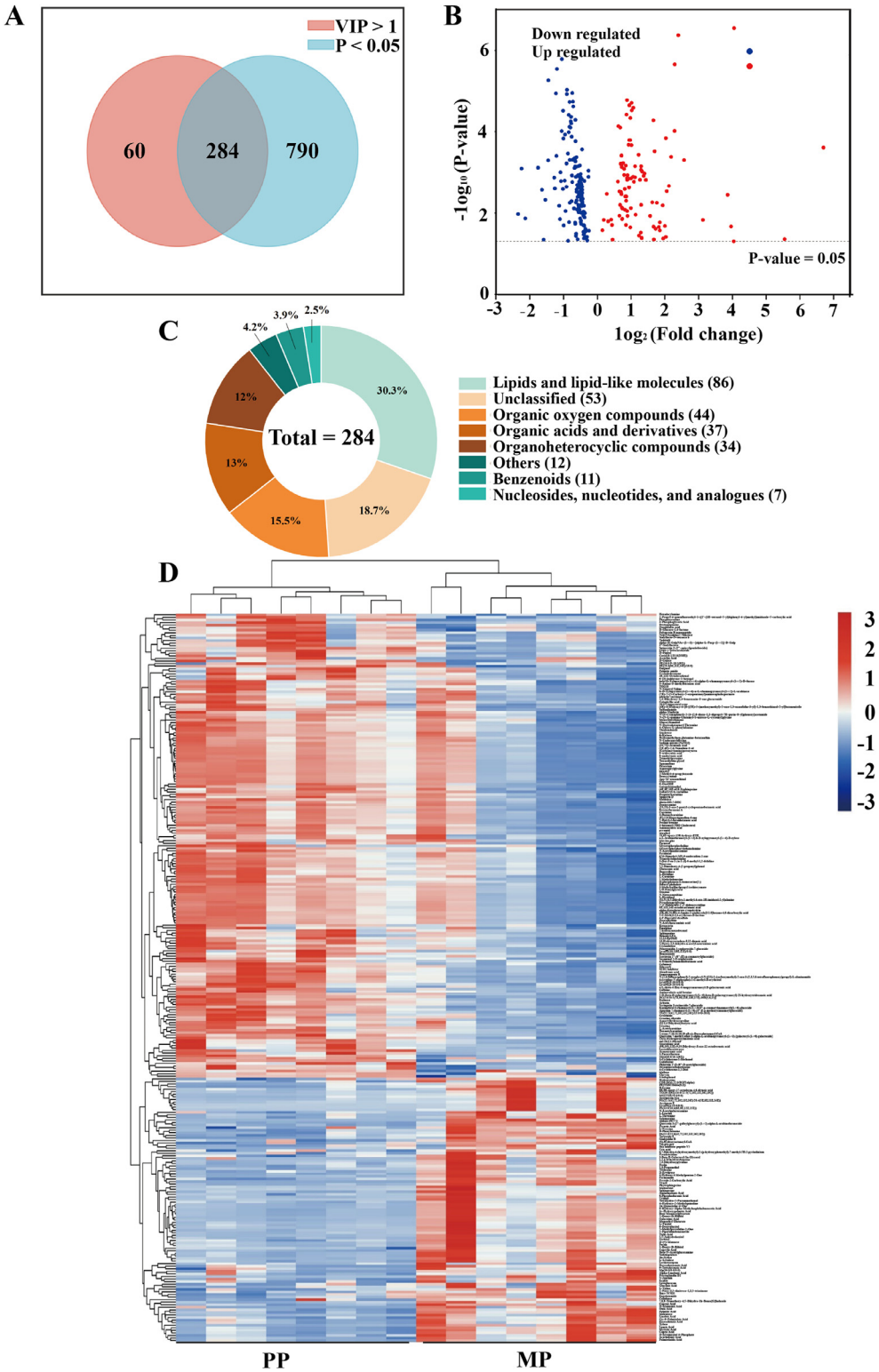

Fig. 5. Bioinformatics analysis of differential metabolites in different parity groups. (A) Venn diagram of variable importance in the projection (VIP) and *P*-value results of the metabolites in mature human milk; (B) Volcano plot of screened by VIP and *P*-value results; (C) Heat map analysis of DEMs; PP represent primiparous group; MP represent multiparous group; (D) The superclass of DEMs between multiparous and primiparous mothers.

Table 2
Identification of differentially expressed metabolites (DEM) in mature human milk (MHM) from multiparous (MP) and primiparous (PP) mothers.

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log2(FC)
LC	6.9461	pos	Solamargine	Organoheterocyclic compounds	1.0838	.00025	6.7036
LC	6.90958	pos	Arctignan E	Organoheterocyclic compounds	1.5640	.04421	5.5477
GC	9.598	-	Pyrrole-2-Carboxylic acid	Organoheterocyclic compounds	2.9209	2.8E-07	4.0527
LC	5.55375	pos	LysoPA (a-13:0/0:0)	Lipids and lipid-like molecules	1.1815	.04989	4.0418
LC	5.55375	pos	PS (13:0/18:4(6Z,9Z,12Z,15Z))	Lipids and lipid-like molecules	1.0058	.02163	3.9606
GC	24.709	-	Prostaglandin D2	Unclassified	1.4076	.00362	3.8628
LC	5.17757	neg	Avermectin A1a	Lipids and lipid-like molecules	1.1428	.01492	3.1255
GC	19.184	-	D-Mannonic acid	Unclassified	2.2714	.0005	2.5717
LC	5.74988	pos	Abbott-195773	Organic oxygen compounds	1.3276	4.2E-07	2.4002
LC	5.74988	pos	Quercetin 3-(2''-galloylglucosyl)-(1->2)-alpha-L-arabinofuranoside	Lipids and lipid-like molecules	1.1428	2.2E-06	2.2965
LC	6.17667	pos	Torvoside E	Lipids and lipid-like molecules	1.1196	9.7E-05	2.2896
GC	22.735	-	D-Myoinositol 4-Phosphate	Unclassified	2.2047	.00042	2.1885
GC	24.778	-	Melezitose	Organic oxygen compounds	1.7259	.00219	2.1203
GC	23.769	-	Docosahexaenoic acid	Lipids and lipid-like molecules	2.4563	.00294	2.0617
GC	21.855	-	3-Beta-D-Galactosyl-Sn-Glycerol	Unclassified	1.5053	.03895	2.0325
LC	5.71465	pos	PE(TXB2/DiMe(9,3))	Unclassified	1.5177	.00014	2.0308
LC	5.18153	pos	MG(TXB2/0:0/0:0)	Unclassified	1.9757	.02107	2.0034
LC	5.76712	pos	PG(22:5(4Z,7Z,10Z,13Z,16Z)/20:4(5Z,8Z,11Z,14Z))	Lipids and lipid-like molecules	2.0802	.04244	1.9549
LC	4.99337	neg	Bax inhibitor peptide V5	Phenylpropanoids and polyketides	1.3423	.00658	1.9421
LC	6.55143	pos	TG(16:1(9Z)/14:0/22:5(7Z,10Z,13Z,16Z,19Z))	Lipids and lipid-like molecules	3.2777	.01714	1.8547
GC	24.469	-	Lormetazepam	Unclassified	1.2078	.02708	1.8534
LC	4.48928	pos	8R,9R-epoxy-17-octadecen-4,6-diynoic acid	Lipids and lipid-like molecules	1.1623	.02236	1.8249
LC	6.26535	pos	CDP-DG(i-22:0/PGF1alpha)	Unclassified	3.3435	.00565	1.7650
GC	23.691	-	Phytosphingosine	Organic nitrogen compounds	1.5415	.02456	1.7278
GC	22.606	-	Arachidonic acid	Lipids and lipid-like molecules	1.6982	.00031	1.6983
GC	23.707	-	Maltotriitol	Unclassified	1.4822	.0449	1.6784
GC	23.137	-	Uridine	Nucleosides, nucleotides, and analogues	1.3620	.02182	1.6646
GC	15.078	-	Xylose	Unclassified	1.7445	5.2E-05	1.6601
GC	22.811	-	6-Phosphogluconic acid	Organic oxygen compounds	1.5431	.00555	1.6304
GC	23.804	-	Mg(16:0/0:0/0:0)	Unclassified	1.8574	.01193	1.5645
GC	15.368	-	Dl-Xylose	Unclassified	1.5364	.00154	1.4430
GC	19.721	-	Palmitelaidic acid	Lipids and lipid-like molecules	1.5316	.00107	1.3989
GC	19.783	-	Cis-9-Palmitoleic acid	Unclassified	1.5469	.00122	1.3734
GC	20.715	-	Beta-N-Acetylglucosamine	Unclassified	1.4848	.00096	1.3296
GC	23.918	-	Cellobiose	Organic oxygen compounds	2.3349	.04142	1.3153
GC	22.813	-	Sphingosine	Unclassified	1.5074	.00071	1.2991
GC	7.554	-	L-Leucine	Organic acids and derivatives	1.3925	.00637	1.2980
GC	20.715	-	Sedoheptulose	Organic oxygen compounds	1.4673	.00087	1.2949
GC	15.856	-	Levoglucosan	Organoheterocyclic compounds	1.5052	.0304	1.2882
GC	22.398	-	7,8,9-Trimethoxy-4,5-Dihydro-1h-Benzo[G]Indazole	Unclassified	1.4269	.00152	1.2668
GC	19.338	-	Gluconic Acid	Organic oxygen compounds	1.1634	.03623	1.2343
GC	6.2	-	Caproic Acid	Lipids and lipid-like molecules	1.4166	.00037	1.2280
LC	4.98908	pos	Val-pro-pro	Organic acids and derivatives	2.4642	.01115	1.1329

(*continued on next page*)

Table 2 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log2(FC)
GC	14.263	-	Prolin	Unclassified	1.4317	.00071	1.1196
GC	14.614	-	1-Deoxy-D-Ribitol	Organic oxygen compounds	1.3552	.00133	1.1191
GC	22.859	-	Eicosadienoic acid	Lipids and lipid-like molecules	1.2963	.00171	1.0885
LC	7.16218	pos	PA(15:0/22:6(4Z,7Z, 10Z,13Z,16Z,19Z))	Lipids and lipid-like molecules	2.5674	2.5E-05	1.0660
GC	27.462	-	Ginkgolide B	Lipids and lipid-like molecules	1.3818	.0013	1.0638
GC	18.297	-	L-Tyrosine	Organic acids and derivatives	1.4007	3E-05	1.0184
GC	15.12	-	Lauric acid	Lipids and lipid-like molecules	1.3727	1.9E-05	1.0159
GC	21.502	-	Oleic acid	Lipids and lipid-like molecules	1.3441	.00022	1.0130
GC	13.856	-	D-Phenylalanine	Unclassified	1.3555	.00021	1.0035
GC	9.76	-	L-Threonine	Organic acids and derivatives	1.2836	.00141	0.9997
LC	14.3125	neg	S-Japonin	Lipids and lipid-like molecules	2.8254	.0077	0.9977
GC	17.687	-	Myristic acid	Lipids and lipid-like molecules	1.3331	4.6E-05	0.9838
GC	21.54	-	Alpha-Linolenic acid	Unclassified	1.2603	.02261	0.9838
GC	10.496	-	Uracil	Organoheterocyclic compounds	1.3436	.00016	0.9775
GC	14.299	-	D-Erythrose	Unclassified	1.3651	2.2E-05	0.9552
GC	21.283	-	6-Hydroxy-Alpha-Methylnaphthaleneacetic acid	Unclassified	1.3362	.00016	0.9514
GC	7.366	-	5-Methylpyrrolidin-2-One	Unclassified	1.2517	.00237	0.9424
GC	5.068	-	Tiglic acid	Lipids and lipid-like molecules	1.2271	.00142	0.9362
GC	21.256	-	3r-Hydroxypalmitic acid	Unclassified	1.3145	.00021	0.9290
GC	21.252	-	Beta-Mannosylglycerate	Unclassified	1.3145	.00021	0.9290
GC	16.701	-	L-Fucitol	Unclassified	1.1352	.01184	0.8970
GC	21.474	-	Linoleic acid	Lipids and lipid-like molecules	1.2176	.00117	0.8970
GC	16.484	-	6-Deoxyglucitol	Unclassified	1.1287	.00903	0.8884
GC	6.499	-	L-Valine	Organic acids and derivatives	1.2299	.00153	0.8863
LC	7.70162	pos	(S)-Hydroxyoctanoyl-CoA	Lipids and lipid-like molecules	1.1138	.00138	0.8815
GC	19.981	-	Palmitic acid	Lipids and lipid-like molecules	1.2911	1.7E-05	0.8807
GC	12.306	-	Capric acid	Lipids and lipid-like molecules	1.2528	3.8E-05	0.8686
GC	11.162	-	Monoethyl Glutarate	Unclassified	1.1771	.00399	0.8602
GC	18.022	-	D-(+)-Mannose	Unclassified	1.1191	.00508	0.8579
GC	17.565	-	9-Tetradecenoic acid	Unclassified	1.1161	.01984	0.8525
GC	14.393	-	2-Deoxy-D-Ribitol	Unclassified	1.2204	.00068	0.8482
GC	13.501	-	1,2,3-Trihydroxybenzene	Benzenoids	1.1186	.00399	0.8332
GC	18.824	-	Pectin	Organic oxygen compounds	1.0806	.00845	0.8215
GC	6.263	-	Pyruvic acid	Organic acids and derivatives	1.1635	.0026	0.8149
GC	17.657	-	1,5-Anhydrohexitol	Unclassified	1.1468	.00083	0.7951
GC	15.358	-	L-Lyxulose	Unclassified	1.1258	.00116	0.7928
GC	14.21	-	4-Hydroxy-2-Methylquinoline	Unclassified	1.1618	.00059	0.7684
GC	10.082	-	2,3-Dihydroxypyridine	Unclassified	1.0470	.00787	0.7471
GC	21.175	-	N-Acetylgalactosamine	Organic oxygen compounds	1.0509	.01403	0.7437
LC	13.5451	neg	Ecabet	Lipids and lipid-like molecules	1.8553	.0143	0.7364
GC	18.556	-	Sorbitol	Organic oxygen compounds	1.0444	.00747	0.7347
GC	19.032	-	Galactonic acid	Organic acids and derivatives	1.1233	.00075	0.7292
GC	13.988	-	1h-Quinazolin-4-One	Unclassified	1.1258	.00065	0.7223
GC	12.498	-	Aminomalonic acid	Organic acids and derivatives	1.0792	.00351	0.7202
GC	5.388	-	4-Hydroxy-4-Methylpentan-2-One	Unclassified	1.1280	.00039	0.7175
GC	16.13	-	2-Piperidinobenzonitrile	Unclassified	1.0579	.0031	0.6987
GC	9.979	-	Tridecane	Unclassified	1.0873	.00062	0.6948
GC	9.247	-	Caprylic acid	Lipids and lipid-like molecules	1.1050	8E-05	0.6801
LC	14.1116	neg	Hydroxyzine	Benzenoids	1.6888	.00636	0.6759
GC	6.603	-	Tetrahydro-2-Furanmethanol	Unclassified	1.0171	.003	0.6576
LC	0.80178	neg	Enzalutamide	Organoheterocyclic compounds	2.0420	.01174	0.6576
GC	6.077	-	Formamide	Organic acids and derivatives	1.0822	7.4E-05	0.6253
GC	5.877	-	1,3-Propanediol	Unclassified	1.0024	.00169	0.6114
LC	11.0486	neg	6,7-Dihydro-4-(hydroxymethyl)-2-(p-hydroxyphenethyl)-7-methyl-5H-2-pyrindinium	Benzenoids	1.0095	.04544	0.4561

(*continued on next page*)

Table 2 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log2(FC)
LC	0.61968	pos	D-Lysine	Organic acids and derivatives	1.0104	.01975	0.4354
LC	0.64447	neg	3-Iodophenol	Unclassified	1.0754	.01471	0.4141
LC	0.78505	neg	Pseudouridine	Nucleosides, nucleotides, and analogues	2.2563	.01619	0.3227
LC	0.81708	neg	Bms-707035	Organoheterocyclic compounds	3.0076	.00342	0.2828
LC	0.76843	neg	Uric acid	Organoheterocyclic compounds	1.0116	.0149	0.1921
LC	0.6922	neg	1-Nitro-3,5-dinitroso-1,3,5-triazinane	Organoheterocyclic compounds	10.7919	.02672	0.1542
LC	0.78505	neg	Oxoglutaric acid	Organic acids and derivatives	9.0375	.0275	−0.2456
LC	0.70858	neg	Desaminosulfamethazine	Benzenoids	4.6828	.00091	−0.2576
LC	0.70858	neg	Phloretin 2′-O-(6′′-O-acetylglucoside)	Lipids and lipid-like molecules	8.1744	.00129	−0.2600
LC	0.83818	pos	(7R)-7-(5-Carboxy-5-oxopentanoyl) aminocephalosporinate	Organoheterocyclic compounds	1.6097	.04754	−0.2971
LC	0.7615	pos	beta-D-Xylopyranosyl-(1->4)-alpha-L-rhamnopyranosyl-(1->2)-D-fucose	Organic oxygen compounds	3.7261	.03848	−0.3081
LC	0.7615	pos	3-Amino-3-methylbutanoic acid	Organic acids and derivatives	1.3361	.04028	−0.3220
LC	0.74685	pos	alpha-Viniferin	Phenylpropanoids and polyketides	1.7010	.03885	−0.3354
LC	0.83818	pos	2,4-Dihydroxy-1,4-benzoxazin-3-one glucuronide	Organic oxygen compounds	1.7303	.0261	−0.3385
LC	0.83818	pos	adenylo-succinate	Nucleosides, nucleotides, and analogues	1.9166	.02889	−0.3443
LC	0.57318	pos	Monochlorobimane	Organoheterocyclic compounds	1.9575	.02478	−0.3499
LC	0.74685	pos	N-(4-Cyanophenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-7H-purin-8-yl)phenoxy]acetamide	Organoheterocyclic compounds	1.5283	.04116	−0.3577
LC	0.80732	pos	N-(N-L-gamma-Glutamyl-S-nitroso-L-cysteinyl)glycine	Organic acids and derivatives	1.2436	.02766	−0.3638
LC	0.73207	pos	N-Acetylmannosamine	Organic oxygen compounds	2.9888	.0102	−0.3656
LC	0.7615	pos	Pyridoxal	Organoheterocyclic compounds	1.1995	.01299	−0.3737
LC	0.72345	neg	3-Fucosyllactose	Organic oxygen compounds	11.3893	.00164	−0.3771
LC	10.4489	pos	Sphinganine	Organic nitrogen compounds	1.0144	.02851	−0.3827
LC	0.66997	pos	Ketanserin	Organic oxygen compounds	1.8119	.0319	−0.3838
LC	14.2531	pos	N-Stearoyl Valine	Organic acids and derivatives	1.0894	.021	−0.3854
LC	0.71722	pos	Calophyllic acid	Lipids and lipid-like molecules	2.3381	.04202	−0.3895
LC	0.68672	pos	a-L-Arabinofuranosyl-(1->3)-b-D-xylopyranosyl-(1->4)-D-xylose	Organic oxygen compounds	4.2169	.0343	−0.3976
LC	0.68672	pos	6-Kestose	Organic oxygen compounds	6.1371	.00675	−0.3981
LC	0.74685	pos	20:0 Campesterol ester	Lipids and lipid-like molecules	1.8845	.01991	−0.4023
LC	0.68672	pos	Stachyose	Organic oxygen compounds	6.6474	.01045	−0.4044
LC	0.80732	pos	Hydramethylnon	Unclassified	3.1664	.00933	−0.4061
LC	12.8514	pos	Farnesol	Lipids and lipid-like molecules	1.2117	.02938	−0.4076
LC	0.80732	pos	glutamine-betaxanthin	Organic acids and derivatives	3.0453	.01164	−0.4122
LC	0.70148	pos	Glucosylisomaltol	Organic oxygen compounds	1.7925	.00451	−0.4292
LC	0.73207	pos	Aminoadipic acid	Organic acids and derivatives	1.4565	.00573	−0.4424
LC	10.7742	pos	2,5-Dimethoxy-4-(2-propenyl)phenol	Benzenoids	1.0775	.00274	−0.4446
LC	11.0604	pos	Emulphor	Organic oxygen compounds	1.0635	.00671	−0.4458
LC	14.0614	pos	3-(but–3-en-1-yn-1-yl)-6-methyl-1,2-dithiine	Lipids and lipid-like molecules	2.4204	.00325	−0.4476
LC	14.3057	pos	2′,3′-Didehydro-2′,3′-dideoxycytidine	Nucleosides, nucleotides, and analogues	1.3447	.00518	−0.4479

(*continued on next page*)

Table 2 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log2(FC)
LC	0.6922	neg	2-Propyl-4-pentafluoroethyl-1-((2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl)imidazole-5-carboxylic acid	Benzenoids	4.7537	.00028	−0.4496
LC	0.70148	pos	Melibiose	Organic oxygen compounds	10.3135	.00527	−0.4515
LC	14.0961	pos	(1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid	Organic acids and derivatives	4.1076	.00229	−0.4516
LC	14.0961	pos	O-phosphonato-L-homoserine(2-)	Organic acids and derivatives	1.1537	.00147	−0.4544
LC	15.2074	pos	3-hexanoyl-NBD Cholesterol	Unclassified	7.5752	.00687	−0.4549
LC	0.73207	pos	pyramid	Organoheterocyclic compounds	1.4288	.00614	−0.4588
LC	0.74685	pos	Glutaconic acid	Organic acids and derivatives	2.9549	.00346	−0.4591
LC	13.2405	pos	8Z,11Z,14Z-octadecatrienoic acid	Lipids and lipid-like molecules	1.2240	.00387	−0.4598
LC	14.0961	pos	alpha-Ketoglutarate cyanohydrin	Organic acids and derivatives	4.7580	.00205	−0.4655
LC	14.3237	pos	Deoxycytidine	Nucleosides, nucleotides, and analogues	4.0935	.00347	−0.4663
LC	11.7881	pos	6,10-Dimethyl-5(E),9-undecadien-2-one	Lipids and lipid-like molecules	1.1295	.00294	−0.4665
LC	12.0368	pos	Dibutyl phthalate	Benzenoids	2.0053	.00395	−0.4685
LC	0.73207	pos	Deoxyribose	Organic oxygen compounds	2.5408	.00239	−0.4703
LC	15.4599	pos	Spinosyn D	Unclassified	1.5491	.00146	−0.4704
LC	14.3057	pos	Pyroglutamylglycine	Organic acids and derivatives	1.3363	.00259	−0.4713
LC	0.82227	pos	(4R)-4-Hydroxy-4-[6-[(5R)-5-(methoxymethyl)-2-oxo-1,3-oxazolidin-3-yl]−1,3-benzothiazol-2-yl]butanenitrile	Organoheterocyclic compounds	2.4928	.00448	−0.4719
LC	13.4517	pos	Nitarsone	Benzenoids	1.0237	.00225	−0.4738
LC	9.4266	pos	2-hydroxyhexadecanal	Lipids and lipid-like molecules	2.0295	.01	−0.4739
LC	0.71722	pos	N-Acetylneuraminic acid	Organic oxygen compounds	2.4251	.01866	−0.4741
LC	4.71167	pos	2-Methyl-4-propyloxazole	Organoheterocyclic compounds	1.1794	.00321	−0.4745
LC	0.74685	pos	(S)-3,4-Dihydroxybutyric acid	Organic acids and derivatives	1.3193	.00188	−0.4748
LC	14.3057	pos	Proxyphylline	Organoheterocyclic compounds	1.1059	.00215	−0.4752
LC	14.3057	pos	(S)-N-(4,5-Dihydro-1-methyl-4-oxo-1H-imidazol-2-yl)alanine	Organic acids and derivatives	2.0236	.00223	−0.4759
LC	12.7637	pos	4,4′-Dipyridyl disulfide	Organoheterocyclic compounds	3.5274	.00204	−0.4772
LC	0.66997	pos	Glycerylphosphoryleth-anolamine	Lipids and lipid-like molecules	1.0612	.01558	−0.4780
LC	0.7615	pos	N-Docosahexaenoyl Threonine	Unclassified	4.8987	.00348	−0.4797
LC	4.27028	pos	4-Chloro-L-phenylalanine	Organic acids and derivatives	5.0222	.00238	−0.4814
LC	14.745	pos	Trimethylolmelamine	Organoheterocyclic compounds	1.0517	.00149	−0.4818
LC	14.2361	pos	Erectusfuranone A	Lipids and lipid-like molecules	2.0477	.00396	−0.4820
LC	4.27028	pos	Thiabendazole	Organoheterocyclic compounds	2.9061	.00236	−0.4821
LC	0.74685	pos	2-C-Methyl-1,4-erythrono-D-lactone	Organoheterocyclic compounds	2.5054	.00234	−0.4822
LC	14.5329	pos	Sodium nitrate (NaNO3)	Mixed metal/nonmetal compounds	1.3897	.0049	−0.4829
LC	14.5329	pos	L-Histidinol	Organic nitrogen compounds	3.2633	.00192	−0.4832
LC	0.98103	neg	D-Glucaro-1,4-lactone	Organoheterocyclic compounds	1.7656	.01685	−0.4860
LC	14.3404	pos	3-Butenylcarnitine	Lipids and lipid-like molecules	1.1078	.00438	−0.4863

(*continued on next page*)

Table 2 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log2(FC)
LC	11.5922	pos	Apo-11-zeaxanthinal	Lipids and lipid-like molecules	2.0125	.00253	−0.4876
LC	13.6113	pos	bhas#32	Lipids and lipid-like molecules	2.8469	.00224	−0.4880
LC	0.96752	pos	(Carbamoylamino) peroxyurea	Lipids and lipid-like molecules	1.6517	.0029	−0.4883
LC	14.5329	pos	Oxazine	Organoheterocyclic compounds	3.4129	.0011	−0.4889
LC	12.8334	pos	3-Methylsulfinylpropyl isothiocyanate	Organosulfur compounds	2.8480	.00132	−0.4899
LC	14.2531	pos	Tetraethylene glycol	Organic oxygen compounds	1.5603	.00192	−0.4908
LC	7.23447	pos	8-undecynoic acid	Lipids and lipid-like molecules	1.2402	.00329	−0.4915
LC	9.2295	pos	(2E,4E)-2,4-Nonadien-1-ol	Lipids and lipid-like molecules	1.2984	.00364	−0.4929
LC	14.3057	pos	Piracetam	Organic acids and derivatives	8.8622	.00178	−0.4930
LC	8.0057	pos	N-Undecanoylglycine	Organic acids and derivatives	2.6365	.00309	−0.4933
LC	14.4453	pos	Carcinine	Organoheterocyclic compounds	1.1794	.00385	−0.4946
LC	9.10383	pos	8-tridecynoic acid	Lipids and lipid-like molecules	3.5871	.00227	−0.4954
LC	11.4141	pos	(E)-5,8-Megastigmadien-4-one	Organic oxygen compounds	1.3164	.00223	−0.4960
LC	14.5505	pos	Squamolone	Organoheterocyclic compounds	2.0310	.00161	−0.4961
LC	14.3404	pos	N-Nitrosoanatabine	Organoheterocyclic compounds	3.4233	.00139	−0.4962
LC	14.8337	pos	Trimethylpyrazine	Organoheterocyclic compounds	1.0069	.00175	−0.4977
LC	14.4453	pos	Asparaginylglycine	Organic acids and derivatives	3.0213	.00152	−0.4990
LC	11.2905	pos	(1S,2S)-3-oxo-2-pentyl-cyclopentanebutanoic acid	Lipids and lipid-like molecules	4.2013	.00312	−0.4998
LC	6.32005	pos	1-O-Pentylglycerol	Lipids and lipid-like molecules	1.5031	.00222	−0.5010
LC	0.6922	neg	Neu5NAcalpha2->6lactose	Organic oxygen compounds	6.9152	.01377	−0.5011
LC	11.6284	pos	5-Hexyl-2-furanhexanoic acid	Lipids and lipid-like molecules	1.0017	.0019	−0.5125
LC	11.699	pos	Sulfanilamide	Benzenoids	1.0353	.00068	−0.5148
LC	0.71722	pos	Aspartylhydroxyproline	Organic acids and derivatives	2.2192	.00697	−0.5217
LC	0.74685	pos	Proline betaine	Organic acids and derivatives	1.7248	.00399	−0.5231
LC	11.7881	pos	Cyclandelate	Benzenoids	1.1902	.00174	−0.5294
LC	14.5329	pos	Geranylcitronellol	Lipids and lipid-like molecules	3.1094	.00842	−0.5297
LC	11.5211	pos	(4E,8E,10E-d18:3)sphingosine	Lipids and lipid-like molecules	1.3112	.00171	−0.5361
LC	11.184	pos	Cyclododecanone	Organic oxygen compounds	1.4838	.00348	−0.5463
LC	0.71722	pos	2-Deoxy-2,3-dehydro-n-acetyl-neuraminic acid	Organic acids and derivatives	2.0121	.0007	−0.5501
LC	0.78505	neg	Protoapigenone	Organoheterocyclic compounds	2.1210	.00123	−0.5527
LC	0.5885	pos	Benzoxazole	Organoheterocyclic compounds	1.0294	.00711	−0.5530
LC	8.04055	pos	(3S,7S)-Jasmonic acid	Lipids and lipid-like molecules	1.9757	.0032	−0.5545
LC	4.52245	pos	5S,6S-epoxy-15R–hydroxy-ETE	Organic oxygen compounds	1.1545	.00965	−0.5547
LC	0.66997	pos	Glycerophosphocholine	Lipids and lipid-like molecules	13.6710	.00386	−0.5556
LC	12.0731	pos	4beta-OH-7-DHC	Lipids and lipid-like molecules	1.5993	.00643	−0.5612
LC	0.7615	pos	1-Methyladenosine	Nucleosides, nucleotides, and analogues	1.1738	.00117	−0.5663
LC	0.66997	pos	Met-leu-phe	Organic acids and derivatives	3.6835	.0024	−0.5717
LC	0.70148	pos	Creatinine	Organic acids and derivatives	1.6253	.00121	−0.5773
LC	0.71722	pos	b-D-Xylopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-L-arabinose	Organic oxygen compounds	1.9453	.04474	−0.5776
LC	3.1252	pos	Isobutyryl-L-carnitine	Lipids and lipid-like molecules	2.4091	.0041	−0.5805
LC	0.7615	pos	Daunosamine	Organic nitrogen compounds	1.0950	.00206	−0.5956
LC	5.05858	pos	Tetraglyme	Organic oxygen compounds	1.2014	.00831	−0.5970
LC	1.35185	pos	Propionylcarnitine	Lipids and lipid-like molecules	1.7050	.00501	−0.6063
LC	0.71722	pos	Creatine riboside	Organic oxygen compounds	1.5365	.00305	−0.6079
LC	0.61968	pos	L-Histidine	Organic acids and derivatives	1.1563	.00269	−0.6089
LC	11.6815	pos	LysoPE(P-16:0/0:0)	Lipids and lipid-like molecules	1.6519	.00043	−0.6111
LC	0.65987	neg	Sialyllacto-N-tetraose b	Organic oxygen compounds	4.6163	.03262	−0.6230
LC	11.9482	pos	12,13-EpOME	Lipids and lipid-like molecules	1.2756	.00073	−0.6233
LC	13.2044	pos	Palmitic amide	Lipids and lipid-like molecules	1.5429	.03667	−0.6259

(*continued on next page*)

Table 2 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log2(FC)
LC	0.66997	pos	alpha-D-GalpNAc-(1->3)-[alpha-L-Fucp-(1->2)]-D-Galp	Organic oxygen compounds	9.9366	.00559	−0.6288
LC	10.7197	pos	9-OxoODE	Lipids and lipid-like molecules	1.6984	.0013	−0.6331
LC	12.6212	pos	LysoPE(P-18:0/0:0)	Lipids and lipid-like molecules	1.3355	.00044	−0.6364
LC	0.73815	neg	Lactodifucotetraose	Lipids and lipid-like molecules	4.4583	.00017	−0.6420
LC	0.94952	pos	Cefminox	Organoheterocyclic compounds	2.1766	5E-05	−0.6447
LC	11.0604	pos	LysoPE(18:2(9Z,12Z)/0:0)	Lipids and lipid-like molecules	1.1068	.00054	−0.6472
GC	24.71	-	Chrysin	Unclassified	1.1843	.01852	−0.6526
LC	0.64447	neg	Tadalafil	Organoheterocyclic compounds	2.0655	.01744	−0.6549
LC	0.67643	neg	Phosphocreatine	Organic acids and derivatives	3.8164	.00073	−0.6564
LC	0.71722	pos	Creatine	Organic acids and derivatives	3.7526	.00078	−0.6762
LC	0.7615	pos	L-Acetylcarnitine	Lipids and lipid-like molecules	6.6875	.00046	−0.6798
LC	12.7102	pos	6-[3]-ladderane-1-hexanol	Lipids and lipid-like molecules	2.3726	.00771	−0.6907
LC	0.65342	pos	Isovitexin 2′′-(6′′′-(E)-p-coumaroylglucoside)	Phenylpropanoids and polyketides	11.3455	.00785	−0.6915
LC	0.94952	pos	N-(2,6-Difluorophenyl)-2-oxoglycyl-N-((1S)-1-(carboxymethyl)-2-oxo-3-(2,3,5,6-tetrafluorophenoxy)propyl)-L-alaninamide	Organic oxygen compounds	3.8872	2.4E-05	−0.7097
LC	0.94952	pos	4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol	Nucleosides, nucleotides, and analogues	2.2067	1.8E-05	−0.7167
LC	11.5036	pos	13-Hydroxyoctadeca-9,12-dienoic acid	Lipids and lipid-like molecules	1.2475	.00049	−0.7307
LC	0.68672	pos	Cadabicine	Organic oxygen compounds	2.0720	.00174	−0.7371
LC	0.79097	pos	Samarangenin B	Phenylpropanoids and polyketides	3.0074	1.1E-05	−0.7373
LC	12.568	pos	Behenoyl-EA	Lipids and lipid-like molecules	2.3860	.00119	−0.7466
LC	0.94952	pos	Alendronic acid	Organic acids and derivatives	1.0959	.00013	−0.7574
LC	14.3755	pos	Enigmol	Lipids and lipid-like molecules	1.7203	.00521	−0.7613
LC	0.63677	pos	Quercetin 7-methyl ether 3-alpha-L-arabinopyranosyl-(1->3)-[galactosyl-(1->6)-galactoside]	Lipids and lipid-like molecules	1.2978	6.3E-05	−0.7731
LC	0.94952	pos	Zilascorb	Organoheterocyclic compounds	2.0389	4.3E-05	−0.7741
LC	0.7615	pos	Aminovaleric acid betaine	Lipids and lipid-like molecules	1.1258	5.6E-05	−0.7865
LC	5.69677	pos	Hexanoylcarnitine	Lipids and lipid-like molecules	1.1725	8E-05	−0.7964
LC	0.65342	pos	Galactotriose	Organic oxygen compounds	2.1022	.0005	−0.8147
LC	13.4697	pos	3E,13Z-Octadecadienal	Lipids and lipid-like molecules	6.6777	.00567	−0.8149
LC	0.71722	pos	Arbutin	Organic oxygen compounds	1.5285	1.8E-05	−0.8176
LC	0.94952	pos	SCD1 Inhibitor	Benzenoids	1.6137	7.7E-05	−0.8234
GC	24.658	-	Maltose	Unclassified	1.3760	.0169	−0.8334
LC	0.68672	pos	L-Carnitine	Organic nitrogen compounds	5.5237	.0001	−0.8425
LC	5.48317	pos	2-O-(beta-D-galactopyranosyl-(1->6)-beta-D-galactopyranosyl) 2S-hydroxytridecanoic acid	Lipids and lipid-like molecules	1.2691	3.8E-05	−0.8436
LC	13.435	pos	PE(16:0/P-18:1(9Z))	Lipids and lipid-like molecules	1.9810	.04836	−0.8604
LC	0.66997	pos	Lewis y Tetrasaccharide	Organic oxygen compounds	6.4036	.00035	−0.8652
LC	0.70148	pos	Rutinose	Organic oxygen compounds	1.8222	9.3E-06	−0.8946
GC	7.234	-	3-Cyclohexene-1-Methanol	Unclassified	1.2996	1.2E-05	−0.8996
LC	0.63677	pos	Sesaminol 2-O-triglucoside	Organic oxygen compounds	1.5456	.00061	−0.9083
LC	0.70148	pos	TOG10	Lipids and lipid-like molecules	4.7725	.00229	−0.9126
LC	13.6467	pos	PE(20:3(8Z,11Z,14Z)/18:0)	Lipids and lipid-like molecules	1.2712	.00903	−0.9414

(*continued on next page*)

Table 2 (*continued*)

Data class	Retention time (min)	Ion mode	Metabolites	Super class	VIP	*P*-value	log2(FC)
LC	0.63677	pos	Spinacetin 3-(2′′-apiosylgentiobioside)	Phenylpropanoids and polyketides	1.1919	.00012	−0.9515
LC	11.1489	pos	13(S)-Hydroperoxylinolenic acid	Lipids and lipid-like molecules	1.9288	.00161	−0.9621
GC	10.021	-	4-Cyclohexene-1,2-Diol	Unclassified	1.4648	.02033	−0.9908
LC	14.0103	pos	SM(d18:0/16:1(9Z))	Lipids and lipid-like molecules	6.7964	.00015	−1.0026
LC	0.66997	pos	4-Trimethylammoniobutanoic acid	Lipids and lipid-like molecules	12.3605	3E-05	−1.0103
LC	0.70148	pos	a-L–threo-4-Hex-4-enopyranuronosyl-D-galacturonic acid	Organic oxygen compounds	1.7982	.00261	−1.0160
LC	0.7615	pos	PC(2:0/20:5(7Z,9Z,11E,13E,17Z)-3OH(5,6,15))	Unclassified	1.6286	1.6E-06	−1.0496
LC	0.61968	pos	2-trans-7,10,13,16,19-all-cis-Docosahexaenoyl-CoA	Lipids and lipid-like molecules	1.5000	9.7E-05	−1.0519
LC	0.63677	pos	TG(22:5(4Z,7Z,10Z,13Z,16Z)/15:0/O-18:0)	Lipids and lipid-like molecules	1.6814	.00166	−1.0729
LC	11.3258	pos	ent-16-L1-PhytoP	Lipids and lipid-like molecules	1.1760	.001	−1.0898
GC	21.213	-	Hexadecylamine	Unclassified	1.6652	.01427	−1.1035
GC	19.197	-	D-Gulose	Unclassified	1.3949	.00673	−1.1279
LC	0.71722	pos	Kaempferol 3-rhamnosyl-(1->3)(4′′′-p-coumarylrhamnosyl)(1->6)-glucoside	Lipids and lipid-like molecules	5.9875	2.9E-06	−1.1923
LC	0.61968	pos	Pelargonidin 3-sophoroside-7-glucoside	Lipids and lipid-like molecules	1.9890	.0004	−1.2066
LC	0.63677	pos	Apigenin 7-rhamnosyl-(1->6)-(4′′-E-p-methoxycinnamoylglucoside)	Lipids and lipid-like molecules	3.2833	1.1E-05	−1.2226
LC	11.6633	pos	Stearidonic acid	Lipids and lipid-like molecules	1.3012	.00254	−1.2546
LC	0.68672	pos	3′-Sialyllactose	Organic oxygen compounds	7.2430	.00135	−1.2757
LC	0.63677	pos	Syringetin 3-rutinoside-7-glucoside	Lipids and lipid-like molecules	1.1072	5.5E-06	−1.4461
LC	0.63677	pos	Cer(d18:1/35:0(35OH))	Lipids and lipid-like molecules	1.3142	.00051	−1.4530
LC	6.9461	pos	Polymyxin B nonapeptide	Organic acids and derivatives	1.7685	.00482	−1.5399
GC	18.875	-	Ascorbic Acid	Organoheterocyclic compounds	2.3018	.04535	−1.5861
LC	8.92388	pos	(9R,10S,12Z)-9,10-Dihydroxy-8-oxo-12-octadecenoic acid	Lipids and lipid-like molecules	1.1947	.00273	−1.6362
LC	0.74685	pos	Aceneuramic acid	Organic oxygen compounds	1.2109	.00077	−1.7513
LC	4.95395	pos	Caffeine	Organoheterocyclic compounds	1.0028	.0138	−2.1302
GC	18.855	-	D-Pinitol	Unclassified	2.4637	.00081	−2.2412
GC	17.169	-	3-Phosphoglyceric acid	Organic oxygen compounds	2.2064	.01079	−2.3419

levels of lacto-N-neotetraose and lacto-N-tetraose but lower levels of 3-fucosyllactose [53]. Although the level of HMOs is predominantly determined by maternal genetics, Se and Le genes, other variables including parity number also contribute to differences in HMOs [54]. For example, PP mothers were found to have lower contents of 3-fucosyllactose, 6′-galactosyllactose, lacto-N-fucopentaose II, and lacto-N-fucopentaose but higher levels of disialyllacto-N-tetraose and lacto-N-neotetraose compared with MP mothers [28]. Research across Asia, Europe, and South America has established a connection between parity and the composition of HMOs [55–58]. Similar effects related to parity have been observed in milk oligosaccharides across other species, although the precise mechanisms remain unclear [59]. Possible contributing factors include maternal immune responses, hormonal fluctuations, mammary gland structural differences, or variations in milk microbiota [52]. It is worth noting that successive pregnancies may lead to changes in the structure or function of the mammary gland, resulting in changes in the nutrient content of HM.

HM contains high levels of HMOs, with colostrum at 20–25 g/L and mature milk at 5-20 g/L [60]. These HMOs, which include over 200 structurally diverse types, provide significant benefits such as immune modulation, microbiota regulation, cognitive improvement, antiallergy effects, and infection prevention [61,62]. However, infant formulas which is primarily composed of cow's milk, goat's milk, whey protein, lactose, and lipids, in-

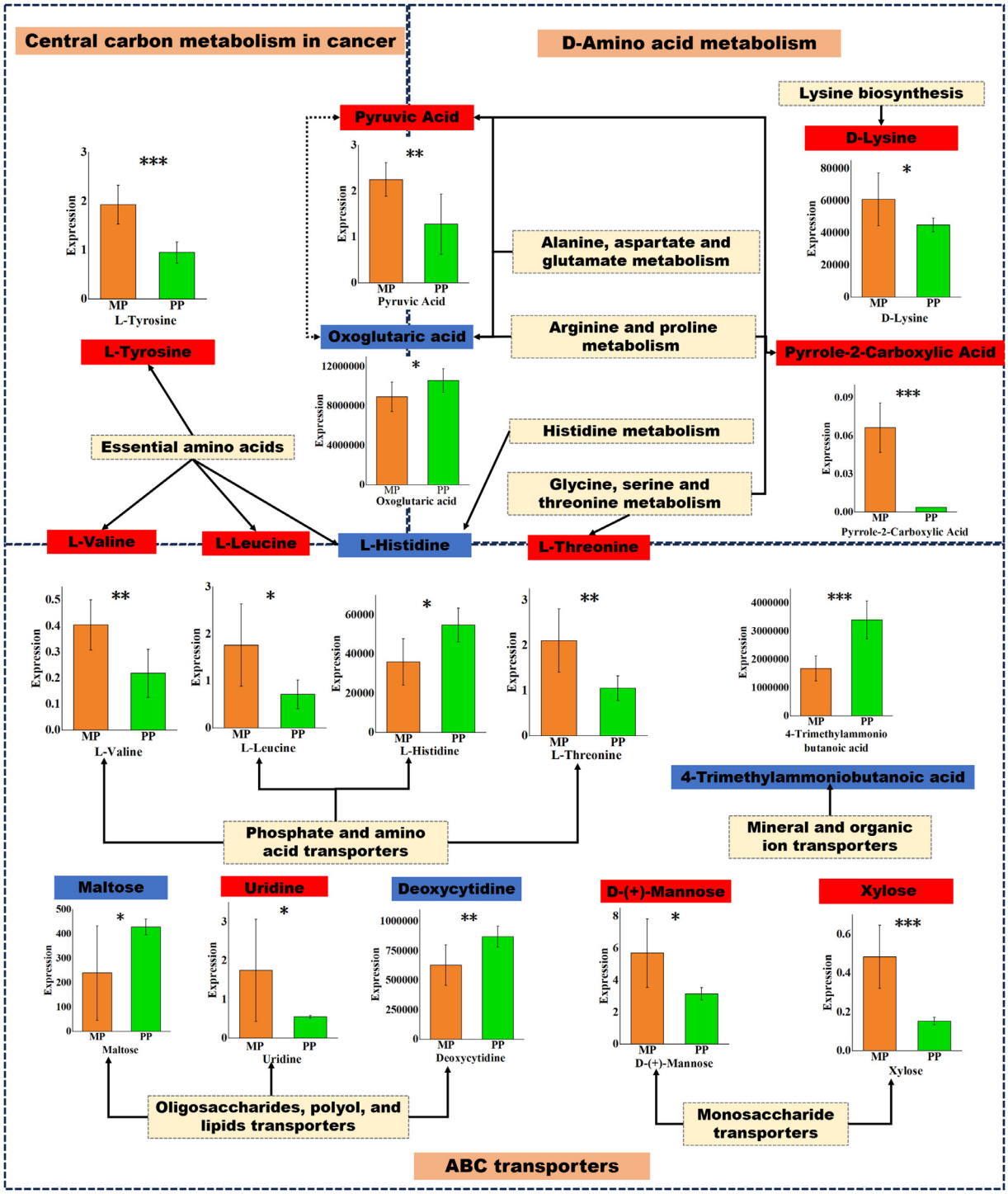

Fig. 6. Hypothesized scheme pathway most related to mature human milk metabolite changes between different parity groups. The y-axis represents the relative abundance of metabolites. Error bars indicate SEM. *means $P<.05$, **means $P<.001$, ***means $P<.001$.

herently lack HMOs. Eight HMOs have already been considered Generally Recognized as Safe (GRAS) substances by the American Food and Drug Administration (FDA), including 2′-fucosyllactose, 3-fucosyllactose, difucosyllactose, lactose-N-neotetraose, lacto-N-tetraose, 3′-sialyllactose, 6′-sialyllactose and Lacto-N-triose II [63]. In 2019, FDA set maximum doses at 2.4 g/L for 2′-fucosyllactose and 0.6 g/L for lacto-N-neotetraose [61]. According to the European Food Safety Authority (EFSA), the maximum allowable concentrations of HMOs in infant formula were: 2′-fucosyllactose 2.4 g/L, a combination of 2′-fucosyllactose and difucosyllactose 1.6 g/L, 3′-sialyllactose 0.2 g/L, 6′-sialyllactose 0.4 g/L, lacto-N-neotetraose 0.6 g/L, and lacto-N-tetraose 0.8 g/L [64]. Although some commercial products have begun incorporating HMOs, the types and amounts added remain limited [65].

As Fig. 3C-D shows, we identified 23 significantly enriched differential metabolic pathways ($P<.05$). Figure 6 shows three of

the most significant metabolic pathways, including ABC transporters (10 DEMs: L-Leucine, L-Histidine, D-(+)-Mannose, Xylose, L-Valine, L-Threonine, Maltose, Uridine, Deoxycytidine, 4-Trimethylammoniobutanoic acid), central carbon metabolism in cancer (6 DEMs: Pyruvic Acid, Oxoglutaric acid, L-Tyrosine, L-Leucine, L-Histidine, and L-Valine), and D-Amino acid metabolism pathways (6 DEMs: Pyruvic Acid, Oxoglutaric acid, D-Lysine, Pyrrole-2-Carboxylic Acid, L-Histidine, and L-Threonine). DEMs involved in ABC transporter pathway are mainly phosphate and amino acid transporters; oligosaccharides, polyol, and lipids transporters; mineral and organic ion transporters; and monosaccharide transporters. By hydrolyzing ATP, ABC transporters transport a wide range of substrates across cell membranes, including metabolites, lipids and drugs [66]. D-amino acid metabolism is another significant pathway related to parity, which is consistent with a previous study that found parity could influence amino acid metabolism [67]. Previous genomic studies on dairy cows of different parities showed KEGG enrichment in pathways like Alanine, aspartate and glutamate metabolism and Tyrosine metabolism, potentially linked to milk production performance [68].

Among the three metabolic pathways, we identified four essential amino acids (L-Leucine, L-Valine, L-Threonine, and L-Histidine), one nonessential amino acid (L-Tyrosine), and one D-type amino acid (D-Lysine). Notably, L-Histidine [$\log_2$(FC)=−0.6089] showed downregulation in MHM from MP mothers, whereas L-Leucine [$\log_2$(FC)=1.2980], L-Valine [$\log_2$(FC)=0.8863], L-Threonine [$\log_2$(FC)=0.9997], L-Tyrosine [$\log_2$(FC)=1.0184], D-Lysine [$\log_2$(FC)=0.4354] have significant upregulation. Leucine, isoleucine, valine, and phenylalanine are positively correlated with changes in length-for-age Z-scores in infants aged 5–12 months [69].

At 1 month of age, the urinary excretion of L-Threonine was significantly lower in breast-fed infants compared to formula-fed infants [70]. Dietary histidine inversely correlates with pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-1, interleukin-6, and the inflammation biomarker C-reactive protein [71]. Leucine acts as a nutrient signal through the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway and independently regulates protein synthesis in mammary epithelial cells [72]. Reduced levels of L-Leucine may contribute to the decline in milk protein synthesis observed in the mammary glands of PP mothers. Maternal circulating levels of glutamine and serine are associated with an increase in offspring birth weight, while elevated levels of leucine and phenylalanine are linked to a decrease in offspring birth weight [73]. Essential amino acids provide nitrogen sources for growing infants and are vital for neurological and physical development, suggesting that protein supplementation or additional essential amino acids in the diet are needed to meet the metabolic needs of PP mothers and infants [74]. However, these observations remain to be elucidated and their physiological significance for breastfed infants needs to be assessed in future research.

4. Conclusion

This study utilized untargeted metabolomics to analyze the impact of delivery mode and parity on the composition of MHM metabolites. The findings revealed that lipids and lipid-like molecules were significantly upregulated in the MHM of CS mothers, such as palmitelaidic acid, DHA, arachidonic acid, linoleic acid, myristic acid, oleic acid, lauric acid, cis-gondoic acid, 12,13-EpOME, and 9-OxoODE.

Whereas, VB mothers exhibited higher levels of HMOs like 3-fucosyllactose, lactodifucotetraose, and lacto-N-difucopentaose II, which are crucial for infant gut microbiota regulation, immune function, and brain development. These differences may stem from variations in maternal hormone levels, maternal diet, and fetal sex associated with different delivery modes. KEGG pathway analysis indicated that DEMs were significantly enriched in pathways such as unsaturated fatty acid biosynthesis, fatty acid biosynthesis, linoleic acid metabolism, ABC transporters, center carbon metabolism in cancer, and D-amino acid metabolism, suggesting that delivery mode and parity regulate milk composition through metabolic pathways. Additionally, essential amino acids like L-Leucine, L-Valine, and L-Threonine were significantly upregulated in MP mothers, whereas L-Histidine was downregulated. These amino acids play vital roles in protein synthesis, immune modulation, and neurological development in infants, suggesting that supplementing dietary proteins or essential amino acids may be essential to fulfill the metabolic requirements of PP mothers and their infants. These findings highlighted how delivery mode and parity influenced infant growth and development by modulating milk metabolite composition. Future research is needed to further explore the underlying mechanisms and long-term effects on infant health.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jiayue Tang: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cai Shen:** Formal analysis, Data curation. **Dan Yao:** Supervision, Formal analysis, Data curation. **Jingwen Yu:** Supervision, Formal analysis, Data curation. **Yanan Liu:** Formal analysis, Data curation. **Maolin Tu:** Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Hong Zhang:** Formal analysis, Data curation, Conceptualization. **Xuebing Xu:** Formal analysis, Data curation, Conceptualization. **Oi-Ming Lai:** Formal analysis, Data curation. **Ling-Zhi Cheong:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis.

Acknowledgments

The authors acknowledge the support of the Zhejiang Key R&D Program (2022C04009). Jiayue Tang acknowledged funding support from the General Research Project of Zhejiang Provincial Department of Education (Y202455443).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2025.109967.

References

[1] Picciano MF. Nutrient composition of human milk. Pediatr Clin N Am 2001;48:53–67.
[2] Andres SF, Scottoline B, Good M. Shaping infant development from the inside out: bioactive factors in human milk. Semin Perinatol 2023;47:151690.
[3] Zhao JH, Liu YY, Shen C, Lai OM, Tan CP, Cheong LZ. Enhanced storage stability and in-vitro digestibility of powdered-solid lipid nanoparticles with high-algae oil-load. Food Biosci 2023;55:102810.
[4] Henrick BM, Rodriguez L, Lakshmikanth T, Pou C, Henckel E, Arzoomand A, et al. Bifidobacteria-mediated immune system imprinting early in life. Cell 2021;184:3884–98.
[5] Wada Y, Lönnerdal B. Bioactive peptides derived from human milk proteins: an update. Curr Opin Clin Nutr Metab Care 2020;23:217–22.

[6] Perrone S, Biologist ML, Zollino I, Biologist FB, Biologist MT, Biologist AV, et al. Breast milk: to each his own. From metabolomic study, evidence of personalized nutrition in preterm infants. Nutrition 2019;62:158–61.
[7] Meng FY, Uniacke-Lowe T, Lanfranchi E, Meehan G, O'Shea CA, Dennehy T, et al. A longitudinal study of fatty acid profiles, macronutrient levels, and plasmin activity in human milk. Front Nutr 2023;10:1172613.
[8] Niwa S, Kawabata T, Shoji K, Ogata H, Kagawa Y, Nakayama K, et al. Investigation of maternal diet and *FADS1* Polymorphism associated with long-chain polyunsaturated fatty acid compositions in Human milk. Nutrients 2022;14:2160.
[9] Emwas A-HM. The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research. Methods Mol Biol 2015;1277:161–93.
[10] Emwas AH, Roy R, McKay RT, Tenori L, Saccenti E, Gowda GAN, et al. NMR spectroscopy for metabolomics research. Metabolites 2019;9.
[11] Drouin N, Ramautar R. Capillary electrophoresis-mass spectrometry for metabolomics: possibilities and perspectives. In: Separation Techniques Applied to Omics Sciences: from Principles to Relevant Applications, 1336; 2021. p. 159–78.
[12] Yao D, Shen C, Zhang X, Tang J, Yu J, Tu M, et al. Untargeted metabolomics study of mature human milk from women with and without gestational diabetes mellitus. Food Chem 2024;460:140663.
[13] Zeki OC, Eylem CC, Recber T, Kir S, Nemutlu E. Integration of GC-MS and LC-MS for untargeted metabolomics profiling. J Pharm Biomed Anal 2020;190:113509.
[14] Wang W, Kou J, Long J, Wang T, Zhang MM, Wei M, et al. GC/MS and LC/MS serum metabolomic analysis of Chinese LN patients. Sci Rep 2024;14:1523.
[15] Wang ZY, Sun YG, Wu YZ, Chen R, Xu YA, Cai YF, et al. Metabonomic analysis of human and 12 kinds of livestock mature milk. Food Chem-X 2023;17:100581.
[16] Wen L, Wu Y, Yang Y, Han TL, Wang WL, Fu HJ, et al. Gestational diabetes mellitus changes the metabolomes of Human colostrum, transition milk and mature milk. Med Sci Monit 2019;25:6128–52.
[17] Li KL, Jiang JJ, Xiao HL, Wu KJ, Qi C, Sun J, et al. Changes in the metabolite profile of breast milk over lactation stages and their relationship with dietary intake in Chinese women: HPLC-QTOFMS based metabolomic analysis. Food Funct 2018;9:5189–97.
[18] Garwolinska D, Hewelt-Belka W, Kot-Wasik A, Sundekilde UK. Nuclear magnetic resonance metabolomics reveals qualitative and quantitative differences in the composition of Human breast milk and milk formulas. Nutrients 2020;12:921.
[19] Komatsu Y, Kumakura D, Seto N, Izumi H, Takeda Y, Ohnishi Y, et al. Dynamic associations of milk components with the infant gut microbiome and fecal metabolites in a mother-infant model by microbiome, NMR metabolomic, and time-series clustering analyses. Front Nutr 2022;8:813690.
[20] Tang J, Yao D, Shen C, Yu J, Zhang H, Xu X, et al. Effects of maternal and perinatal factors on human milk fat globule membrane proteome: a data independent acquisition approach. Food Biosci 2024;58:103791.
[21] Liu ZY, Liu HT, Chen ZJ, Deng C, Zhou L, Chen SY, et al. Identification of a novel plasma metabolite panel as diagnostic biomarker for hepatocellular carcinoma. Clin Chim Acta 2023;543:117302.
[22] Qian HY, Guo HM, Zhang X, Liu MZ, Zhao GD, Xu AY, et al. Metabolic characterization of hemolymph in Bombyx mori varieties after Bombyx mori nucleopolyhedrovirus infection by GC-MS-based metabolite profiling. Arch Virol 2022;167:1637–48.
[23] Li WH, Zeng WK, Zhang YP, Ma ZJ, Fang XY, Han YC, et al. A comparative metabolomics analysis of domestic yak (*Bos grunniens*) milk with human breast milk. Front Vet Sci 2023;10:1207950.
[24] Zhang H, Wang L, Wang X, Deng L, He B, Yi X, et al. Mangiferin alleviated poststroke cognitive impairment by modulating lipid metabolism in cerebral ischemia/reperfusion rats. Eur J Pharmacol 2024;977:176724.
[25] Zhang Y, Liu CH, Liu JH, Liu XM, Tu ZH, Zheng YP, et al. Multi-omics reveals response mechanism of liver metabolism of hybrid sturgeon under ship noise stress. Sci Total Environ 2022;851:158348.
[26] Bai YL, Song YX, Zhang J, Fu SX, Wu L, Xia C, et al. GC/MS and LC/MS based serum metabolomic analysis of dairy cows with ovarian inactivity. Front Vet Sci 2021;8:678388.
[27] Mahmood MH, Sultan M, Miyazaki T. Significance of temperature and humidity control for agricultural products storage: overview of conventional and advanced options. Int J Food Eng 2019;15:20190063.
[28] Samuel TM, Binia A, de Castro CA, Thakkar SK, Billeaud C, Agosti M, et al. Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy European mothers. Sci Rep 2019;9:11767.
[29] Sun W, Tao L, Qian C, Xue P, Tong X, Yang L, et al. Human milk oligosaccharides and the association with microbiota in colostrum: a pilot study. Arch Microbiol 2024;206:58.
[30] Du ZH, Li ZY, Guang CE, Zhu YY, Mu WM. Recent advances of 3-fucosyllactose in health effects and production. Arch Microbiol 2024;206:378.
[31] Newburg DS, Tanritanir AC, Chakrabarti S. Lactodifucotetraose, a human milk oligosaccharide, attenuates platelet function and inflammatory cytokine release. J Thromb Thrombolysis 2016;42:46–55.
[32] Hu MM, Miao M, Li KW, Luan QM, Sun GL, Zhang T. Human milk oligosaccharide lacto-N-tetraose: physiological functions and synthesis methods. Carbohydr Polym 2023;316:121067.
[33] Oliveros E, Vazquez E, Barranco A, Ramirez M, Gruart A, Delgado-Garcia JM, et al. Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats. Nutrients 2018;10:1519.
[34] Tang J, Yao D, Zhao J, Tu M. Inflammatory modulation effects of human milk oligosaccharides: from screening in-vitro to application in the milk powders. Food Biosci 2025;65:106111.
[35] Vass RA, Zhang MM, Sarkadi LS, Ueveges M, Tormási J, Benes EL, et al. Effect of holder pasteurization, mode of delivery, and infant's gender on fatty acid composition of donor breast milk. Nutrients 2024;16:1689.
[36] Samuel TM, Thielecke F, Lavalle L, Chen C, Fogel P, Giuffrida F, et al. Mode of neonatal delivery influences the nutrient composition of Human milk: results from a multicenter European cohort of lactating women. Front Nutr 2022;9:834394.
[37] Calvo-Lerma J, Cabrera-Rubio R, Lerin C, González S, Selma-Royo M, Martínez–Costa C, et al. Comprehensive targeted and quantitative profiling of the Human milk metabolome: impact of delivery mode, breastfeeding practices, and maternal diet. Mol Nutr Food Res 2024;68:2400424.
[38] Mansell T, Vlahos A, Collier F, Ponsonby AL, Vuillermin P, Ellul S, et al. The newborn metabolome: associations with gestational diabetes, sex, gestation, birth mode, and birth weight. Pediatr Res 2022;91:1864–73.
[39] Khelouf N, Haoud K, Meziani S, Fizir M, Ghomari FN, Khaled MB, et al. Effect of infant's gender and lactation period on biochemical and energy breast milk composition of lactating mothers from Algeria. J Food Compos Anal 2023;115:104889.
[40] Miliku K, Richelle J, Becker AB, Simons E, Moraes TJ, Stuart TE, et al. Sex-specific associations of human milk long-chain polyunsaturated fatty acids and infant allergic conditions. Pediatr Allergy Immunol 2021;32:1173–82.
[41] Vass RA, Zhang M, Simon Sarkadi L, Üveges M, Tormási J, Benes EL, et al. Effect of holder pasteurization, mode of delivery, and infant's gender on fatty acid composition of donor breast milk. Nutrients 2024;16:1689.
[42] Bobinski R, Bobinska J. Fatty acids of human milk - a review. Int J Vitam Nutr Res 2022;92:280–91.
[43] Einerhand AWC, Mi W, Haandrikman A, Sheng XY, Calder PC. The impact of linoleic acid on infant health in the absence or presence of DHA in infant formulas. Nutrients 2023:15.
[44] Lien EL, Richard C, Hoffmann DR. DHA and ARA addition to infant formula: current status and future research directions. Prostaglandins Leukot Essent Fat Acids 2018;128:26–40.
[45] Rosa F, Matazel KS, Bowlin AK, Williams KD, Elolimy AA, Adams SH, et al. Neonatal diet impacts the large intestine luminal metabolome at weaning and post-weaning in piglets fed formula or Human milk. Front Immunol 2020;11:607609.
[46] da Costa, Souza F, Grodzki ACG, Morgan RK, Zhang Z, Taha AY, et al. Oxidized linoleic acid metabolites regulate neuronal morphogenesis in vitro. Neurochem Int 2023;164:105506.
[47] Schaefer E, Demmelmair H, Horak J, Holdt L, Grote V, Maar K, et al. Multiple micronutrients, lutein, and docosahexaenoic acid supplementation during lactation: a randomized controlled trial. Nutrients 2020;12:3849.
[48] de Sousa IRM, Wang ZX, Hu R, Stahl B, Jin Y, Eussen S, et al. Dietary intake of Chinese lactating women is associated with the fatty acid profile of their milk. Ann Nutr Metab 2022;78:33–45.
[49] Wang FM, Yu JH, Wang L, Wang S, Jin QZ, Wang QY, et al. Fatty acids and their *sn*-2 positional distribution in breast milk and their association with edible oils in maternal diet: a study of five regions in China. Food Funct 2023;14:5589–605.
[50] Petersohn I, Hellinga AH, van Lee L, Keukens N, Bont L, Hettinga KA, et al. Maternal diet and human milk composition: an updated systematic review. Front Nutr 2024;10:1320560.
[51] Srinivas V, Varma S, Kona SR, Ibrahim A, Duttaroy AK, Basak S. Dietary omega-3 fatty acid deficiency from pre-pregnancy to lactation affects expression of genes involved in hippocampal neurogenesis of the offspring. Prostaglandins Leukot Essent Fat Acids 2023;191:102566.
[52] Matharu D, Ponsero AJ, Lengyel M, Meszaros-Matwiejuk A, Kolho K-L, de Vos WM, et al. Human milk oligosaccharide composition is affected by season and parity and associates with infant gut microbiota in a birth mode dependent manner in a Finnish birth cohort. eBioMedicine 2024;104:105182.
[53] Azad MB, Robertson B, Atakora F, Becker AB, Subbarao P, Moraes TJ, et al. Human milk oligosaccharide concentrations are associated with multiple fixed and modifiable maternal characteristics, environmental factors, and feeding practices. J Nutr 2018;148:1733–42.
[54] Han SM, Derraik JGB, Binia A, Sprenger N, Vickers MH, Cutfield WS. Maternal and infant factors influencing Human milk oligosaccharide composition: beyond Maternal genetics. J Nutr 2021;151:1383–93.
[55] Tonon KM, de Morais MB, Abrao A, Miranda A, Morais TB. Maternal and infant factors associated with Human milk oligosaccharides concentrations according to Secretor and Lewis phenotypes. Nutrients 2019;11:1358.
[56] Lahdenperä M, Galante L, Gonzales-Inca C, Vahtera J, Pentti J, Rautava S, et al. Residential green environments are associated with human milk oligosaccharide diversity and composition. Sci Rep 2023;13:216.
[57] Ferreira AL, Alves R, Figueiredo A, Alves-Santos N, Freitas-Costa N, Batalha M, et al. Human milk oligosaccharide profile variation throughout postpartum in healthy women in a Brazilian cohort. Nutrients 2020;12:790.
[58] Xun YP, Feng XY, Xue YL, Feng LL, Zhang D, Ma R, et al. Profile of twenty-three Human milk oligosaccharides in Han Chinese mothers throughout postpartum 1 year. J. Food Qual 2022;2022:6230832.

[59] Durham SD, Wei Z, Lemay DG, Lange MC, Barile D. Creation of a milk oligosaccharide database, MilkOligoDB, reveals common structural motifs and extensive diversity across mammals. Sci Rep 2023;13:10345.
[60] Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology 2012;22:1147–62.
[61] Dinleyici M, Barbieur J, Dinleyici EC, Vandenplas Y. Functional effects of human milk oligosaccharides (HMOs). Gut Microbes 2023;15:2186115.
[62] Wicinski M, Sawicka E, Gebalski J, Kubiak K, Malinowski B. Human milk oligosaccharides: health benefits, potential applications in infant formulas, and pharmacology. Nutrients 2020;12:266.
[63] Zhu Y, Zhang W, Mu W. Human milk oligosaccharides: the new gold standard for premium infant formula. J Agric Food Chem 2022;70:2061–3.
[64] Duman H, Bechelany M, Karav S. Human milk oligosaccharides: decoding their structural variability, health benefits, and the evolution of infant nutrition. Nutrients 2025;17:118.
[65] Urashima T, Ajisaka K, Ujihara T, Nakazaki E. Recent advances in the science of human milk oligosaccharides. Bba Adv 2025;7:100136.
[66] Garcia-Lino AM, Alvarez-Fernandez I, Blanco-Paniagua E, Merino G, Alvarez AI. Transporters in the mammary gland-contribution to presence of nutrients and drugs into milk. Nutrients 2019;11:2372.
[67] Hu LR, Brito LF, Luo HP, Chen SK, Johnson JS, Sammad A, et al. Differential responses of physiological parameters, production traits, and blood metabolic profiling between first- and second-parity Holstein cows in the comparison of spring versus summer seasons. J Agric Food Chem 2023;71:11902–20.
[68] Zhu H, Lu XB, Jiang H, Yang ZP, Xu TL. Descriptive statistics and genome-wide copy number analysis of milk production traits of Jiangsu Chinese holstein cows. Animals 2024;14:17.
[69] Tang MH, Weaver NE, Berman LM, Brown LD, Hendricks AE, Krebs NF. Different blood metabolomics profiles in infants consuming a meat- or dairy-based complementary diet. Nutrients 2021;13:388.
[70] Shoji H, Taka H, Kaga N, Ikeda N, Kitamura T, Miura Y, et al. A pilot study of the effect of human breast milk on urinary metabolome analysis in infants. J Pediatr Endocrinol Metab 2017;30:939–46.
[71] Li YC, Li CL, Qi JY, Huang LN, Shi D, Du SS, et al. Relationships of dietary histidine and obesity in Northern Chinese adults, an internet-based cross-sectional study. Nutrients 2016;8:420.
[72] Yang Y, Zheng N, Zhao X, Zhang Y, Han R, Yang J, et al. Metabolomic biomarkers identify differences in milk produced by Holstein cows and other minor dairy animals. J Proteom 2016;136:174–82.
[73] Zhao J, Stewart ID, Baird D, Mason D, Wright J, Zheng J, et al. Causal effects of maternal circulating amino acids on offspring birthweight: a mendelian randomisation study. Ebiomedicine 2023;88:104441.
[74] Liu ZX, Jiang AY, Lv XK, Zhou CS, Tan ZL. Metabolic changes in serum and milk of Holstein cows in their first to fourth parity revealed by biochemical analysis and untargeted metabolomics. Animals 2024;14:407.