

Electronic supplementary information (ESI)

Materials and Methods

Lipid extraction

1 mL ammonium hydroxide was added to 5 mL breast milk, shaken at 65 ± 5 °C water for 20 min, followed by the addition of 5 mL ethanol, 12.5 mL ether, and 12.5 mL petroleum ether. The mixture was vortexed fully, kept static until the two layers separated, then the supernatant was collected and extracted again with half volume of solvents as described above. The final supernatant was gathered and dried with nitrogen.

Analysis of total FAs

Fatty acid methyl esters (FAMES) were prepared using hexane and potassium hydroxide-methanol referring to the method in AOCS Ce-1b 89 (2007). Subsequently, FAMES were analyzed by fast-gas chromatography equipped with flame ionization detection and a Trace TR-FAME capillary column (60 m $\times$ 0.25 mm $\times$ 0.25 μ m). The speed of nitrogen was set at 1.2 mL/min. The temperature of detection and the injection port were both 250 °C. The heating procedure was as follows: initial column temperature of 60 °C maintained for 3 min, increased to 175 °C at 5°C/min and maintained for 15 min, finally increasing to 220 °C at 2°C/min and held for 10 min.

Analysis of sn-2 FAs

Sn-2 monoglyceride (MAG) was hydrolyzed from triglyceride (TAG) as reported by Luddy. The hydrolysis product was separated by silica gel plates with hexane/ether/acetic acid (50:50:1, vol/vol/vol). The MAG band was scraped and extracted with ether. FAME was prepared and detected with fast-gas chromatography as described above.

Table S1 Fat content (g/dL) according to clinical characteristics

	Increased weight during pregnancy (kg)			Maternal age (years)		Delivery mode	
	≥15	9-15	≤9	≥30	<30	Vaginal	Cesarean
Colostrum	2.85±0.41 ^a	2.38±0.64 ^a	2.96±0.38 ^a	2.70±0.84 ^a	2.47±0.48 ^a	2.55±0.73 ^a	2.77±0.64 ^a
Transitional	3.90±0.89 ^a	3.92±0.68 ^a	3.80±0.83 ^a	3.69±0.68 ^a	3.92±0.77 ^a	3.63±0.76 ^a	3.64±0.88 ^a
1 month	3.34±0.83 ^a	3.99±0.90 ^a	3.75±0.50 ^a	3.58±0.65 ^a	3.35±1.07 ^a	3.82±0.82 ^a	3.39±0.77 ^a
	Pre-pregnancy BMI (kg/m2)			Parity			
	<18.5	18.5-23.9	≥24	1	2		
Colostrum	2.35±0.74 ^a	2.63±0.60 ^a	2.73±0.37 ^a	2.76±0.86 ^a	2.47±0.52 ^a		
Transitional	3.57±1.02 ^a	4.04±0.68 ^a	3.74±0.44 ^a	3.87±0.75 ^a	3.68±0.73 ^a		
1 month				3.65±1.01 ^a	3.30±0.73 ^a		

Data were expressed as mean ± SD. Lower case letters indicate differences among groups under the same classification (different letters indicate significant differences, according to Bonferroni or Dunnett's T post-hoc test, significance: p<0.05).

Table S2. Sn-2 fatty acid composition (% wt/wt) according to the stage of lactation and gestational age

Fatty acids	Gestation group	Colostrum(n=44)	Transitional(n=43)	1 month(n=39)	2 month(n=22)	3 month(n=13)
			)			
MCSFAs	EPT	16.19 ± 2.78 ^{a#}	34.99 ± 3.11 ^{b#}	29.81 ± 5.47 ^{c#}	24.12 ± 3.79 ^{d#}	26.85 ± 2.35 ^{cd#}
	MPT	18.80 ± 2.47 ^{a#&}	32.36 ± 2.60 ^{b#}	29.76 ± 3.76 ^{b#}	28.82 ± 4.68 ^{b#}	20.53 ± 0.89 ^{a&}
	NT	19.41 ± 3.57 ^{a&}	35.45 ± 3.81 ^{b#}	27.14 ± 3.94 ^{c#}	21.99 ± 4.39 ^{ad#}	24.86 ± 2.98 ^{cd#&}
LCSFAs	EPT	58.56 ± 3.02 ^{a#}	49.27 ± 3.86 ^{b#}	50.67 ± 3.25 ^{bc#}	54.70 ± 3.27 ^{c#}	53.75 ± 1.43 ^{bc#}
	MPT	60.11 ± 2.22 ^{a#}	49.18 ± 3.17 ^{b#}	49.82 ± 4.24 ^{b#}	54.41 ± 3.31 ^{c#}	56.35 ± 3.69 ^{ac#}
	NT	59.67 ± 4.78 ^{a#}	46.65 ± 4.51 ^{b#}	51.96 ± 3.88 ^{c#}	50.77 ± 4.17 ^{c#}	54.92 ± 2.21 ^{ac#}
SFAs	EPT	75.19 ± 3.66 ^{a#}	81.63 ± 3.28 ^{b#}	81.00 ± 2.63 ^{bc#}	77.26 ± 2.90 ^{ac#}	80.60 ± 2.07 ^{bc#}
	MPT	79.06 ± 1.95 ^{a&}	81.31 ± 2.32 ^{b#}	80.13 ± 2.78 ^{ab#}	78.42 ± 1.96 ^{ab#}	78.36 ± 3.38 ^{ab#}
	NT	78.57 ± 3.02 ^{a&}	81.71 ± 2.86 ^{b#}	79.66 ± 2.66 ^{ab#}	74.44 ± 3.00 ^{c#}	78.03 ± 3.06 ^{ac#}
C18:1n-9	EPT	9.69 ± 2.25 ^{a#}	5.64 ± 1.61 ^{b#}	6.35 ± 1.48 ^{b#}	7.20 ± 1.28 ^{b#}	6.34 ± 1.17 ^{b#}
	MPT	7.44 ± 1.24 ^{a&}	5.56 ± 1.33 ^{b#}	6.19 ± 2.50 ^{ab#}	6.46 ± 1.91 ^{ab#}	7.04 ± 0.96 ^{ab#}
	NT	7.41 ± 1.22 ^{a&}	5.68 ± 1.70 ^{b#}	6.17 ± 1.27 ^{bc#}	7.14 ± 2.25 ^{ac#}	7.68 ± 2.24 ^{ac#}
C24:1n-9	EPT	0.08 ± 0.03 ^{a#}	0.06 ± 0.02 ^{b#}	0.05 ± 0.02 ^{b#}	0.04 ± 0.02 ^{b#}	0.03 ± 0.01 ^{b#}
	MPT	0.06 ± 0.02 ^{a#}	0.06 ± 0.03 ^{a#}	0.05 ± 0.02 ^{ab#}	0.05 ± 0.01 ^{ab#}	0.03 ± 0.01 ^{b#}
	NT	0.07 ± 0.04 ^{a#}	0.06 ± 0.03 ^{ab#}	0.05 ± 0.02 ^{bc#}	0.04 ± 0.01 ^{bc#}	0.03 ± 0.01 ^{c#}
MUFAs	EPT	13.94 ± 2.29 ^{a#}	9.64 ± 2.44 ^{b#}	10.76 ± 2.24 ^{b#}	11.07 ± 1.59 ^{b#}	9.85 ± 1.56 ^{b#}
	MPT	11.88 ± 1.56 ^{a&}	9.64 ± 1.94 ^{b#}	10.46 ± 1.50 ^{ab#}	10.82 ± 2.60 ^{ab#}	11.52 ± 1.21 ^{ab#}
	NT	11.45 ± 1.46 ^{a&}	9.69 ± 2.41 ^{b#}	10.70 ± 1.83 ^{ab#}	11.25 ± 2.98 ^{ab#}	12.14 ± 2.39 ^{a#}
C18:3n-3	EPT	0.38 ± 0.18 ^{a#}	0.41 ± 0.27 ^{a#}	0.52 ± 0.29 ^{a#}	0.66 ± 0.34 ^{a#}	0.64 ± 0.31 ^{a#}
	MPT	0.35 ± 0.11 ^{a#}	0.57 ± 0.22 ^{ab#}	0.70 ± 0.47 ^{b#}	0.73 ± 0.23 ^{b#}	0.64 ± 0.35 ^{ab#}
	NT	0.39 ± 0.22 ^{a#}	0.46 ± 0.12 ^{a#}	0.67 ± 0.34 ^{b#}	0.76 ± 0.43 ^{bc#}	1.00 ± 0.42 ^{c#}
C22:5n-3	EPT	0.16 ± 0.03 ^{a#}	0.10 ± 0.03 ^{b#}	0.08 ± 0.01 ^{b#}	0.09 ± 0.04 ^{b#}	0.07 ± 0.01 ^{b#}
	MPT	0.16 ± 0.05 ^{a#}	0.09 ± 0.02 ^{b#}	0.11 ± 0.07 ^{b#}	0.08 ± 0.02 ^{b#}	0.09 ± 0.02 ^{b#}
	NT	0.13 ± 0.04 ^{a#}	0.10 ± 0.05 ^{b#}	0.09 ± 0.04 ^{b#}	0.09 ± 0.04 ^{b#}	0.07 ± 0.03 ^{b#}
C22:6n-3	EPT	0.31 ± 0.09 ^{a#}	0.26 ± 0.07 ^{ab#}	0.23 ± 0.13 ^{ab#}	0.19 ± 0.13 ^{b#}	0.15 ± 0.07 ^{b#}
	MPT	0.27 ± 0.08 ^{a#}	0.28 ± 0.08 ^{a#}	0.24 ± 0.10 ^{a#}	0.21 ± 0.06 ^{a#}	0.19 ± 0.10 ^{a#}
	NT	0.28 ± 0.13 ^{a#}	0.27 ± 0.07 ^{a#}	0.24 ± 0.09 ^{a#}	0.20 ± 0.13 ^{ab#}	0.12 ± 0.04 ^{b#}
n-3LCPUFAs	EPT	0.48 ± 0.12 ^{a#}	0.36 ± 0.09 ^{b#}	0.31 ± 0.14 ^{b#}	0.27 ± 0.16 ^{b#}	0.23 ± 0.09 ^{b#}
	MPT	0.43 ± 0.12 ^{a#}	0.37 ± 0.09 ^{ab#}	0.35 ± 0.17 ^{ab#}	0.29 ± 0.08 ^{b#}	0.27 ± 0.12 ^{b#}
	NT	0.41 ± 0.15 ^{a#}	0.37 ± 0.11 ^{ab#}	0.33 ± 0.12 ^{abc#}	0.29 ± 0.16 ^{bc#}	0.20 ± 0.07 ^{c#}
n-3PUFAs	EPT	0.86 ± 0.21 ^{a#}	0.77 ± 0.31 ^{a#}	0.83 ± 0.34 ^{a#}	0.94 ± 0.38 ^{a#}	0.87 ± 0.23 ^{a#}
	MPT	0.43 ± 0.12 ^{a#}	0.37 ± 0.09 ^{ab#}	0.35 ± 0.17 ^{ab#}	0.29 ± 0.08 ^{b#}	0.27 ± 0.12 ^{b#}
	NT	0.80 ± 0.28 ^{a#}	0.82 ± 0.17 ^{ab#}	0.99 ± 0.29 ^{abc#}	1.05 ± 0.42 ^{bc#}	1.20 ± 0.41 ^{c#}
C18:2n-6	EPT	7.55 ± 2.25 ^{ab#}	6.92 ± 2.31 ^{a#}	7.33 ± 2.20 ^{ab#}	9.78 ± 2.75 ^{b#}	8.26 ± 1.38 ^{ab#}
	MPT	7.21 ± 1.03 ^{a#}	7.65 ± 1.38 ^{a#}	7.81 ± 1.89 ^{a#}	8.34 ± 1.80 ^{a#}	8.76 ± 0.60 ^{a#}
	NT	7.85 ± 1.99 ^{ac#}	6.98 ± 1.81 ^{a#}	8.00 ± 2.39 ^{abc#}	9.90 ± 3.19 ^{b#}	9.59 ± 2.62 ^{bc#}
C20:4n-6	EPT	0.36 ± 0.14 ^{a#}	0.23 ± 0.09 ^{b#}	0.19 ± 0.09 ^{b#}	0.19 ± 0.03 ^{b#}	0.15 ± 0.03 ^{b#}
	MPT	0.32 ± 0.10 ^{a#}	0.21 ± 0.13 ^{b#}	0.24 ± 0.08 ^{b#}	0.19 ± 0.06 ^{b#}	0.16 ± 0.01 ^{b#}
	NT	0.29 ± 0.09 ^{a#}	0.27 ± 0.06 ^{a#}	0.20 ± 0.07 ^{b#}	0.18 ± 0.05 ^{b#}	0.14 ± 0.03 ^{b#}
C22:5n-6	EPT	0.09 ± 0.01 ^{a#}	0.05 ± 0.02 ^{b#}	0.03 ± 0.01 ^{c#}	0.02 ± 0.00 ^{c#}	0.03 ± 0.02 ^{c#}
	MPT	0.09 ± 0.03 ^{a#}	0.05 ± 0.01 ^{b#}	0.04 ± 0.01 ^{c#}	0.02 ± 0.00 ^{cd#}	0.01 ± 0.00 ^{d#}
	NT	0.10 ± 0.03 ^{a#}	0.06 ± 0.02 ^{b#}	0.04 ± 0.02 ^{b#}	0.02 ± 0.01 ^{c#}	0.02 ± 0.01 ^{c#}
n-6LCPUFAs	EPT	1.29 ± 0.34 ^{a#}	0.65 ± 0.14 ^{b#}	0.53 ± 0.10 ^{bc#}	0.44 ± 0.05 ^{bc#}	0.37 ± 0.09 ^{c#}
	MPT	1.15 ± 0.30 ^{a#&}	0.62 ± 0.18 ^{b#}	0.59 ± 0.11 ^{bc#}	0.43 ± 0.08 ^{bc#}	0.37 ± 0.02 ^{c#}
	NT	0.99 ± 0.26 ^{a&}	0.71 ± 0.13 ^{b#}	0.58 ± 0.18 ^{c#}	0.44 ± 0.07 ^{cd#}	0.37 ± 0.06 ^{d#}

n-3PUFAs	EPT	8.85 ± 2.40 ^{ab#}	7.57 ± 2.39 ^{a#}	7.86 ± 2.21 ^{ab#}	10.22 ± 2.78 ^{b#}	8.64 ± 1.40 ^{ab#}
	MPT	8.36 ± 1.08 ^{a#}	8.27 ± 1.38 ^{a#}	8.40 ± 1.89 ^{a#}	8.77 ± 1.85 ^{a#}	9.13 ± 1.40 ^{a#}
	NT	8.84 ± 2.06 ^{ab#}	7.69 ± 1.84 ^{a#}	8.58 ± 2.36 ^{a#}	10.68 ± 2.54 ^{b#}	9.95 ± 2.62 ^{ab#}
PUFAs	EPT	9.70 ± 2.43 ^{ab#}	8.34 ± 2.53 ^{a#}	8.68 ± 2.49 ^{ab#}	11.16 ± 3.10 ^{b#}	9.50 ± 1.49 ^{ab#}
	MPT	9.14 ± 1.13 ^{a#}	9.21 ± 1.52 ^{a#}	9.44 ± 1.97 ^{a#}	9.78 ± 2.07 ^{a#}	10.04 ± 1.65 ^{a#}
	NT	9.64 ± 2.27 ^{ab#}	8.51 ± 1.93 ^{a#}	9.57 ± 2.58 ^{ab#}	11.68 ± 2.52 ^{b#}	11.15 ± 2.70 ^{b#}

Data were expressed as mean ± SD. EPT-early preterm, MPT-moderate preterm, NT-near term, representing that infants' gestational age are 28^{0/7}–31^{6/7}, 32^{0/7}–33^{6/7} and 34^{0/7}–36^{6/7} weeks, respectively. xM, x-month mature milk. SFAs, saturated fatty acids. MCSFAs, medium chain SFAs. LCSFAs, long chain SFAs. MUFAs, monounsaturated fatty acids. PUFAs, polyunsaturated fatty acids. LCPUFAs, long chain PUFAs. Lower case letters and symbols indicate differences among five stages of lactation milk and among gestational age groups respectively (different letters or symbols indicate significant differences, according to Bonferroni or Dunnett's T post-hoc test, significance: p<0.05).

Table S3. The main difference of sn-2 fatty acids caused by maternal characteristics (PLS-DA, VIP>1)

Factors		R ² X	R ² Y	Q ²	FA and VIP												
Lactation time	C vs T	0.34	0.859	0.8	10:0	12:0	16:0	22:4n6	22:2n6	22:5n-6	18:1n7	20:0	22:5n-3	18:0	18:1n9	14:0	
					1.78	1.76	1.51	1.49	1.40	1.38	1.33	1.33	1.19	1.18	1.15	1.12	
	C vs M	0.315	0.858	0.817	22:5n6	22:4n6	22:2n6	10:0	12:0	20:3n6	20:4n6	22:5n3	20:0	18:1n7	16:0	24:1n9	
					1.82	1.72	1.70	1.62	1.50	1.40	1.27	1.25	1.22	1.17	1.11	1.08	
	T vs M	0.307	0.573	0.398	22:5n6	22:2n6	18:0	20:3n6	22:4n6	14:0	12:0	6:0	16:1n9	18:1n9	24:1n9	16:0	10:0
					1.90	1.43	1.39	1.36	1.32	1.32	1.25	1.22	1.09	1.08	1.07	1.04	1.04
Gestational age	EPT vs MPT	0.276	0.895	0.549	20:3n6	22:0	12:1	22:4n6	15:0	18:1n7	22:5n3	13:0	22:2n6	6:0	14:1n5	22:6n3	18:3n6
					1.66	1.52	1.42	1.42	1.40	1.40	1.39	1.29	1.25	1.21	1.14	1.01	1.01
	EPT vs NT	0.261	0.861	0.489	20:1n9	18:3n6	18:1n9	20:3n6	22:5n3	18:1n7	22:4n6	6:0	20:4n6	22:1n9	22:5n6	22:6n3	
					2.12	1.67	1.56	1.52	1.41	1.38	1.32	1.30	1.19	1.18	1.06	1.03	
Maternal BMI	LB vs NB	0.424	0.788	0.245	16:1n7	18:1n7	17:0	12:0	15:0	22:5n6	10:0	22:1n9	18:2n6	20:1n9			
					1.76	1.74	1.67	1.64	1.35	1.30	1.29	1.18	1.16	1.06			
Maternal age	LA vs HA	0.283	0.659	0.188	18:3n3	22:6n3	22:5n3	22:5n6	20:2n6	18:1n7	14:1n5	20:4n6	13:0	8:0	24:1n9	22:4n6	22:1n9
					1.81	1.70	1.48	1.33	1.31	1.29	1.25	1.10	1.09	1.05	1.05	1.04	1.00

C, colostrum (4 ± 3 days); T, transitional milk (11 ± 3 days); M, mature milk (27–63 days). EPT-early preterm, MPT-moderate preterm, NT-near term, representing that infants' gestational age are 28^{0/7}-31^{6/7}, 32^{0/7}-33^{6/7} and 34^{0/7}-36^{6/7} weeks respectively. BMI, body mass index; LB, lower BMI (<19); NB, normal BMI (19–24). LA, lower age (< 30 years old); HA, higher age (≥ 30 years old); PLS-DA, partial least-squares-discriminate analysis; VIP, variables important in the projection.

Table S4. Sn-2 fatty acids composition in preterm colostrum milk from women of different age

Maternal age (years)	<30	≥30
18:3n-3	0.30±0.11 ^b	0.47±0.21 ^a
22:5n-3	0.12±0.03 ^b	0.16±0.04 ^a
22:6n-3	0.22±0.07 ^b	0.34±0.12 ^a
ΣLCPUFAs n-3	0.34±0.09 ^b	0.49±0.15 ^a
ΣLCPUFAs n-6	1.11±0.27 ^a	0.95±0.24 ^a

Data were expressed as mean ± SD. LCPUFAs, long chain PUFAs. Lower case letters and symbols indicate differences between 2 age groups (different letters indicate significant differences, according to Bonferroni or Dunnett's T post-hoc test, significance: p<0.05)

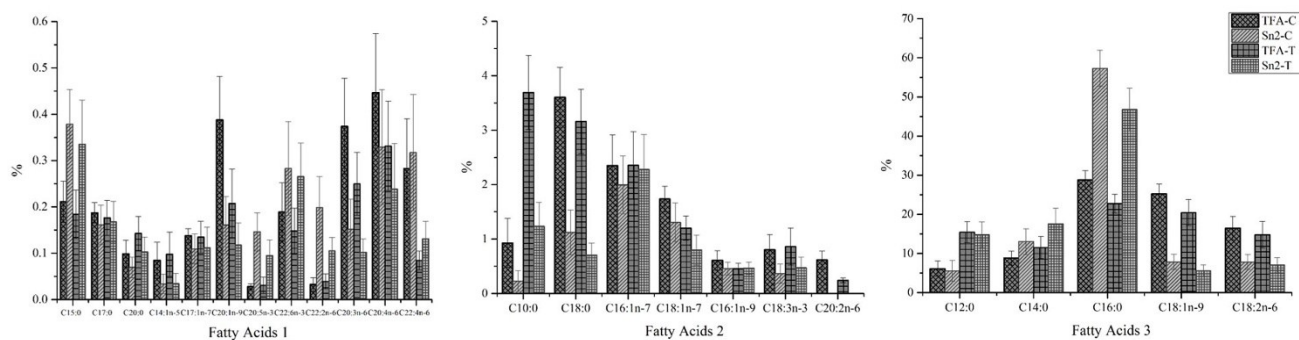


Figure S1 The positional distribution of fatty acids. C, colostrum; T, transitional milk; TFA, total fatty acids; Sn2, sn-2 fatty acids.

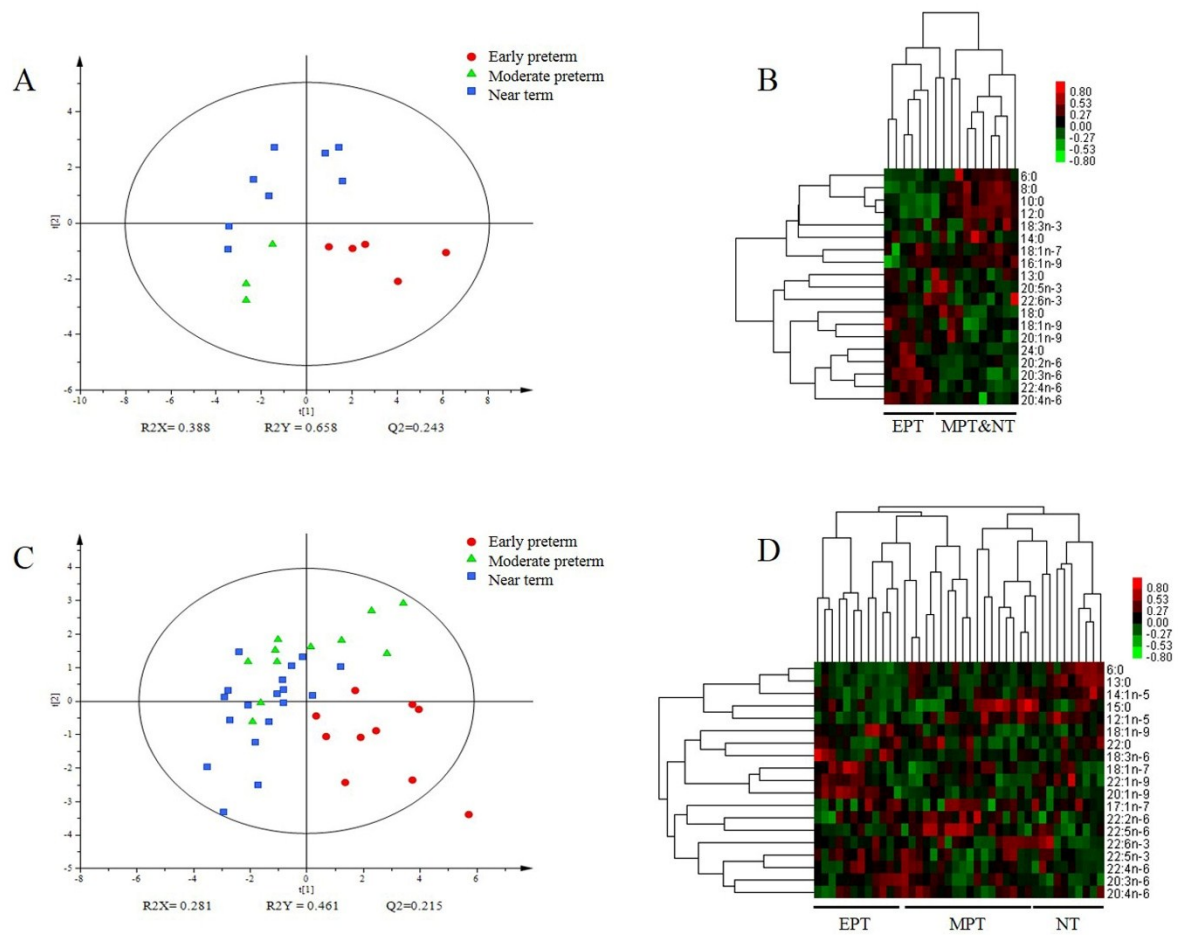


Figure S2 Score plots of PLS-DA and dendrogram based on total (A, B) or sn-2 (C, D) fatty acids composition of preterm milk according to gestational age. EPT, early preterm; MPT, moderate preterm; NT, near term.

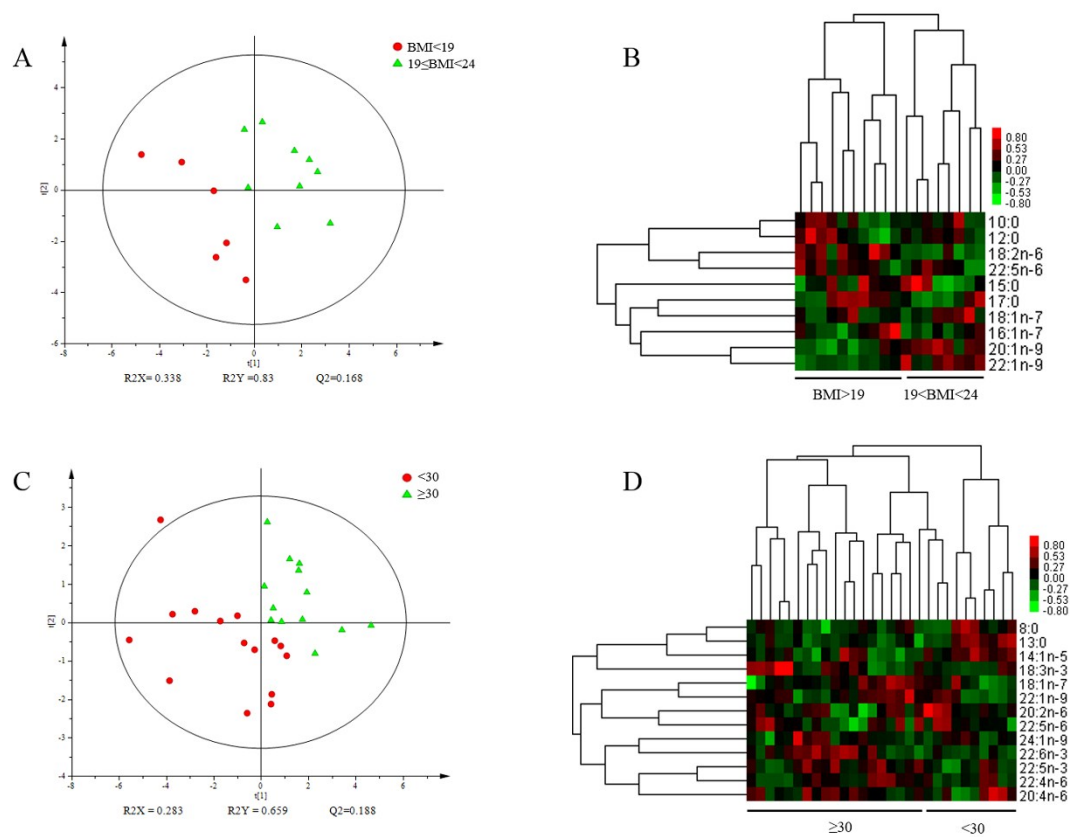


Figure S3 Score plots of PLS-DA and dendrogram based on sn-2 fatty acids composition of preterm milk according to pre-pregnancy BMI (A, B) and age (C, D).

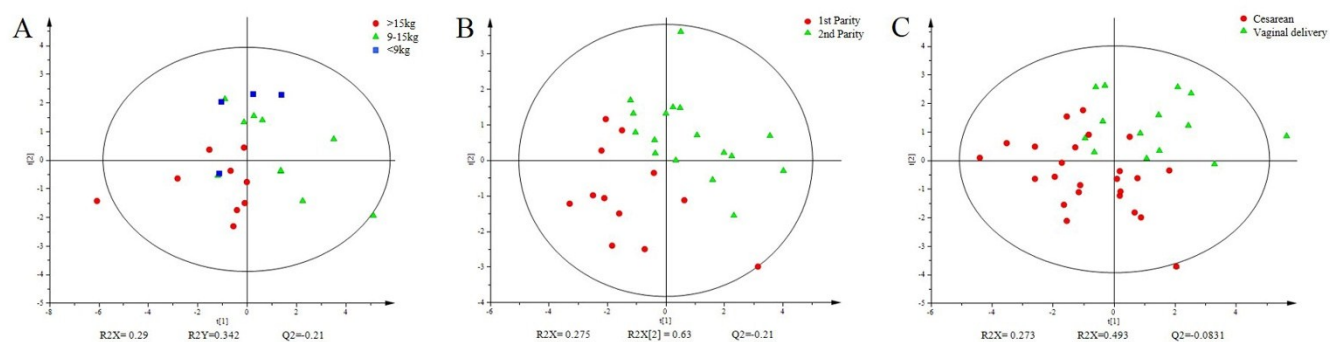


Figure S4 Score plots of PLS-DA based on sn-2 fatty acids composition of preterm milk according to maternal weight gain during pregnancy (A), parity (B) and delivery mode(C).

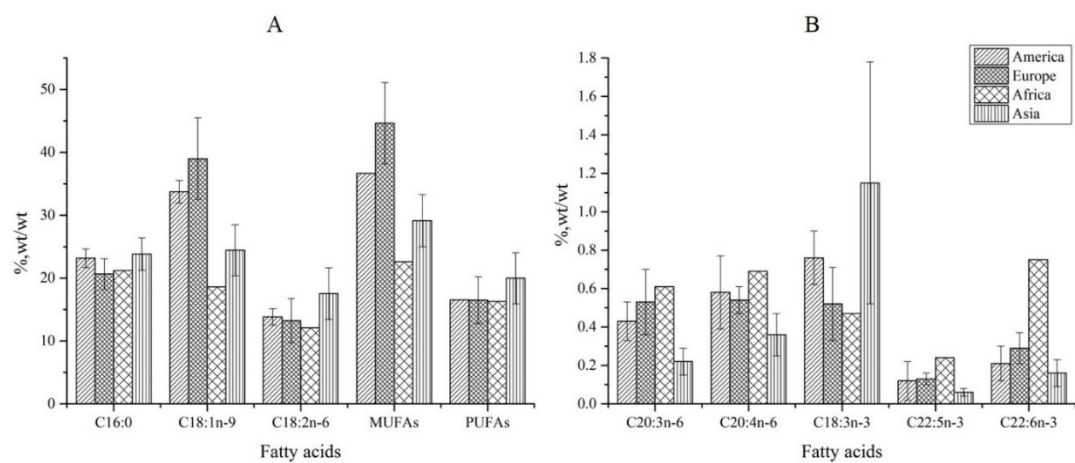


Figure S5 Comparison of the main (A) and key (B) fatty acids composition in preterm milk from different regions.