Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

Effects of excessive body fat on colostrum lipid patterns: overweight/obese *vs*. normal weight mothers

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ARTICLE INFO

Article history:
Received 16 November 2023
Received in revised form 25 December 2023
Accepted 4 February 2024

Keywords:
Overweight/obesity
Colostrum
Maternal diet
Fat reserves
Glycerides
Phospholipids
Sphingolipids

ABSTRACT

Prenatal overweight/obesity (OW/OB) can alter colostrum lipid patterns, thereby affecting the lipid metabolism and even the cognitive and healthy development of infants. However, studies on changes in colostrum lipids in the context of OW/OB are limited, particularly for glycerides and polar lipids. Therefore, this study investigated the influence of maternal prenatal weight on colostrum in lipid subclasses and molecular species. The concentration of triacylglycerols (TAGs) in the colostrum of the OW/OB group (35 894.43 mg/L) was higher than that of the normal weight (NW) group (26 639.20 mg/L), suggesting that colostrum from OW/OB mothers could provide more energy to their infants. Further analysis of the fatty acid composition of TAGs revealed that elevated maternal body weight enhanced the concentration of TAGs containing saturated or *n*-6 fatty acids and shortened the carbon number of TAGs. Docosahexaenoic acid (DHA)/arachidonic acid (AA)/choline-containing lipids, such as DHA-containing TAGs, AA/DHA-containing phosphatidylethanolamine, and choline-containing phospholipids, were present in higher levels in the colostrum of OW/OB mothers than NW mothers. However, the concentrations of palmitic acid-containing TAGs, linoleic acid-containing TAGs, dihomo-*γ*-linolenic acid-containing TAGs, and polar lipids and the ratio of TAGs containing *n*-6 fatty acid/*n*-3 fatty acid were significantly higher in the colostrum of OW/OB mothers than in that of NW mothers. The fatty acid composition and sphingoid bases of sphingolipids were also altered due to elevated body weight. In conclusion, OW/OB affects colostrum lipids with respect to composition, concentration, and percentage. Although the colostrum of healthy OW/OB mothers can provide sufficient DHA/AA/choline-containing lipids to their infants, normalization of body weight and fat reserves should be considered as a strategy for high-quality human milk lipids.

1. Introduction

Overweight/obesity (OW/OB) conditions in mothers present significant maternal health concerns worldwide because they increase the risks of embryo arrest, intrauterine distress, preterm birth, maternal diabetes, hypertension, and eclampsia. OW/OB is caused by an energy imbalance between the calories consumed and calories expended[1]. Significant differences in dietary habits and fat reserves are observed between OW/OB and normal weight (NW) individuals. Among human milk components, lipids exhibit the greatest variations because of differences in maternal diet, body mass index (BMI), parity and duration of pregnancy[2-3]. Maternal diet has a persistent and significant influence on milk lipids, whereas fat reserves only significantly affect lipids at the initial stages of lactation[4]. OW/OB

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Peer review under responsibility of Tsinghua University Press.

Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2024.9250193

can induce metabolic changes in the liver, leading to a reduction in the ability of ingested lipids to undergo oxidation due to maternal insulin resistance and postprandial inflammation[5]. In addition, OW/OB decreases the levels and synthesis of polyunsaturated fatty acids (PUFAs) due to liver steatosis and oxidative stress[6] and also impairs the capacity for maternofetal lipid transfer, particularly for PUFAs[7]. Colostrum is the first milk secreted by mammary glands within 0–5 days after childbirth. Notably, pregnant women begin producing colostrum between 12 and 18 weeks of pregnancy. The colostrum amount produced by most mothers ranges from 15 mL to 30 mL within the first 24 h of delivery. Therefore, colostrum lipids are primarily influenced by maternal prenatal diet, fat storage in adipose tissue, and liver metabolism[8-9].

Colostrum is formed by the action of pregnancy hormones produced by the placenta[10]. It is an ideal nutritional supplement for newborns, and its concentrated nutrients help establish a healthy gut by preventing the absorption of harmful bacteria[11]. Additionally, it is rich in antibodies that enhance the baby's immune system[12]. As the first food of the newborn, colostrum constitutes the initial postnatal substrate supply to exclusively breastfed infants, and the abundant immune and growth factors and other bioactive compounds in colostrum are essential for the overall health, growth, and vitality of newborns[13]. Colostrum is easier to digest because of the lower lipid constituents than mature milk (2% *vs*. 3.5%); therefore, it is easier to digest[14]. The lipids in colostrum are contained within fat globules composed of a glyceride core and a membrane consisting of three layers of polar lipids (PLs), including glycerol phospholipids and sphingolipids. The predominant lipids in colostrum are triacylglycerols (TAGs), whereas the main functional lipids are glycerol phospholipids and sphingolipids. Glycerides, including TAGs and diacylglycerols (DAGs), provide 45%–50% of the energy required by breastfed infants. PLs are structural components of biological membranes, which maintain cell permeability, regulate cell cycle signaling, promote lipid metabolism, enhance immune regulation, and improve cognitive ability[15]. Additionally, lipids in colostrum serve as an important means to deliver some of its beneficial biologically active substances[16]. Colostrum lipids also play a critical role in maternal-infant communication and can strongly impact adipose tissue development, inflammatory response, and central nervous system development and function in infants[8,17-21]. Although the effects of the maternal weight status on the secretion and quality of lipids in colostrum are superficial, the effects on fetal and infant health, nervous system development, and cognitive ability are considerably great[8,22]. Hence, this study selected colostrum to investigate the effect of prenatal OW/OB on lipid patterns.

Currently, limited data are available on the effect of the maternal weight status on colostrum lipids, and the available studies have mostly investigated the fatty acid content and composition. However, although free fatty acids are important intermediates of lipid metabolism, they constitute only a small proportion of colostrum lipids (0.08%–0.40%)[15,23]. As colostrum lipids play a key role in the immune system, cognitive development, and bioactive compounds delivery of the newborns, the effect of maternal weight status on the composition, content, and structure of colostrum glycerides, glycerol phospholipids, and sphingolipids must be analyzed beyond the limitation of fatty acids. Therefore, the present study aimed to compare the molecular species, composition, structure, and concentration of glycerides, glycerol phospholipids, and sphingolipids in the colostrum of OW/OB and NW mothers to determine the impact of maternal weight status on colostrum lipid patterns.

2. Materials and methods

2.1 Sample collection

Colostrum samples were provided by 86 volunteers who participated in the China maternal and infant nutrition health cohort study. The participants were categorized into three categories based on their BMI (kg/m^2) as per the definition established by the World Health Organization: NW (18 to < 25), OW (25 to < 30), and OB (≥ 30). Of the 86 participants, 40 were classified as NW and 46 were classified as OW/OB. All participants had normal physical indicators, except for their body weight, did not participate in any weight-loss treatments, and had a healthy lifestyle (no regular smoking, drinking, or drug use), and their infants were delivered at term without congenital or genetic diseases. Before collecting milk, the participants were instructed to empty one breast from 6 AM to 7 AM. Then, milk from the same breast was collected between 9 AM and 11 AM after warming the breast with a hot towel. The milk was collected at home or the hospital using a breast pump. The collected milk was mixed, subpackaged into 1 mL frozen storage tubes, and stored at –80 °C. The samples were transported *via* a cold chain to the laboratory for analysis.

The study has been ongoing since 2016 and was approved by the Ethics Committee of Beijing Ditan Hospital Affiliated with Capital Medical University (#2015-027-01). All participants provided informed consent.

2.2 Reagents and standards

All reagents used in the study were of mass spectroscopic grade and purchased from Thermo Fisher Scientific (USA). ProElut Silica solid phase extraction columns (1 g/6 mL, Cat No.: 63006) were purchased from Dikma Scientific and Technical Corporation (USA). Phospholipid standards, including phosphatidylcholine (PC; 18:0-18:2 *vs*. 14:1-17:0), phosphatidylethanolamine (PE; 18:0-18:2 *vs*. 14:1-17:0), phosphatidylinositol (PI; 16:0-18:1 *vs*. 14:1-17:0), phosphatidylglycerol (PG; 16:0-18:1 *vs*. 14:1-17:0), sphingomyelin (SM; d18:1-24:0 *vs*. d18:1-17:0), and ceramides (Cer; d18:1-24:1 *vs*. d18:2-24:0), were purchased from Avanti Polar Lipids Inc. (USA). The TAG 16:0-18:1-18:1 and TAG 16:0-18:1-18:1 (d5) standards were purchased from Larodan AB (Sweden) and Shanghai ZZBIO Co., Ltd. (China), respectively.

2.3 Lipid extraction from colostrum samples

Lipid extraction was conducted according to a previously described procedure[23]. Briefly, the standards were first dissolved in methanol-dichloromethane (1:1, *V*/*V*) containing 10 mmol/L ammonium acetate at concentrations similar to those in human milk. Internal standards, including 20 μL of 4.00 g/L TAG 16:0-18:1-18:1 (d5), 20 μL of 10.38 mg/L PC 14:1-17:0, 20 μL of 9.64 mg/L PE 14:1-17:0, 8 μL of 10.22 mg/L PI 14:1-17:0, 4 μL of 9.81 mg/L PG 14:1-17:0, 20 μL of 10.00 mg/L SM d18:1-17:0, and 4 μL of 10.00 mg/L

Cer d18:2-24:0, were added to 200 μL of colostrum sample. 2 mL of methanol, 900 μL of dichloromethane, and 200 μL of ultrapure water were added to the mixture, which was then agitated by shaking for 10 s. The organic and aqueous phases were separated by subsequent addition of 900 μL dichloromethane and 200 μL ultrapure water, followed by shaking for 10 s and centrifugation at 3 300 × *g* for 15 min. The organic phase was isolated and collected using a pipette and the phase separation step was repeated by mixing the organic phase with 2.2 mL of methanol, 600 μL of dichloromethane, and 1 mL of ultrapure water, followed by centrifugation at 3 000 × *g* for 10 min. The aqueous phase was mixed with 1.8 mL dichloromethane and centrifuged at 3 300 × *g* for 15 min to obtain the remaining organic phase.

The organic phase samples were dried under a nitrogen gas flow and dissolved in 1 mL methanol-dichloromethane (1:1, *V*/*V*) containing 10 mmol/L ammonium acetate. For glyceride analysis, the organic phase samples were further diluted (200 times) by adding 50 μL sample to methanol-dichloromethane (1:1, *V*/*V*) containing 10 mmol/L ammonium acetate.

ProElut Silica (1 g/6 mL) solid-phase extraction columns were activated with 3 mL of *n*-hexane, and 950 μL of the diluted samples were poured into the columns. Non-polar lipids were removed by 3 mL of hexane and diethyl-ether elution (8:2 and 1:1, *V*/*V*, respectively), and PLs were obtained by adding 4 mL of methanol followed by 2 mL of methanol and 2 mL of a dichloromethane-methanol-water elution (3:5:2, *V*/*V*)[24]. The recovered fractions were dried under a gentle stream of nitrogen and dissolved in 190 μL methanol-dichloromethane (1:1, *V*/*V*) containing 10 mmol/L ammonium acetate for PL analysis.

2.4 Detection conditions of lipids

Glycerides and PLs were separated based on two different liquid phase conditions using ultra-performance liquid chromatography (AB Sciex, ExionLC) and phenomenex liquid chromatography columns with dimensions of 50 mm × 3 mm and 150 mm × 4.6 mm (Kinetex® 2.6 μm C_{18} 100 Å), respectively. The injected volume was 2 μL, the flow rates were 0.3 and 0.8 mL/min, and the column temperature was set at 50 and 30 °C for glycerides and PL analysis, respectively. Mobile phase A was methanol/acetonitrile/water (1:1:1, *V*/*V*) containing 5 mmol/L ammonium acetate, and mobile phase B was isopropanol containing 5 mmol/L ammonium acetate. The elution gradient is shown in Table S1[25].

Glycerides and PLs were detected using quadrupole time-of-flight (Q-TOF) mass spectrometry (MS, AB Sciex, 6600) with electrospray ionization (ESI), and the MS analysis was performed coupled to liquid chromatography. The positive ionization mode was used to detect glycerides and sphingolipids, whereas the negative ionization mode was used for glycerol phospholipids. The ESI ion source and MS were operated in a scan range of 50–1 300 Da. The ion source pressure, curtain gas pressure, and ion source temperature were 60 psi, 35 psi, and 650 °C, respectively. The declustering potential, collision energy, and ion source spray voltage were +80 and –80 V, +10 and –10 V, and +5 500 and –4 500 V in the positive and negative ionization modes, respectively.

2.5 Qualitative and quantitative analysis of lipids

Glyceride data were qualitatively and quantitatively analyzed based on fragment ions using mass spectrometry-data independent analysis (MS-DIAL) software. Glycerides were detected in positive mode with the adduct ion of NH_4^+. In positive mode, the neutral loss fragments of fatty acyls were observed and presented as $[DAG]^+$ fragments. Therefore, TAGs were identified based on positively charged molecular ions $[M + NH_4]^+$ and $[DAG]^+$ (or $[M - RCOO + H]^+$) fragments with neutral loss of fatty acyl moieties. Pearkview (version 2.2) and Multiquant (version 3.0.2) software were used for qualitative and quantitative analysis of PC, PE, PI, PG SM, and Cer, respectively. PE, PI, and PG containing H^- adducts and PC containing CH_3COO^- adducts were identified based on the adductive ion mass, head group fragments, and fatty acyl residues in the negative ion mode. SM was detected based on the mass of the parent ion and a characteristic fragment ion of *m*/*z* 184, which was obtained by the neutral loss of the collision-activated sphingolipids. Cer were identified based on the parent ion mass and specific product ions *m*/*z* 264 or 266 produced by the neutral loss of fatty acyl and the dissociation of the amide bond. Regression equations built with internal and external standards were used to quantify the concentrations, as shown in Table S2[25].

2.6 Structure analysis of DAGs

The positional structure of DAGs could not be directly identified using the lipid analysis method adopted in this study. However, the distribution of fatty acyls on the glycerol skeleton could be indirectly analyzed based on the different dissociation tendencies of fatty acyls at the *sn*-2 and *sn*-1,3 positions in a DAG[26]. The fatty acyls located in the outer position (*sn*-1,3 position) tend to be more easily lost than those in the inner position[27]. Monoacylglycerols (MAGs) can be obtained by the dissociation of $[DAG + NH_4]^+$, which loses a fatty acyl group. One DAG molecule species can produce two types of MAG dissociation products, defined here as MAG^+1 and MAG^+2. In general, when fatty acyls are located at the *sn*-1 and *sn*-3 positions of DAGs, the two fatty acyls have the same tendency to dissociate, thus presenting a similar MAG^+ fragment intensity during MS analyses. When DAG contains an *sn*-2 fatty acyl, the fragment intensity of MAG^+ containing an *sn*-2 fatty acyl is higher than that of MAG^+ containing *sn*-1/3 fatty acyl because the *sn*-2 fatty acyl does not dissociate as easily as the *sn*-1/3 fatty acyl in DAGs. Therefore, the ratio of MAG^+1 to MAG^+2 was used to infer the location distribution of fatty acyls in DAGs. Specifically, a ratio of MAG^+1 to MAG^+2 close to "1" indicates that the DAG contains two fatty acyls at *sn*-1 and *sn*-3 positions, whereas ratios that deviate from "1" indicate that the DAG contains *sn*-2 and *sn*-1/3 fatty acyl. Moreover, ratio values of MAG^+1 to MAG^+2 showing greater deviateions from "1" indicate more *sn-2* fatty acyl in DAGs.

2.7 Statistical analysis

The colostrum concentration data from the OW/OB and NW groups were analyzed by performing one-factor statistical analysis using Metaboanalyst (https://www.metaboanalyst.ca/). The population characteristics and lipid species and their concentrations and percentages were compared between the OW/OB and NW groups using IBM SPSS Statistics software (version 25.0), with an independent sample *t*-test or nonparametric test to analyze the two sample groups and a chi-square test to analyze percentage differences of infant sex. The results are presented as the mean ± standard deviation or percentages. Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1 Molecular species, concentrations, and percentages of lipid subclasses

The maternal and infant characteristics are listed in Table 1. The maternal age and cesarean delivery rates in the OW/OB group were significantly higher than those in the NW group, which may be attributed to subfertility and a reduction in the birth canal space due to fat accumulation in the OW/OB group[28]. The predominant lipid forms or functional lipids in the colostrum of OW/OB and NW mothers, including TAGs, DAGs, PC, PE, PI, PG, SM, and Cer, were identified and analyzed using ultra-performance liquid chromatography/Q-TOF MS. The species numbers of TAGs, DAGs, PC, PE, PI, PG, SM, and Cer in the colostrum collected from the OW/OB and NW mothers are illustrated in Fig. 1A. Only the species numbers of PI and Cer showed significant differences.

Table 1
Maternal and infant characteristics.

Study population	OW/OB ($n = 46$)	NW ($n = 40$)	Significance
Mother			
Age (years)	32.87 ± 4.01	29.36 ± 5.05	*
Height (kg)	1.62 ± 0.16	1.61 ± 0.06	NS
Pre-pregnancy weight (kg)	74.27 ± 11.76	59.62 ± 9.50	**
Pre-pregnancy BMI (kg/m^2)	28.23 ± 3.45	23.24 ± 3.58	**
Gestational weight gain (kg)	12.21 ± 5.32	13.35 ± 5.55	NS
Postpartum weight loss (kg)	10.16 ± 3.15	6.74 ± 2.10	**
Cesarean delivery (%)	45.00	31.25	*
Infant			
Birth height (cm)	49.90 ± 2.80	50.45 ± 1.27	NS
Birth weight (kg)	3.38 ± 0.70	3.38 ± 0.67	NS
Male infant (%)	51.66	59.18	*

Note: ** Represents a highly significant difference ($P < 0.01$), * represents a significant difference ($P < 0.05$), NS represents no significant difference. The results are presented as mean ± standard deviation or percentages.

The concentration of TAGs and DAGs in the colostrum of OW/OB mothers ((36 147.51 ± 12 532.7) mg/L) was significantly higher than that in the colostrum of NW mothers ((26 798.61 ± 17 269.12) mg/L), as shown in Fig. 1B, suggesting that the colostrum derived from OW/OB mothers can provide higher energy than that from NW mothers. According to the Institute of Medicine guidelines, gestational weight gain is associated with pre-pregnancy BMI, in which NW, OW, and OB females should gain 11.5–16.0, 7.0–11.5, and 5.0–9.0 kg, respectively. In the present study, the gestational weight gain was (12.21 ± 5.32) kg in the OW/OB group and (13.35 ± 5.55) kg in the NW group (Table 1), with no significant differences. The OW/OB mothers had gestational weight gain exceeding the recommended values, indicating prenatal excess

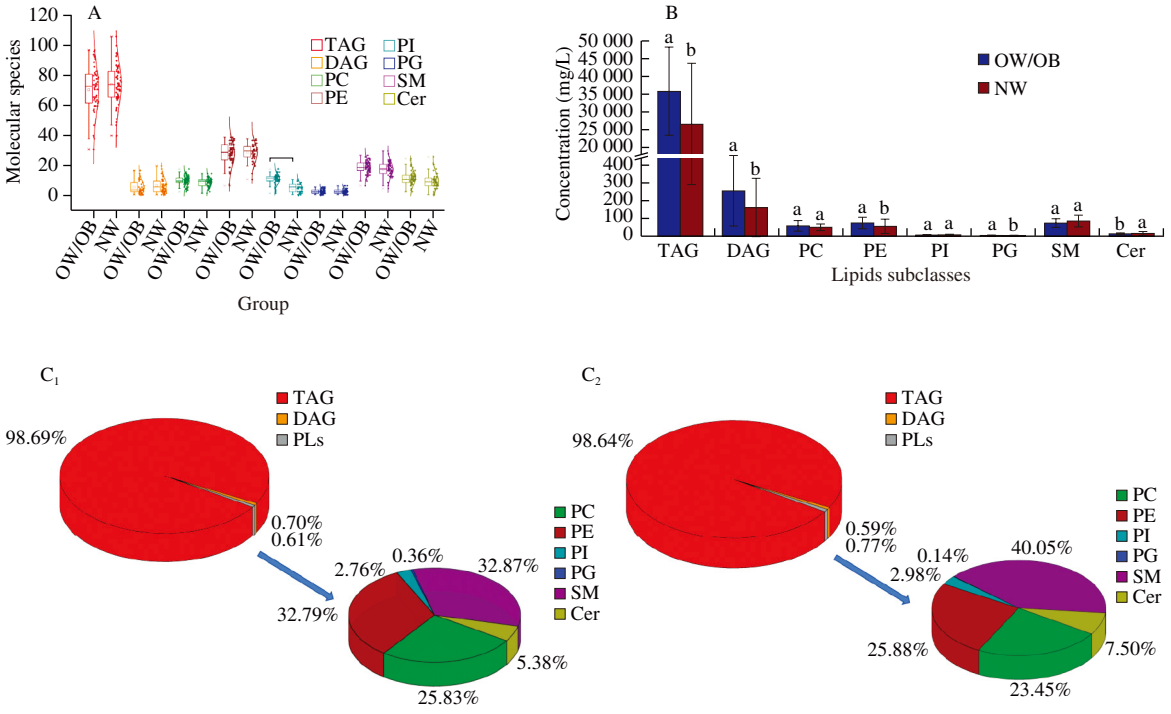


Fig. 1 Molecular species number (A), concentration (B), and proportion (C) of lipid subclasses in the colostrum of OW/OB (C_1) and NW (C_2) mothers. * Represents a significant difference ($P < 0.05$). Different lowercase letters represent significant differences ($P < 0.05$) between two groups in the same subclass. PC includes LPC and PC; PE includes LPE and PE; PI includes LPI and PI; and PG includes LPG and PG.

nutrition, which may have contributed to the significantly higher levels of TAGs and DAGs in their colostrum. The concentrations of PC, PE, PI, PG, SM, and Cer in the colostrum were (57.41 ± 28.82), (72.88 ± 32.40), (6.14 ± 3.15), (0.81 ± 0.88), (73.05 ± 24.01), and (11.95 ± 5.86) mg/L in the OW/OB group and (48.51 ± 19.06), (53.55 ± 60.61), (6.16 ± 3.66), (0.29 ± 0.25), (82.87 ± 35.83), and (15.52 ± 10.74) mg/L in NW group, respectively (Fig. 1B). The concentrations of PE and PG and that of Cer were significantly higher and lower ($P < 0.05$) in the colostrum of OW/OB mothers, respectively. Milk lipids take the form of dispersed fat globules with a glyceride core enveloped by a PL trilayer-structured membrane[29]. The concentration of glycerides and PLs in the colostrum of OW/OB mothers was 1.35 and 1.07 times higher than that of NW mothers, respectively. Therefore, the proportion of PLs to glycerides was correspondingly reduced. In male mice, intake of infant formula with a large amount of PL-coated lipid droplets during early life reduces the risk of OB in adult life by preventing excessive hypertrophic growth of white adipose tissue in adulthood, despite unaltered adipogenesis[19]. Infants undergo rapid growth and fat accumulation during the first few months of life, and approximately 45%–50% of the energy consumed by infants is derived from TAGs. The concentration of TAGs in the colostrum of the OW/OB group ((35 894.42 ± 12 333.74) mg/L) was higher than that in the colostrum of NW group ((26 639.20 ± 17 103.63) mg/L), and the physical structure of the fat globules changed. Although no significant differences were observed in the height, weight, and head circumference of infants at birth between the OW/OB and NW groups (Table 1), the effects of these parameters on the short- and long-term health of infants must still be monitored.

The percentages of lipid subclasses in glycerides or PLs are illustrated in Fig. 1C. The maternal body weight status did not affect the percentage of glyceride subclasses but significantly affected the percentage of PL subclasses, especially PE, SM, and Cer.

3.2 Fatty acid composition, concentration, and structure of lipid molecules

3.2.1 Glycerides

TAGs are the primary energy sources and functional substances for the growth and development of infants, and their fatty acids composition and structure affect their nutritional value. The stereo-specific positioning of fatty acids is highly conserved and unusual in human milk TAGs[30], indicating that the fatty acid composition and concentration are the main factors determining their nutritional value.

The results of the *t*-test analysis of TAGs and DAGs with different fatty acid compositions in the colostrum of the OW/OB and NW groups based on one-factor statistical analyses in metaboanalyst are illustrated in Fig. 2A. A total of 33 TAGs were significantly higher in the colostrum of the OW/OB group compared with that of the NW group. Among these, the concentrations of saturated fatty-acyl TAGs (such as TAG 12:0-12:0-14:0, TAG 12:0-16:0-18:0, TAG 14:0-16:0-18:0, 12:0-14:0-16:0, TAG 12:0-14:0-18:0, TAG 16:0-16:0-18:0, and TAG 16:0-18:0-18:0) were significantly higher in the OW/OB group ((1 693.71 ± 753.44) mg/L) than the NW group ((888.52 ± 608.25) mg/L). Similarly, the concentrations of total saturated fatty-acyl TAGs and DAGs in the colostrum were significantly higher in the OW/OB group ((2 004.32 ± 1 207.62) mg/L) than the NW group ((1 014.74 ± 715.36) mg/L) (Fig. 2B). The concentrations of DAGs and TAGs initially increased and then decreased with increasing unsaturated fatty acids of the colostrum of both the OW/OB and NW groups (Fig. 2B). When the unsaturation was < 2, the concentration of colostrum DAGs and TAGs in the OW/OB group was significantly higher than that in the NW group; however, when the unsaturation reached 3, the concentrations of colostrum DAGs and TAGs did not significantly differ between the two groups. These results indicate that the concentration of saturated fatty acyls was significantly higher in the colostrum DAGs and TAGs of OW/OB mothers than in those of NW mothers. The dietary patterns of OW/OB individuals are characterized by high intake of saturated fats, refined carbohydrates, low dietary fiber, and low natural antioxidants[31], which may induce an imbalance in fatty acid intake and thereby influence the concentration of saturated fatty-acyl TAGs or DAGs in human milk[31]. Studies on lipid nutrition have shown that saturated-fat-rich diets can increase the concentration of saturated fatty-acyl-containing TAGs[32]. Additionally, lactating women consuming high-carbohydrate diets exhibit an increase in the synthesis and secretion of $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$ into human milk, whereas the synthesis and secretion of C_{18} unsaturated fatty acids derived from uptake from the plasma are decreased[30]. A high-saturated fat diet, especially in palmitic acid, can modulate the activity of desaturase enzymes via liver steatosis, a common metabolic condition associated with OB that directly affects the synthesis of PUFAs[8]. Furthermore, fatty acyls at the *sn*-1,3 positions of TAGs are digested by gastric lipases pancreatic lipase-related protein 2 (PLRP2) and bile salt-stimulated lipase (BSSL), which produce *sn*-2 MAGs and free fatty acids. The released free fatty acids are re-esterified to TAGs and incorporated into chylomicrons to be transferred into the bloodstream and absorbed by various organs[15,33]. However, saturated free fatty acids ($C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$) have melting points above body temperature, are poorly absorbed upon release, and form calcium soap with Ca^{2+} in infants. Therefore, elevated saturated fatty acids in the TAGs of OW/OB mothers might impair the absorption of fatty acids and calcium, which causes constipation, especially in newborns[15].

When the carbon number of TAGs was from 38 to 50, the concentration of colostrum TAGs in the OW/OB group was significantly higher than that in the NW group. When the carbon number was between 52 and 58, no significant differences were observed between the two groups. However, when the carbon number of TAGs reached 60, the concentration of colostrum TAGs in the OW/OB group was significantly lower than that in the NW group (Fig. 2C). These findings suggest that maternal weight influenced the chain length of fatty acyls in TAGs. Additionally, the carbon number of TAGs in the colostrum of OW/OB mothers was significantly lower than that of NW mothers, which is consistent with the known characteristics of the carbon chain length of fatty acids in human milk from OW/OB and NW mothers[22,30].

Maternal dietary habits and metabolic status are associated with the quality of human milk lipid. However, specific fatty acids, such as palmitic acid, linoleic acid, linolenic acid, and docosahexaenoic acid (DHA), particularly affect the quality of human milk. Therefore, the concentrations of TAGs containing these fatty acids in the colostrum of the OW/OB and NW groups were analyzed (Fig. 2D). The concentration of palmitic acid-containing TAGs was significantly

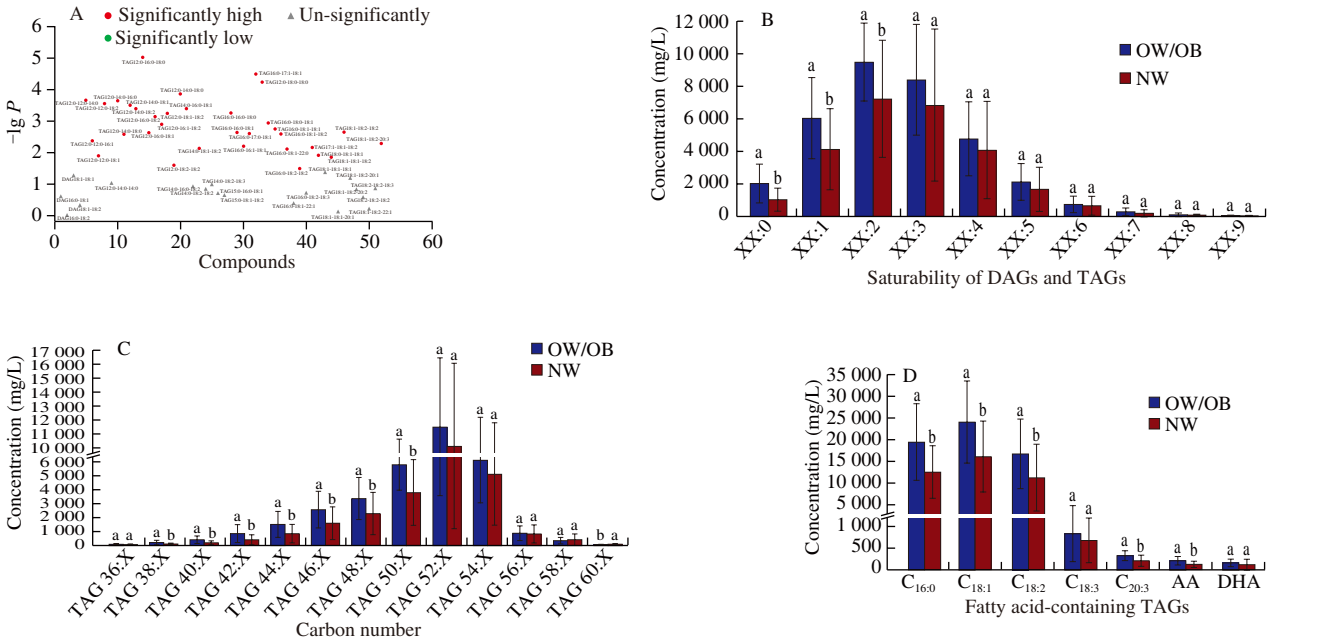


Fig. 2 *t*-Test analysis of TAGs and DAGs with different fatty acid compositions in the colostrum of OW/OB and NW mothers (A). Red, green, and gray dots indicate significantly higher, significantly lower, and not significantly changed lipid concentrations between the colostrum of OW/OB and NW mothers, respectively. Concentrations of DAGs and TAGs with different saturability in the colostrum of the OW/OB and NW groups (B). Concentrations of DAGs and TAGs with different carbon numbers in the colostrum of the OW/OB and NW groups (C). Concentrations of TAGs containing certain fatty acids in the colostrum of the OW/OB and NW groups (D).

higher in the OW/OB group ((19 349.66 ± 8 889.01) mg/L) than in the NW group ((12 404.13 ± 6 097.10) mg/L). This finding is consistent with a previous study that showed that the palmitic acid content in the human milk of OW/OB mothers was significantly higher than that of NW mothers (Table S3)[22]. The concentration of linoleic acid-containing TAGs was significantly higher in the OW/OB group ((16 594.03 ± 8 026.51) mg/L) than in the NW group ((11 077.23 ± 7 823.12) mg/L). Notably, excessive linoleic acid in colostrum is associated with poorer cognitive development in children at 2 and 3 years of age[21]. The concentrations of DHA-containing TAGs in the colostrum were 161.00 and 120.96 mg/L in the OW/OB and NW groups, respectively. Moreover, the concentrations of linolenic acid-containing TAG (DHA precursor) were 838.52 and 680.06 mg/L in the colostrum of the OW/OB and of NW groups, respectively. Although the differences were not statistically significant, the levels of *n*-3 fatty acid-containing TAGs in the colostrum were higher in the OW/OB group than the NW group. These results indicate that the maternal weight status (OW/OB) did not decrease the concentration of *n*-3 family fatty acids in colostrum, which is contrary to previous findings[34-35]. This discrepancy may be due to previous studies comparing percentages rather than concentrations. Furthermore, the concentrations of *n*-6 fatty acid-containing TAGs, such as $C_{18:2}$, $C_{20:3}$, and arachidonic acid (AA), were significantly higher in the OW/OB group than the NW group; however, the concentrations of *n*-3 fatty acid-containing TAGs, such as $C_{18:3}$ and DHA, were not significantly different between the two groups (Fig. 2D). The ratio of TAGs containing *n*-6 fatty acid and *n*-3 fatty acid was 17.14 and 14.25 in the colostrum of OW/OB and NW mothers, respectively. These results are consistent with a previous study showing that the ratio of *n*-6/*n*-3 fatty acid was higher in the human milk of OB mothers than in that of NW mothers (Table S3)[35].

Dihomo-γ-linolenic acid (DGLA, $C_{20:3n-6}$), a PUFA that is less abundant in food, has recently gained importance in distinguishing between healthy and inflammatory tissues[36]. OB is associated with an increase in DGLA levels, and previous studies have demonstrated that the levels of DGLA in the plasma total lipids, PLs, and cholesteryl esters are significantly higher in OW/OB individuals than the controls[36-37]. The concentration of DGLA-containing TAGs and PLs, including TAG 18:1-18:2-20:3 (Fig. 2A) and PE 18:0-20:3 (PE 38:3, Fig. 3), was significantly higher in the OW/OB group than the NW group (Fig. 2D). A similar result was observed in a study of human milk fatty acid profiles from OW/OB and NW mothers by Ellsworth et al.[22]. Therefore, OW/OB status may influence the levels of certain DGLA-containing TAGs or PLs, thus affecting the quality of human milk lipids.

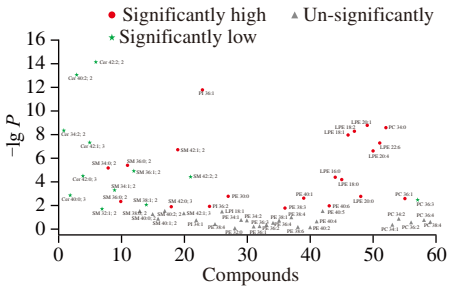


Fig. 3 *t*-Test analysis of PLs with different fatty acid compositions in the colostrum of OW/OB and NW mothers. Red, green, and gray dots indicate significantly higher, significantly lower, and not significantly changed lipid concentrations between the colostrum of OW/OB and NW mothers, respectively.

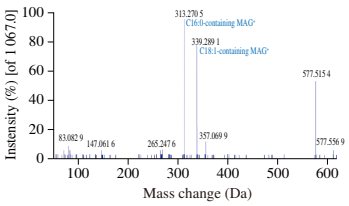
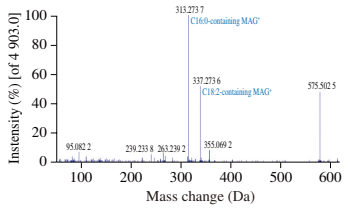
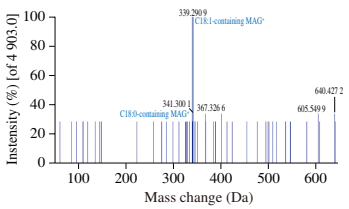
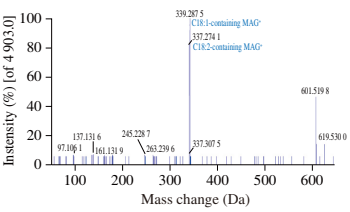
Dietary *sn*-1,3 DAGs promote the β-oxidation of fatty acids and regulate the expression of genes involved in lipid metabolism, thereby reducing the risk of OB, diabetes, and cardiovascular diseases[38]. The

structural differences in DAGs, rather than the fatty acid composition, markedly affect the nutritional behavior of lipids[38]. Therefore, exploring the structural characteristics of DAGs from mothers with different bodyweights is crucial to determine the nutritional value of DAGs. In the present study, the MS^2 fragment intensity and dissociation characteristics of DAGs in the OW/OB and NW groups were determined to understand the positional structure of DAGs (Table 2). The MAG^+ ratios were similar for DAG 34:1, DAG 34:2, and DAG 36:3 in both groups, indicating that maternal BMI has no effect on the position distribution of DAGs on the glycerol skeleton in colostrum (Table 2). The MAG^+ ratio of DAG 36:1 and DAG 36:3 was close to "1," suggesting that fatty acyls are mainly distributed in the *sn*-1 and *sn*-3 positions. In addition, the ratio of MAG^+ of palmitate acid-containing DAG 34:1 and DAG 34:2 deviated from "1", which is consistent with the greater likelihood that palmitate acid in human milk TAGs will be esterified at the *sn*-2 position. This finding indicates that palmitate acyls in DAGs also tend to be esterified at the *sn*-2 position of the glycerol skeleton. Thus, the position distribution of DAGs in the colostrum was not affected by maternal BMI, and the distribution characteristics of palmitate acyls in DAGs were similar to those of TAGs.

3.2.2 Phospholipids

Results from the *t*-test analysis of PLs with different fatty acid compositions in the colostrum showed that 21 and 12 PLs had significantly higher and lower concentrations in the OW/OB and NW groups, respectively (Fig. 3). Most of lysophosphatidylethanolamines (LPEs) and a fraction of the PEs in the colostrum were significantly higher in the OW/OB group compared with the NW group (Fig. 3). The total PE and LPE concentration in the OW/OB group was 19.33 mg/L higher than that in the NW group, and the PE and LPE proportion in the total PL increased by 6.91% (Figs. 1B-C). A recent study on the impact of OB on the PL composition in breast adipose tissue in mice reported that breast adipose tissue from obese mice without tumors had significantly elevated amounts of PE[39]. OB could increase inflammation in breast adipose tissue through dyslipidemia, which may further increase PE level[39]. Phospholipids in human milk are derived from the endoplasmic reticulum, cytoplasm of lactating cells, and apical plasma membrane of mammary cells during its biosynthesis[15]. Therefore, elevated PE in obese breast adipose tissue may contribute to the high level of PE in the colostrum of OW/OB mothers. In addition, DGLA, an indicator of inflammation, in the colostrum was higher in the OW/OB group than the NW

Table 2
MS^2 fragment characteristics of colostrum DAG in the OW/OB and NW mothers under positive ion mode.

Molecular species	Mass (Da)	MAG^+ fragment (MAG^+1 and MAG^+2)	MAG^+ containing fatty acyl	MAG^+1/MAG^+2 of intensity		MS^2 spectrum
				OW/OB	NW	
DAG 34:1	594.52	313.27	$C_{16:0}$	1.30	1.30	
		339.29	$C_{18:1}$			
DAG 34:2	592.51	313.27	$C_{16:0}$	1.50	1.61	
		337.27	$C_{18:2}$			
DAG 36:1	622.55	339.29	$C_{18:1}$	0.99	1.09	
		341.30	$C_{18:0}$			
DAG 36:3	618.52	337.28	$C_{18:2}$	0.97	0.96	
		339.29	$C_{18:1}$			

group. DHA and AA are required for brain tissue incorporation and optimal neurological development in infants, and DHA-containing PLs, especially DHA-containing lysophospholipids, may cross the blood-brain barrier and directly participate in brain biochemical reactions[40-41]. Moreover, PE is the main molecular carrier of phospholipids containing AA or DHA in human milk[23]. The concentrations of PE 40:6, LPE 20:4, and LPE 22:6 in the colostrum were significantly higher in the OW/OB group than the NW group, indicating that the colostrum of healthy OW/OB mothers could provide adequate AA- or DHA-containing PE to their infants.

PC and SM are primarily located in the outer bilayer, the structural material of the cell membrane that maintains cell permeability and signal transmission[15]. Approximately 17% of the choline required by neonates is derived from PC and SM in colostrum[42]. The concentrations of PC and SM in the colostrum did not significantly differ between the OW/OB and NW groups (Fig. 1B), although the total percentage of PC and SM in the colostrum from the OW/OB group was higher than that from the NW group (Fig. 1C). Therefore, although maternal BMI did not affect the content of choline-containing PLs, it did affect the percentage of such PLs in the colostrum.

The primary sphingolipid in human milk is SM, followed by Cer, both of which have beneficial effects on infants, such as reducing inflammatory responses and regulating cell growth, differentiation, and apoptosis[15]. The concentrations of sphingolipids containing different fatty acid compositions or sphingoid bases in the colostrum differed between the two groups (Fig. 3). The predominant sphingoid base was sphingosine (d18:1), followed by dihydrosphingomyelin (d18:0) and 4-hydroxysphingosine (t18:0). The content of t18:0 represents an indicator of a plant-based diet[43]. In the present study, the colostrum of the OW/OB group had significantly lower levels of t18:0-containing Cer, including Cer 40:0;3 (t18:0-22:0), Cer 42:0;3 (t18:0-24:1), and Cer 42:1;3 (t18:0-24:1), which may have been caused by the refined carbohydrates and low fiber diet of OW/OB mothers. However, the colostrum of the OW/OB group showed significantly higher levels of d18:0-containing SM (such as SM 34:0;2, SM 36:0;2, SM 36:0;3, and SM 42:0;3) but significantly lower levels of d18:1-containing SM compared with that of the NW. This suggests that the maternal weight status affected the sphingoid base composition in sphingolipids.

An OB status, or a high-fat diet increases the expression of sphingomyelinases, leading to the accumulation of Cer in plasma, liver, muscle, and other tissues[44-45]. However, the level of Cer in the colostrum was significantly lower in the OW/OB group than the NW group (Fig. 1B), which suggests that the activity of sphingomyelinases was affected by the availability of SM substrates at low concentrations (Fig. S1). Furthermore, dihydroceramide desaturase (Degs1) expression is decreased in adipose tissues of individuals with OB and murine models of genetic and nutritional OB[46], which may block the *de novo* synthesis pathway of Cer (Fig. S1). Ceramide synthases 2 (CerS2) and Degs1 facilitate the synthesis of Cer with very long acyl chains varying from $C_{20:0}$ to $C_{26:0}$, whereas CerS6 and Degs1 catalyze the formation of Cer containing $C_{14:0}$ and $C_{16:0}$ fatty acids[45]. A study on the adipose tissue of C57BL mice reported that OB induced by a high-fat diet increased the levels of C_{16}- or C_{18}-containing Cer but decreased the levels of C_{24}-containing Cer[47]. Therefore, the effect of OB on Cer content may be associated with the length of the acyl chains because in the colostrum of the OW/OB group, significantly low levels of Cer almost always contained C_{22} or C_{24}, including Cer 42:2;2 (d18:1-24:1) and Cer 40:2;2 (d18:1-22:1), as illustrated in Fig. 3.

3.3 Strengths and limitations

The major strengths of this study include the collection of rare colostrum samples (especially from OW/OB mothers) and the analysis of TAG and PL subclasses and molecular species, thus providing data beyond that of the fatty acid level. However, a major limitation of the study is the lack of complete and detailed dietary information about the volunteers, which prevented a correlation analysis of lipids in the colostrum.

4. Conclusions

This study analyzed the characteristics of the colostrum of OW/OB and NW mothers to determine the influence of maternal prenatal weight status on colostrum glycerides and PLs. Although the maternal body weight status did not affect the percentage of glyceride subclasses, it significantly affected the percentage of PL subclasses, especially PE, SM, and Cer. The colostrum of OW/OB mothers could provide more TAGs, saturated fatty acids, and *n*-6 fatty acids to infants than the colostrum of NW mothers. DHA/AA/choline-containing lipids were present in higher levels in the colostrum of OW/OB mothers than NW mothers, whereas the percentages of these lipids were lower in the OW/OB group than the NW group. Furthermore, the maternal weight status did not influence the position distribution of fatty acyls in DAGs, which showed similar distribution characteristics as TAGs. Overall, our findings indicate that BMI altered the colostrum lipid pattern in terms of fatty acid composition, concentration, and percentage but not the position distribution of fatty acyls. Furthermore, the continuous observation of the development, health status, and body fat changes of the offspring of our study volunteers will provide deeper insights into the influence of early lipid nutrition on long-term health status.

Conflicts of interest

The authors declare no conflicts of interest in the submission and publication of this manuscript.

Acknowledgements

This work was supported by the Guangxi Science and Technology Project (AD20297088); the National Natural Science Foundation of China (32272316), and the Beijing Innovation Team of Livestock Industry Technology System (BAIC05-2022).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250193.

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