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Lipid composition and structural characteristics of bovine, caprine and human milk fat globules

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ABSTRACT

Milk fat is widely accepted to be the major nutrient in human milk. Commercial infant formulas are usually based on mammalian milk such as bovine or caprine milk, but the differences in milk fat globules (MFGs) between human, bovine and caprine milk remain unclear. We showed that saturated fatty acid content was higher in bovine and caprine MFGs (>60%) than in human MFG (<40%), but that content of the unsaturated fatty acids C18:2 in human MFG was >7 times higher than in bovine and caprine MFGs. The cholesterol content of human milk was ~20% higher than that of bovine and caprine milk. Triacylglycerol molecular species and polar lipids also differed between bovine, caprine and human MFGs. Confocal laser scanning microscopy images of MFGs revealed that the shape of the liquid-ordered domains varied by species.

1. Introduction

Human milk is an important nutritional resource for newborn infants and provides 40–60% of energy from lipids (Abrahamse et al., 2012). Although human milk is recommended as the optimal food for infants, not every mother is able to breastfeed her baby, for a variety of reasons. In such cases, commercial infant formulas are the best substitutes to fulfil the nutritional needs of infants. Bovine and caprine milk and ingredients derived therefrom are the main ingredients in the infant formulas currently on the market (Prosser, McLaren, Frost, Agnew, & Lowry, 2008). However, the composition and structure of milk fat globules (MFGs) differ between bovine, caprine and human milk. It is thus necessary to study the MFGs of these types of milk, to explore a more suitable breast milk substitute than the currently available products.

MFGs are composed of a core of triacylglycerols (TAGs) and an MFG membrane (MFGM) consisting of proteins, glycoproteins, enzymes, phospholipids and sterols. The nutritional and functional characteristics of MFGs depend not only on their chemical composition, but also on their lateral membrane organisations (Garcia-Saez, Chiantia, & Schwille, 2007). Recently, confocal laser scanning microscopy (CLSM), using lipophilic fluorescent probes, has been used to investigate the features of MFGs (Gallier, Gragson, Jimenez-Flores, & Everett, 2010b). The microstructure of MFGs is associated with the phospholipid and fatty acid composition. Thus, the different proportions of phospholipids and the distributions of fatty acids in each phospholipid among the species could result in significant differences in the microstructure and nutritional aspects of MFGs. Therefore, the structure and, physical and chemical properties of bovine, caprine and human milk should be thoroughly studied.

The purpose of this study was to compare the lipid composition of bovine, caprine and human MFGs on the basis of fatty acids of TAG and polar lipids, fatty acids of individual polar lipids, TAG, polar lipids, and sterols. The size distribution, zeta potential and microstructure of the MFGs were also evaluated. Such comparative studies may contribute to the improvement of infant formula.

2. Materials and methods

2.1. Samples and reagents

Bovine milk samples from 10 Chinese Holstein cows (mature milk, 30–60 d in lactation, third quarter of 2015) were kindly

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provided by a local producer (Tian zi Dairy Industry Co., Ltd., Wuxi, Jiangsu, China). Ten pure-bred Boer caprine milk samples (mature milk, 30–60 d in lactation, third quarter of 2015) were purchased from Zhengxing animal husbandary Co., Ltd. (Zhejiang, China). Ten human milk samples (mature milk, 16–30 d in lactation, third quarter of 2015) were kindly donated by healthy Chinese woman, 28–32 y old, in the Wuxi Maternal and Child Health Hospital. The mothers had been well informed before participating in this study, which was approved by the medical ethics committee of Wuxi Maternal and Child Health Hospital (ethics approval number 2013022). The milk samples were characterised within 24 h after collection for particle size, zeta-potential and CLSM. Other samples were then stored at −20 °C until further chemical analysis. The 37-component fatty acid methyl ester (FAME), cholesterol, phospholipids standards, boron triflouride-methanol solution and the high performance thin-layer chromatography (HPTLC) plates were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents used were all of high-performance liquid chromatograph purity (Sinopharm Chemical Reagent Co., Ltd., Beijing, China).

2.2. Analysis of lipid composition

2.2.1. Milk fat extraction

Milk fat was extracted following the method described by Folch, Lees, and Sloane-Stanley (1957). Briefly, 10 mL of milk was mixed with 200 mL of chloroform: methanol (2:1, v/v). The mixture was shaken for 15 min and then centrifuged at 3500 × *g* for 5 min. The clear lower chloroform layer was mixed with 50 mL 0.86% NaCl solution. The organic phase was evaporated by rotary vacuum evaporator. The extracted lipid was stored at −20 °C until further analysis.

2.2.2. Analysis of fatty acids of total lipids and polar lipids

For fatty acid analysis of total lipids (Christopherson & Glass, 1969), milk fat (20 mg) was added to a sealable tube and hexane (2 mL) and methanolic KOH (2 M, 500 μL) were added. The mixture was vortexed for 5 min, after which then deionised water (5 mL) was added for extraction. After shaking, the upper layer was collected and dried over anhydrous sodium sulphate, and the resulting FAME solution (1 μL) was analysed by gas chromatography (GC).

Non-polar and polar fractions were separated by HPTLC with a solvent system of hexane/diethyl ether/acetic acid (80:20:1, by vol) (Benoit et al., 2010). The baseline band containing the polar lipids fraction was scraped off the plates and placed into screw-capped tubes. Then, polar lipids were extracted three times with the mixture of chloroform/methanol/water (5:5:1, by vol). Fatty acid methyl esters of phospholipids were used to characterise the long-chain fatty acids. BF_3 (14% in methanol, 500 μL) solution was added for methylation, and the screw-capped tubes were kept at 100 °C for 90 min. Hexane (600 μL) and deionised water (1.5 mL) were subsequently added, after which the mixture was centrifuged at 2100 × *g* for 10 min at 20 °C, and the solvent phase was collected and dried over anhydrous sodium sulphate. After centrifugation (2100 × *g* for 10 min), the upper layer was injected into GC for fatty acid analysis.

The analysis of fatty acid composition was performed with an Agilent 7820A GC (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an autosampler, a flame ionisation detector, and a Trace TR-FAME capillary column (60 m × 0.25 mm × 0.2 μm, ThermoFisher Scientific, Waltham, MA, USA). The temperatures of injector and detector were 230 and 250 °C respectively. Nitrogen carrier gas at 1 mL min^{-1} was used and the split ratio was 1:100. The oven temperature was held at 60 °C for 3 min, then raised to 175 °C at 5 °C min^{-1} and held for 15 min at this temperature, followed by an increase to 220 °C at 2 °C min^{-1} and held for 10 min (Yao, Zhao, Zou, Huang, & Wang, 2015).

2.2.3. Fatty acid analysis of the different polar lipids species

A HPTLC method was carried out to separate the phospholipids classes. The mobile phase was composed of methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9, by vol). Iodine vapour was applied overnight for the revelation of spots. The different phospholipid species were scraped off and fatty acid content was determined as described above for total polar lipids. The fatty acids of sphingomyelin were converted to the corresponding methyl ester by treatment with methanolic HCl (0.5 M, 400 μL) at 80 °C for 20 h (Sanchez-Juanes, Alonso, Zancada, & Hueso, 2009). The procedure was then continued as described above for total fatty acids analysis.

2.2.4. Triacylglycerol analysis

The separation and identification of TAG was performed as previously described by Zou et al. (2012a). TAG species were separated by reverse-phase high-performance liquid chromatography (RP-HPLC) and the identification of TAG was carried out on a HPLC–atmospheric pressure chemical ionisation mass spectrometry (HPLC-APCI–MS).

2.2.5. Polar lipid composition

Polar lipid composition was determined according to the method described by Rombaut, Camp, and Dewettinck (2005). Separation of the polar lipids was carried out on a HPLC equipped with an evaporative light scattering detector (ELSD). A silica column (4.6 mm × 250 mm, 5 μm particle size; Phenomenex, Inc., Torrance, CA, USA) was used in this study. One hundred milligrams milk fat was dissolved with 1 mL chloroform/methanol (88:12, v/v) and transferred into capped test tubes for HPLC analysis. Nitrogen was used as the nebulising gas at a flow rate of 1 L min^{-1}, and the evaporating temperature was 85 °C. The set of the program was followed as Zou et al. (2012a).

2.2.6. Sterols content

The sterols in all samples were determined according to the method of Fraga et al. (2000). Milk samples (100 mg) were saponified in capped tubes with 2 M KOH by heating at 85 °C for 1 h, and the unsaponifiable fraction was extracted with hexane. The hexane phase was then dried and silylated by 400 μL N,O-Bis (trimethylsilyl) trifluoroacetamide +1% trimethylchlorosilane (BSTFA + TMCS) at 70 °C for 30 min; the residue was dissolved in 1 mL of hexane, and 1 μL of product was analysed by gas chromatography-mass spectrometry (GC–MS) equipped with a DB-5 MS capillary column (30 m; 0.25 mm i.d., 0.52 μm film thickness; Agilent Corp.). The carrier gas was helium, with a flow rate of 1 mL min^{-1} and the split ratio was 1:50. The oven temperature was held at 150 °C for 1 min and then raised to 300 °C at a rate of 10 °C min^{-1} and then held for 15 min at 300 °C. Scan time and mass range were 1 s and 50–500 (*m/z*), respectively (Li et al., 2011).

2.3. Physical and structural analysis

2.3.1. Particle size

The particle size distribution of milk samples was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK), equipped with an He/Ne laser (λ = 633 nm) and an electroluminescent diode (λ = 466 nm). The refractive index of milk fat was taken to be 1.460 at 466 nm and 1.458 at 633 nm, and the aqueous phase was 1.33. The apparatus and method for particle size analysis have been described in detail by Michalski, Briard, and Michel (2001).