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Evaluation of *sn*-2 fatty acid composition in commercial infant formulas on the Chinese market: A comparative study based on fat source and stage

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ABSTRACT

The *sn*-2 fatty acid composition of 180 commercial infant, follow-on and growing-up formulas with three fat sources (plant oil, cows' milk and goats' milk) was investigated and compared with mature human milk (MHM). *Sn*-2 fatty acids in formulas were mostly dependent on fat source and stage. Compared with MHM, all types of formulas contained lower levels of palmitic acid (PA), saturated fatty acid and long-chain polyunsaturated fatty acids (LC-PUFA), and higher levels of oleic acid (OA), linoleic acid (LA) and α-linolenic acid (LNA) at the *sn*-2 position. Even some formulas were supplemented with 1,3-dioleoyl-2-palmitoylglycerol, the proportions of relative PA at the *sn*-2 position in formulas were much lower than that in MHM. Moreover, formulas had higher proportions of relative OA, LA and LNA, and lower LC-PUFAs at the *sn*-2 position. This study indicated that there were significant differences in the positional distribution of fatty acids between formulas and MHM.

1. Introduction

There is no doubt that human milk is regarded as the best food for full-term infants. Human milk fat (HMF) provides 40–50% of the energy requirements for infants, and also provides essential fatty acids and fat-soluble vitamins (Yuhas, Pramuk, & Lien, 2006). HMF is made up of triacylglycerols (TAG, 98%), phospholipids (0.8%), cholesterol (0.5%) and many others (Jensen, 1999). For oils and fats, it is accepted that the functional and nutritional properties of TAGs depend not only on their fatty acid composition, but also on positional distribution of fatty acids. The TAGs in HMF have special composition and structure. Saturated fatty acids (SFA), especially palmitic acid (C16:0, PA), are mainly located at the *sn*-2 position of the TAGs, whereas unsaturated fatty acids (UFA), such as oleic acid (C18:1n-9, OA) and linoleic acid (C18:2n-6, LA), are mainly located at the *sn*-1,3 positions (Haddad, Mozzon, & Frega, 2012; Lopez-Lopez, Lopez-Sabater, Campoy-Folgoso, Rivero-Urgell, & Castellote-Bargallo, 2002). In contrast, PA is predominantly esterified at the *sn*-1,3 positions in commercial infant formulas (Lopez-Lopez et al., 2002; Straarup, Lauritzen, Faerk, Hoy, & Michaelsen, 2006). Clinical studies (Bracco, 1994; Decker, 1996) have provided increasing evidences that these differences in the positional distribution of fatty acids between formulas and human milk affected intestinal fat absorption in infants. Specifically, the high level of PA at the *sn*-2 position plays a key role on increasing the absorption of fatty acids and calcium, and improving bone matrix quality and stool consistency (Havlicekova, Jesenak, Banovcin, & Kuchta, 2016; Lien, Boyle, Yuhas, Tomarelli, & Quinlan, 1997; Lopez-Lopez et al., 2001). Therefore, it is of great importance to investigate and evaluate the differences in the *sn*-2 fatty acid composition between commercial formulas and human milk.

Over the last two decades, there have been limited studies reporting on the differences in the *sn*-2 fatty acid composition between commercial formulas and human milk (Lopez-Lopez et al., 2002; Prosser, Svetashev, Vyssotski, & Lowry, 2010; Straarup et al., 2006), which is characterized by a large number of human milk samples and few of formulas. In addition, most of studies are focused on formulas in Europe and New Zealand, whereas few studies are focused on formulas in China. Hence, it is essential to analyze the *sn*-2 fatty acid composition in commercial formulas on the Chinese market in a sufficient sample size.

According to our previous study (Sun et al., 2016), there are three fat sources stated on the labels in commercial formulas at present, which involves plant-oil formula (POF), cows' milk formula (CMF) and goats' milk formula (GMF). In general, CMF and GMF are not pure milkfat, which are combined with small amounts of vegetable oils to mimic the fatty acid composition of HMF. As is well known, the positional distribution of fatty acids in cow milk and goat milk are similar to HMF (Zou et al., 2013), whereas vegetable oils, which are characterized by high proportions of UFAs at the *sn*-2 position and SFAs at the *sn*-1,3 positions (Mattson & Volpenhein, 1961), are different from HMF. Therefore, formulas with different fat sources may contribute to

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differences in the *sn*-2 fatty acid composition. In addition, commercial formulas are generally divided into infant formula (IF, for infants 0–6 months), follow-on formula (FF, for older infants 6–12 mouths) and growing-up formula (GF, for young children 12–36 months). The three stages of formulas meet the different nutritional demands for the corresponding infants, respectively. It is necessary to understand the differences among formulas with different fat sources and stages, and compare each type with human milk.

At present, more and more formula manufacturers have realized the importance of PA at the *sn*-2 position. Commercial formulas should mimic HMF not only in the total fatty acid composition, but also in the *sn*-2 fatty acid composition. Therefore, many studies (Lee, Son, Akoh, Kim, & Lee, 2010; Wei, Feng, Zhang, Ca, & Feng, 2015; Zou, Jin, Guo, Xu, & Wang, 2016) are focused on the preparation of human milk fat substitutes (HMFS), especially HMFS rich in 1,3-dioleoyl-2-palmitoylglycerol (OPO). In some studies (Lee et al., 2010; Wei et al., 2015), OPO-enriched HMFS contain higher PA content at the *sn*-2 position than HMF. Betapol® and Infat® are two examples of commercial HMFS rich in OPO, which contain more than 50% PA at the *sn*-2 position (Alvarez & Akoh, 2015). OPO can be added into commercial formulas as an important ingredient to enhance the PA content at the *sn*-2 position. Currently, some formulas with high price are supplemented a certain amount of OPO. However, whether it reaches the abundance of PA at the *sn*-2 position in HMF after supplementation remains uncertain. Thus, it is significant to compare the differences in the *sn*-2 fatty acid composition between formulas with OPO and human milk.

The objectives of this study were to investigate the *sn*-2 fatty acid composition of commercial formulas on the Chinese market, compare the profile differences with different fat sources and stages, and evaluate the positional distribution of fatty acids in commercial formulas by comparison with HMF.

2. Materials and methods

2.1. Reagents and standards

Porcine pancreatic lipase (type II) and thin layer chromatography (TLC) plates were purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). Methanol (> 95.0% purity) and hexane (> 99.9% purity) were obtained from J & K Scientific, Ltd. (Beijing, China). The other solvents and reagents were all of analytical grade (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China). The standard mixture of fatty acid methyl esters (FAME) was purchased from Sigma-Aldrich Chemical Co. Ltd. (Shanghai, China).

2.2. Samples

A total of 180 commercial formulas were selected based on some principles and purchased from major supermarkets and official flagship stores in Wuxi between 2013 and 2014 (Sun et al., 2016). There were 27 brands, including 16 local brands and 11 imported brands, which were totally accounted for more than 75% market share on the Chinese market.

Ten mature human milk (MHM) samples were kindly donated by healthy Chinese mothers in Wuxi Maternal and Child Health Hospital (Wuxi, China). The mothers had been well explained before participating in this study and were approved by the medical ethics committee of Wuxi Maternal and Child Health Hospital.

2.3. Fat extraction

An adapted protocol of the extraction procedure developed from the Röse-Gottlieb method was used for the extraction of total fat, as detailed by Sun et al. (2016). This method was performed for formulas and MHM.

2.4. Sn-2 fatty acid composition

Fat extracted from formulas and MHM were hydrolyzed to 2-monoacylglycerol (2-MAG) by means of the method described by Sahin, Akoh, and Karaali (2005). Pancreatic lipase (porcine pancreatic lipase, 20 mg), Tris buffer (pH 8.0, 1 mL), bile salts (0.05%, 0.25 mL), and calcium chloride (2.2%, 0.1 mL) were added to a test tube containing 100 mg fat. The mixture was incubated in a water bath (40 °C) for 3 min with shaking. Then HCl solution (6 M, 1 mL) and diethyl ether (1 mL) were added to stop the enzyme reaction. Diethyl ether was evaporated to a 200 μL volume by nitrogen gas. The hydrolytic product was separated on a silica gel G TLC plate, and the developing solvents were hexane/diethyl ether/acetic acid (50:50:1, v/v/v). The band corresponding to 2-MAG was scrapped off and extracted twice with diethyl ether (1 mL). The solvent was removed by nitrogen gas, and the residue was methylated and analyzed by gas chromatograph (GC) (Sun et al., 2016).

The GC was an Agilent 7820A (Agilent, California, USA) equipped with a hydrogen flame ionization detector and a Trace TR-FAME capillary column (60 m × 0.25 mm × 0.25 μm, Thermo Fisher, USA). Both injector and detector temperatures were 250 °C. Nitrogen carrier gas at 1.0 mL/min was used, and split ratio was 1:20. The oven temperature was held at 60 °C for 3 min, then raised to 175 °C at the rate of 5 °C/min and maintained for 15 min at this temperature, followed by an increase to 220 °C at a rate of 2 °C/min and held at 220 °C for 10 min. The FAMEs were identified by comparing retention times of sample peaks with those of a mixture of FAME standards.

The relative percentage of each fatty acid at the *sn*-2 position was calculated as (M/T × 3) × 100, where M is the percentage of fatty acid at the *sn*-2 position and T is the percentage of fatty acid in TAG (Bracco, 1994). The fatty acid composition in TAG in formulas and MHM was cited from our previous paper (Sun et al., 2016).

2.5. Statistical analysis

All analysis of formulas were carried out in duplicate, while those of MHM were made in triplicate. The results were expressed as means (%) ± standard deviations (SD) and performed using the SPSS 19.0 (SPSS, Chicago, IL, USA). Data were firstly checked for normality with the Kolmogorov-Smirnov test. When the variables were normally distributed, one-way analysis of variance (ANOVA) was adopted to identify differences; otherwise, a non-parametric Kruskal-Wallis test was used. *P* value was set at 5% for all analyses. Differences among different types of formulas were compared by use of Tukey's test at $P < 0.05$. Two-factor ANOVA was analyzed for evaluating the effects of fat source and stage on the *sn*-2 fatty acid composition of formulas. Fat source and stage were regarded as independent variables and each fatty acid as the dependent variable.

3. Results and discussion

3.1. Characteristics of formulas

The collected formulas were comprised of POF (n = 90), CMF (n = 66) and GMF (n = 24), and of IF (n = 60), FF (n = 60) and GF (n = 60). OPO could be found on the labels of 54 samples (including 11 domestic brands and 2 international brands), among which POF, CMF and GMF accounted for 44.4%, 38.9% and 16.7%, respectively. The additive amounts of OPO in IF ranged from 32 g/kg to 64 g/kg, which were generally higher than those in FF (24–50.6 g/kg) and GF (24–50 g/kg) from the same brand.

3.2. Sn-2 fatty acid composition of formulas and mature human milk

The *sn*-2 fatty acid composition in the selected formulas and MHM is shown in Tables 1–3: SFA in Table 1, monounsaturated fatty acids