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Triacylglycerol containing medium-chain fatty acids (MCFA-TAG): The gap between human milk and infant formulas

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ABSTRACT

The high concentration of medium-chain fatty acids (MCFAs) in human milk has special benefits for infants. The study compared the characteristic of triacylglycerol containing MCFAs (MCFA-TAG) in human milk and infant formulas with different fat sources including plant oil, cows' milk and goats' milk (POF, CMF and GMF, respectively). Significant differences were observed in both the concentration and composition of the MCFA-TAG. The MCFAs naturally present in human milk were medium-and-long chain triacylglycerols (MLCTs), especially the TAG with one MCFA and two long-chain fatty acids (MLL type), whereas POF contained higher proportions of the TAG with three MCFAs (MMM type) and GMF contained higher proportions of the TAG with two MCFAs and one LCFA (MML type) than that in human milk. The results indicated that deeper research is needed to narrow the gap in MCFA-TAGs between human milk and infant formulas.

1. Introduction

In nature, dietary medium-chain fatty acids (MCFAs) mainly come from milk and dairy products, and oils from fruits of tropical regions, like coconut, palm kernel oil. Human milk fat, in which about 50% of the energy is provided by triacylglycerol (TAG), is an important source of MCFAs for infants. There are various definitions of MCFAs: C_6–C_{10} (Nagao & Yanagita, 2010), C_6–C_{12} or C_8–C_{10} (Dayrit, 2014), C_8–C_{12} (Mumme & Stonehouse, 2015) and C_8–C_{14} (Neville & Picciano, 1997). The definition of MCFA as C_6–C_{12} proposed by Bach and Babayan (1982) was based on the physicochemical and metabolic properties.

However, it was found that research on the fatty acid (FA) composition of milk defined C_8–C_{14} as MCFA, which are mainly derived from de novo synthesis in the endogenous mammary glands (Boersma, Offringa, Muskiet, Chase, & Simmons, 1991; Granot, Ishay-Gigi, Malaach, & Flidel-Rimon, 2016; Haddad, Mozzon, & Frega, 2012; Wu et al., 2019). During lactation, the mammary epithelial cell is the primary site for de novo FA synthesis. However, the presence of an acyl thioester-hydrolase (thioesterase II) limits the FA synthesis to those with 8–14 carbons (Neville & Picciano, 1997) and thus the FAs synthesised de novo in mammary gland such as caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), and myristic acid (14:0) are known as MCFAs (Boersma et al., 1991) on the basis of the source of FAs. Taking into consideration that the mammary gland can synthesise FAs predominantly with 8–14 carbons in human milk (Dils, 1986; Hachey, Silber, Wong, & Garza, 1989), C_8–C_{14} FAs were selected as MCFAs in this study.

The FA and TAG composition of human milk and other mammal milk has been extensively investigated (Gastaldi et al., 2011; Haddad, Mozzon, Strabbioli, & Frega, 2011a; Teng, Wang, Yang, Ma, & Day, 2017). For human milk, it was found that the content of MCFAs increased from colostrum to transitional and mature milk, irrespective of the region, gestational age of mothers (Bitman, Wood, Hamosh, Hamosh, & Mehta, 1983; Giuffrida et al., 2016), and preterm milk contained significantly higher MCFA content than term milk (Bitman et al., 1983; Genzel-Boroviczényy, Wahle, &

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Koletzko, 1997). Human milk was observed to be better absorbed due to its TAG composition in clinical studies (Straarup, Lauritzen, Faerk, Hoy, & Michaelsen, 2006; Tomarelli, Meyer, Weaber, & Bernhart, 1968). The TAG composition, with regard to physiology and nutrition, plays important roles in improving digestion and absorption in infants. For TAG containing MCFAs (MCFA-TAG), it has been reported that almost half of the TAG molecules contained at least one MCFA (8:0–14:0) in human milk (Kallio, Nylund, Bostrom, & Yang, 2017). The majority of previous research has indicated that MCFAs in human milk exist as medium-chain triacylglycerols (MCTs) (Jacobi & Odle, 2012; Klenoff-Brumberg & Genen, 2002), but few MCTs was detected in human milk. Up to now, the specific and detailed MCFA-TAGs in human milk are much less well understood. Advanced, significant differences in TAGs with short-chain FAs have been detected between human milk and infant formulas (Sun et al., 2018b), whereas the specific differences of the MCFA-TAGs composition in human milk and infant formulas are still unclear. And to the best of our knowledge, no papers focusing on the differences in the MCFA-TAGs between human milk and infant formulas have been yet published.

Although it has been reported that MCT was readily absorbed, the benefit of MCT for infants has been questioned (Borum, 1992) and more recently discussed (Billeaud et al., 2018; Klenoff-Brumberg & Genen, 2002). In most of the clinical trials for MCT, the comparison has been made in separate groups of infants fed either medium-chain or long-chain TAG formulas. Some reports, however, indicated no improvement in fat digestibility, nitrogen retention or weight gain of neonates when MCT oil was added to the fat (Borum, 1992; Hamosh et al., 1989). As an easily accessible energy source, MCT could stimulate the production of ketone bodies by the liver (Jensen & Jensen, 1992). Furthermore, administration of MCFAs at concentrations exceeding the neonate's requirements in a short time may prevent the administration of other essential FAs in neonates (Pettei, Daftary, & Levine, 1991) and may produce metabolites that are potentially toxic for some organs (Borum, 1992). In preterm infants receiving human milk supplemented with MCT, significantly increased levels of n-9 monounsaturated FAs were recently observed (Billeaud et al., 2018). A meta-analysis showed there was no evidence of difference between MCT and long-chain triacylglycerol (LCT) formula in short-term growth, gastrointestinal intolerance, or necrotising enterocolitis of infants (Klenoff-Brumberg & Genen, 2002). The nutritional importance of MCT for infants has long been studied, while the controversy still remains on their effects of improve short-term growth or other adverse effects.

MCFAs are considered an important source of energy due to the special nutritional and metabolic properties, including digestion, absorption and oxidation rates, for neonates to meet the high growth rate demand. Additionally, MCFAs and monoacylglycerols possess antibacterial activity, which may have functional effects on the establishment of the gut microbiota and gut-associated immune function in early life (Nejrup, Licht, & Hellgren, 2017). Recently, the TAG structure has obtained more attention with respect to nutritional importance in lipid digestion and metabolism. The different positional distribution in TAG molecules could lead to different metabolic fates, resulting in the change of FA composition in liver and inguinal adipose TAG in rats (Pons, Bargalló, Folgoso, & López-Sabater, 2000). The structured MCFA-TAGs in human milk may have some different actions from identical physical mixtures of MCT and LCT in infant formulas. According to the previous reports, the structured TAG was significantly correlated with improved fat digestibility, lymphatic transport, lymphatic absorption in experimental animals (Jensen et al., 1994; Straarup & Høy, 2000) and nitrogen retention, protein economy, liver function in patients receiving parenteral nutrition (Puiggros et al., 2009; Wu, Zaniolo, Schuster, Schlotzer, & Pradelli, 2017; Zhao & Wang, 2017). In addition, the TAG structure also plays a pivotal role in regulating visceral fat accumulation. Lee et al. (2018) found mice fed enzymatically interesterified medium-and-long chain TAG (MLCT) had significantly less body fat accumulation compared with physical blend having similar FA composition.

This study systematically compared the MCFAs in human milk at different lactation stage and commercial infant formulas with different fat sources (Sun et al., 2018b; Yuan et al., 2019), with particular attention to the molecular structure of MCFA-TAGs.

2. Material and methods

2.1. Samples

The samples were described in detail previously (Qi et al., 2018); in short, 309 human milk samples (103 samples each for colostrum, transitional human milk and mature human milk) were obtained from 103 healthy mothers who delivered their babies full term in Wuxi Maternal and Child Health Hospital (Wuxi, China) (WXM201560). One hundred and eighty commercial infant formulas with different fat sources including plant oil, cows' milk and goats' milk were included; this comprised 27 brands (16 domestic and 11 international brands), representing more than 75% of the Chinese market share as stated in our previous study (Sun et al., 2016).

2.2. Data

The data were derived from our previous studies that investigated the FAs and TAG composition in human milk from three different lactations and commercial infant formulas with different fat sources and stages on the Chinese market (Qi et al., 2018; Sun et al., 2018b, 2016; Sun, Wei, Su, Zou, & Wang, 2018a; Yuan et al., 2019). Since there were no significant differences in the total and *sn*-2 FA and TAG composition of infant formulas between the three stages (Sun et al., 2016, 2018a,b), the stages of infant formulas were not distinguished in the present study. In this paper, we revealed the special feature of MCFA-TAGs in human milk and the profile differences between human milk and infant formulas with different fat sources.

2.3. Analytical methods

The extraction procedure of fat and the method for total and *sn*-2 FA composition of lipid in human milk and infant formulas was described previously in detail by Qi et al. (2018); TAG composition of human milk and infant formulas was determined by the method Zou et al. (2013). In view of the same method being used for human milk and infant formulas, comparisons were made possible; differences in the composition of MCFA-TAGs identified in human milk and infant formulas were revealed.

2.4. Statistical analysis

Differences in characteristics of MCFA between human milk and infant formulas were performed using one-way analysis of variance (ANOVA) using IBM SPSS Statistics, version 20.0. The results were expressed as means ± standard deviations. Duncan post-tests were adopted to identify the difference. For all analyses, *P*-values less than 0.05 were considered statistically significant. A heat map, generated using the R function heatmap giving a graphical representation of data where the individual values contained in a matrix

are represented as colours was used to determine the specific change in individual MCFA-TAG.

The comparison of characteristics of MCFA-TAG in human milk and infant formulas was performed with box plots that display variation in samples of a statistical population without making any assumptions of the underlying statistical distribution. The spacing between the different parts of the box indicate the degree of dispersion (spread) and skewness in the data. The top and bottom of each box plot represents the 25th and 75th percentiles, and whisker (lines extending vertically from the boxes) indicate 1.5 times the median servings.

3. Results

3.1. Medium-chain fatty acids composition in human milk and infant formulas

The MCFAs (8:0–14:0) composition in human milk (colostrum, transitional and mature milk) and infant formulas (plant-oil formulas, POF; cows' milk formulas, CMF; goats' milk formulas, GMF) are shown in Table 1. The odd carbon FAs (9:0, 11:0 and 13:0) were not included here considering their very low content and not being detected in the *sn*-2 position and TAGs. Table 1 showed that the MCFAs in human milk accounted for 7.98–13.62% of total FAs with 12:0 and 14:0 being the dominant MCFAs. Compared with colostrum, transitional and mature milk contained a higher level of MCFA. The 8:0 level in human milk was lower than that in all three types of infant formulas. The MCFA in CMF was comparable with human milk. One distinct feature of POF was that it contained a higher level of 8:0, 12:0 and total MCFAs than CMF, GMF and human milk.

The level of total *sn*-2 MCFAs in infant formulas was comparable with human milk (Supplementary material Table S1). Specifically, GMF presented the highest percentage of 8:0 and 10:0, which might be one mark of GMF, and POF contained the most 12:0 at the *sn*-2 position, while the transitional and mature milk showed a great higher content of 14:0 at the *sn*-2 position compared with colostrum and infant formulas.

3.2. Human milk fats are enriched in medium-and long triacylglycerols

Though the TAG composition in human milk had been reported before, the characterisation of MCFA-TAGs molecules was limited. The MCFA-TAGs composition and structure in human milk fat were found to be quite distinctive; as shown in the first row of Table 2 the total MCFA-TAGs represented 22.57% of all TAG in colostrum, whereas it increased to 34.73% and 31.90% in transitional milk and mature milk respectively. As the lactation progressed, the content of MCFA-TAGs in transitional and mature milk was higher than that in colostrum ($P < 0.01$), which was consistent with the change in level of MCFAs in human milk during lactation. According to the number of MCFAs in the TAG molecule, the MCFA-TAGs could be divided into three type groups in more detail: MMM (three MCFAs), MML (two MCFAs and one LCFA), and MLL (one MCFA and two LCFAs). Only a few MMM were detected in human milk, with a relatively low content of 0.80% (colostrum), 1.13% (transitional milk) and 0.97% (mature milk). The content of MLL type TAG, the most abundant TAG type in human milk, was approximately 20.89–31.47%. In more detail, the MCFA in MLL type TAG was occupied mostly by 12:0 and 14:0, followed by 10:0 and some 8:0. The most abundant MCFA-TAGs in human milk were 12:0/16:0/18:1, 18:1/14:0/18:2, 18:1/12:0/18:2, 10:0/16:0/18:1, 18:1/12:0/18:1 and 10:0/16:0/18:2 (Table 2). Particularly, it was recognised that the MCFAs mainly existed together with 16:0 and C18 FAs (18:0, 18:1 n-9, 18:2 n-6).

3.3. Infant formulas are enriched in medium-chain triacylglycerols

The composition of MCFA-TAGs of infant formulas with different fat sources compared with human milk during different lactation was visualised in a heat map (Fig. 1). There were remarkable differences in the structure of MCFA-TAGs between the infant formulas with three kinds of fat source and human milk. The main MCFA-TAGs were 12:0/12:0/12:0 and 12:0/12:0/14:0 in the POF, 14:0/18:1/18:1 and 14:0/16:0/18:1 in the CMF, and 14:0/18:1/18:1, 12:0/12:0/18:1 and 10:0/12:0/16:0 in the GMF, while in human milk that were 12:0/16:0/18:1 and 18:1/14:0/18:2.

The relative content of three main types MCFA-TAGs (MMM, MML and MLL) in infant formulas were compared with human milk with different lactation stages (Fig. 2). The POF contained high concentrations of MMM, which were about 9.14% of the total TAGs, while the MMM type TAG content was significantly lower in CMF (1.79%), GMF (1.52%) and human milk (less than 2%) ($P < 0.01$). And the average content of MLL type TAG was just the opposite, the most in human milk (31.47%, 28.54% and 20.88% in colostrum, transitional and mature milk respectively), second in GMF (15.08%), next in CMF (11.73%), and lowest in POF (0.83%) ($P < 0.01$). The predominant structure of MCFA-TAGs in CMF was MLL type TAG and that in GMF was MML type TAG and MLL type TAG. It seemed that GMF was characterised by MML type TAG, which contained a higher concentration of MML type TAG compared with POF, CMF and human milk.

In addition to the three types of MCFA-TAGs, a portion of MCFAs was combined with one short FA; as MSL type in CMF (0.94%) and GMF (3.68%), i.e., one short FA, one MCFA and one LCFA in the same TAG molecule (Supplementary material Fig. S1). This unique TAG was essentially due to the abundance of short-chain FA in cow milk and goat milk. The same type TAG structure (such as 4:0/10:0/16:0, 4:0/12:0/18:1, 4:0/14:0/18:1) were also found in cows' milk and goats' milk by Gastaldi et al. (2011).

To reveal more clearly the special composition of MCFA-TAGs, the MLL type TAG, as the main MCFA-TAG molecules in human milk, was further analysed. From Fig. 3 [8:0-MLL(a), 10:0-MLL(b),

Table 1
Fatty acid composition of medium-chain fatty acids (MCFAs) in human milk and infant formulas.[a]

MCFA	Colostrum	Transitional	Mature	POF	CMF	GMF
	(n = 103)	(n = 103)	(n = 103)	(n = 90)	(n = 66)	(n = 24)
8:0	0.15 ± 0.11^{a}	0.19 ± 0.05^{a}	0.20 ± 0.05^{a}	1.48 ± 1.36^{d}	0.73 ± 0.49^{b}	1.16 ± 0.31^{c}
10:0	0.59 ± 0.33^{a}	1.47 ± 0.29^{b}	1.35 ± 0.50^{b}	1.47 ± 1.14^{b}	1.29 ± 0.64^{b}	3.35 ± 1.20^{c}
12:0	2.98 ± 1.44^{a}	6.07 ± 1.11^{c}	5.50 ± 1.32^{c}	8.99 ± 4.14^{d}	4.20 ± 2.91^{b}	4.56 ± 1.91^{b}
14:0	4.26 ± 1.30^{a}	5.89 ± 1.07^{c}	5.04 ± 1.51^{b}	4.63 ± 1.79^{b}	5.11 ± 1.51^{b}	5.82 ± 1.16^{c}
Sum	7.98 ± 2.28^{a}	13.62 ± 1.76^{c}	12.09 ± 2.29^{b}	16.57 ± 7.28^{d}	11.33 ± 4.45^{b}	14.89 ± 2.68^{c}

[a] Data (%) are expressed as mean value ± standard deviation; different superscript letters indicate significant differences in each row between the colostrum, transitional, mature human milk, plant oil formula (POF), cows' milk formula (CMF) and goats' milk formula (GMF) ($P < 0.05$).

Table 2
Characteristics of the TAG containing MCFAs identified in human milk (% of total TAG).[a]

Study	Area/country	Sample	MCFA-TAG							The five most abundant MCFA-TAG
			Sum	MMM	MML	MLL				
						8:0	10:0	12:0	14:0	
Yuan et al. (2019)	Wuxi, China	colostrum	22.57	0.80	0.88	–	2.99	11.83	6.07	12:0/16:0/18:1 (8.08%), 18:1/14:0/18:2 (4.83%), 18:1/12:0/18:2 (2.32%), 10:0/16:0/18:1 (2.21%), 18:1/12:0/18:1 (1.42%)
		transitional	34.73	1.13	2.13	–	7.97	13.95	9.55	12:0/16:0/18:1 (7.38%), 18:1/14:0/18:2 (7.27%), 10:0/16:0/18:1 (5.39%), 18:1/12:0/18:2 (4.32%), 10:0/16:0/18:2 (2.58%)
		mature	31.90	0.97	2.38	–	7.09	13.61	7.84	12:0/16:0/18:1 (7.23%), 18:1/14:0/18:2 (6.06%), 18:1/12:0/18:2 (4.60%), 10:0/16:0/18:1 (4.59%), 10:0/16:0/18:2 (2.50%)
Zhao et al. (2018)	Beijing, China	colostrum	16.95	0.22	2.42	–	1.04	3.91	9.35	18:1/14:0/18:1 (4.46%), 14:0/16:0/18:1 (2.01%), 18:1/12:0/18:1 (1.65%), 18:1/14:0/18:2 (1.26%), 12:0/16:0/18:1 (1.08%)
		mature	22.54	0.65	4.09	–	1.74	6.84	9.22	18:1/14:0/18:1 (4.01%), 18:1/12:0/18:1 (2.58%), 12:0/16:0/18:1 (2.53%), 14:0/16:0/18:1 (2.05%), 18:1/14:0/18:2 (1.34%)
Tu et al. (2017)	Hubei, China	transitional	27.10	0.07	3.69	–	2.90	9.14	11.30	18:1/14:0/18:1 (4.01%), 12:0/16:0/18:1 (2.53%), 14:0/16:0/18:1 (2.05%), 14:0/16:0/18:2 (1.95%), 18:1/14:0/18:2 (1.34%)
	Sichuan, China		31.53	0.17	5.82	–	3.27	10.93	11.34	12:0/16:0/18:1 (3.50%), 14:0/16:0/18:1 (3.48%), 18:1/14:0/18:1 (3.40%), 18:1/14:0/18:2 (2.99%), 18:1/12:0/18:1 (2.57%)
	Beijing, China		28.29	0.20	2.23	–	3.31	9.26	13.29	14:0/16:0/18:1 (4.47%), 18:1/14:0/18:2 (3.37%), 12:0/16:0/18:1 (3.02%), 18:1/14:0/18:1 (2.93%), 14:0/16:0/18:2 (2.52%)
	Hubei, China	mature	47.11	2.16	14.26	–	5.51	15.40	9.78	12:0/16:0/18:2 (4.06%), 18:1/12:0/18:2 (3.50%), 12:0/16:0/18:1 (3.48%), 18:1/14:0/18:2 (3.26%), 12:0/12:0/18:1 (2.65%)
	Sichuan, China		42.26	0.99	10.25	–	4.65	15.41	10.96	12:0/16:0/18:1 (3.79%), 12:0/16:0/18:2 (3.47%), 14:0/16:0/18:1 (3.46%), 18:1/14:0/18:2 (2.74%), 18:1/14:0/18:1 (2.58%)
	Beijing, China		38.56	0.68	7.53	–	4.52	15.00	10.83	12:0/16:0/18:1 (4.26%), 12:0/16:0/18:2 (3.48%), 14:0/16:0/18:1 (3.06%), 18:1/14:0/18:2 (2.94%), 14:0/16:0/18:2 (2.50%)
Kallio et al. (2017)	Beijing, China	mature	31.51	0.21	6.05	0.74	5.06	11.61	7.84	12:0/16:0/18:1 (2.12%), 18:1/14:0/18:2 (1.92%), 18:1/14:0/18:1 (1.61%), 18:1/12:0/18:2 (2.16%), 18:1/12:0/18:1 (1.61%)
	Finland	mature	40.54	1.19	10.46	0.60	5.40	11.36	11.53	12:0/16:0/18:1 (3.53%), 10:0/16:0/18:1 (1.75%), 18:1/14:0/18:1 (3.17%), 14:0/16:0/18:1 (1.82%), 18:1/12:0/18:1 (2.52%)
Zou et al. (2013)	Denmark	colostrum	31.15	0.77	11.44	–	–	17.21	1.73	18:1/12:0/18:1 (7.89%), 12:0/16:0/18:1 (7.30%), 14:0/14:0/18:0 (4.59%), 12:0/14:0/18:1 (4.13%), 18:1/12:0/18:2 (2.02%)
		transitional	46.20	3.72	17.34	–	–	20.15	4.99	12:0/16:0/18:1 (12.72%), 12:0/14:0/18:1 (8.23%), 18:1/12:0/18:1 (6.05%), 12:0/16:0/14:0 (3.74%), 12:0/12:0/18:1 (3.05%)
		mature	40.35	3.00	15.32	–	–	19.23	2.80	12:0/16:0/18:1 (10.89%), 12:0/14:0/18:1 (7.27%), 18:1/12:0/18:1 (5.96%), 12:0/12:0/18:1 (2.93%), 18:1/12:0/18:2 (2.38%)
Gastaldi et al. (2011)	Italy	mature	30.80	0.57	3.51	0.29	3.14	10.45	12.84	18:1/14:0/18:1 (5.28%), 14:0/16:0/18:0 (5.22%), 12:0/16:0/18:1 (3.83%), 18:1/12:0/18:1 (2.71%), 14:0/16:0/18:1 (2.34%)

[a] The data cited from the literature are presented as mean values; values are weight percent except for those from Zhao et al. (2018) and Kallio et al. (2017), which are mole percent. MCFA-TAG indicates the TAG containing MCFAs including MMM, MML and MLL. Abbreviations are: TAG, triacylglycerol; MCFAs, medium-chain fatty acids; LCFAs, long-chain fatty acids; MMM, the TAG with three MCFAs; MML, the TAG with two MCFAs and one LCFA; MLL, the TAG with one MCFA and two LCFAs.

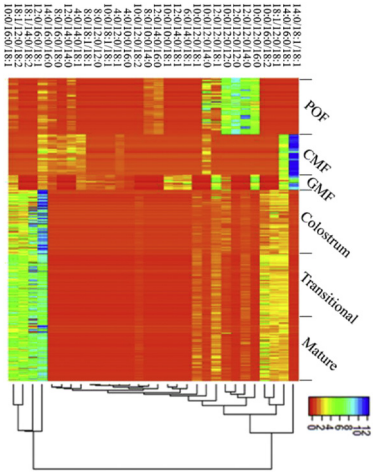

Fig. 1. Hierarchical clustering analysis of 309 human milk (colostrum, transitional and mature milk) and 180 commercial infant formula samples [plant oil formula (POF), cows' milk formula (CMF) and goats' milk formula (GMF)]. The difference in the composition and relative content of triacylglycerols containing medium-chain fatty acids identified in human milk and infant formulas were shown in the heatmap.

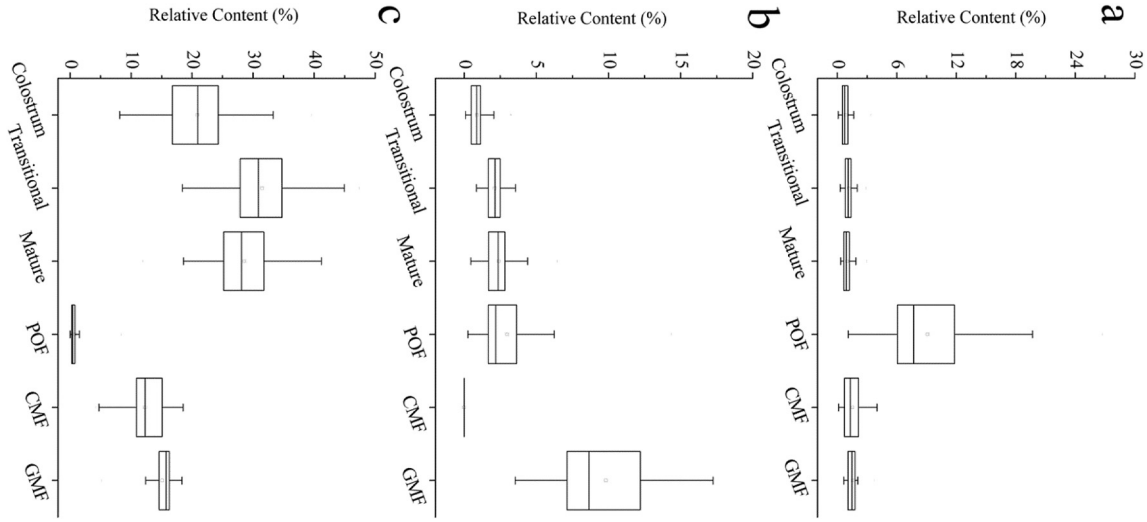

Fig. 2. Box plots of the relative content of three types of triacylglycerol (TAG) containing medium-chain fatty acids (MCFAs): a, TAG with three MCFAs (MMM); b, TAG with two MCFAs and one LCFA (MML); c, TAG with one MCFA and two LCFAs (MLL) in human milk (colostrum, transitional and mature milk) and infant formulas (POF, plant oil formula; CMF, cows' milk formula; GMF, goats' milk formula).

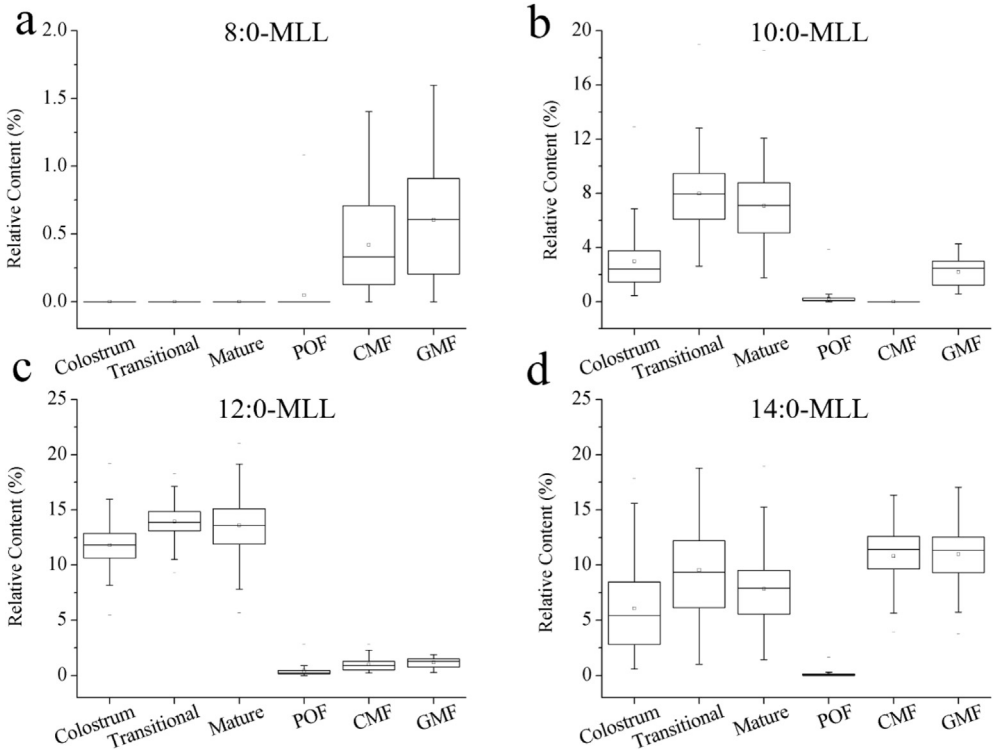

Fig. 3. Box plots of the relative content of triacylglycerol (TAG) with one MCFA and two LCFAs (MLL): a, b, c and d show TAGs with 8:0, 10:0, 12:0 and 14:0 with two LCFAs respectively, relative to all TAGs in human milk (colostrum, transitional and mature milk) and infant formulas (POF, plant oil formula; CMF, cows' milk formula; GMF, goats' milk formula).

12:0-MLL(c) and 14:0-MLL(d)], the composition of MLL type TAG in the infant formulas differed significantly from that in human milk during the different lactation. Although the percentage of MLL type in CMF was comparable with that in human milk, the MLL composition was different. In CMF and GMF the predominant MLL type TAG was comprised of 14:0-MLL, such as 14:0/18:1/18:1 and 14:0/16:0/18:1, which was far higher than that in human milk. Human milk had a significantly higher content of MLL type TAG, with 10:0 and 12:0, than all infant formulas. The percentage of MLL type TAG with 8:0 and 14:0 in CMF and GMF was higher than POF and human milk. In human milk, about 13.13% of TAG comprised 12:0-MLL (11.83% in colostrum, 13.61% in transitional milk and 13.94% in mature milk), while in the infant formulas the 12:0-MLL content was significantly lower (only 0.41% in POF, 0.97% in CMF and 1.19% in GMF on average).

4. Discussion

Fat, of which ~98% is TAG, is the main source of energy for infants. MCFAs can provide some benefits and cover this requirement as they are more easily absorbed by the newborn infant with an immature digestive system. For the MCFAs in human milk, it was generally accepted that the content of MCFAs in colostrum was lower than transitional and mature milk (López-López, López-Sabater, Campoy-Folgoso, Rivero-Urgell, & Castellote-Bargalló, 2002; Molto-Puigmarti, Castellote, Carbonell-Estrany, & Lopez-Sabater, 2011), due partly to the immature metabolism of mammary gland. Compared with that in human milk, the MCFA profiles of infant formulas with different fat source showed great differences, with 8:0 and 10:0 being higher in GMF and 12:0 being higher in POF. The 8:0 level in human milk was lower than that in all three types of infant formulas, which was also found by Straarup et al. (2006).

From the relative FA at the *sn*-2 position, and taking into account of the total percentage of each FA (Supplementary material Table S2), we could better determine the distribution of MCFAs (López-López et al., 2002); 8:0 and 10:0 appeared to be mainly esterified in the *sn*-1,3 position in the infant formulas, while 12:0 (except transitional milk) and 14:0 was preferentially located at the *sn*-2 position in human milk and infant formulas (Haddad et al., 2012). In our study, 8:0 and 10:0 did not showed the *sn*-2 positional preference in human milk except 8:0 in mature milk (46.29%), but variations have been found in the different lactations. The study of Wu et al. (2019) showed the relative 8:0 content at the *sn*-2 position in human milk from Shanghai ranged from 6.23 to 10.61% (the data might contain outliers and need to be validated), whereas that in mature milk from Finland and Beijing were 41.03 and 31.52% respectively (Kallio et al., 2017). Furthermore, similar to previous studies, it was also found that the relative percentage of MCFA at the *sn*-2 position increased from 10:0 to 14:0 (Haddad et al., 2012; Wu et al., 2019), which was not discovered in the PMF and GMF (López-López et al., 2002).

Regarding MCFA-TAGs in human milk, it was shown in our study that about half of TAG molecules contained MCFA and MCFAs mainly existed together with LCFAs as MLCT. The information of previous studies of others reporting the composition of MCFA-TAGs identified in the human milk are summarised in Table 2. Some studies were not present as there was no quantification of specific TAG reported or the content of MCFA-TAGs, which were not separated from other TAGs, was unable to be calculated (Beccaria et al., 2014; Kim, Park, & Shim, 2015; Morera, Castellote, Jauregui, Casals, & Lopez-Sabater, 2003). Although the results of previous studies could not be compared due to the different analytical techniques

applied [such as gas chromatography (Haddad, Mozzon, Strabbioli, & Frega, 2011b), non-aqueous reverse-phase high-performance liquid chromatography (Zou et al., 2013) and supercritical fluid chromatography (Tu, Ma, Bai, & Du, 2017)], which could result in differences in the composition and concentration of TAG in human milk, and variations in human milk samples, the common features were still found. The MCFAs (8:0–14:0) were identified mainly as MLL type TAG in which 12:0 was the main MCFAs, with a relative low content of MMM and MML type. On the whole, MCFAs predominated in human milk as MLCT, especially the MLL type. Another characteristic was that the main MLCT molecule found in human milk comprised one or two unsaturated FA, such as the dominant MCFA-TAG components 12:0/16:0/18:1, 18:1/12:0/18:2, 10:0/16:0/18:1, 18:1/12:0/18:1 as listed in Table 2. The special structure of TAG might be a significant finding. The common TAG in the five most abundant MCFA-TAGs of these studies was 12:0/16:0/18:0 (Table 2).

There was a similar increasing trend as the lactation prolonged that the content of MCFA-TAGs in transitional and mature milk was higher than that in colostrum (Zhao et al., 2018; Zou et al., 2013). The increase was consistent with the change in level of MCFAs in human milk during lactation. This could be explained by the maturity of the mammary gland during lactation and therefore more ready for the de novo synthesis of these FAs (Neville & Picciano, 1997). This indicates the changes reflected the development of the mammary gland to accommodate for the changing needs in the developmental stages of infants, as growth will increase the energy requirements. It may be an advantage for infant as the MCFAs could be a rapidly utilised source of energy.

Differences in the MCFA-TAGs in human milk from different areas and countries could show the effect of dietary habits on the TAG composition (Kallio et al., 2017). The content of 18:1/12:0/18:1 (2.52%) in Finnish milk samples was higher compared with Chinese samples (1.61%), the same as 18:1/14:0/18:1 (3.17% in Finnish and 1.61% in Chinese). On the contrary, a lower proportion of 18:2/12:0/18:2 in Finnish samples (0.26%) than in Chinese samples (1.34%) was observed. One explanation for this might be the difference in FA 18:2 (9.31% in Finnish and 23.29% in Chinese) and FA 18:1 (32.32% in Finnish and 28.73% in Chinese). As was the case with 18:1/16:0/18:1 and 18:1/16:0/18:2 observed before (Kallio et al., 2017; Yuan et al., 2019), the most abundant TAG was 18:1/16:0/18:1 in human milk of Europeans (Morera et al., 2003; Ten-Domenech, Beltran-Iturat, Herrero-Martinez, Sancho-Llopis, & Simo-Alfonso, 2015) and 18:1/16:0/18:2 in Chinese (Tu et al., 2017).

Overall, the total and sn-2 MCFA composition of infant formulas was close to that in human milk, but there were huge gaps in the content and composition of MCFA-TAGs between infant formula and human milk. In most POFs, the MCFA-TAGs were characterised by MMM (Supplementary material Fig. S1), which was abundant with 12:0/12:0/14:0, 12:0/12:0/12:0, 10:0/12:0/12:0 (Fig. 1). However, there were only small amount of MMM type in human milk, which were less than 2% of the total TAG (Table 2). As known that the POF was usually prepared with supplement of coconut oil or other oils rich with MCFA to make the similar MCFAs with human milk or to provide energy directly for newborn babies, which might be responsible for this distinction. It is important to realise the issue of whether the high concentration of MMM type TAG in POF is necessary or beneficial for the growth and development of infants. It was well-known that MCFAs could be oxidised rapidly and provided energy immediately for premature neonates, MCT was generally added into formulas, especially for premature and low birth weight infants. However, in previous study it reviewed that the formulas with MCT could not improve short-term growth of premature infants (Klenoff-Brumberg & Genen, 2002). The administration of MCFAs as MCT at concentrations exceeding the neonate's requirements in a short time may prevent the administration of other essential FAs in neonates (Pettei et al., 1991). In addition, high level of MTC can potentially lead to gastrointestinal intolerance and dicarboxylic aciduria (Wu et al., 1993). The concerns that what effect of the high content of MCT in infant formulas would have on infants, how much MCT is appropriate for infants, and the effect of structure of MCFA-TAG on infants should be noticed.

The significant higher 12:0-MLL content compared with all infant formulas was a feature of human milk. This feature might have some specific functions for infants. MCFAs, especially 10:0 and 12:0, might have an important role in modifying the establishment of the gut microbiota and preventing gut infection (Arsenault et al., 2019; Nejrup et al., 2017). Furthermore, among the saturated FAs, 12:0 has the most active against Gram-positive bacteria and 1-monolaurin acid was more active than FA (Kabara, Swieczkowski, Conley, & Truant, 1972). More investigations will be required to the functional effects of this structure of TAG on the composition of the gut microbiota in relation to the health of infants.

5. Conclusions

As far as we know, this is the first time that the MCFAs, as well as the MCFA-TAGs in both human milk and infant formulas, have been systematically studied. Although the MCFAs composition of infant formulas was similar with that in human milk, there were marked differences in the content and composition of MCFA-TAGs in the infant formulas and human milk. In human milk, MCFAs mainly exist together with LCFAs as MLCTs, which is rarely present in infant formula. With regard to the different brands of commercial formulas, the composition of MCFA-TAGs in infant formulas was strongly affected by the fat source. Compared with human milk, POF contained higher content of MMM type TAG such as 12:0/12:0/14:0 and 12:0/12:0/12:0, and CMF and GMF contained lower content of MLL type TAG in which 14:0/18:1/18:1 was the most abundant TAG. The results could be useful for designing the formulas adjusted suitably for infants. The gap between human milk and infant formula regarding the MCFAs should be studied further. More research is needed concerning the nutritional functions of the specific MLCTs in human milk and the effect of the differences in MCFA-TAGs structure between human milk and infant formulas on infants. The nutritional effects of MLCT may promote the MCFAs use in infant formula as well as food for special medical purposes, in the treatment of infant with special fat absorption requirements, such as preterm infants, short bowel syndrome (SBS) and atopic disorders.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2019.104545.

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