Available online at www.sciencedirect.com

ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology 52 (2013) 527–533

www.tjog-online.com

Original Article

Comparison of free fatty acid content of human milk from Taiwanese mothers and infant formula

Chih-Kuang Chuang [a,b,c,*,1], Chun-Yan Yeung [b,d,e,1], Wai-Tim Jim [d,e], Shuan-Pei Lin [a,d,e], Tuen-Jen Wang [f,g], Sung-Fa Huang [f,g], Hsuan-Liang Liu [b,**]

[a] *Division of Genetics and Metabolism, Department of Medical Research, Mackay Memorial Hospital, 92 Chung-Shan North Road, Section 2, Taipei 10449, Taiwan*

[b] *Institute of Biotechnology, National Taipei University of Technology, 1 Zhongxiao East Road, Section 3, Taipei 10608, Taiwan*

[c] *College of Medicine, Fu-Jen Catholic University, 510 Chung Cheng Road, Hsinchuang, Taipei County 24205, Taiwan*

[d] *Department of Pediatrics, Mackay Memorial Hospital, 92 Chung-Shan North Road, Section 2, Taipei 10449, Taiwan*

[e] *Department of Early Childhood Care and Education, Mackay Junior College of Medicine, Nursing and Management, 92 Shengjing Road, Beitou District, Taipei 11260, Taiwan*

[f] *Department of Laboratory Medicine, Mackay Memorial Hospital, 92 Chung-Shan North Road, Section 2, Taipei 10449, Taiwan*

[g] *School of Medical Laboratory Science and Biotechnology, Taipei Medical University, 250 Wu-Hsing Street, Taipei 11031, Taiwan*

Accepted 26 December 2012

Abstract

Objective: Few studies on the free fatty acid (FFA) content of milk from non-Caucasian mothers have been published. We compared the FFA concentrations in human milk (HM) from Taiwanese mothers of preterm (PTHM) and full-term infants (FTHM) and in infant formula (IF).
Materials and methods: Thirty-eight HM samples were collected from 23 healthy lactating mothers and 15 mothers who gave birth prematurely (range 29–35 weeks, mean 33 weeks). The regular formula and preterm infant formula (PTIF) for three brands of powdered IF were also evaluated. Milk samples were extracted and methylated for analysis by gas chromatography/mass spectrometry (GC/MS).
Results: Reference values for individual FFAs in breast milk from Taiwanese mothers were determined. The mean total FFAs were significantly higher in IF (21,554 μmol/L) and PTIF (19,836 μmol/L) than in FTHM (8,540 μmol/L) and PTHM (9,259 μmol/L) ($p < 0.05$). Saturated FAs were predominant in all types of milk (43.1% for FTHM, 42.8% for PTHM, 45.5% for IF and 45.3% for PTIF). Monounsaturated FAs were significantly higher in IF and PTIF (42.6% and 43.9%) than in FTHM and PTHM (37.7% and 39.5%), and polyunsaturated FAs in FTHM and PTHM (20% and 18.2%) were higher than in IF and PTIF (11.9% and 10.9%). HM had a more desirable linoleic acid/α-linolenic acid ratio than IF. No significant differences in individual FFAs in FTHM were observed among three lactating periods.
Conclusion: FFA levels in HM from Taiwanese mothers are in agreement with results for different geographically distinct populations. Nevertheless, the FFA content in IF did not meet well with HM, particularly, the excess additives of saturated and monounsaturated FAs, and the shortage of polyunsaturated FAs. The effect of variations in FFA content in IF on future unfavorable outcomes such as obesity, atopic syndrome, and less optimal infant neurodevelopment should be further investigated.

Keywords: free fatty acids; human milk; infant formula; gas chromatography/mass spectrometry

* Corresponding author. Department of Medical Research, Mackay Memorial Hospital, 92 Chung-Shan North Road, Section 2, Taipei 10449, Taiwan.
** Corresponding author. Institute of Biotechnology, National Taipei University of Technology, 1 Zhongxiao East Road, Section 3, Taipei 10608, Taiwan.
E-mail addresses: cck@ms1.mmh.org.tw (C.-K. Chuang), f10894@ntut.edu.tw (H.-L. Liu).
[1] These authors contributed equally to this work.

1028-4559/$ - see front matter
http://dx.doi.org/10.1016/j.tjog.2013.10.013

Introduction

Breast milk is an essential and natural nutrient source for the normal growth and development of infants. The composition of breast milk is recognized as the gold standard for the manufacture of infant formula (IF). Since early 2000, long-chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (22:6 ω3; DHA) and eicosapentaenoic acid (20:5 ω3; EPA) have been added to IF and the effectiveness of LCPUFA supplementation in infancy is of great interest. In particular, two LCPUFA groups have attracted special interest. Homologs of linoleic acid (18:2 ω6; LA) of the n-6 series are precursors of arachidonic acid (20:4 ω6; AA), and homologs of α-linolenic acid (18:3 ω3; ALA) of the n-3 series are precursors of EPA and DHA. Breast milk contains the essential FA precursors (LA and ALA) and adequate AA and DHA, and the crucial role of these FAs in central nervous system development and retinal function has been verified in many studies [1–4].

FAs in human milk, particularly LCPUFAs, contribute significantly to early infant development. According to Jensen et al, FAs provide approximately 50% of an infant's energy requirements [5]. Inadequate FA supply, especially of LCPUFAs, may have negative effects on neurologic function, visual acuity, psychomotor function, and immunological development [6–8]. There is also evidence that infants who receive DHA or EPA supplementation score significantly higher on the Bayley Psychomotor Development Index at the age of 30 months [9]. Birch and colleagues suggested that infants fed IF not fortified with DHA and/or AA exhibit lower visual acuity. This is because a reduction in DHA exposure can alter nerve cell signaling, leading to permanent changes in neural membrane function and cortical visual acuity [10]. In addition, some studies reported that atopy is associated with a higher n-6 LCPUFA status and a low n-3 LCPUFA status. Fish oil supplementation in infancy may decrease the risk of developing some manifestations of allergic disease [11,12]. Fish and fish oils are sources of long-chain n-3 PUFAs and these FAs act to oppose the actions of n-6 PUFAs [12].

Breastfeeding in Taiwan has been promoted since the 1980s. According to a Taiwan Bureau of Health Promotion survey in 2004, only 29.4% of lactating mothers started exclusive breastfeeding, which is lower than in the USA (>70%) and Norway (99%). Only 13.1% of mothers were practicing restricted breastfeeding 6 months after delivery, and 21.2% of mothers were feeding children under 6 months of age with a mix of human milk (HM) and IF. This means up to 80% of young children in Taiwan rely on IF [13,14]. To evaluate the effect of food on infant growth, it is important to know the FFA content in milk. However, there have been very few studies on this topic in Taiwanese women. Clarifying the FFA content in HM and IF may help industry to manufacture IF with a composition closer to that of HM. We designed this study to compare HM from mothers with preterm and full-term infants, and to compare HM with three brands of IF in both regular and preterm infant formulations. In addition, we investigated the changes in FFA composition in HM through the development from colostrum to transitional milk to mature milk.

Materials and methods

Preparation of fatty acid methyl esters (FAMEs) is by far the most common approach for lipid analysis [15,16]. FAMEs were separated and quantified on an Agilent 5975C Inert GC/MSD system using a capillary column equipped and a flame ionization detector (Agilent Technologies, Inc., Santa Clara, CA., USA). FFAs in milk were derivatized to FAMEs using 3N methanolic HCl, (MeHCl) as an acid catalyst for gas chromatography/mass spectrometry (GC/MS) analysis. Individual FFAs in HM and IF were quantified according to individual FA calibration curves, a known concentration of an internal standard and its peak area, and the internal response factor (IRF).

Standards and reagents

All fatty acid standards, including decanoic acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2 ω6), α-linolenic acid (C18:3 ω3), γ-linolenic acid (C18:3 ω6), arachidic acid (C20:0), homo-γ-linolenic acid (C20:3), arachidonic acid (C20:4 ω6), eicosapentaenoic acid (20:5 ω3), docosanoic acid (C22:0), docosahezaenoic acid (C22:6 ω3), and tetracosanoic acid (C24:0), were purchased from Sigma (St. Louis, MO, USA). FA standard solutions (10 mL) were prepared at a concentration of 1 mM in 1:1 (vol/vol) chloroform/methanol. Aliquots of each FA standard solution were stored at −20 °C. Chloroform, methanol, and hexane were purchased from Merck (Darmstadt, Germany); 3 N MeHCl was from Supelco (Bellefonte, PA. USA), and HiPerSolv methanol was from BDH

Table 1
Molecular weight, major ion fragments, and retention time (RT) for individual fatty acid methyl esters detected by SIM-GC/MS.

Fatty acid	Mass	Major ions	RT (min)
Decanoic acid (C10:0)	186	74, 87, 143, 186	5.522
Lauric acid (C12:0)	214	74, 87, 171, 183, 214	6.811
Myristic acid (C14:0)	242	74, 87, 143, 199, 211, 242	9.367
Palmitoleic acid (C16:1)	268	55, 69, 194, 236, 268	12.276
Palmitic acid (C16:0)	270	74, 87, 143, 227, 239, 270	12.652
α-Linolenic acid (C18:3 ω3)	292	55, 67, 79, 150, 292	15.243
Linoleic acid (C18:2 ω6)	294	55, 67, 81, 95, 264, 294	15.561
Oleic acid (C18:1)	296	55, 69, 83, 180, 222, 264, 296	15.738
Stearic acid (C18:0)	298	74, 87, 143, 199, 255, 298	16.091
Arachidonic acid (C20:4 ω6)	318	55, 67, 133, 150, 203, 318	18.364
Eicosapentaenoic acid (20:5 ω3)	316	55, 67, 133, 147, 201, 316	18.417
Homo-γ-linolenic acid (C20:3)	320	67, 79, 121, 135, 222, 320	18.581
Arachidic acid (C20:0)	326	55, 143, 283, 326	19.459
Docosahezaenoic acid (C22:6 ω3)	342	55, 67, 79, 131, 145, 342	22.298
Docosanoic acid (C22:0)	354	74, 87, 143, 311, 354	24.400
Tetracosanoic acid (C24:0)	368	74, 87, 143, 325, 368	27.971