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A novel infant milk formula concept: Mimicking the human milk fat globule structure

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ABSTRACT

Human milk (HM) provides all nutrients to support an optimal growth and development of the neonate. The composition and structure of HM lipids, the most important energy provider, have an impact on the digestion, uptake and metabolism of lipids. In HM, the lipids are present in the form of dispersed fat globules: large fat droplets enveloped by a phospholipid membrane. Currently, infant milk formula (Control IMF) contains small fat droplets primarily coated by proteins. Recently, a novel IMF concept (Concept IMF) was developed with a different lipid architecture, Nuturis®, comprising large fat droplets with a phospholipid coating. Confocal laser scanning microscopy (CLSM), with appropriate fluorescent probes, and transmission electron microscopy were used to determine and compare the interfacial composition and structure of HM fat globules, Concept IMF fat droplets and Control IMF fat droplets. The presence of a trilayer-structured HM fat globule membrane, composed of phospholipids, proteins, glycoproteins and cholesterol, was confirmed; in addition exosome-like vesicles are observed within cytoplasmic crescents. The Control IMF fat droplets had a thick protein-only interface. The Concept IMF fat droplets showed a very thin interface composed of a mixture of phospholipids, proteins and cholesterol. Furthermore, the Concept IMF contained fragments of milk fat globule membrane, which has been suggested to have potential biological functions in infants. By mimicking more closely the structure and composition of HM fat globules, this novel IMF concept with Nuturis® may have metabolic and digestive properties that are more similar to HM compared to Control IMF.

1. Introduction

Human milk (HM) is the sole source of nutrients and energy in the form of lipids, proteins, carbohydrates, vitamins and minerals, and is considered to be the gold standard to support optimal growth and development of the newborn. Lipids in HM produced by the epithelial cells of the mammary gland represent up to 55% of the total caloric intake of the newborn [1]. The World Health Organization recommends to exclusively breast-feed during the first 6 months of life and to complement breastfeeding with appropriate infant foods up to 2 years of age or beyond. Breast-fed infants have a reduced risk of obesity and metabolic disease later in life compared to infants fed infant milk formula (IMF) [2,3]. Breastfeeding also has a positive effect on cognitive and immune functions [2,3].

HM (and mammalian raw milk in general) has a distinct lipid architecture as a result of the way the fat globules are produced and secreted from the mammary gland cells. The milk fat globule is composed of a triglyceride core enveloped by a trilayer, the milk fat globule membrane (MFGM), composed mainly of phospholipids, specific proteins and also cholesterol [4,5]. The size of the fat globules in mature HM varies from 0.1 μm up to 15 μm with a mode diameter between 3 and 5 μm, based on volume [6]. Approximately 98–99% of HM lipids are in the form of triglycerides [7]. HM contains 3–4.5 g fat/100 mL of which 0.4–0.5% are in the form of phospholipids and composed of sphingomyelin (36–42%), phosphatidylcholine (25–28%), phosphatidylethanolamine

Abbreviations: HM, human milk; IMF, infant milk formula; MFGM, milk fat globule membrane; WGA, wheat germ agglutinin; Rd-DOPE, rhodamine-dioleoyl-phosphatidylethanolamine; CLSM, confocal laser scanning microscopy; TEM, transmission electron microscopy; MUC, mucin; l_o, liquid-ordered; l_d, liquid-disordered.

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(17–23%), phosphatidylserine (7–10%) and phosphatidylinositol (4–7%) [8]. The main proteins in MFGM are xanthine oxidase, adipophilin, fatty acid binding proteins and glycoproteins such as mucins, lactadherin, butyrophilin and CD36 [9]. MFGM glycosphingolipids comprise cerebrosides and gangliosides, which have been shown to contribute to the development of the brain and gut immune system of infants [1]. The glycoproteins and glycolipids, with their carbohydrate residues protruding into the aqueous phase, form the MFGM glycocalyx, a rich source of bacterial and viral ligands [10]. The MFGM lipids and proteins mentioned have been shown to have important biological functions such as antimicrobial, antibacterial and antiviral activities [1,10–12]. Sphingomyelin and its metabolites play a role in gut maturation as well as myelination of the developing central nervous system in the newborn [11]. The supply of long chain fatty acids from HM phospholipids is crucial for neural membrane synthesis [8].

Currently, the IMF design is based on the nutrient composition of mature HM to deliver adequate levels of macronutrients (carbohydrates, lipids and proteins) and micronutrients (vitamins and minerals) to support growth. In current IMF (Control IMF), fat droplets have a mode diameter of 0.4 μm, based on volume, and proteins are the main emulsifier of fat droplets. This results in a stable and reproducible product with a long shelf life but with a fat droplet architecture strongly different from that in HM. Studies showed that the type of emulsifier as well as the size of emulsion droplets affect lipolysis [13–15]. In preterm infants, hydrolysis of human milk triglycerides was higher than that of triglycerides of a standard IMF with small fat droplets coated by proteins [16]. This effect was attributed to the difference in size and interfacial coating of the fat droplets in addition to a different fatty acid profile between human milk and standard IMF. Given the emerging interest in the bioactivity of MFGM compounds and the potential effect of food structure on digestion, absorption of lipids and metabolic fate, we have developed a novel IMF concept (Concept IMF) mimicking more closely the composition and structure of HM fat globules. To this end, the production process was modified and adapted and a phospholipid-enriched dairy source was used. Our Concept IMF (patent EP2825062A1) contains Nuturis®, large fat droplets coated by added phospholipids that are present in similar amount and profile as in HM [17]. In previous rodent studies, mouse pups were fed this Concept IMF or Control IMF from infancy until postnatal Day 42. Mice fed Concept IMF until early adulthood showed reduced adiposity when subsequently challenged by a moderately high-fat Western-style diet in adolescence and adulthood despite similar food intake [18,19]. These nutritional programming models using rodent pups [18,19] suggested that the altered lipid structure of the Concept IMF in early life affected adult adipocyte functionality but not adipocyte number. Also, the metabolic response to this diet was improved in Concept IMF-fed mice, with lower body weight and lower plasma insulin despite similar food intake. In the present study, we characterized the structure of the fat droplets of the Concept IMF (Nuturis®) in more detail and compared it with the structure of HM fat globules as well as Control IMF fat droplets using advanced microscopic techniques.

2. Materials and methods

2.1. Milk samples

Expressed mature HM (less than 15 mL) was collected in the morning using a breast milk pump from healthy donors (four for particle size measurement, three for confocal laser scanning microscopy (CLSM) and two for transmission electron microscopy (TEM)) between 2 and 12 months postpartum. Each freshly expressed milk sample was kept at room temperature until analysis. The particle size measurement was carried out maximum 2 h after collection and the sample preparation for CLSM and TEM was carried out just after collection.

The Control IMF (powder) was produced according to current standard stage I IMF recipe and processing procedures (Danone Nutricia Research, Utrecht, The Netherlands). The Concept IMF (powder) was produced by adding bovine milk phospholipids (as beta-serum, Fonterra Co-operative Group Ltd., New Zealand) to an amount of 1.5% of total fat (corresponding to 0.5 g of milk phospholipid in 1 L of reconstituted Concept IMF), and processing procedures were modified as to yield larger fat droplets than in Control IMF. The beta-serum was added to the aqueous phase along with proteins, lactose, vitamins and minerals. The aqueous phase was pasteurized at 85 °C for 6 min and then homogenized with the vegetable oil blend (i.e., the lipid phase) with an inline mixer to obtain large and phospholipid-coated fat droplets [17]. Concept and Control IMF powders were freshly reconstituted as follows: 13.7 g of powder was mixed with 90 g of tap water at 40 °C. The reconstituted Concept and Control IMFs contained 33.5 g/L of fat, 13.2 g/L of proteins and 70.0 g/L of lactose. As an additional control, 2 g of beta-serum, also commonly called butter serum, was reconstituted in 10 mL of water at room temperature for imaging purpose.

2.2. Particle size

The particle size distribution of HM, Concept IMF and Control IMF was determined using a laser light scattering instrument (Mastersizer 2000—Hydro 2000G, Malvern Instruments, UK). The refractive index for the dispersed phase was 1.460 and the absorbance was 0.001; the parameters used for the continuous phase were those of water (i.e., viscosity 0.8872 cP; refractive index 1.330). Measurements were done in duplicate.

2.3. Confocal laser scanning microscopy

Nile Red (Sigma–Aldrich, Zwijndrecht, The Netherlands), a lipophilic fluorescent probe, was diluted at 1 mg/mL in acetone (Fisher Scientific, Landsmeer, The Netherlands). Fast Green FCF (Sigma–Aldrich) was diluted at 1 mg/mL in deionized water. The fluorescent phospholipid analogue 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (Rd-DOPE, Avanti Polar Lipids, Alabaster, AL, USA) was diluted at 1 mg/mL in chloroform (Merck, Houten, The Netherlands). Filipin complex from *Streptomyces filipinensis* (Sigma–Aldrich) was diluted at 2 mg/mL in ethanol (VWR International, Amsterdam, The Netherlands). The Alexa Fluor 488 conjugate of wheat germ agglutinin (WGA, 1 mg/mL in 0.2 M PBS, pH 7.4) was purchased from Invitrogen (Carlsbad, CA, USA). All solvents were of analytical grade.

The samples were single- or dual-stained with Nile Red (1:100 v/v), Fast Green FCF (1:100 v/v), Rd-DOPE (1:100 v/v) and WGA (5:100 v/v) as described previously in Gallier et al. [20]. The samples were allowed to react for 20 min in the dark at room temperature. The staining with filipin (1:10 v/v) was carried out at 40 °C for 1 h in the dark. 40 μL of stained sample was quickly mixed with 70 μL of 1% melted agarose (Sigma–Aldrich) solution. A coverslip was placed rapidly on top of the sample before gelling.

An inverted confocal laser scanning microscope LSM 700 Axio Observer Z1 (Zeiss, Oberkochen, Germany) was used to image the HM and reconstituted IMF samples with a Plan-Apochromat 63×/1.40 Oil DIC (WD = 0.19 mm) objective. The images were analyzed with the proprietary Zeiss Zen 2011 software.

Nile Red and WGA were each excited with the 488 nm laser line and the filters were set to collect the emitted light between 493 and 550 nm. Dual-stained samples with Nile Red and WGA were excited with the 555 nm laser line (for Nile Red) and the emitted light was collected between 560 and 800 nm. The same latter parameters