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Size Distribution of Fat Globules in Human Colostrum, Breast Milk, and Infant Formula

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ABSTRACT

Only a few results are available on the size of human milk fat globules (MFG), despite its significance regarding fat digestion in the infant, and no data are available at <24 h postpartum (PP). We measured the MFG size distribution in colostrum and transitional human milk in comparison with fat globules of mature milk and infant formula. Colostrum and transitional milk samples from 18 mothers were collected regularly during 4 d PP and compared with mature milk samples of 17 different mothers and 4 infant formulas. The size distribution was measured by laser light scattering. For further characterization, the ζ-potential of some mature MFG was measured by laser Doppler electrophoresis. The MFG diameter decreased sigmoidally in the first days. At <12 h PP, the mode diameter was 8.9 ± 1.0 μm vs 2.8 ± 0.3 μm at 96 h PP. Thus, the surface area of MFG increased from 1.1 ± 0.3 to 5.4 ± 0.7 m^2/g between colostrum and transitional milk. In mature milk, the MFG diameter was 4 μm on average and increased with advancing lactation, whereas the droplets in infant formula measured 0.4 μm. The ζ-potential of mature MFG was –7.8 ± 0.1 mV. The fat globules are larger in early colostrum than in transitional and mature human milk and in contrast with the small-sized fat droplets in infant formula. Human MFG also have a low negative surface charge compared with bovine globules. These structural differences can be of nutritional significance for the infant. (**Key words:** human milk, milk fat globule, size distribution, ζ-potential)

Abbreviation key: $\mathbf{d_{32}}$ = volume-surface average diameter, $\mathbf{d_{43}}$ = volumic average diameter, **MFG** = milk fat globule, **PP** = postpartum, **SSA** = specific surface area.

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INTRODUCTION

Fat is a major component of human milk and is composed of >98% triacylglycerols (Mulder and Walstra, 1974; Hamosh et al., 1985; Jensen et al., 1990). The nonpolar nature of milk lipids prevents solubility in the aqueous phase, within the mammary secretory cells before secretion, as well as in milk (Hamosh et al., 1999). Thus, fat globules are formed throughout the mammary epithelial cell, grow in size as they move toward the apical cell membrane, and are extruded into the alveolar lumen (Jensen et al., 1990; Keenan and Dylewski, 1995; Keenan, 2001; Ollivier-Bousquet, 2002). During the extrusion process, the globule is enveloped by portions of the cell membrane, which becomes the milk fat globule (**MFG**) membrane. The core of the MFG contains triacylglycerols (Figure 1), and the membrane has the general composition of biological membranes, i.e., phospholipid, cholesterol, glycoproteins, enzymes, etc. (Anderson and Cawston, 1975; McPherson and Kitchen, 1983; Christie, 1995; Keenan and Dylewski, 1995; Hamosh et al., 1999). The physico-chemical properties [structure, size distribution, electrokinetic potential (or so-called ζ-potential), and thermal behavior] of bovine MFG have been widely studied (Walstra, 1969; Mulder and Walstra, 1974; Christie, 1995; Walstra, 1995; Lopez et al., 2000; Michalski et al., 2002d; Briard et al., 2003).

Many studies concern the chemical nature and biochemical significance of human milk lipids (Emmett and Rogers, 1997; Francois et al., 1998; Villalpando and del Prado, 1999; Fidler and Koletzko, 2000; Francois et al., 2003; Schweigert et al., 2004). However, despite the great significance of the physical structure of milk fat regarding lipase acitivity, cholesterol availability and lipid absorption in the gastrointestinal tract of neonates (Bitman et al., 1983; Armand et al., 1996; Armand, 1998; Armand et al., 1999; Hamosh et al., 1999; Lönnerdal, 2003), only a few researchers have studied the size distribution of human MFG (Whittlestone and Perrin, 1954; Rüegg and Blanc, 1981, 1982; Simonin et al., 1984). Since the latter studies, in which a microscope or a particle counter were

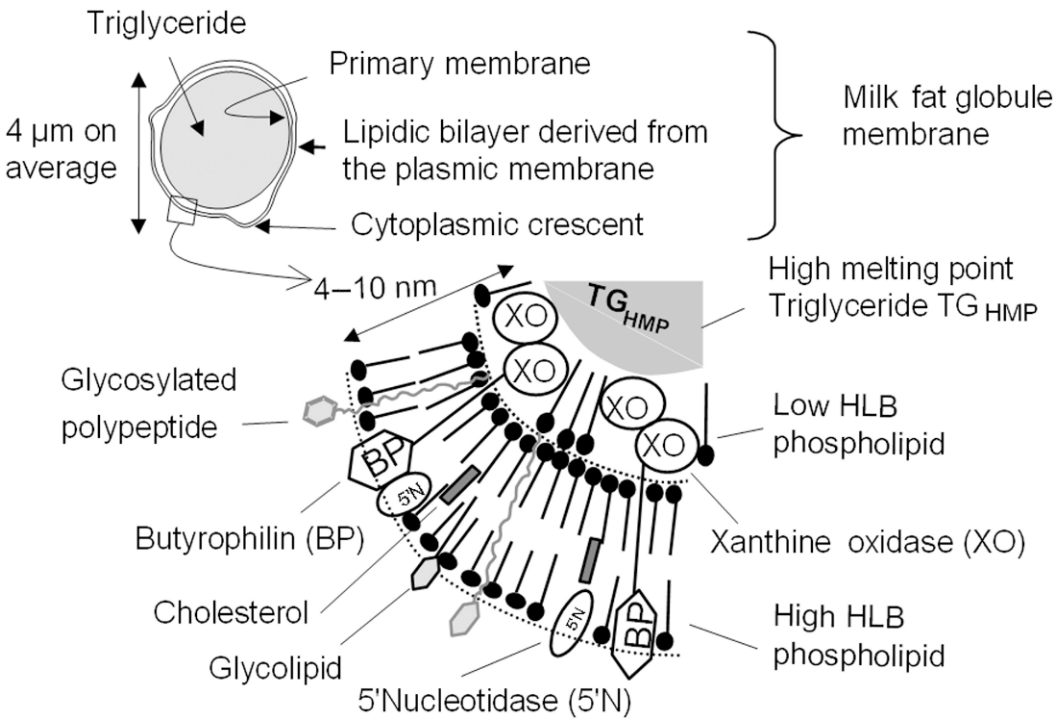

Figure 1. Typical structure of the native milk fat globule. Schemes are not to scale.

used, laser light scattering devices have been developed (McCrae and Lepoetre, 1996); the newest ones allow a better study of MFG size distribution from submicronic to micrometric particles thanks to 2 different laser beams (Michalski et al., 2001). To our knowledge, no MFG size data are available to date regarding colostrum during the first 24 h after delivery, although it is known that colostrum composition varies greatly within the first 4 d postpartum (**PP**) (Jensen et al., 1990). Also, the surface properties of human MFG, such as their electrokinetic- or ζ-potential, which accounts for the surface charge of the globules and can affect globule interactions with proteins and lipases, have not been yet characterized.

The aim of the present study was to measure the fat globule size distribution in colostrum and transitional milk from mothers having delivered term infants during the first 4 d of lactation and to compare it with fat globule size measured from 1) mature human milk samples from other mothers corresponding to 1 to 37 mo of lactation and 2) infant formula for term infants. Typical physico-chemical parameters of the fat globules were characterized (average diameters, specific surface area, and span and ζ-potential and fat content for the mature milk of one mother) and compared with literature results regarding human, cow, and homogenized cow milk.

MATERIALS AND METHODS

Breast Milk Samples

Colostrum and breast milk samples from 18 volunteer mothers, who had delivered at term and signed an informed agreement for participating in the study, were collected twice a day [once in the morning and once in the evening from delivery (colostrum) to the end of the 4-d stay in the hospital (transitional milk)]. Consistent characteristics of the volunteer mothers are shown in Table 1. Thirteen mothers (71%) delivered with epidural analgesia; one of them delivered by caesarean. All samples were collected with midwife supervision by manual expression in microtubes without preservative. For ethical reasons, samples were collected from the sucked breast at the end of nursing so that the fat milk could be analyzed (Aksit et al., 2002). This method is physiologically consistent as the milk obtained by manual or mechanical expression may not have the same composition as milk consumed during breastfeeding itself (Lucas et al., 1977; Aksit et al., 2002). Some samples were also collected from the opposite breast before nursing to check that fat globule size did not vary throughout the single feeding. The samples were stored at 4°C until they were analyzed in the laboratory the day after collection. Preliminary experiments in our laboratory had shown that