Organellar Contacts of Milk Lipid Droplets

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Abstract
Milk-secreting epithelial cells of the mammary gland are functionally specialized for the synthesis and secretion of large quantities of neutral lipids, a major macronutrient in milk from most mammals. Milk lipid synthesis and secretion are hormonally regulated and secretion occurs by a unique apocrine mechanism. Neutral lipids are synthesized and packaged into perilipin-2 (PLIN2) coated cytoplasmic lipid droplets within specialized cisternal domains of rough endoplasmic reticulum (ER). Continued lipid synthesis by ER membrane enzymes and lipid droplet fusion contribute to the large size of these cytoplasmic lipid droplets (5–15 μm in diameter). Lipid droplets are directionally trafficked within the epithelial cell to the apical plasma membrane. Upon contact, a molecular docking complex assembles to tether the droplet to the plasma membrane and facilitate its membrane envelopment. This docking complex consists of the transmembrane protein, butyrphilin, the cytoplasmic housekeeping protein, xanthine dehydrogenase/oxidoreductase, the lipid droplet coat proteins, PLIN2, and cell death-inducing DFFA-like effector A. Interactions of mitochondria, Golgi, and secretory vesicles with docked lipid droplets have also been reported and may supply membrane phospholipids, energy, or scaffold cytoskeleton for apocrine secretion of the lipid droplet. Final secretion of lipid droplets into the milk occurs in response to oxytocin-stimulated contraction of myoepithelial cells that surround milk-secreting epithelial cells. The mechanistic details of lipid droplet release are unknown at this time. The final secreted milk fat globule consists of a triglyceride core coated with a phospholipid monolayer and various coat proteins, fully encased in a membrane bilayer.

Keywords
cell biology, contact, electron microscopy, endoplasmic reticulum, lipid droplet, membrane

Introduction
Lipids are key macronutrients of milk and a major source of calories and essential bioactive molecules required for neonatal growth and development in many mammalian species (Ofteød, 1984). Physiological conditions that reduce the quantity or alter the composition of lipids secreted into milk, such as maternal obesity, are linked to impaired lactation outcomes and altered neonatal metabolic function in humans and animal models (Wahlig et al., 2012; Rudolph et al., 2017). In mice, genetic deletion of key proteins regulating the synthesis or secretion of milk lipids have been shown to produce lactation deficiency or failure (Vorbach et al., 2002; Cases et al., 2004; Ogg et al., 2004; Beigneux et al., 2006; Russell et al., 2011; Monks et al., 2016).

Milk lipids are composed primarily of neutral lipids (98%–99%) in the form of triglycerides, diglycerides, and cholesteryl esters (Jensen et al., 1990; Jensen, 1999), which are packaged into cytoplasmic lipid droplets within specialized milk-secreting mammary epithelial cells. Unlike serum lipids, which are secreted as soluble lipoprotein particles by vesicle-mediated exocytosis, milk lipids are secreted by a unique apocrine mechanism in which cytoplasmic lipid droplets are secreted intact as membrane-coated structures, referred to as milk fat globules (McManaman, 2012). Evidence is emerging that milk lipid biogenesis and secretion involve specific membrane-
and inter-organelle contacts, with novel molecular and structural features.

**Lipid Droplet Synthesis in the Mammary Epithelial Cell Begins During Pregnancy**

Milk-secreting mammary epithelial cells undergo functional differentiation and the capacity to synthesize milk substances during mid-pregnancy in most species. One of the earliest morphological features of this differentiation process, termed lactogenesis I, is the accumulation of cytoplasmic lipid droplets (Russell et al., 2007). These early droplets are synthesized primarily from fatty acids liberated from circulating lipoproteins by lipoprotein lipase located in the vascular bed of the mammary gland or by adipose-derived circulating fatty acids bound to albumin. However, after parturition and the onset of milk secretion, de novo synthesis of medium chain fatty acids by the milk-secreting epithelial cells from glucose occurs and synthesis of triglycerides increases precipitously, accompanying other changes in the gland such as closure of the tight junctions, milk protein and lactose synthesis and secretion, which define lactogenesis II (Rudolph et al., 2010; Lv et al., 2015).

The triglycerides that comprise the neutral lipid core of these cytoplasmic lipid droplets are synthesized by resident endoplasmic reticulum (ER) membrane enzymes, which catalyze sequential steps in the fatty acid esterification of glycerol-3-phosphate (Kennedy, 1957) or of sn2-monoacylglycerol (Coleman and Lee, 2004). Evidence from mouse knockout models has demonstrated the importance of triglyceride synthesis in the development and lipid secretion functions of mammary epithelial cells. Loss of glycerol-3-phosphate acyltransferase-4 (GPAT4), which catalyzes acylation of glycerol-3-phosphate, the first step in triglyceride synthesis, depletes milk-secreting mammary epithelial cells of cytoplasmic lipid droplets. Its loss also impairs mammary epithelium development and inhibits milk lipid secretion (Beigneux et al., 2006; Takeuchi and Reue, 2009). Similarly, loss of acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1), one of two DGAT enzymes that catalyze the final acylation step in TAG synthesis (Yen et al., 2008), in milk-secreting mammary epithelial cells impairs their functional differentiation and CLD accumulation, and inhibits milk secretion in mice (Cases et al., 2004). Interestingly, the observation that mammary gland expression of DGAT2, a major contributor to TAG synthesis from de novo synthesized fatty acids in many mammalian tissues (Yen et al., 2008), does not compensate for the effects of DGAT1 loss on mammary gland functional differentiation and CLD accumulation indicates that the two DGAT enzymes have different physiological functions as originally proposed by Robenek et al. (2006) in the

**Lipid Droplets Interact With ER**

Cytoplasmic lipid droplets originate from the ER in eukaryotic cells, and connections between these structures are necessary for initial lipid droplet expansion (Robenek et al., 2006; Wilfling et al., 2013). It is generally thought that cytoplasmic lipid droplets form from specific tubular microdomains of the smooth ER, based on structural considerations and evidence that disrupting the function of proteins responsible for smooth ER structure interferes with lipid droplet formation/expansion (Kassan et al., 2013; Walther et al., 2017). However, unlike other highly lipogenic cells, such as hepatocytes and adrenal cortical cells (Baumann and Walz, 2001; Shibata et al., 2006), milk-secreting epithelial cells are highly enriched in rough ER (Jarasch et al., 1977; Wooding, 1977), which possess the enzymes required for neutral lipid synthesis (Bauman and Davis, 1974) and can form extensive connections with lipid droplets (Stemberger et al., 1984; Figure 1(a)).

Evidence that lipid droplets originate from rough ER in milk-secreting epithelial cells, and that they are secreted into milk by a distinct mechanism, was obtained as early as 1967 by Stein and Stein. Using radioautography and electron microscopy (EM), these investigators showed that within 1 to 3 minutes after injecting radioactive palmitic or oleic acid into tail veins of lactating mice, labeled esters were first localized over rough ER cisternae in milk-secreting epithelial cells before being incorporated into rough ER-localized lipid droplets and then secreted into milk. Label was not observed in the Golgi or any secretory granules, indicating that unlike lipoproteins, milk lipids are not packaged into vesicles and secreted by an exocytic mechanism. Subsequent proteomic analyses of membrane-free preparations of lipid droplets from lactating mice by Wu et al. (2000) identified ER lumenal and membrane proteins, which provided the first biochemical evidence of an ER origin of these structures.

Variable degrees of contact, ranging from discrete contacts at single or multiple sites to more extensive contacts that follow the contour of the lipid droplet surface, exist between lipid droplet and ER membranes (Salo and Ikonen, 2019). Using electron tomography and high-pressure freezing and freeze substitution approaches that preserve cellular structures in near native states (McIntosh, 2001), ER cisternae in milk-secreting epithelial cells from lactating rats have been shown to form unique, concentric, multilayered contacts with lipid droplets, which can cover large areas of the droplet surface (Ladinsky et al., 2019; Figure 1(b)).
An egg cup model of cytoplasmic lipid droplet expansion, this organization is ideally configured for transferring neutral lipids and proteins from their sites of synthesis on ER membranes to support production and growth of lipid droplets, which may be particularly important during lactation when demand for lipid formation is increased. Ribosomes on these cisternae are located on outer membrane leaflets facing the cytoplasm or on cisternal membranes distal to sites of contact. However, ribosomes are absent from cisternal membranes that...
contact the lipid droplet surface or other cisternae. Despite their lack of direct contact, sufficient numbers of ribosomes remain associated with the lipid droplet upon secretion that many groups now use this milk fraction to noninvasively sample the milk-secreting cell transcriptome (Maningat et al., 2007; Brenaut et al., 2012; Lemay et al., 2013). In addition, the abundance of ribosomal proteins in the proteome of milk fat globules is so great that they are routinely ignored in pathway analyses (Honvo-Houeto et al., 2016).

Cytoplasmic lipid droplets are hypothesized to originate by the accumulation of neutral lipids between leaflets of the ER membrane by a *lensing* mechanism (Walther et al., 2017). Some of the earliest evidence of this lensing concept was obtained from electron micrographs of milk-secreting epithelial cells in lactating mammary glands (Long and Patton, 1978; Keenan and Dylewski, 1985; Zacek and Keenan, 1990), which showed lipids within distended ER membranes that were contiguous with ribosome-studded rough ER. More recently, Ladinsky et al. (2019) showed via EM tomography in milk-secreting epithelial cells from pregnant rats the presence of nascent lipid droplets within cisternal domains of rough ER, which are continuous with, and have the same density as, the ER lumen (Figure 1(c)). An ER luminal origin of lipid droplets is consistent with data from yeast indicating that neutral lipids accumulate in the ER lumen prior to be incorporated into droplets (Choudhary et al., 2011; Choudhary et al., 2015; Mishra et al., 2016). It is also supported by proteomic data that show selective enrichment of ER luminal proteins on cytoplasmic lipid droplets isolated from milk-secreting epithelial cells relative to those obtained from hepatocytes (Wu et al., 2000). Whether neutral lipid accumulation in the ER lumen ultimately leads to lipid droplet formation and how this transition occurs is not known. However, dynamic remodeling of ER-lipid droplet interacting domains involving fission and fusion of ER membranes (Walther et al., 2017) may provide a possible mechanism. Intriguingly, defects in lipid droplet formation in milk-secreting epithelial cells have been linked to abnormalities in ER membrane morphology associated with decreased levels of atlastin-2 (Le Guillou et al., 2019), a member of a family of GTPases previously shown to regulate ER fusion and lipid droplet size in *Caenorhabditis elegans* (Klemm et al., 2013).

### Lipid Droplets in Mammary Epithelial Cells Are Coated With PLIN2

Lipid droplet–ER interactions are mediated by specific protein interactions (Walther et al., 2017). Lipid droplets in milk-secreting epithelial cells are coated by perilipin-2 (PLIN2, also known as adipophilin/ADPH and adipose differentiation-related protein/ADRP) (Wu et al., 2000), which is a prominent, constitutively associated lipid droplet coat protein whose actions have been shown to regulate droplet size and to promote neutral lipid accumulation in multiple cell types including milk-secreting epithelial cells during functional differentiation of the mammary gland (Listenberger et al., 2007; Russell et al., 2007, 2011; Orlicky et al., 2019). PLIN2 is detected at sites of contact between lipid droplets and ER membranes (Fujimoto and Parton, 2011; Ladinsky et al., 2019), and its depletion has been shown to increase their interaction (Ozeki et al., 2005). At ER and lipid droplet contact sites, PLIN2 is hypothesized to stabilize the forming lipid droplet at the ER membrane, drawing it into the cytoplasm, instead of into the lumen of the ER (Robenek et al., 2006). PLIN2 is also thought to stabilize lipid droplets by inhibiting lipolysis (Listenberger et al., 2007). Several lipases are known to be associated with the secreted milk fat globule, including bile salt-stimulated lipase (CEL), lipoprotein lipase, and patatin-like phospholipase domain containing 2 (PNPLA2, a.k.a. ATGL; Monks et al., 2016). Silencing PNPLA2/ATGL in milk-secreting epithelial cells increases cytoplasmic lipid droplet accumulation and cellular tri-glyceride levels (Li et al., 2015). In mice, PLIN2 loss is associated with increased PNPLA2/ATGL binding to lipid droplets in milk-secreting epithelial cells, an effect that is reversed by adenoviral expression of GFP-tagged PLIN2 (Russell et al., 2011). Although experiments with PLIN2-deficient mice show a major role for PLIN2 in stabilization of lipid droplets, details about mechanisms remain uncertain and compensation by other members of the perilipin family, such as Plin3, is likely (Sztalryd et al., 2006; Russell et al., 2011; Monks, unpublished). Experiments exploring the role of PLIN2 in lipid droplet growth, stabilization, transport, and secretion in the mammary gland are ongoing.

### Lipid Droplets Associate With Other Organelles in Mammary Epithelial Cells

Lipid droplets in milk-secreting epithelial cells also interact with other organelles and cellular structures, including Golgi and mitochondria (Stemberger et al., 1984; Ladinsky et al., 2019). Large percentages of lipid droplets in the apical portion of milk-secreting epithelial cells are in contact with Golgi (21%), secretory vesicles (74%), and mitochondria (34%) (Stemberger et al., 1984). Benador et al. (2018) demonstrated that mitochondria bound to lipid droplets, termed peridroplet mitochondria (PDM) have functional and morphological properties that are distinct from cytoplasmic mitochondria. For example, PDM exhibit increased area contact with the lipid droplet surface and have metabolic properties that increase adenosine triphosphate (ATP)
and several lines of evidence indicate that this movement is mediated by interactions with microtubules (Spandl et al., 2009; Welte, 2009; Orlicky et al., 2013). In milk-secreting epithelial cells, the secretion of lipid droplets as milk fat globules requires their transport from their site of synthesis on ER in the basolateral portion of the cell to the apical surface for release. Analysis of fixed specimens by EM showed that the majority of microtubules in milk-secreting epithelial cells of lactating rats are oriented perpendicular to the apical plasma membrane with minus ends directed toward the apically located centriole (Dylewski and Keenan, 1984), suggesting the possible involvement of microtubules for lipid droplet trafficking. Directed movement of lipid droplets in lactating mouse mammary gland was measured via intravital imaging and was shown to be slower than that reported for droplets moving on microtubules in cultured cells (Masedunskas et al., 2017). This motility difference may suggest that lipid droplets in milk-secreting epithelial cells hitchhike with other organelles on microtubules (Guimaraes et al., 2015). However, the microtubule motor proteins kinesin and dynein were both found in the proteomics data sets of mouse milk fat globules and lipid droplets isolated from milk-secreting epithelial cells of lactating mice (Wu et al., 2000; Monks et al., 2016). The association of secretory vesicles with lipid droplets has been observed by many labs (Figure 2(b)) and may be the source of these motor proteins. Definitive demonstration of lipid droplet trafficking on specific cytoskeletal elements and motor proteins is, unfortunately, still lacking.

Lipid Droplets Dock at the Apical Plasma Membrane

As the cytoplasmic lipid droplets come into contact with the apical plasma membrane, docking and progressive membrane envelopment occurs prior to release as milk fat globules by an acocrine mechanism of milk secretion (Figure 2(a); Mather and Keenan, 1998; Heid and Keenan, 2005). Ultrastructural analyses revealed that docked lipid droplets are connected to the cytoplasmic face of the apical membrane by a 10- to 20-nm wide hexagonally ordered electron dense layer (Mather and Keenan, 1998), and biochemical studies indicate that this material is composed of the cytoplasmic tail of the transmembrane protein butyrophilin (BTN), the cytosolic protein xanthine dehydrogenase/oxidoreductase (XDH/XOR), and the lipid droplet coat protein PLIN2, linked covalently by disulfide bonding (McManaman et al., 2002; Heid and Keenan, 2005). Several protein disulfide isomerases (P4hb, Pdia3, Pdia6, and Pdia4) are found associated with secreted milk fat globules (Monks et al., 2016). Although many details remain to be elucidated,
direct binding of XDH/XOR to the B30.2 domain located in the cytoplasmic tail of BTN may form the basic structure (Jeong et al., 2009) and PLIN2, which contains distinct lipid droplet and phospholipid-binding domains (McManaman et al., 2003; Chong et al., 2011b), may act as a bridge between the lipid droplet and the membrane (Chong et al., 2011a). Our laboratory has also shown that CIDEA concentrates in the dock (Figure 2(c)), but it is unknown whether it interacts with BTN, XOR, or PLIN2, or perhaps self-associates, bridging between the apical plasma membrane and lipid droplet like PLIN2. Super-resolution microscopy (15 nm resolution) or cryoEM of the lattice would help with the elucidation of these structures.

Surprisingly, docking at the apical membrane is not absolutely required for milk lipid secretion. In mice in which XDH/XOR was specifically deleted from milk-secreting epithelial cells, lipid droplets fail to dock at the apical membrane, but they still undergo apocrine secretion and lactating dams are able to support their litters through weaning (Monks et al., 2016). Similarly in BTN and CIDEA knockout mice, which do exhibit lactation failure, lipid droplets appear to be secreted during the initial phase of lactation, although the process of secretion is impaired (Ogg et al., 2004; Wang et al., 2012). These observations suggest that lipid droplet–membrane docking may be an evolutionary adaptation of the apocrine mechanism to facilitate milk lipid secretion.

**Lipid Droplets Are Secreted Into the Milk Surrounded by Membrane**

Using intravital imaging, Masedunskas et al. (2017) showed that lipid droplets remain within the cytoplasm of milk-secreting epithelial cells in association with the
apical plasma membrane until oxytocin-stimulated myoepithelial cell contraction induces their secretion. In the absence of oxytocin stimulation, membrane docked lipid droplets become progressively enveloped by the apical membrane but do not undergo release (Monks, unpublished). However, within 1 minute of exposure to oxytocin, myoepithelial cells rhythmically squeeze the entire alveolus and membrane-docked lipid droplets pinch off into the milk (Masedunskas et al., 2017). The mechanism of this release is unclear; however, the milk fat globule that is released is completely surrounded by membrane. It has been noted that the function of the membrane is “stabilization of milk fat in the dispersed form, prevention of flocculation and coalescence of globules, as well as protection against adverse effects of lipases” (Smoczynski, 2017, p. 120). Indeed, secretion of improperly docked droplets in BTN or XOR knockout mice produces structures that seem to have fragile membranes and greater tendency to aggregate, resulting in clogging of milk ducts (Ogg et al., 2004; Monks et al., 2016). Analysis of the complement of proteins associated with the milk fat globule by untargeted proteomics suggests that the origin of the surrounding membrane is likely a combination of apical plasma membrane, ER, and secretory vesicle membrane, with possible contribution by Golgi (Wu et al., 2000; Chat et al., 2011; Wooding and Sargeant, 2015; Honvo-Houeto et al., 2016). The membrane fusion and fission events mediating the apocrine secretion of milk fat globules are, as yet, unknown.

**Lipid Droplets Are Degraded in Lysosomes During Mammary Gland Involution**

Upon weaning of the neonate, the remaining lipid droplets and milk fat globules in the gland must be cleared away. Sargeant et al. (2014) have shown that cathepsin-D positive autophagic vacuoles containing LipidTox-stained droplets appear in the gland within 24 hours of removing litters from lactating dams, suggesting that lipid degradation occurs by lysosomal-mediated processes within milk-secreting epithelial cells. These investigators also obtained evidence that fatty acids released during lysosomal-mediated lipolysis of lipid droplets cause leakage of cathepsin D, which triggers apoptosis of milk-secreting epithelial cells during mammary gland involution (Sargeant et al., 2014). It is not clear whether the lipids seen within the lysosomes were cytoplasmic droplets undergoing lipophagy or milk fat globules which had undergone efferocytosis and fusion with lysosomes. Further studies are necessary to resolve this conundrum.

**Summary**

Lipid droplets begin to form in earnest in milk-secreting epithelial cells in mid pregnancy, as small droplets within the ER. PLIN2 associates with these droplets early in their formation and may be necessary for stabilization. Lipid droplets in the milk-secreting epithelial cells may stay closely associated with ER, but cytoskeletal elements may also be involved in their trafficking to the apical part of the cell. Upon contacting the apical plasma membrane, several proteins rearrange and become posttranslationally modified to stabilize a lipid droplet docking complex. In this tethered position, Golgi, mitochondria, and secretory vesicles are often seen in close contact with lipid droplets, although the functional significance of this association is unknown. Final secretion of the lipid droplet from the cell as a milk fat globule is stimulated by contraction of the surrounding myoepithelial cells. The final globule, transported to the neonate, is composed of a neutral lipid core completely surrounded by a membrane bilayer originating from the plasma membrane, with possible contributions of ER and secretory vesicle membranes as well.

**Future Directions**

Nearly, all the mechanisms presented here have been determined by (a) fixed tissue image analysis of the mammary gland, especially electron micrographs, (b) composition analyses of secreted milk fat globules, and (c) cell fractionation of whole mammary gland with some inferred from other eukaryotic systems. Major gaps exist in our knowledge including which cytoskeletal elements and motors are responsible for transport. Is there potential hitchhiking of lipid droplets for directional trafficking within the milk-secreting epithelial cells? What are the molecular details and energetics of dock formation? How does the cell regulate the phospholipid monolayer upon droplet fusion? How does the ultimate release of the final droplet occur, including signaling, membrane engulfment and fission? As so beautifully executed by Mather et al. (2019), the technology is finally available to use intravital imaging to probe the live, secreting, lactating mammary gland directly, and to begin to answer these questions.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by NIH grant R01HD093729.
Keenan TW, Dylewski DP (1985). Aspects of intracellular transit of serum and lipid phases of milk. J Dairy Sci 68, 1025–1040. doi: 10.3168/jds.S0022-0302(85)80925-5
Kennedy EP (1957). Metabolism of lipides. Ann Rev Biochem 26, 119–148. doi: 10.1146/annurev.bi.26.070157.001003
Klemm RW, Horton JP, Cole RA, Li CS, Park SH, Crane MM, Li L, Jin D, Boye-Doe A, Liu TY, et al. (2013). A conserved role for atlastin GTPases in regulating lipid droplet size. Cell Rep 3, 1465–1475. doi: 10.1016/j.celrep.2013.04.015
Ladinsky MS, Mardones GA, Orlicky DJ, Howell KE, McManaman JL (2019). Electron tomography reveals that milk lipids originate from endoplasmic reticulum domains with novel structural features. J Mammary Gland Biol Neoplasia. In Press. doi: 10.1007/s10911-019-09438-y
Le Guillou S, Laubier J, Pechoux C, Aujean E, Castille J, Ladinsky MS, Mardones GA, Orlicky DJ, Howell KE, Lemay DG, Ballard OA, Hughes MA, Morrow AL, Horseman Kennedy EP (1957). Metabolism of lipides. Ann Rev Biochem 36, 119–148. doi: 10.1146/annurev.bi.36.070157.001003
Monks J, Dzieciatkowska M, Bales ES, Orlicky DJ, Wright RM, McManaman JL (2016). Xanthine oxidoreductase mediates membrane docking of milk-fat droplets but is not essential for apocrine lipid secretion. J Physiol 594, 5899–5921. doi: 10.1113/JPHYSIOL.2015.283183
Ottedal OT (1984). Milk composition, milk yield and energy output at peak lactation: a comparative review. Symp Zool Soc Lond 51, 33–85.
Ogg SL, Weldon AK, Dobbie L, Smith AJ, Mather IH (2004). Expression of butyrophilin (Btn1a1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. Proc Natl Acad Sci U S A 101, 10084–10089. doi: 10.1073/pnas.0402930101
Orlicky DJ, Libby AE, Bales ES, McMahan RH, Monks J, La Rosa FG, McManaman JL (2019). Perilipin-2 promotes obesity and progressive fatty liver disease in mice through mechanistically distinct hepatocyte and extra-hepatocyte actions. J Physiol 597, 1565–1584. doi: 10.1113/JPHYSIOL.2018.146502
Rudolph MC, Monks J, Burns V, Phistry M, Marians R, Rudolph MC, Monks J, Stefanski AL, McManaman JL (2013). Dynamics and molecular determinants of cytoplasmic lipid droplet clustering and dispersion. PLoS One 8, e66837. doi: 10.1371/journal.pone.0066837
Ozeki S, Cheng J, Tauchi-Sato K, Hatano N, Taniguchi H, Fujimoto T (2005). Rab18 localizes to lipid droplets and induces their close apposition to the endoplasmic reticulum-derived membrane. J Cell Sci 118, 2601–2611. doi: 10.1242/jcs.02401
Robenek H, Hofnagel O, Buers I, Robenek MJ, Troyer D, Severs NJ (2006). Adiprophilin-enriched domains in the ER membrane are sites of lipid droplet biogenesis. J Cell Sci 119, 4215–4224. doi: 10.1242/jcs.03191
Rudolph MC, Monks J, Burns V, Phistry M, Marians R, Foote MR, Bauman DE, Anderson SM, Neville MC (2010). Sterol regulatory element binding protein and dietary lipid regulation of fatty acid synthesis in the mammary epithelium. Am J Physiol Endocrinol Metab 299, E918–E927. doi: 10.1152/ajpendo.00376.2010
Rudolph MC, Young BE, Lemas DJ, Palmer CE, Hernandez TL, Barbour LA, Friedman JE, Krebs NF, MacLean PS (2017). Early infant adipose deposition is positively associated with the n-6 to n-3 fatty acid ratio in human milk
independent of maternal BMI. Int J Obes (Lond) 41, 510–517. doi: 10.1038/ijo.2016.211

Russell TD, Palmer CA, Orlicky DJ, Fischer A, Rudolph MC, Neville MC, McManaman JL (2017). Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. J Lipid Res 49, 2283–2301. doi: 10.1194/jlr.R800018-JLR200

Vorbach C, Scriven A, Capecechi MR (2002). The housekeeping gene xanthine oxidoreductase is necessary for milk fat droplet enveloping and secretion: gene sharing in the lactating mammary gland. Genes Dev 16, 3223–3235. doi: 10.1101/gad.1032702

Wahlig JL, Bales ES, Jackman MR, Johnson GC, McManaman JL, Maclean PS (2012). Impact of high-fat diet and obesity on energy balance and fuel utilization during the metabolic challenge of lactation. Obesity (Silver Spring) 20, 65–75. doi: 10.1038/oby.2011.196

Walther TC, Chung J, Farese RV Jr (2017). Lipid droplet biogenesis. Annu Rev Cell Dev Biol 33, 491–510. doi: 10.1146/annurev-cellbio-100616-060608

Wang W, Ly N, Zhang S, Shui G, Qian H, Zhang J, Chen Y, Ye J, Xie Y, Shen Y, et al. (2012). Cidea is an essential transcriptional coactivator regulating mammary gland secretion of milk lipids. Nat Med 18, 235–243. doi: 10.1038/nm.2614

Welle MA (2009). Fat on the move: intracellular motion of lipid droplets. Biochem Soc Trans 37, 991–996. doi: 10.1042/BST0370991

Willimg F, Wang H, Haas JT, Krahmer N, Gould TJ, Uchida A, Cheng JX, Graham M, Christiano R, Frohlich F, et al. (2013). Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. Dev Cell 24, 384–399. doi: 10.1016/j.devcel.2013.01.013

Wooding FB (1977). Comparative Mammary Fine Structure. In: Comparative Aspects of Lactation, ed. M. Peaker, London: Academic Press, 1–41.

Wooding FB, Sargeant TJ (2015). Immunocytochemical evidence for Golgi vesicle involvement in milk fat globule secretion. J Histochem Cytochem 63, 943–951. doi: 10.1369/0022155415608918

Wu CC, Howell KE, Neville MC, Yates JR III, McManaman JL (2000). Proteomics reveal a link between the endoplasmic reticulum and lipid secretory mechanisms in mammary epithelial cells. Electrophoresis 21, 3470–3482. doi: 10.1002/1522-2683(20001001)21:16<3470::AID-ELPS3470>3.3.CO;2-7

Wu L, Zhou L, Chen C, Gong J, Xu L, Ye J, Li D, Li P (2014). Cidea controls lipid droplet fusion and lipid storage in brown and white adipose tissue. Sci China Life Sci 57, 107–116. doi: 10.1007/s11427-013-4585-y

Yen CL, Stone SJ, Kolivad S, Harris C, Farese RV Jr (2008). Themetic review series: glycolipids. DGAT enzymes and triacylglycerol biosynthesis. J Lipid Res 49, 2283–2301. doi: 10.1194/jlr.R800018-JLR200

Zaczek M, Keenan TS (1990). Morphological evidence for an endoplasmic reticulum origin of milk lipid globules obtained using lipid-selective staining procedures. Protoplasma 159, 179–182. doi: 10.1007/BF01322600