

Chapter 1

The Lipid Droplet: a Dynamic Organelle, not only Involved in the Storage and Turnover of Lipids

Sven-Olof Olofsson, Pontus Boström, Jens Lagerstedt, Linda Andersson, Martin Adiels, Jeanna Perman, Mikael Rutberg, Lu Li, and Jan Borén

Abstract Neutral lipids such as triglycerides are stored in cytosolic lipid droplets. These are dynamic organelles and consist of a core of neutral lipids surrounded by amphipathic lipids and proteins. The surface is complex and contains proteins involved in lipid biosynthesis and turnover and proteins involved in sorting and trafficking events in the cell. Lipid droplets are formed at microsomes as primordial droplets, which increase in size by fusion. In this chapter, we review the assembly and fusion of lipid droplets. We also discuss a possible mechanism to explain the link between lipid accumulation in muscle cells and the development of insulin resistance. Triglycerides are secreted as milk globules from the epithelial cells of the mammary glands, as chylomicrons from enterocytes, and as very low-density lipoproteins (VLDL) from hepatocytes. We review the processes involved in the formation of milk globules and VLDL, and we discuss the clinical consequences of overproduction of VLDL.

Abbreviations ADRP, adipocyte differentiation related protein; ATGL, adipose triglyceride lipase; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; ERGIC, ER Golgi intermediate compartment; ERK2, extracellular signal regulated kinase 2; GPAT, glycerol-3-phosphate acyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NSF, N-ethylmaleimide-sensitive factor; PA, phosphatidic acid; PAP, phosphatidic acid phosphohydrolase; PC, phosphatidylcholine; PLD1, phospholipase D1; SNAP23, synaptosomal-associated protein of 23 kDa; α -SNAP, α -soluble NSF adaptor protein; SNARE, SNAP receptor; VAMP, vesicle-associated protein; VLDL, very low-density lipoprotein

S.-O. Olofsson, P. Boström, J. Lagerstedt, L. Andersson,
M. Adiels, J. Perman, M. Rutberg, L. Li, and J. Borén
The Wallenberg Laboratory, Sahlgrenska Center for Metabolic and Cardiovascular Research,
Department of Molecular and Clinical Medicine, Sahlgrenska University Hospital, Sahlgrenska
Academy, University of Göteborg, 41345 Göteborg, Sweden
e-mail: Sven-Olof.Olofsson@wlab.gu.se

1.1 Introduction

Neutral lipids, such as triglycerides and cholesterol esters, are stored in the cells within so-called cytosolic lipid droplets. The neutral lipids form the core of the lipid droplet and are surrounded by an outer layer of amphipathic lipids, such as phospholipids and cholesterol (Brown 2001; Martin and Parton 2006; Fig. 1.1). The surface of the lipid droplet is generally considered to be a monolayer of lipids (Robenek et al. 2005).

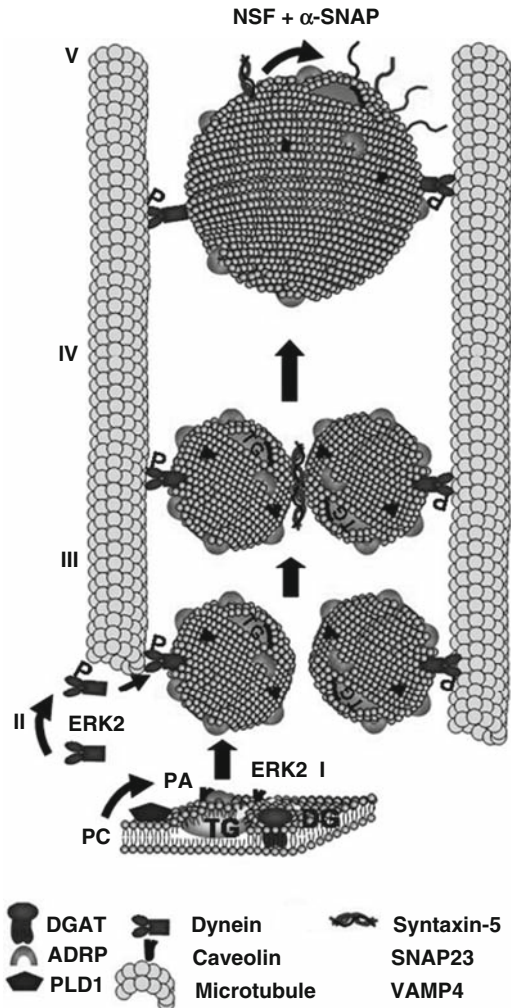


Fig. 1.1 A model for the formation of lipid droplets. Diglycerides (*DG*) are catalyzed by DGAT to form triglycerides (*TG*) in the microsomal membrane. *TG* have limited solubility in the amphipathic monolayer, and therefore oil out between the leaflets of the membrane to form a *TG* lens, which will become the core of the lipid droplet (*I*). Lipid droplet assembly also requires the production of phosphatidic acid (*PA*) from phosphatidylcholine (*PC*) catalyzed by PLD1 and an active ERK2 (*II*).

Specific proteins that are essential for the formation, structure and function of the lipid droplet are bound to its surface (Brasaemle 2007; Martin and Parton 2006). As a result of recent advances in our knowledge about the structure and function of lipid droplets, they are now considered to be dynamic organelles that can interact with other organelles and have a key role in the cellular turnover of lipids (Martin and Parton 2006).

In today's increasingly overweight society, the problems associated with excess levels of triglycerides are now well recognized. Accumulation of triglycerides, particularly in the liver and muscles, is highly correlated with the development of insulin resistance and type 2 diabetes, which are important risk factors for arteriosclerosis and cardiovascular diseases (Taskinen 2003). Triglycerides are stored to a variable extent in most cells, but they are only efficiently secreted by certain organs, i.e. liver and intestine (Olofsson and Boren 2005) and mammary glands (McManaman et al. 2007). In this article, we review the storage and secretion of triglycerides.

1.2 Lipid Droplets Form as Primordial Structures at Microsomal Membranes

The nature of the site of assembly of lipid droplets has not been conclusively determined. Results from a cell-free system indicate that they can be formed from a microsomal fraction enriched in markers for the endoplasmic reticulum (ER) and Golgi apparatus but lacking markers for the plasma membrane (Marchesan et al. 2003). An ER localization of the assembly of lipid droplets is also suggested by results showing that lipid droplets are associated with adipocyte differentiation related protein (ADRP)-enriched regions of the ER (Robenek et al. 2006). However, other results (Ost et al. 2005) indicate that the plasma membrane may be a source of droplets: triglycerides accumulate in the plasma membrane of adipocytes and this accumulation seems to be a precursor for the formation of cytosolic lipid droplets.

1.2.1 Microsomal Membrane Proteins Involved in Lipid Droplet Formation

The formation of lipid droplets is highly linked to the biosynthesis of triglycerides (Marchesan et al. 2003). Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the first step, i.e. the formation of lyso-phosphatidic acid. GPAT exists in several

Fig. 1.1 (continued) The assembly process first forms a primordial droplet with a diameter <0.5 μm . ERK2 phosphorylates the motor protein dynein, which is then sorted to droplets allowing them to transfer on microtubules (II). This allows long-distance transport of the droplet in the cell and is also required for lipid droplet fusion (III–V). The fusion process is catalyzed by the SNAREs SNAP23, syntaxin-5 and VAMP4 (IV). After the fusion, the four-helix bundle formed by the SNARE domains of these three SNAREs is recognized by α -SNAP which, together with the ATPase NSF, unwinds the bundle, allowing new fusions to occur (V)

isoforms. GPAT1 and GPAT2 are present on mitochondria and GPAT3 and GPAT4 are present on ER (Gonzalez-Baro et al. 2007; for a review, see Coleman et al. 2000). The mitochondrial isoforms of GPAT were first identified and cloned and most information is from studies of these isoforms. Overexpression of GPAT1 has been shown to increase the accumulation of triglycerides in the cell and promote the formation of steatosis (Gonzalez-Baro et al. 2007). GPAT3 has also been cloned (Cao et al. 2006). This isoform is highly upregulated during adipocyte differentiation and overexpression leads to lipid accumulation in the cell (Cao et al. 2006).

The formation of phosphatidic acid from lyso-phosphatidic acid is catalyzed by 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT). This enzyme exists in several isoforms of which 1 and 2 are confined to microsomes. AGPAT is a membrane-spanning protein that catalyzes the reaction on the cytosolic side of the ER (for reviews with references, see for example Agarwal and Garg 2003; Leung 2001).

When associated with microsomal membranes, the amphipathic enzyme phosphatidic acid phosphohydrolase hydrolyzes phosphatidic acid, forming diacylglycerol (for reviews, see Carman and Han 2006; Coleman et al. 2000). Diacylglycerol acyltransferase (DGAT), an integral membrane protein of microsomes (Stone et al. 2006), then catalyzes the conversion of diacylglycerol to triglycerides. There are two mammalian forms of DGAT: DGAT1 and DGAT2. DGAT1 is a multifunctional enzyme (Yen et al. 2005) whereas DGAT2 has been shown to be more potent and specific for triglyceride synthesis (Stone et al. 2004). In addition to its localization on the ER membrane, DGAT2 has also been identified on lipid droplets (Kuerschner et al. 2008). However, DGAT2 is a membrane protein that spans the bilayer twice; and it remains to be clarified how it is integrated into the amphipathic monolayer that surrounds lipid droplets. Alternatively, there may be a very tight interaction between lipid droplets and the ER allowing the DGAT2 product formed in the microsomal membrane to enter into the droplets. Such a tight interaction has been demonstrated and shown to be dependent on the GTPase Rab18 (for a review, see Martin and Parton 2006).

Lipid droplet assembly is also dependent on phospholipase D (PLD) activity and the formation of phosphatidic acid (Marchesan et al. 2003). Using intact cells, we showed that the active isoform is PLD1 and not PLD2 (Andersson et al. 2006), which is consistent with the localization of the two isoforms: PLD1 is present in ER and Golgi membranes (Andersson et al. 2006; Freyberg et al. 2001) while PLD2 is confined to the plasma membrane (Andersson et al. 2006; Du et al. 2004).

The observation that most of the identified enzymes associated with lipid droplet assembly are localized on microsomes supports the idea that lipid droplets are formed at the ER and/or Golgi apparatus.

1.2.2 Model for the Assembly of Lipid Droplets

The lipid droplets formed at the isolated microsomal membranes in a cell-free system have a diameter of 0.1–0.4 μm (Marchesan et al. 2003). This corresponds well to the size of the smallest droplets observed in cells by electron microscopy

(Marchesan et al. 2003). The newly formed droplets recovered from the cell-free system contain ADRP and are rich in caveolin and vimentin; and we propose that they represent the first primordial structures formed during the assembly process (Marchesan et al. 2003).

Although no experimental results have been obtained to date demonstrating how lipid droplets are formed, a tentative model for their assembly has been proposed (see for example Brown 2001; Fig. 1.1). Triglycerides (formed from diglycerides and acyl-CoA by the DGAT reaction in the microsomal membranes) are highly hydrophobic and have limited solubility in the monolayer of the membrane. The formed triglycerides will therefore “oil out” as a separate phase between the two leaflets, forming a lens structure that is the core of the lipid droplets. One problem is that the formed triglycerides may rapidly diffuse laterally in the ER and Golgi membranes and saturate these organelles before the oiling out occurs. However, this could be prevented if the regions of triglyceride synthesis are sealed off from the rest of the organelle.

1.3 Lipid Droplet Size Increases by Fusion

We have shown that droplets can increase in size by a fusion process, which is independent of triglyceride biosynthesis (Bostrom et al. 2005; Fig. 1.1). Approximately 15% of all droplets in the cells are engaged in fusion events at any given time (Bostrom et al. 2005) and thus fusion is a frequently occurring event that represents an important mechanism by which lipid droplets increase in size.

Lipid droplets are transported relatively long distances on microtubules (Welte et al. 1998) and motor proteins such as dynein have been shown to be present on droplets (Bostrom et al. 2005). We demonstrated that dynein is sorted to the droplets following phosphorylation by the cytosolic protein extracellularly regulated kinase 2 (ERK2; Andersson et al. 2006; Fig. 1.1). Both dynein and microtubules are essential for the fusion between droplets (Andersson et al. 2006; Bostrom et al. 2005).

1.3.1 *SNAREs are Involved in Lipid Droplet Fusion*

We have shown that the fusion between lipid droplets is catalyzed by N-ethylmaleimide-sensitive factor adaptor protein receptors (SNAREs), the synaptosomal-associated protein of 23 kDa (SNAP 23), syntaxin-5 and vesicular-associated protein 4 (VAMP4; Fig. 1.1). In addition, the fusion requires the ATPase N-ethylmaleimide-sensitive factor (NSF) and α -soluble NSF adaptor protein (α -SNAP; Fig. 1.1).

The role of these proteins has been extensively described for the fusion process between transport vesicles and target membranes (see for example Jahn and Scheller 2006). The SNAREs present on the target membrane (t- or Q-SNAREs) interact with a SNARE on the transport vesicle (v- or R-SNARE) to form a SNARE complex that causes fusion. A central feature in this process is the formation of a superhelix bundle, formed by four α -helical SNARE domains from the different SNAREs. The formation of the four-helix bundle forces the two membranes together, promoting their fusion.



<http://www.springer.com/978-3-642-00299-1>

Cellular Lipid Metabolism

Ehnholm, C. (Ed.)

2009, XVIII, 376 p., Hardcover

ISBN: 978-3-642-00299-1