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Mini-review

Triglyceride-rich lipoproteins and cytosolic lipid droplets in enterocytes: Key players in intestinal physiology and metabolic disorders

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ABSTRACT

During the post-prandial phase, intestinal triglyceride-rich lipoproteins (TRL) *i.e.* chylomicrons are the main contributors to the serum lipid level, which is linked to coronary artery diseases. Hypertriglyceridemia can originate from decreased clearance or increased production of TRL. During lipid absorption, enterocytes produce and secrete chylomicrons and transiently store lipid droplets (LDs) in the cytosol. The dynamic fluctuation of triglycerides in cytosolic LDs suggests that they contribute to TRL production and may thus control the length and amplitude of the post-prandial hypertriglyceridemia. In this review, we will describe the recent advances in the characterization of enterocytic LDs. The role of LDs in chylomicron production and secretion as well as potential previously unsuspected functions in the metabolism of vitamins, steroids and prostaglandins and in viral infection will also be discussed.

Fat absorption by the small intestine is a very efficient process. Triglycerides (TG) are exported from enterocytes as chylomicrons that are triglyceride-rich lipoproteins (TRL). They are subsequently hydrolyzed in the circulation to provide the body with fatty acids. Thus, serum lipid levels result from the production of lipoproteins by liver and intestine and from their clearance. Chylomicrons are important contributors to the circulating lipids during the post-prandial state and it is well established that post-prandial serum lipid levels have a positive correlation with coronary artery diseases [1,2]. Recent studies have shown that a transient formation of lipid droplets (LDs) in the cytosol of enterocytes may be a part of the physiological process contributing to chylomicron assembly and secretion after a meal, and thus to the control of the amplitude and duration of postprandial hypertriglyceridemia [3,4]. Despite their promising impact in the understanding of metabolic disorders, the molecular mechanisms governing intestinal cytosolic LD formation and mobilization and triglyceride distribution between the cytosol for transient storage and the endoplasmic reticulum (ER) lumen for chylomicron assembly, are only beginning to be explored. Although chylomicrons and cytosolic LDs are both composed of a core of neutral lipids (TG and cholesterol esters) surrounded by a monolayer of amphipathic lipids (phospholipids and cholesterol), they differ in many aspects as summarized in Table 1.

In this review, we will present the links between the production and metabolism of chylomicrons and the risk factors of cardiovascular diseases. We will review the present knowledge on enterocyte cytosolic LDs including their dynamics and composition, and we will discuss their potential roles in the control of chylomicron assembly and production, as well as their potential functions in the metabolism of hydrophobic molecules (summarized in Fig. 1).

Abbreviations: ABHD5/CGI-58, α/β hydrolase domain 5/comparative gene identification-58; Apo, apolipoprotein; ATGL, adipose triglyceride lipase; CIDE, cell death-inducing DNA fragmentation factor 45-like effector; Cox-2, cyclooxygenase-2; DGAT, diacylglycerol: acylCoA transferase; ER, endoplasmic reticulum; HDL, high density lipoprotein; LD, lipid droplet; LDL, low density lipoprotein; LPCAT, lysophosphatidylcholine acyltransferase; LPS, lipopolysaccharide; MGAT, monoacylglycerol: acylCoA transferase; MTP, microsomal triglyceride transfer protein; PC, phosphatidylcholine; TG, triglycerides; TRL, triglyceride-rich lipoprotein; UBXD8/FAF2, UBX domain-containing protein 8/FAS-associated factor 2; VLDL, very low density lipoprotein.

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Table 1
Differences between cytosolic lipid droplets and chylomicrons.

	Cytosolic lipid droplet	Chylomicron
Size	Up to 6 μm (non adipocyte LDs)	75–1200 nm
Localization	Cytosol	Lumen of the secretory pathway (endoplasmic reticulum, Golgi apparatus) and extracellular
Specific protein coat		
Apolipoprotein B	No	Yes
Perilipin family	Yes	No
Function	Intracellular lipid storage	Lipid distribution to the body

1. Chylomicrons and risk factors of cardiovascular diseases

Chylomicrons are mainly produced by the jejunum after a meal, during the post-prandial phase. They are low density particles ($d < 1.006$ g/mL) and are very heterogeneous in size (diameters 75–1200 nm) [5,6]. A chylomicron is composed of a core of neutral lipids (more than 90%), predominantly TG, with traces of cholesteryl ester, stabilized by a shell of amphipathic lipids (phospholipids, cholesterol) and one structural protein, the apolipoprotein (apo) B48, as well as other exchangeable apolipoproteins [7]. In the blood circulation, TG derived from the chylomicrons are hydrolyzed by lipoprotein lipase into fatty acids that are taken up by organs such as the skeletal muscles and the heart for energy supply, or the adipose tissue for storage. The chylomicron remnants are taken up primarily by the liver, especially by the low density lipoprotein (LDL) receptor that has a high affinity for apoE, but other routes are involved including the LDL receptor-related protein 1 (LRP1) and the heparin sulfate proteoglycan (HSPG) pathway (for review, see Refs. [8–10]).

Cardiovascular diseases, type 2 diabetes and dyslipidemia are comorbidities of obesity [11]. Chylomicron metabolism can contribute to the development of these diseases. First, being directly exposed to dietary fat, the small intestine is instrumental in the control of the amount of lipids that enter the body and might contribute to the development of obesity. Second, liver and intestine produce TRL, called very low density lipoproteins (VLDL) and chylomicron respectively, and, under post-prandial conditions, the chylomicrons are important contributors to the circulating lipids. Furthermore, post-prandial serum lipid levels have a stronger positive correlation with coronary artery diseases than the fasting serum lipid levels [1,2]. Third, chylomicron remnants can rapidly penetrate the arterial wall suggesting a contribution in atherosclerosis development [12,13]. Fourth, an accumulation of lipoproteins in the plasma can result from decreased catabolism but also from increased production rates. Intestinal apoB48 lipoprotein overproduction was demonstrated for the first time in an insulin-resistant animal model, the fructose fed hamster [14]. This demonstrates that the intestine is not just an absorptive organ: it is also able to modulate lipoprotein production, a capacity previously strictly devoted to the liver.

Overall, establishing strategies to optimize chylomicron metabolism in order to reduce postprandial lipemia and chylomicron remnant accumulation would be a promising avenue for the prevention of cardiovascular diseases.

2. Chylomicron assembly and secretion

2.1. Chylomicron assembly: a two-step process

Absorption of dietary lipids by the enterocytes of the jejunum is a highly specialized and complex process (Fig. 1) (for reviews, see Refs. [15–17]). The hydrolysis of TG, which is the main dietary lipid, initiates in the stomach by the gastric lipase then in the lumen of the upper part of the small intestine by pancreatic lipase and leads

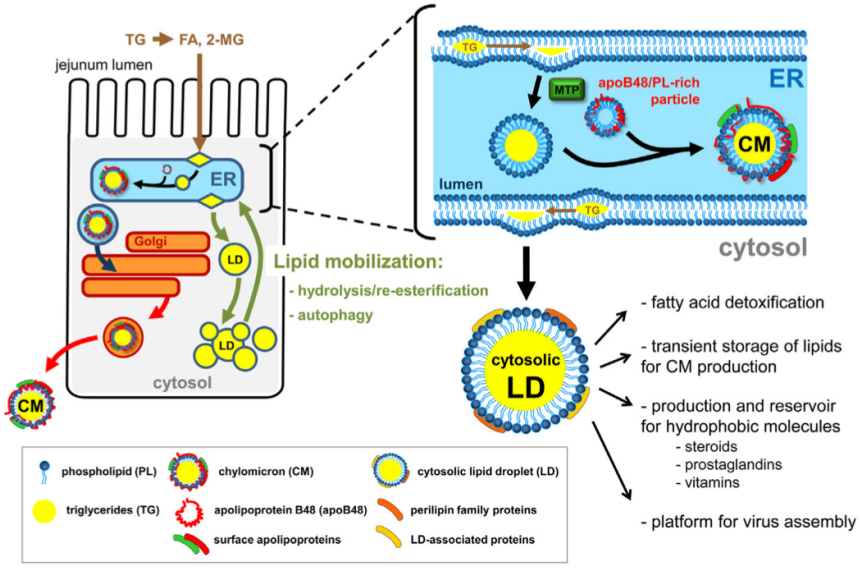

Fig. 1. Schematic model of neutral lipid distribution and trafficking in enterocytes, with a focus on chylomicron (CM) and cytosolic lipid droplets (LDs) biogenesis, fate, and functions. Triglycerides (TG), the main dietary lipid, are hydrolyzed in the lumen of the jejunum into fatty acids (FA) and 2-monoacylglycerols (2-MG), which are taken up by enterocytes and metabolized into TG at the endoplasmic reticulum (ER) membrane. The newly synthesized TGs accumulate between the two leaflets of the ER phospholipid (PL) membrane. A nascent LD buds off the ER in the ER lumen and fuses with an apoB48/PL-rich particle to form a CM that will traffic along the Golgi apparatus and will be secreted at the basal pole of enterocytes to provide lipids to the body. Microsomal triglyceride transfer protein (MTP) is required for CM assembly. After an acute dietary lipid load, the nascent LDs also bud off the ER in the cytosol for transient lipid storage. They will be mobilized between meals for CM formation through hydrolysis/re-esterification and/or autophagy. Additional potential functions of cytosolic LDs in enterocytes are indicated.

to fatty acid and 2-monoacylglycerol production. Phospholipids originating from both the bile and the diet are hydrolyzed by the pancreatic phospholipase A2 into lysophosphatidylcholine (lysoPC) and fatty acids. These lipids are taken up by the enterocytes by passive diffusion and/or transporters. TG and phospholipids are subsequently synthesized at the ER membrane. TG are mainly synthesized by the monoacylglycerol pathway. Monoacylglycerol: acylCoA transferase (MGAT) converts monoacylglycerol and fatty acids into diacylglycerol, which is in turn converted to TG by diacylglycerol: acylCoA transferase (DGAT). As documented for VLDL assembly in hepatocytes, chylomicron assembly occurs via a two-step process in the secretory pathway [18,19]. Dense apoB48 phospholipid-rich particles are first produced, and they subsequently fuse with a LD formed independently in the ER lumen, giving rise to large-sized chylomicron particles (Fig. 1). The microsomal triglyceride transfer protein (MTP) is necessary for both steps. Once matured and trafficked through the Golgi apparatus, the chylomicrons are secreted at the basal pole of the enterocytes. Exchangeable apos, including apoA-IV and apoC-III, are suggested to facilitate different stages of chylomicron assembly and trafficking through the ER–Golgi secretory compartments [20]. The maturation and the subsequent secretion of chylomicrons are also dependent on proper ER-to-Golgi and intra/post-Golgi trafficking steps. In this context, several membrane trafficking regulators of the biosynthetic pathway have been directly involved in lipoprotein lipidation and/or maturation, such as COPII complex [21,22] or ADP-ribosylation factor related protein 1 (ARFRP1) [23], a small GTPase. ARFRP1 has been also proposed to act in lipid droplet growth in adipocytes [24].

2.2. The chylomicron: a versatile lipoprotein particle

Lipid availability in the ER lumen plays a pivotal role in the regulation of chylomicron production. An increase of the TG load transported by the chylomicrons can result from the production of an increased number of chylomicrons of similar sizes, or from the production of a similar number of chylomicrons with an increased particle size, or from the increase of both size and number of chylomicrons. It is important to make the distinction between particle number and particle size, because the production of an increased number of particles will lead to an increased number of chylomicron remnants, hence potentially to an increased risk of atherosclerosis [12,25].

The impact of insulin and of insulin resistance on lipoprotein production has been studied (for reviews, see Refs. [12,17,25–27]). Insulin inhibits TRL production in the liver and in the intestine. Under conditions of insulin resistance, intestinal TRL production is stimulated because of an elevated flux of free fatty acids into the intestine, accompanied by the down-regulation of intestinal insulin signaling and the upregulation of MTP levels. In diabetic animal models, alteration of the number and/or of the size of chylomicrons has been reported. However, except for the role of MTP, the molecular mechanisms governing the formation of the LDs in the ER lumen and the control of the TG core size of chylomicrons are still unclear.

Small dense apoB-48 phospholipid-rich particles (*i.e.* TG poor) are secreted by enterocytes from hamsters or rabbits fed a low fat-diet [25]. Similarly, Caco-2 cells, an in vitro model for human enterocytes, secrete a substantial amount of small dense apoB48 phospholipid-rich particles [28–30].

3. Cytosolic lipids droplets in enterocytes

As underlined by recent reviews [31–33], cytosolic LDs are composed of a core of TG and cholesterol esters surrounded by a monolayer of phospholipids, cholesterol and of a variety of proteins (Fig. 1). Recent advances have revealed LDs to be dynamic structures that are able to communicate and interact with other intracellular compartments [32,34]. The onset of LD biogenesis is widely accepted as being the accumulation of newly synthesized TG between the two leaflets of the ER phospholipid bilayer [35–37]. Several models have been proposed for the later stages of biogenesis [38,39]. In all cell types, the nascent LD buds off the ER into the cytosol, while in hepatocytes and enterocytes it can bud also towards the ER lumen where TRL assembly takes place leading to lipid secretion. The mechanisms that control the lipid distribution between the cytosol and the ER lumen are still unclear.

LDs are coated by a variety of proteins, as demonstrated by several proteomics studies that have been undertaken on LDs isolated from various sources [40,41]. The proteome of the LD includes structural proteins of the perilipin family (perilipins 1–5, previously known as PAT family proteins), proteins involved in lipid metabolism, membrane trafficking, signaling, cytoskeleton and chaperones. These studies have revealed the complexity of the LD coat. It can differ from one cell type to another as well as within a single cell where different populations of LDs can co-exist, and can vary depending on the physiopathology of the cell.

3.1. Dynamics of cytosolic lipid droplets in enterocytes

Cytosolic LDs have been observed in jejunal enterocytes during lipid absorption in rats [42], mice [43], golden hamsters [44], rabbits [45], pigs [46] and humans [47]. Moreover, they have recently been shown to be very dynamic organelles. By CARS (coherent anti-stokes Raman scattering) imaging [3,48] and electron microscopy [4], mouse jejunal enterocytes were shown to exhibit many cytosolic LDs at their apical pole after oil gavage. Remarkably, the LDs are absent 12 h later. Since their lipid content is exported later as chylomicrons, cytosolic LDs can thus be considered as important contributors to the control of postprandial triglyceridemia.

In the human jejunum, LDs of up to 6 μm diameter are present in enterocytes 6 h after a fat load. As compared to water, ingestion of glucose leads to a lower intracellular TG content and increased chylomicron secretion, suggesting that carbohydrate and fat influence each other's metabolism [47]. Recent studies conducted in ob/ob mice and high-fat diet-fed mice showed an altered TG handling by the small intestine compared to lean mice *i.e.* the accumulation of mucosal TG possibly secondary to a decreased lipid secretion [49,50].

3.2. Proteome of lipid droplets isolated from enterocytes

Several studies have highlighted recently the proteins associated with cytosolic LDs in enterocytes. Perilipin 2 (ADRP) and perilipin 3 (TIP47) are the only members of the perilipin family expressed in mouse enterocytes *in vivo* [48]. Unfortunately, the proteome of LDs isolated from the jejunal enterocytes has not yet been investigated. However, the LD proteome of Caco-2 cells, a cell culture model of human enterocytes that is able to secrete TRL upon lipid supply [29,30,51], has been described. Of the proteins identified in LD fractions isolated from Caco-2 cells, one-quarter were directly involved in lipid metabolism pathways potentially relevant to enterocyte-specific functions including fatty acid activation, triglyceride hydrolysis, phospholipid metabolism, lipoprotein metabolism, steroid metabolism, etc [4]. More recently, by a differential proteomic approach, the proteins associated with LDs that were altered by hepatitis C virus (HCV) core protein expression were identified [52]. HCV core protein is known to localize onto LDs and to impair TG secretion in hepatocytes [53]. A set of 16 proteins was characterized and most of these hits were involved in lipid

metabolism. Because the HCV core protein led to decreased TRL secretion, the array of proteins identified might be involved in the balance between lipid storage as LDs and lipid secretion as TRL.

A diversity of proteins known to be associated with organelles such as the ER, mitochondria or peroxisomes were identified in the LD fractions isolated from the Caco-2 enterocytes [4,52]. This feature has been previously reported in LD fractions isolated from other cell types [41] and these organelles are particularly abundant in the cytoplasm of Caco-2 cells. Caution should be used in classifying these proteins as contaminants because it has been demonstrated recently that proteins with a hairpin-like monotopic topology are targeted to both the ER membrane and the LD hemimembrane [54,55], and because the previous localization studies were generally undertaken in the absence of added fatty acids, which are required to induce clearly visible LD formation.

4. Functions of the cytosolic lipid droplets in enterocytes

4.1. Role in transient lipid storage and lipoprotein production between meals

Observations made in enterocytes *in vivo* during lipid absorption have clearly shown dynamic accumulation and depletion of TG in cytosolic LDs during the process of fat absorption [3,4,47]. To what extent these processes are controlled and may contribute to intestinal adaptation/modulation of lipoprotein production is unclear.

In Caco-2 cells, TG stored as cytosolic LDs can be mobilized to contribute to TRL production [29] and TG distribution between cytosol and ER can be modulated by nutrients [30]. By differential proteomics, Beilstein et al. [52] identified proteins potentially involved in the control of the balance between lipid storage and secretion among which the two proteins of the perilipin family that are expressed by enterocytes, perilipin 2 and perilipin 3. Perilipin 2 is unstable when not bound to LDs while perilipin 3 is present in the cytosol when LDs are absent. Studies to elucidate the functions of perilipins include gain- and loss-of-function experiments in cells and gene targeting and silencing in mice (for reviews, see Refs. [56,57]). The effects of gain- and loss-of-function differ from cell type to cell type and from tissue to tissue. The function(s) of perilipin 2 and 3 in jejunum has not yet been investigated. Overall, perilipin 2 is suggested to stabilize long term-stored TG by limiting the interactions of lipases with the LDs. Although less effective, perilipin 3 is suggested to have a similar function. Moreover, perilipin 3 is predominantly localized on nascent LDs and has been suggested to be involved in LD synthesis from newly synthesized TG. In mice, perilipin 2 and 3 are expressed differently in enterocytes depending upon the diet and whether an acute or chronic high fat diet is ingested [48]. In hepatic cells, perilipin 2 overexpression results in increased accumulation of cytosolic LDs and a corresponding decrease of VLDL secretion [58]. Overall, in enterocytes, one can hypothesized that, by controlling lipolysis at the LD surface, perilipins control lipid availability for TG synthesis at the ER membrane. Furthermore, Beilstein et al. [52] showed an alteration of the amount of ER-associated proteins, suggesting that the interactions that occur between LD and the ER, where TRL assembly occurs, are important. Unfortunately, the potential interactions between LDs and various organelles, and their functional consequences, are still difficult to observe and to interpret [59] but they may be crucial for chylomicron assembly.

The mobilization of cytosolic triglycerides for the production of lipoproteins may occur through two processes: hydrolysis/re-esterification and/or autophagy (Fig. 1).

4.1.1. Lipid mobilization for lipoprotein assembly by a mechanism of hydrolysis/re-esterification

In enterocytes, the TGs present in cytosolic LDs are thought to contribute to chylomicron assembly through a mechanism of hydrolysis/re-esterification as it occurs in hepatocytes (for review, see Ref. [60] and references therein). We will focus here on the proteins identified in cytosolic LD fractions that may be involved in this process (Table 2). For the factors localized in the ER lumen including MTP and apos, readers are referred to recent reviews ([15] for enterocytes; [20,61] for hepatocytes).

TG hydrolysis involves several enzymes: adipose triglyceride lipase (ATGL) that hydrolyzes TG into diacylglycerols and fatty acids, hormone-sensitive lipase (HSL) that hydrolyzes diacylglycerol into monoacylglycerol and fatty acids, and monoacylglycerol lipase (MGL) that hydrolyzes monoacylglycerol into glycerol and fatty acids (for review, see Ref. [62]). These lipids can be substrates of MGATs and DGATs for re-synthesis of TG at the ER membrane for chylomicron assembly.

ATGL and ABHD5/CGI-58, a coactivator of ATGL, were identified on the LDs of Caco-2 enterocytes [4] and mouse intestinal mucosa [63]. In addition to being an activator of ATGL, ABHD5/CGI-58 has been suggested to play a role in TRL production in hepatocytes and this should now be examined in enterocytes [64–66]. Furthermore, it has been shown recently in the liver that knockdown of ABHD5/CGI-58 leads to the sequestration of diacylglycerol in LDs/ER, preventing diacylglycerol association with the plasma membrane, activation of PKCε, and induction of hepatic insulin resistance [67]. Thus, it should be determined whether a similar mechanism occurs in the intestine, which is also an insulin sensitive organ [68].

Table 2
Proteins associated with LDs from enterocytic cells that may be involved in lipid mobilization for lipoprotein assembly by a mechanism of hydrolysis/re-esterification.

Name	Protein name	Function in hydrolysis/re-esterification	References
ACSL 3	Long chain acyl-CoA synthetase 3	Fatty acid activation	[4,52]
ACSL 4	Long chain acyl-CoA synthetase 4	Fatty acid activation	[4]
ACSL 5	Long chain acyl-CoA synthetase 5	Fatty acid activation	[52]
ABHD5/CGI-58	α/β Hydrolase domain 5/comparative gene identification-58	ATGL activation	[4,52]
ATGL	Adipose triglyceride lipase	Triglyceride hydrolysis	[4,52,63]
CCT-α	Choline-phosphate cytidylyltransferase A	Phosphatidylcholine synthesis	[4]
HSL	Hormone sensitive lipase	Diglyceride hydrolysis	[63]
LPCAT2	Lysophosphatidylcholine acyltransferase 2	Phosphatidylcholine synthesis, phospholipid remodeling	[4,52]
MGAT2	Monoacylglycerol: acylCoA transferase 2	Diglyceride synthesis	[63]
MGL	Monoglyceride lipase	Monoglyceride hydrolysis	[4,52]
MTP	Microsomal triglyceride transfer protein	Lipoprotein assembly (luminal lipid droplet formation, apolipoprotein B stabilization)	[4,52,63]
PLIN2	Perilipin 2/ADRP	Interactions of lipases with LD restricted	[4,48,52,63]
PLIN3	Perilipin 3/TIP47	Interactions of lipases with LD restricted	[4,48,52]
UBXD8/FAF2	UBX domain-containing protein 8/FAS-associated factor 2	ATGL inhibition	[4,52]

UBXD8/FAF2, which has also been identified in the proteome of LDs isolated from Caco-2 enterocytes [4,52], localizes to both the ER and to LDs and it appears to be involved in multiple functions related to lipid and lipoprotein metabolism. It is a sensor for unsaturated fatty acids and a regulator of TG synthesis [69]. It also plays a role in the dislocation and degradation of lipidated apoB100 in Huh-7 hepatic cells [70]. Furthermore, it was recently shown that UBXD8/FAF2 binds directly to ATGL and promotes its dissociation from ABHD5/CGI-58 resulting in inhibition of lipase activity [71]. The specific role of UBXD8/FAF2 in the control of lipid and lipoprotein metabolism would be very interesting to investigate in the intestine.

DGAT2 has been found previously to associate with LDs during TG synthesis in fibroblasts and adipocytes [72] whereas it was not found in LD fractions isolated from Caco-2 enterocytes [4]. Interestingly, whereas the mouse intestine expresses both DGAT1 and DGAT2, the human intestine only expresses DGAT1 [73] suggesting that TG synthesis and hydrolysis are more strictly compartmentalized in humans than in mice. Also, the human liver expresses both DGATs, suggesting that the control of hydrolysis/re-esterification might be different in the intestine and the liver.

Because several proteins/enzymes that are involved in TG metabolism are localized at the surface of LDs and because the TG core volume of LDs can vary significantly, all proteins/enzymes that impact the size of LDs can in turn control TG metabolism including TRL production. Proteins of the cell death-inducing DNA fragmentation factor 45-like effector (CIDE) family, localized on LDs and ER, appear to play a unique role in controlling the size of cytosolic LDs in various cell types (see for review [74]). In the liver, VLDLs secreted from Cideb deficient hepatocytes contain significantly less TG, indicating an impairment in VLDL lipidation [74], and Cideb and perilipin 2 were shown to play opposing roles in controlling VLDL lipidation and hepatic lipid homeostasis [75]. However, although Cideb is expressed markedly in the liver it is expressed much less in the small intestine [74] and may not be as important in the control of chylomicron lipidation in the enterocytes. Other candidates able to impact the size of LDs are the enzymes controlling the phospholipid monolayer of LDs, mainly composed of phosphatidylcholine (PC) [76]. Choline-phosphate cytidylyltransferase A (CCT-α), which catalyzes the rate-limiting step for the synthesis of PC in the choline pathway, translocates to the LD surface upon oleate addition and its knockdown leads to fewer LDs of larger size [77]. Liver-specific CCT-α KO mice display impaired VLDL secretion in the plasma [78]. Lysophosphatidylcholine acyltransferases (LPCAT) 1 and 2 have been shown to localize both to the ER and the LDs in different cell lines including Caco-2 enterocytes [4,79]. In Huh-7 cells, LPCAT1 and 2 knockdowns resulted in an increase of LD size [79] and, in Caco-2 enterocytes, the amount of LPCAT2 on LDs was found to be altered by HCV core protein expression [52]. Recently, knockdown of LPCAT3 in the liver was shown to promote VLDL production by inducing MTP expression through the accumulation of lysoPC [80]. In the intestine, a large source of lysoPC comes from the lumenal hydrolysis of dietary and biliary PC. By controlling the amount of lysoPC, LPCATs may contribute to intestinal TG homeostasis and the regulation of chylomicron production. The role of these proteins in the hydrolysis/re-esterification process and chylomicron production remains to be explored.

4.1.2. Lipid mobilization for lipoprotein assembly by autophagy

Intracellular lipid pools can also be regulated by the macroautophagy degradative pathway (here referred as autophagy) [81]. In autophagy, which is induced by stress, nutrient starvation or infection, a double membrane structure, called a phagophore is formed from the ER and engulfs a portion of the cytoplasm containing the material to be degraded. Once this structure is closed, the autophagosome is mature and it can fuse with lysosomes to promote degradation of the phagocytosed material [82], with the potential degradation of lipids by lysosomal acid lipases [62]. This degradative pathway, which is crucial for cellular homeostasis and which is required during development, has been extensively studied in human physiopathological contexts including diabetes or obesity [83,84]. Recently, it has been demonstrated that autophagy participates in lipid metabolism in hepatocytes, and a novel concept of "lipophagy" has been invoked [85]. In that study, the authors showed that the induction of autophagy can lead to LD breakdown by direct and specific interaction with autophagosomes, and that the inhibition of autophagy increases the total TG content and LD number. Together, these data suggest that LDs are bona fide and specific targets for autophagy-mediated degradation, and that autophagy can act as regulators of TG levels in cells that must process large amounts of lipids [86,87]. Knowing that apoB also has been reported to be a putative target for autophagosomal degradation [88], it is tempting to postulate that autophagy participates in the control of storage and secretion in TRL-secreting cells. This remains to be explored in enterocytes, which process extensive amounts of dietary lipids coming from the intestinal lumen.

4.2. Lipid droplets as platforms for the metabolism of hydrophobic molecules

The proteome of LDs is more complex than initially thought and the nature of the proteins identified on LDs isolated from enterocytes suggests more extensive functions than simply transient dietary lipid storage during the process of lipid absorption (Fig. 1). Some examples are given below.

One of the main functions of LDs is to remove toxic concentrations of free fatty acids in the cytosol. High concentrations of fatty acids present in the intestinal lumen during the absorptive phase enter into cells by passive diffusion or specific transporters [89]. Therefore, it is particularly important for enterocytes to have the capacity to rapidly synthesize TGs and to produce LDs to detoxify the fatty acids when the capacity to assemble and secrete chylomicrons is saturated.

It has been demonstrated recently that retinol dehydrogenase Rdh10, which is essential for retinoic acid (vitamin A) biosynthesis, localizes to LDs during acyl ester biosynthesis and that its specific activity is strongly increased when targeted to LDs [90]. Rdh10, which catalyzes the conversion of retinol into retinal, the first step of *all-trans*-retinoic acid, was identified in the proteome of LDs isolated from Caco-2 enterocytes as well as the retinal reductase dhrs3 [4]. Jiang and Napoli [90] suggested that these enzymes are part of a metabolon that controls retinol homeostasis to/from LDs. Although this remains to be demonstrated *in vivo*, the LDs of enterocytes may as well contribute to retinol homeostasis in the organism.

Short chain dehydrogenase/reductase (SDR) family members metabolize various compounds including steroids, retinoids, fatty acid derivatives and xenobiotics [91]. The small intestine is a xenobiotic-metabolizing organ [92], although this role is usually devoted to the liver. We have recently shown that 17β-hydroxysteroid dehydrogenase 2, which catalyzes the conversion of 17-keto (e.g. estrone, testosterone) to 17β-hydroxysteroid (e.g. estradiol, androstenedione) is localized to LDs and interferes with triglyceride secretion, probably through its capacity to inactivate testosterone [52]. Indeed, 17β-hydroxysteroid dehydrogenase 2 is also expressed in the epithelial cells of the small intestine in humans [93].

4.3. Role of lipid droplets in inflammation

The gut harbors a microbial ecosystem all along its length, including in the jejunum [94]. Gram-negative bacteria, present in

the intestinal microflora, release lipopolysaccharide (LPS) upon lysis, which is a source of circulating LPS. The transfer of LPS into the circulatory system is favored by dietary lipids [95]. Paracellular and transcellular routes contribute to LPS movement from intestine lumen into circulation [96]. Concerning the latter pathway, lipid micelles formed from the hydrolyzed alimentary lipids and bile products are important for LPS uptake and enterocytes secrete LPS on chylomicrons [95,97]. Although of lipophilic nature, it has not been investigated yet whether LPS can also distribute into cytosolic LDs. LDs may also play an active role in the production of inflammatory mediators in response to a variety of inflammatory stimuli. In intestinal epithelial cells such as Caco-2 cells or IEC-6 cells, it has been shown that cyclooxygenase-2 (Cox-2) localizes to LDs [98,99]. Cox-2 synthesizes prostaglandins from arachidonic acid released from hydrolysis of phospholipids by cytosolic phospholipase A2. Interestingly, in microglia, cytosolic phospholipase A2 was shown to colocalise with LDs induced by LPS but not with LDs induced by oleic acid treatment [100]. Finally, Cox-2 appears to play an important role in the repair response upon intestinal injury [101]. Overall, LDs may be sites for prostaglandin production, especially upon LPS trigger.

4.4. Lipid droplets as platform assemblies for viruses

It is now well documented that members of the *Flaviviridae* family, *i.e.* hepatitis C virus as well as the Dengue virus, use LDs as a platform for assembly of nascent virions and that this association is necessary for efficient viral replication [102,103].

Rotaviruses, which belong to the *Reoviridae* family, infect epithelial cells of the intestine and are a major cause of acute gastroenteritis in infants and young children worldwide. Rotaviruses have been shown recently to associate with LDs and the inhibition of LD formation negatively affects viral replication [104]. Taken together, manipulating the LDs or the proteins acting in lipid metabolism might be instrumental for the development of antiviral strategies.

5. Conclusion

Research on LDs that forms in the cytosol of enterocytes during lipid absorption lagged behind the interest for understanding the mechanisms of the assembly and the secretion of intestinal lipoproteins. Because they are usually detected in enterocytes after a fat load and are very dynamic, LDs are believed to contribute to the control of the fate of newly synthesized neutral lipids and thus to control post-prandial hypertriglyceridemia, a known risk factor for atherosclerosis. The mechanisms that govern the lipid distribution in the various cellular compartments of enterocytes are still poorly understood. Indeed, the characterization of the proteome of LDs isolated from Caco-2 enterocytes highlighted candidate proteins that may be involved in lipid storage, a stock of molecules made available later for the production of lipoproteins at periods distant from meals. This remains to be demonstrated. Interestingly, proteins/enzymes involved in the metabolism of vitamins, steroids and prostaglandins were identified on LDs and this opens new avenues of research as to the role of LDs as a platform for the metabolism of hydrophobic molecules and the production of inflammatory mediators in the intestine (Fig. 1).

Obesity and diabetes lead to altered chylomicron secretion, a process that is linked to cytosolic LD metabolism. It will be of tremendous interest to determine whether in these pathological backgrounds the capacity of the enterocytes to regulate LD composition and function is affected. The recent observation that the specific activity of the Rdh10 enzyme is strongly increased after translocation from the ER membrane to the surface of LDs further highlights the emerging role of LD dynamics in the control of cell metabolism. We are just beginning to appreciate the full complexity and importance of the LDs in enterocytes.

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