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Review Article

Characteristics and functions of lipid droplets and associated proteins in enterocytes

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ABSTRACT

Cytosolic lipid droplets (LDs) are observed in enterocytes of jejunum during lipid absorption. One important function of the intestine is to secrete chylomicrons, which provide dietary lipids throughout the body, from digested lipids in meals. The current hypothesis is that cytosolic LDs in enterocytes constitute a transient pool of stored lipids that provides lipids during interprandial period while lowering chylomicron production during the post-prandial phase. This smoothens the magnitude of peaks of hypertriglyceridemia. Here, we review the composition and functions of lipids and associated proteins of enterocyte LDs, the known physiological functions of LDs as well as the role of LDs in pathological processes in the context of the intestine.

Contents

Abbreviations: 3BHS1, 3-β-hydroxysteroid dehydrogenase 1; ACSL, acyl-CoA synthetase long-chain; apoB48, apolipoprotein B48; ATGL, adipose triglyceride lipase; CE, cholesterol esters; CGI-58, comparative gene identification-58; CIDE, cell death-inducing DFF45-like effector; DGAT, diacylglycerol acyltransferase; DHB2, 17β-hydroxysteroid dehydrogenase 2; ELMOD2, ELMO domain-containing protein 2; ER, endoplasmic reticulum; FA, fatty acid; HCV, hepatitis C virus; HDL, high density lipoproteins; HSL, hormone sensitive-lipase; LD, lipid droplet; MGL, monoacyglycerol lipase; MGAT, monoacylglycerol acyltransferase; MTP, microsomal triglyceride transfer protein; Plin, perilipin; PL, phospholipids; SCFA, short chain fatty acid; TG, triglycerides; TRL, triglyceride-rich lipoproteins

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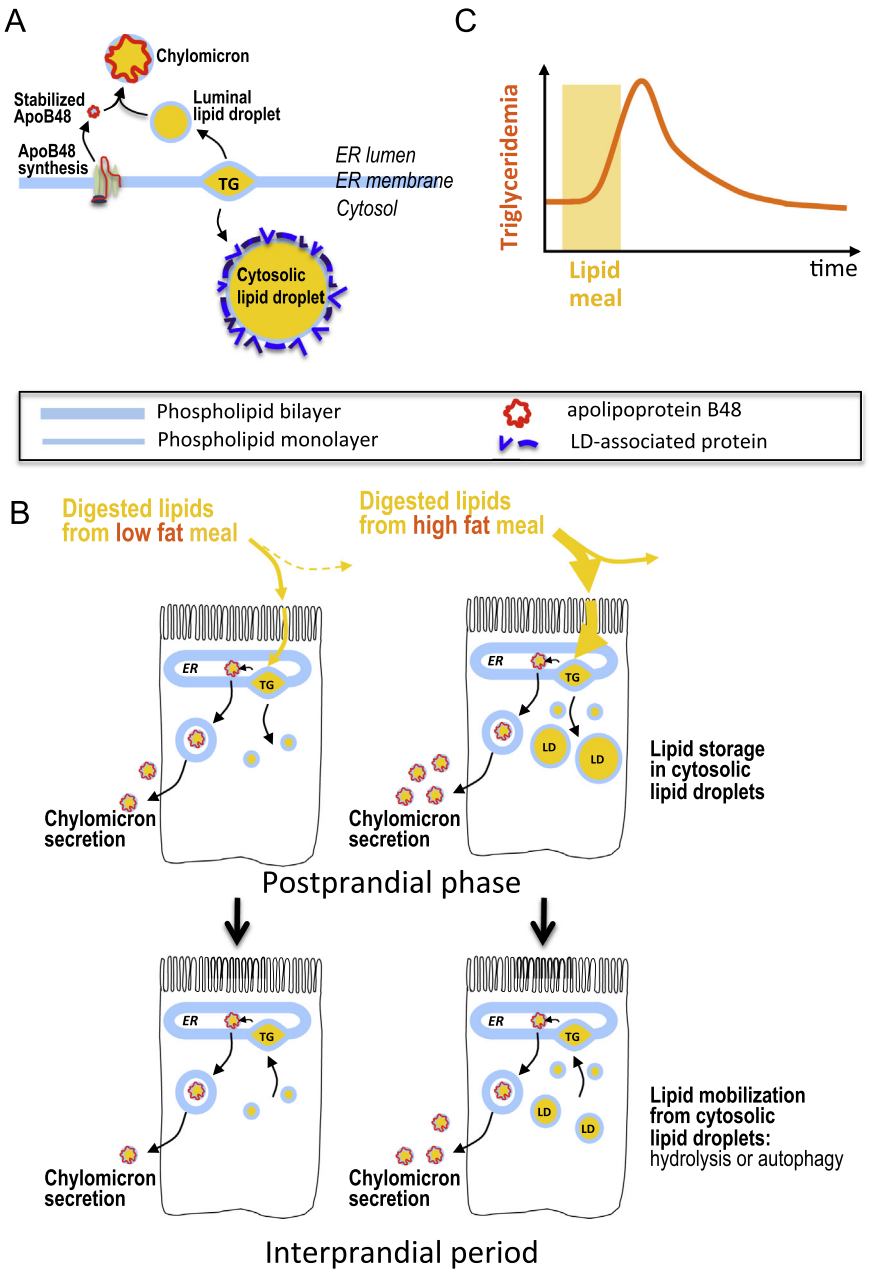

Fig. 1. Biogenesis and fate of cytosolic lipid droplets (LDs) and chylomicrons in enterocytes. (A) Biogenesis of cytosolic LDs and chylomicrons. Newly synthesized triglycerides (TG) accumulate between the two leaflets of the endoplasmic reticulum (ER) membrane. Nascent LDs bud off the ER membrane either into the cytosol to give cytosolic LDs or into the ER lumen to give luminal lipid droplets. Luminal lipid droplets fuse with apolipoprotein B48 (apoB48) to form chylomicrons. LD-associated proteins surround cytosolic LDs. (B) During the postprandial phase (top panels), digested lipids are taken up by enterocytes, TGs are synthesized at the ER membrane and, after assembly, chylomicrons are secreted at the basal pole of enterocytes in lymphatic vessels. After a high fat meal (right panel), a portion of synthesized lipids is stored in cytosolic LDs. During the interprandial phase (bottom panel), TG from cytosolic LDs are mobilized for chylomicron formation. (C) The physiological function of the transient lipid storage in cytosolic LDs is thought to provide sustained lipid supply to organs and smoothen hypertriglyceridemia curve as a function of time after a high fat meal.

1. Physiological functions of lipid droplets in enterocytes

One of the major functions of the intestine is to absorb nutrients, including lipids. The absorption of dietary lipids occurs mainly in the jejunum, a part of the proximal small intestine, and this is performed by enterocytes as a highly specialized and complex process (for review, see [1,2]). Briefly, dietary lipids (mainly triglycerides (TG), but also cholesterol esters (CE) and phospholipids (PL)) are digested in the upper part of the small intestine into fatty acids (FAs), monoacylglycerols, lysophospholipids and cholesterol. The digested lipids present in the lumen of the jejunum are taken up by enterocytes mostly by passive diffusion but also by transporters. Inside the cell, TG and PL are synthesized at the endoplasmic reticulum (ER) membrane and will contribute to chylomicron formation. Chylomicrons, the intestinal triglyceride-rich lipoprotein (TRL), are formed in the ER lumen by the fusion of one particle composed of apolipoprotein B48 (apoB48) stabilized by a small amount of PL and of a luminal lipid droplet present in the lumen of the ER (Fig. 1A). The nascent chylomicrons are transferred by vesicular transport to the Golgi apparatus then secretory vesicles and are finally secreted at the basal pole of the cell, into the lymphatic system (Fig. 1B). In the circulation, chylomicrons are metabolized by lipoprotein lipase, which provides fatty acids (FAs) to cells, including adipocytes. Chylomicron remnants, depleted from lipids, will be taken up by the liver and degraded.

Table 1
Proteins identified by proteomic analyses or western blot in lipid droplet fractions isolated from enterocytes (animal or cellular models) and involved in metabolism-related processes of lipids and hydrophobe molecules. When the localisation on or around lipid droplets has been visualized by confocal or electron microscopy, proteins are in bold type.

Gene	UniProt	Protein name	Refs
ABHD5/CGI-58	**ABHD5_HUMAN**	**α/β Hydrolase domain 5/comparative gene identification-58**	[9]
ACSL3	**ACSL3_HUMAN**	**Long chain acyl-CoA synthetase 3**	[9,16]
ACSL4	ACSL4_HUMAN	Long chain acyl-CoA synthetase 4	[9]
ACSL5, Acsl5	**ACSL5_HUMAN, ACSL5_MOUSE**	**Long chain acyl-CoA synthetase 5**	[16,17]
Adh1	Q3UKA4_MOUSE	Alcohol dehydrogenase 1	[17]
Aldh1a1	AL1A1_MOUSE	Retinal dehydrogenase 1	[17]
ANXA2, Anax2	ANXA2_HUMAN, ANXA2_MOUSE	Annexin A2	[9,17]
ANXA4, Anax4	ANXA4_HUMAN, Q7TMN7_MOUSE	Annexin A4	[9,17]
Apoa1	Q3V2G1_MOUSE	Apolipoprotein A-I	[17]
APOA4, Apoa4	**APOA4_HUMAN, Q9DBN0_MOUSE**	**Apolipoprotein A-IV**	[9,17]
Apob	APOB_MOUSE	Apolipoprotein B	[17]
APOE	APOE_HUMAN	Apolipoprotein E	[9]
Atp5a1	ATPA_MOUSE	ATP synthase subunit alpha, mitochondrial	[17]
Atp5b	ATPB_HUMAN, ATPB_MOUSE	ATP synthase subunit beta, mitochondrial	[9,17]
B4galnt2	B4GN2_MOUSE	Beta-1,4 N-acetylgalactosaminyltransferase 2	[17]
Bpnt1	BPNT1_MOUSE	3(2),5-bisphosphate nucleotidase 1	[17]
Cat	Q8C6E3_MOUSE	Catalase	[17]
Ces2a	EST2A_MOUSE	Pyrethroid hydrolase	[17]
Ces2c	EST2C_MOUSE	Acylcarnitine hydrolase	[17]
Ces2e	EST2E_MOUSE	Pyrethroid hydrolase	[17]
cideb	**CIDEB_MOUSE**	**Cell death activator CIDE-B**	[46]
Clint	Q5SUH7_MOUSE	Clathrin interactor 1	[17]
Cyb5a	CYB5_MOUSE	Cytochrome b5	[17]
CYB5R3, Cyb5r3	NB5R3_HUMAN, NB5R3_MOUSE	NADH–cytochrome b_5 reductase 3	[9,16,17]
Cyp2b10	Q9WUD0_MOUSE	Cytochrome P450 2B10	[17]
Dbi	Q548W7_MOUSE	Diazepam binding inhibitor, Acyl-CoA-binding protein	[17]
DHCR7	DHCR7_HUMAN	7-Dehydrocholesterol reductase	[9]
DHRS1	DHRS1_HUMAN	Dehydrogenase/reductase SDR family member 1	[9,16,17]
DHRS3	DHRS3_HUMAN	Short-chain dehydrogenase/reductase 3	[9,16]
ELMOD2	ELMD2_HUMAN	ELMO domain-containing protein 2	[9]
ECHS1	ECHM_HUMAN	Enoyl-CoA hydratase, mitochondrial	[9]
EPHX1	HYEP_HUMAN	Epoxide hydrolase 1	[9]
Ephx2	Q3UQ71_MOUSE	Bifunctional epoxide hydrolase 2	[17]
Fabp1	Q3V2F7_MOUSE	Fatty acid-binding protein, liver	[17]
Fabp2	Q53YP5_MOUSE	Fatty acid-binding protein, intestinal	[17]
FAF2/UBXD8	FAF2_HUMAN	FAS-associated factor 2/UBX domain-containing protein 8	[9,16]
Golm1	GOLM1_MOUSE	Golgi membrane protein 1	[17]
Hadh	HCDH_MOUSE	Hydroxyacyl-coenzyme A dehydrogenase	[17]
HSD17B2	**DHB2_HUMAN**	**Estradiol 17-beta-dehydrogenase 2**	[16]
HSD17B7	DHB7_HUMAN	3-Oxo-steroid reductase	[9,16]
HSD17B11, Hsd17b11	DHB11_HUMAN, DHB11_MOUSE	Oestradiol 17-β-dehydrogenase 11	[9,16,17]
HSD3B1	**3BHS1_HUMAN**	**3-β-Hydroxysteroid dehydrogenase**	[9,16]
HSPA8, hspa8	HSP7C_HUMAN, HSP7C_MOUSE	Heat-shock cognate 71 kDa protein	[9,17]
Lipe	LIPS_MOUSE	Hormone-sensitive lipase	[12,17]
LDAH/C2orf43	**LDAH_HUMAN**	**Lipid droplet-associated hydrolase (previously UPF0554 protein C2orf43)**	[9,16]
LPCAT2	**PCAT2_HUMAN**	**Lysophosphatidylcholine acyltransferase 2**	[9,16]
LSS	ERG7_HUMAN	Lanosterol synthase	[9,16]
MGLL	MGLL_HUMAN	Monoglyceride lipase	[9,16]
Mogat2	MGAT2_MOUSE	2-acylglycerol à-acylltransferase 2, Monoacylglycerol: acylCoA transferase 2	[12]
MTTP, Mttp	MTP_HUMAN, MTP_MOUSE	Microsomal triglyceride transfer protein	[9,12,16,17]
NSDHL	NSDHL_HUMAN	Sterol-4-α-carboxylate 3-dehydrogenase, decarboxylating	[9,16]
P4HB	PDIA1_HUMAN	Protein disulphide-isomerase	[9,16,17]
PCYT1A	PCY1A_HUMAN	Choline-phosphate cytidylyltransferase A	[9]
PLIN2, Plin2	**PLIN2_HUMAN, PLIN2_MOUSE**	**Perilipin 2/ADRP**	[7,9,12,16]
PLIN3, Plin3	**PLIN3_HUMAN, PLIN3_MOUSE**	**Perilipin 3/TIP47**	[7,9,16,17]
PNPLA2, Pnpla2	PLPL2_HUMAN, PLPL2_MOUSE	Patatin-like phospholipase domain-containing protein 2	[9,12,16]
Prdx6	Q6GT24_MOUSE	Peroxiredoxin-6	[17]
PTGS2	**PGH2_HUMAN**	**Prostaglandin G/H synthase 2, cyclooxygenase-2**	[15]
Rbp2	RET2_MOUSE	Retinol-binding protein 2	[17]
RDH10	RDH10_HUMAN	Retinol dehydrogenase 10	[9,16]
Scp2	NLTP_MOUSE	Non-specific lipid-transfer protein	[17]
SGPL1	SGPL1_HUMAN	Sphingosine-1-phosphate lyase 1	[9]
SQLE	ERG1_HUMAN	Squalene monooxygenase	[16]
Sult1b1	ST1B1_MOUSE	Sulfotransferase family cytosolic 1B member 1	[17]
Ugt1a7c	UD17C_MOUSE	UDP-glucuronosyltransferase 1–7C	[17]

Cytosolic lipid droplets (i.e. LDs present in the cytosol, which will be referred to as LD throughout this review) are observed in enterocytes of jejunum primarily if not only during lipid absorption (for review, see [3]). The minimal definition of a lipid droplet is a core of neutral lipids surrounded by a monolayer of amphipatic lipids and associated proteins. The current hypothesis is that cytosolic LDs in enterocytes constitute a transient pool of stored lipids that help optimize lipid absorption during food consumption and provide sustained lipid supply during fasting (Fig. 1B and C). Present in different intracellular compartments,

cytosolic LDs and chylomicrons are both composed of a core of neutral lipids (TG and CE) surrounded by a monolayer of PLs and cholesterol, and of associated proteins: LD-associated proteins for cytosolic LDs and apolipoproteins for chylomicrons. For a long time, researchers paid far more attention, in enterocyte, to highly specialized chylomicrons than to cytosolic LDs, considered as inert lipid storage depot. LDs became objects of interests, first in adipocytes, when in 1990 C. Londos showed for the first time that upon induction of lipolysis a LD-associated protein was phosphorylated, indicating a regulatory process on LDs [4]. Nowadays, LDs are recognized as genuine organelles, highly dynamic, in close relation with other organelles [5], with more and more biological functions [6]. Later, in 2009 perilipins, the LD coat proteins, were characterized in enterocytes. In these cells, LDs are strongly altered by meal and diet [7–9].

The biogenesis of LDs is widely accepted as being the accumulation of newly synthesized TG between the two leaflets of the ER phospholipid bilayer [10,11] (Fig. 1A). In any cell type, 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. The mechanisms that control the lipid distribution between the cytosol and the ER lumen are still unclear. Most of our knowledge about LDs came from studies performed in adipocytes, a cell type specialized in lipid storage and fatty acid (FA) supply to the body at distance of meals. In fact, almost any cell type can produce LDs and, while common features exist, it is becoming clear that the LDs composition and functions are different among cell types and need to be clarified.

In this review, we will present the composition of cytosolic LDs in enterocytes then we will focus on the specific physiological functions of the lipids and the LD-associated proteins in the context of the enterocyte. We will restrict our review to the proteins that have been demonstrated to be functionally associated to LDs in enterocytes, or to other cell types when relevant. We will then discuss the potential roles that enterocytes LDs may have in health and diseases.

2. Lipid droplets in enterocytes: composition, specific functions of lipids and associated proteins

2.1. Lipids and hydrophobic molecules

A detailed lipid analysis of LDs isolated from the mucosa of mouse proximal intestine was performed four hours after ingestion of sunflower oil containing 2% cholesterol [12]. TG, diacylglycerols (DAG), free cholesterol and CE were the major lipids, but sphingomyelines, phosphatidylcholines and ceramides were also present in LDs in significant amounts. PC content was similar to values found in LDs isolated from 3T3L1 adipocytes [13]. The size of the LDs was heterogeneous, large LDs contained most of the lipids including DAGs while the smallest LDs were enriched in cholesterol and CE [12]. The length chain of the predominant fatty acid identified in LDs was that of the ingested sunflower oil (C18), which underlines the close connection between dietary fat absorption and LDs showing up [8,9].

Enterocytes, like other cell types, may use FAs and cholesterol of LDs for their own purposes, energy production or membrane biosynthesis. FAs can exert cytotoxicity that is prevented during lipid absorption by enterocytes by the synthesis and storage of TGs into LDs. In cytosolic LDs, there is a clear dynamic accumulation of TG followed by depletion during lipid absorption [8,9,14]. The density and size of LDs along the small intestine has been quantified as a function of time after lipid gavage in mice, by coherent anti-Stokes Raman scattering (CARS) imaging [8]. It suggests that the dynamic, cytosolic LD pool in enterocytes may contribute to the regulation of postprandial blood TG concentrations (Fig. 1B and C). A role of LDs in response to inflammatory stimuli in enterocytes is poorly documented but can be suspected due to links with production of prostaglandins, which are lipid mediators in inflammation [15]. Moreover, the favoured hydrophobic environment of LDs allows to store/metabolise hydrophobic molecules including lipophilic vitamins and xenobiotic compounds, which are abundant in the digested food present in the lumen of the jejunum (for review, see [3]).

2.2. Lipid droplet-associated proteins

Three proteomic studies have been published so far for enterocyte LDs after lipid challenges [9,16,17]. The two first studies were performed from LDs isolated from Caco-2 cells incubated for 24 h with lipid micelles [9,16]. This cell line differentiates into enterocyte-like cells that share many of the characteristics of absorptive enterocytes, including TRL secretion and LD accumulation after lipid challenge [18–20]. The third study was performed from jejunum epithelium of mice two hours after an olive oil bolus [17]. In these studies, up to 200 proteins were identified and 20–25% were linked to lipid metabolic pathways (Table 1), a percentage similar to that found in adipocytes [21,22]. As in other cell types, the vast majority of the identified proteins are involved in biological processes such as membrane trafficking, protein folding, signalling pathways, carbohydrate metabolism, translation, etc…, which suggest LDs functions well beyond lipid metabolism. Moreover, proteins from multiple subcellular compartments including ER, mitochondria, lysosomes, autophagosomes and cytosol were also identified in LDs from enterocytes as in other cell types [5].

From a list of proteins identified by mass spectrometry, it is important to distinguish between contamination and *bona fide* LD proteins. This is particularly true for enterocytes whose cytoplasm is very rich in organelles. On the one hand, contaminants may be present because LDs are in very close interaction or in continuity with organelles. On the other hand, stringent conditions can be used for LDs isolation to avoid contamination but loosely bound LD proteins can then be lost. Moreover, recent studies showed that monotopic hairpin membrane proteins such as diacylglycerol acyltransferase 2 (DGAT2), ancient ubiquitous protein (AUP), lysophosphatidylcholine acyltransferase (LPCAT) 1 and 2, etc. are able to insert into a PL bilayer as well as in a PL monolayer, i.e. to ER or to LDs [23–25]. Accordingly, the association of a protein identified by proteomics to LDs must be validated by other methods.

Table 1 lists the proteins that have been identified so far by mass spectrometry on LDs isolated from enterocytes and that are in connection with lipid and hydrophobic molecules [9,16,17]. In the section below, we will focus on proteins whose association to enterocyte LDs is well documented. These proteins are mainly related to lipid metabolism.

2.2.1. Perilipins, the structural proteins

The perilipin (Plin) family quantitatively represents the most abundant signature of the LD machinery. Perilipins are a multiprotein family (5 members) that targets lipid droplet surfaces and regulates lipid storage and hydrolysis [26]. While Plin1 is almost only expressed in adipocytes, there is no perilipin isoform specific for enterocytes. Plin2 (previously ADRP, adipophilin) and Plin3 (previously TIP47) are ubiquitous, including in enterocytes [7,9]. Plin2 is higher after a chronic than an acute high-fat challenge in mouse enterocytes and Plin2 amount is increased after a 24h-lipid supply to Caco-2 cells [7,9]. Plin3 is present in enterocytes from chow and high-fat challenged mice [7]. Contrary to Plin2, Plin3 amount is higher after an acute than a chronic fat challenge. By

proteomic analysis, D'aquila et al. [17] identified only Plin3 on LDs isolated from jejunum enterocytes after an olive oil bolus to mice. Overall, results suggest that Plin3 is preferentially associated to transient lipid storage while Plin2 is associated with more prolonged lipid storage. Furthermore, recent studies in total Plin2 KO mice fed with low or high fat diet for 4 days showed that Plin2 KO mice had decreased LDs in enterocytes and increased faecal triglyceride levels compared to wild type mice [27]. Interestingly, these Plin2 KO mice had an altered intestinal microbiome as compared to wild type mice. The impact of Plin2 deficiency on chylomicron secretion was not examined. Overall, Plin2 deficiency appears to decrease the lipid storage capacity in LDs, which leads to impaired FA uptake by enterocytes.

2.2.2. Proteins involved in hydrolysis/synthesis of triglycerides, and in lipid secretion

Several enzymes involved in TG metabolism were identified on LDs [9,12,16,17]. For TG hydrolysis, these proteins are the adipose triglyceride lipase (ATGL), and its co-activator CGI-58, that hydrolyses TG into diacylglycerol and FAs, the hormone sensitive-lipase (HSL) that hydrolyses diacylglycerol into monoacyglycerol and FAs, and the monoacyglycerol lipase (MGL) that hydrolyses monoacylglycerol into glycerol and FAs (for review, see [28]). These lipids are substrates of monoacylglycerol acyltransferases (MGATs) and diacylglycerol acyltransferases (DGATs) for re-synthesis of triglycerides. Some of their functions have been studied by invalidation in mouse models.

Specific intestinal KO mice for ATGL had increased intracellular LDs in the intestinal epithelium but unchanged TG absorption from intragastric administration of triolein, suggesting that ATGL hydrolyses TGs from LDs but these lipids are not dedicated to chylomicron production in the small intestine [29]. Moreover, ATGL deficiency led to a down-regulation of target genes of PPARα, which is a transcription factor that is activated by FAs. This resulted in particular in delayed cholesterol absorption [29]. Recently, ELMOD2 (ELMO domain-containing protein 2), a putative noncanonical Arf-GTPase activating protein (GAP) anchored by palmitoylation on LDs isolated from Caco-2 cells was shown to down-regulate ATGL recruitment by suppressing the Arf1-COPI activity in LDs in hepatic Huh-7 cells [30]. Arf1/COPI can bud nano-LDs (~60 nm diameter) from PL-covered oil/water interfaces in vitro. This triggers the formation of LD-ER membranes bridges, enabling rapid monotopic protein targeting to LDs including ATGL and DGAT2 [31]. Accordingly, ELMOD2 depletion leads to decreased density and size of LDs. The role of ELMOD2 in the control of TRL secretion has not been examined yet. Intestinal CGI-58 (ATGL co-activator) knockout mice led to large LDs accumulation in enterocytes even during the fasting state, increased TG and CE intestinal content and decreased postprandial plasma TG concentration [32]. Intestinal CGI-58 appears thus required for efficient postprandial TRL secretion. Global MGL deletion in mice led to a markedly reduced intestinal TG secretion following an oral fat challenge, suggesting delayed lipid absorption [33]. Thus, intestinal depletion of enzymes involved in TG hydrolysis leads to LD accumulation in enterocytes.

In enterocytes, the prominent TAG synthesis pathway during lipid absorption is the MGAT pathway involving MGATs and DGATs [1,2]. These enzyme activities are present at the ER membrane. However, MGAT2, highly expressed in the proximal small intestine, was recently evidenced on isolated LDs from mouse enterocytes by western blotting suggesting that LDs may be a site for DAG synthesis as well [12]. Intestinal MGAT2 deficiency did not impair the amount of fat absorbed but reduced the rate of entry of dietary fat into the circulation [34]. This reduced rate led to more fat reaching the distal intestine and, in mice fed a high-fat diet, clusters of LDs were observed in the mid-intestine of KO mice but not in wild type. In intestine, at least two enzymes are responsible for DGAT activity: DGAT1, exclusively localized at the ER membrane, and DGAT2, which has also been identified on LDs of various cell types including differentiated 3T3-L1 adipocytes [35,24]. Intestinal DGAT2 overexpression in mice resulted in increased TG secretion but TG storage in LDs was similar to wild type mice [36]. However, in the conditions tested, none of the proteomic studies identified DGAT2 on LDs isolated from enterocytes [9,16,17]. The respective contribution of the presence of these enzyme activities on ER and/or LDs to enter cellular metabolic pathways (oxidation for energy production, lipid storage, chylomicron production) needs to be addressed.

FAs need to be activated by ACSLs (acyl-CoA synthetase long-chain) before entering any metabolic pathway. ACSL3 and ACSL5 were identified on LDs isolated from enterocytes (Table 1). Recent studies showed that overexpression of ACSL3a (orthologous to human ACSL3) in enterocytes leads to accumulation of cytoplasmic LDs and delays, in part, the appearance of neutral lipids in the vasculature of zebrafish larvae [37]. In hepatic Huh-7 cells, ACSL3 was shown to be required for incorporation of FA into phosphatidylcholine and ACSL3 depletion resulted in decreased secretion of TRL [38]. Cytosolic LDs were not examined in this study. Thus ACSL3 and ACSL5 functions in intestinal lipid absorption and LDs are still unclear.

Cell death-inducing DFF45-like effector (CIDE) family play a role in regulating various aspects of lipid metabolism [39]. While Cidea and Cidec are expressed in adipose tissue, enterocytes express Cideb but this protein was not found in proteomics studies. However, Cideb was shown localised both on ER and LDs by HA- and GFP- expressed Cideb [40]. After olive oil gavage, Cideb-deficient mice exhibited increased LDs accumulation, decreased lipid secretion and size of chylomicrons, and increased faecal lipids. Conversely, overexpression of Cideb reduced lipid accumulation in Caco-2 cells and enhanced lipid secretion. Overall, Cideb appears to decrease transient lipid storage in LDs and facilitate the lipidation of chylomicrons [40].

Chylomicrons contain apoB, the constitutive apolipoprotein, and apoA-1 and apoA-IV, exchangeable apolipoproteins. Their assembly requires microsomal triglyceride transfer protein (MTP) for apoB stabilisation by lipidation and luminal lipid droplet production in the ER lumen (Fig. 1A). ApoB, apoA-1 and apoA-IV were identified in isolated cytosolic LDs from enterocytes in all proteomic studies (Table 1). ApoA-IV, specifically expressed by enterocytes and induced upon lipid supply, was showed to be closely associated to LDs by confocal microscopy and electron microscopy [9,17]. Although this feature may be due to mere contamination of LDs by ER, we have to keep in mind that LDs are generated from ER and they can remain physically linked to ER or in very close proximity [5,10,11]. The physical interactions between ER and LD may well illustrate the strong functional interactions necessary for lipoprotein production, as suggested by Beilstein et al. [16].

2.2.3. Proteins involved in autophagy of lipid droplets (lipophagy)

Lipophagy, first described in hepatocytes, is now known to contribute to mobilization of intracellular lipid stores in many cell types [41]. Lipophagy occurs in enterocytes too [42,43]. Upon lipid micelles supply, an autophagic response is rapidly induced in Caco-2 cells and autophagy inhibition leads to TG accumulation as intracellular LDs, and to accumulation of CE [42]. Furthermore, while inhibition of autophagy has a mild effect on lipid secretion via TRL, it strongly increased high-density lipoproteins (HDL) production, which is responsible of the reverse transport of cholesterol from peripheral tissues to the liver.

In cell lines and mouse liver *in vivo*, it has been shown recently that degradation of LD-associated proteins (perilipins) by chaperone-mediated autophagy facilitates lipolysis [44]. Heat shock

cognate 71 kDa protein (HSC70), the chaperone involved in this process, was identified in abundance in isolated LDs of Caco-2 cells [9]. Further work needs to be done to decipher the exact role of autophagy in the context of regulation of cellular lipid metabolism of enterocytes [41].

2.2.4. Proteins involved in cholesterol and steroid hormone metabolism

The biosynthesis and intracellular traffic of cholesterol is complex and even more in enterocytes because dietary cholesterol is absorbed from the intestinal lumen and secreted in lipoproteins, and because the body cholesterol is eliminated through trans-intestinal cholesterol efflux (TICE), an alternative route to cholesterol excretion by bile acid secretion [45,46]. Lanosterol synthase and NAD(P)-dependent steroid dehydrogenase-like (NSDHL), which operate in the late steps of cholesterol biosynthesis, were identified on isolated LDs. C2orf43, recently characterized as a cholesterol ester esterase in macrophages and named LD-associated hydrolase (LDAH) [47], is identified and localizes around LDs in Caco-2 cells [16]. These results suggest that LDs contribute to cholesterol metabolism in enterocytes.

It is noteworthy that several proteins identified in isolated LDs from enterocytes are linked to steroid hormones metabolism (Table 1). The localization at the LD surface is confirmed for some of them [NSDHL, 17β-hydroxysteroid dehydrogenase 2 (DHB2), 3-β-hydroxysteroid dehydrogenase 1 (3BHS1)]. The identification of enzymes involved in steroid hormones metabolism was surprising because intestine is not reputed as a steroidogenic organ [48], as is adipose tissue [49,50]. The local production of steroid hormones by peripheral tissues is now thought to play important roles in tissue homoeostasis. We have recently shown that DHB2, 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 TG secretion, probably through its capacity to inactivate testosterone [16]. We thus identified a novel modulator of TRL production.

On top of their function, several of the enzymes belonging to the Short chain/dehydrogenase/reductase (SDR) family metabolize various compounds including steroids, retinoids, fatty acid derivatives and xenobiotic molecules [51]. Though liver is traditionally considered to be the major xenobiotic compounds-metabolizing organ, enterocytes are in contact with a large variety of xenobiotic molecules present in diet and may contribute to the detoxification process.

3. Involvement of lipid droplets of enterocytes in pathological processes

Several genes listed in Table 1 are responsible of human genetic diseases. Most proteins associated to LDs, if not all, are not specific of enterocytes. The diseases affect primarily other organs and little attention has been paid to date to the symptoms affecting the intestine itself. In this section, we chose to focus on diseases affecting specifically enterocytes and intestine.

3.1. Lipids droplets and metabolic health and diseases

As detailed in the previous section, an overexpression or deletion of several proteins associated to enterocyte LDs led to an altered size or density of cytosolic LDs, but also to modifications of lipoprotein secretion (amount or secretion rate) and/or to changes of lipid amounts remaining in the intestinal lumen, i.e. lipids eventually measured in faeces. A positive correlation exists between post-prandial serum lipid levels and coronary artery diseases [52]. The possibility of controlling the lipid output over time in the circulation by controlling the transient storage of the lipids in cytosolic LDs makes LDs as promising potential therapeutic targets (Fig. 1).

The alteration of diet lipid content may alter the LDs characteristics of enterocytes in the small intestine. In turn, the presence of lipids in the lumen of the intestine affects the microbiota composition. A high-fat diet influences the types and amounts of gut microbes, which have been implicated in the development of diet-induced obesity, chronic inflammation and insulin resistance (for review, see [53]). A high-fat palm oil diet induced an up-regulation of lipid metabolism-related genes in the distal small intestine compared to a low fat palm oil diet or high-fat olive oil or safflower diets. The same occurs for many proteins associated to LDs such as DGAT2, ACSL3, ACSL5, HSD17B11, MTTP, apoB, apoA-IV, and PCY1A [54]. Additionally, enterocytes, which have a life of only 5 to 7 days, are eliminated into the intestinal lumen where their content, including LDs, might feed the gut microbiota present downstream.

In Humans, mutations of proteins found on LD have been associated with pathologies. CGI-58 mutation, as an example, causes Chanarin-Dorfman syndrome, an autosomal recessive genetic disease characterized by massive accumulation of TGs in the cytosolic LDs in most cell types, including absorptive enterocytes of small intestine.

A genetic variation in human PLIN2, Ser251Pro polymorphism, is associated with decreased concentrations of plasma TG and very low density of lipoproteins (VLDL), the TRL secreted by hepatocytes [55]. When transfected to HEK293 cells, this variant leads to increased lipid accumulation and decreased lipolysis. Plin2 decreases ATGL access to LD surface and thus TG hydrolysis [26]. It is suggested that this variant may affect lipases and/or their modulators to associate with LDs and hence reduce lipolysis. Furthermore, this variant leads to increased Plin3 expression, the other perilipin expressed in enterocytes. It would be interesting to measure the postprandial triglyceridemia in these human subjects fed a high-fat meal or diet to assess the impact of this Plin2 polymorphism in intestine, and to look for possible correlations with insulin resistance, obesity and cardiovascular diseases.

3.2. Lipid droplets and viral infections

Knowledge on gut virome is still scarce [56]. However, several viral intestinal pathogens have been shown to use LDs for their production, and LDs may thus be involved in their pathogenesis. Rotaviruses and enteroviruses are well known in clinical practice and are known to be responsible for common infectious gastroenteritis.

Rotaviruses, the most common cause of infectious diarrhoea among infants and young children, infect enterocytes on the tips of the intestinal villi. Morphogenesis takes place in cytoplasmic inclusion bodies known as viroplasms, which colocalize and interact with LDs. Compounds disrupting or blocking LDs inhibit viral replication [57–59].

Human parechovirus (HPEV-1) cause mild gastrointestinal or respiratory illness. In infected cells, the non-structural viral proteins 2C and 2BC are mainly localized to the surface of LDs [60]. Like the NS5A protein of hepatitis C virus (HCV), a virus which hijacks hepatic lipid metabolism for its replication, the N-terminal region of the picornaviral 2C protein folds into an amphipathic α-helix, which is responsible for the proteins association with membranes in the viral RNA replication complex [61].

In Caco-2 cells, the expression of HCV core protein, which associates to the LD surface, results in decreased lipoprotein secretion [16]. This is similar to what is observed in hepatocytes and in HCV-infected patients.

Enteroviruses exploit the clathrin-mediated endocytosis to

traffic cholesterol from the plasma membrane and extracellular medium to replication organelles. Here, cholesterol regulates viral polyprotein processing, facilitates and stimulates replication. Disruption of clathrin-mediated endocytosis triggers cholesterol storage in LDs. Consequently, cholesterol cannot be trafficked to replication organelles and replication is inhibited [62].

For intestinal viruses which need LDs for their production, it will be interesting to examine the impact of the diet lipid content on virus propagation and pathology.

4. Conclusion

The mechanisms that control the lipid distribution in the various cellular compartments of enterocytes, and the consequences of their alteration on physiopathology are still poorly understood. LDs are now recognized as a transient storage lipid pool, highly dynamic, and regulated by LD-associated proteins. In the past few years, knowledge on enterocyte LD biology increased due to the identification of LD-associated proteins and to functional studies of several of these proteins in the context of the intestine using animal or cellular models. Results show that alteration of several LD- associated proteins impacts chylomicron production. It can also modify lipid uptake from the intestinal lumen, with possible consequences on gut microbiota. LDs should be considered as a possible target for improving metabolic health by nutritional and nutraceutical recommendations.

LDs are associated to proteins involved in lipid metabolism but also to many other proteins, involved in many pathways. The functional significance of the presence of these proteins on LDs in the intestinal context remains to be studied.

Conflict of interest

The authors declare no conflict of interest.

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