ANNUAL REVIEWS

Annual Review of Nutrition

The Roles of Cytoplasmic Lipid Droplets in Modulating Intestinal Uptake of Dietary Fat

Alyssa S. Zembroski,[1] Changting Xiao,[2] and Kimberly K. Buhman[1]

[1]Department of Nutrition Science, Purdue University, West Lafayette, Indiana 47907, USA; email: kbuhman@purdue.edu

[2]Department of Anatomy, Physiology and Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada

Annu. Rev. Nutr. 2021. 41:79–104

First published as a Review in Advance on July 20, 2021

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

https://doi.org/10.1146/annurev-nutr-110320-013657

Keywords

dietary fat absorption, intestine, cytoplasmic lipid droplet, triacylglycerol, enterocyte

Abstract

Dietary fat absorption is required for health but also contributes to hyperlipidemia and metabolic disease when dysregulated. One step in the process of dietary fat absorption is the formation of cytoplasmic lipid droplets (CLDs) in small intestinal enterocytes; these CLDs serve as dynamic triacylglycerol storage organelles that influence the rate at which dietary fat is absorbed. Recent studies have uncovered novel factors regulating enterocyte CLD metabolism that in turn influence the absorption of dietary fat. These include peroxisome proliferator-activated receptor α activation, compartmentalization of different lipid pools, the gut microbiome, liver X receptor and farnesoid X receptor activation, obesity, and physiological factors stimulating CLD mobilization. Understanding how enterocyte CLD metabolism is regulated is key in modulating the absorption of dietary fat in the prevention of hyperlipidemia and its associated metabolic disorders.

Contents

1. INTRODUCTION

The absorption of dietary fat by the small intestine is a complex process that promotes health but can also contribute to metabolic abnormalities during disease states. For example, dietary fat [mostly in the form of triacylglycerol (TAG)] provides cells of the body with substrates needed to synthesize cellular components and upon storage acts as a source of cellular energy and as insulation for the body. In addition, dietary TAG facilitates the absorption of fat-soluble nutrients such as vitamins A, D, E, and K and is an integral component of infant nutrition for adequate growth and development. However, the absorption of dietary TAG can also contribute to metabolic disease. For example, dietary TAG absorption contributes to weight gain, obesity, and associated metabolic consequences such as dyslipidemia and metabolic-associated fatty liver disease. Further, the secretion of chylomicrons (CMs), the major lipoprotein that carries dietary TAG from the small intestine, is elevated in insulin resistance (113), and elevated postprandial lipemia is an independent risk factor for cardiovascular disease (91). The regulation of dietary TAG absorption is therefore an important aspect mediating the balance between health and disease.

TAG: triacylglycerol

CM: chylomicron

CLD: cytoplasmic lipid droplet

A potential area of regulation in the process of dietary TAG absorption is the formation and mobilization of cytoplasmic lipid droplets (CLDs) in enterocytes, the absorptive cells of the small intestine (30). CLDs are organelles that serve as storage for TAG not immediately secreted in CMs, overall increasing the efficiency of dietary TAG absorption. Although the exact fate of TAG stored in enterocyte CLDs has yet to be determined, at least part of stored TAG is thought to be used for CM synthesis and secretion at later times, contributing to blood lipid concentrations hours after a high-fat meal (133). Therefore, CLDs in enterocytes can influence the rate at which

dietary TAG is absorbed, making CLDs potential targets to modulate blood TAG concentrations in order to prevent and treat metabolic disease.

This review covers recent developments on novel factors and concepts regulating CLD metabolism and dietary TAG absorption in enterocytes. Understanding the contribution and function of CLDs in enterocytes will allow for the development of targeted therapies to modulate the absorption of dietary TAG and the development of disease.

FA: fatty acid

MAG: monoacylglycerol

APOB-48: apolipoprotein B-48

MTTP: microsomal triglyceride transport protein

ER: endoplasmic reticulum

FABP: fatty acid binding protein

MGAT: monoacylglycerol acyltransferase

DAG: diacylglycerol

DGAT1/2: diacylglycerol acyltransferase 1/2

2. PROCESS OF DIETARY TAG ABSORPTION

Ingested dietary TAG is hydrolyzed into fatty acids (FAs) and monoacylglycerol (MAG) by pancreatic lipase, and the digestive products are absorbed at the brush border of the small intestine across the apical membrane of enterocytes (**Figure 1**). Absorption of FAs and MAG is very efficient and is achieved primarily through passive diffusion, but it also involves facilitated transport by FA transport proteins including CD36 and FATP4 (27). FA uptake into enterocytes through these transport proteins plays a relatively minor role in overall uptake; however, it plays an important role in intracellular lipid trafficking. For instance, binding of long-chain FAs to CD36 increases apolipoprotein B-48 (APOB-48) and microsomal triglyceride transport protein (MTTP) levels, promoting the formation of large CMs (121), while mice lacking CD36 have impaired CM formation and secretion as well as lipid accumulation in the proximal intestine, although dietary TAG uptake is generally unaffected (44, 89).

2.1. TAG Synthesis at the ER Membrane: Importance of DGAT Enzymes

Inside the enterocyte, FAs and MAG are transported to the endoplasmic reticulum (ER) membrane, where they are reesterified into TAG that is then packaged on either CMs or CLDs (30). FA-binding proteins (FABPs) mediate the trafficking of FAs and MAG in the cytosol. Both intestinal and liver forms of FABP, IFABP and LFABP, are present in enterocytes (138). They differ in their transcriptional regulation, ligand-binding specificities, and ligand transfer mechanisms. IFABP specifically binds to FAs, while LFABP binds to FAs, fatty acyl-coenzyme A (acyl-CoA), and MAG (1).

The majority of TAG synthesis in enterocytes is via the monoacylglycerol acyltransferase (MGAT) pathway, where MGAT2 first adds a FA to MAG to form diacylglycerol (DAG), then diacylglycerol acyltransferase 1 or 2 (DGAT1/2) adds a second FA to form TAG (141). A minor fraction (~20%) of TAG is synthesized via the glycerol-3-phosphate pathway catalyzed by glycerol-3-phosphate acyltransferase (65).

DGAT1 is imperative for the absorption of dietary fat, accounting for the majority of TAG synthesis in rat enterocytes and Caco-2 cells, a human model of enterocytes (20). DGAT1 deficiency in humans is linked to congenital diarrhea (55), intestinal failure, and aberrant lipid metabolism (127). These consequences may be due to lack of compensatory TAG synthesis by DGAT2, as DGAT2 has not been identified in human intestine. This is in contrast to mice, as mice express both DGAT1 and DGAT2 in the intestine, and DGAT1 and DGAT2 differentially partition TAG between storage and secretion (discussed below). In fact, although both are capable of TAG synthesis, DGAT1 and DGAT2 have distinct roles in mice. Mice deficient in DGAT1 are resistant to diet-induced obesity, show abnormal TAG accumulation in enterocyte CLDs, and have a blunted postprandial TAG response (60). These effects may be in part due to dysregulated flux of autophagy-mediated CLD mobilization and disrupted lysosomal function in enterocytes of *Dgat1*$^{-/-}$ mice (59). In contrast, *Dgat2*$^{-/-}$ mice are not viable due to a lack of systemic TAG

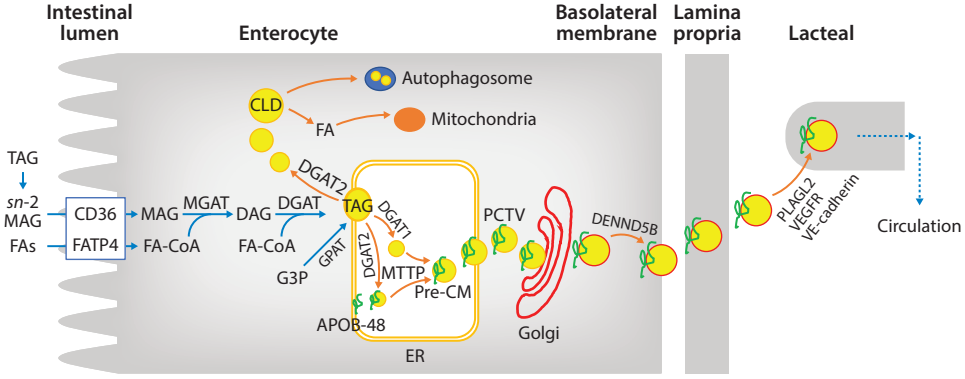

Figure 1

Process of dietary TAG absorption in enterocytes. Dietary TAG is digested in the intestinal lumen into FAs and *sn*-2 MAG. FAs and *sn*-2 MAG are absorbed across the apical membrane of the enterocyte via passive diffusion and transport facilitated by FA transport proteins such as CD36 and FATP4. Trafficking inside the cytosol is mediated by IFABP (FA) and LFABP (FA, FA-CoA, MAG). FA-CoA and *sn*-2 MAG are then reesterified into TAG in the ER membrane, catalyzed sequentially by MGAT and DGAT. DGAT1 and DGAT2 have distinct roles in regulating the metabolic fates of synthesized TAG. DGAT1 is primarily involved in partitioning reesterified TAG into APOB-48-free lumenal lipid droplets within the ER, thereby directing TAG toward secretion rather than retention in CLDs. In contrast, DGAT2 is important for synthesis of the poorly lipidated APOB-48-containing particles while preferentially targeting TAG toward storage in CLDs. A minor portion of TAG is also synthesized via the glycerol-3-phosphate pathway catalyzed by GPAT. Newly synthesized TAG forms lipid droplets that are packaged into pre-CMs through MTTP-mediated lipidation of APOB-48. Pre-CMs are transported in PCTVs from the ER to the Golgi, where they further mature. PCTV budding from the ER, and transport and docking with the Golgi, requires multiple proteins including LFABP, CD36, VAMP7, APOB-48, SAR1B, and SNARE proteins. Mature CMs exit the enterocyte at the basolateral membrane via exocytosis, move through the lamina propria, enter the lacteals, and transport through the lymphatics before joining blood circulation. Post-Golgi CM transport may require DENND5B, while CM entry into the lacteal may require PLAGL2, VEGFR signaling, and VE-cadherin signaling. Synthesized TAG in the ER membrane is also packaged into CLDs. CLDs may be catabolized through lipophagy or cytoplasmic TAG lipolysis, releasing FAs that can undergo beta-oxidation in mitochondria or be recycled back to the ER for TAG and CM synthesis. Abbreviations: APO, apolipoprotein; CLD, cytoplasmic lipid droplet; CM, chylomicron; DAG, diacylglycerol; DENND5B, DENN domain containing protein 5B; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; FA, fatty acid; FA-CoA, fatty acyl-coenzyme A; GPAT, glycerol-3-phosphate acyltransferase; IFABP, intestinal fatty acid binding protein; LFABP, liver fatty acid binding protein; MAG, monoacylglycerol; MGAT, monoacylglycerol acyltransferase; MTTP, microsomal triglyceride transport protein; PCTV, prechylomicron transport vesicle; PLAGL2, pleiomorphic adenoma-like protein 2; pre-CM, prechylomicron; TAG, triacylglycerol; VE-cadherin, vascular endothelial cadherin; VEGFR, vascular endothelial growth factor receptor. Figure adapted with permission from Reference 135 (CC BY-NC-ND 4.0).

content and abnormal skin barrier function (115). These phenotypes suggest a clear species difference in TAG metabolism between mice and humans.

DGAT1 and DGAT2 have been identified as key regulators of lipid partitioning between storage in CLDs and secretion in CMs in mice (60). They are encoded by two separate genes, and although both are found on the ER lumen, DGAT2 also localizes to CLDs (141). In mice, DGAT1 is primarily involved in partitioning reesterified TAG into APOB-48-free lumenal lipid droplets within the ER, thereby directing TAG toward CM formation rather than retention in CLDs. In contrast, DGAT2 is important for synthesis of TAG directed to poorly lipidated

APOB-48-containing particles while preferentially targeting TAG toward storage in CLDs (60). Whether a similar mechanism exists in human enterocytes without the contribution of DGAT2 is unclear.

PCTV: prechylomicron transport vesicle

Recently, the structure and catalytic mechanism of human DGAT1 was characterized in two independent studies (118, 128). DGAT1 has nine transmembrane helices and can form homodimers or homotetramers. A hypothetical model of DGAT1-mediated TAG synthesis features a reaction chamber with an opening to the cytosolic side of the ER for acyl-CoA binding and an opening to the hydrophobic core of the ER membrane for DAG binding. The acyl chain from acyl-CoA is transferred to DAG and the reaction products are released to either the cytoplasm (CoA) or the membrane bilayer (TAG) (118, 128). Recognizing the structure of DGAT1 is crucial in understanding the consequences of human DGAT1 mutations and the mechanism of DGAT1 inhibitors.

2.2. Chylomicron Formation and Transport

CM synthesis and secretion represent the major fate of resynthesized TAG (81). First, ER lumenal lipid droplets fuse with a lipid-poor APOB-48-containing particle at the inner ER membrane to form the pre-CM particle. MTTP plays a crucial role in lipidation of APOB-48. Loss-of-function mutation in *MTTP* results in defective CM synthesis and abetalipoproteinemia (75). Interestingly, conditional intestinal deletion of *Mttp* in mice improved hepatic glucose metabolism, likely involving adaptive modifications in bile acid signaling and gut microbial composition (137). Also added to pre-CMs in the ER is APOA-IV, which is primarily synthesized in the intestine and has multiple physiological roles, including affecting CM assembly, glucose homeostasis, satiety, and atherosclerosis (98).

2.2.1. Chylomicron transport from ER to Golgi in prechylomicron transport vesicles. Pre-CMs are transported in prechylomicron transport vesicles (PCTVs) from the ER to the Golgi apparatus. PCTV budding from the ER is initiated by LFABP and facilitated by several other proteins, including CD36, VAMP7, and APOB-48, and is considered the rate-limiting step for intracellular TAG trafficking (81). Transport of PCTVs from the ER to Golgi requires SNARE proteins (111). Other proteins such as APOA-I are transported by coat protein complex II vesicles from the ER to PCTV-delivered CMs in the Golgi.

2.2.2. Chylomicron modification in Golgi and transport out of enterocytes. CMs are further matured in the Golgi with APOB-48 glycosylation and acquisition of APOA-I (81). Mature CMs exit the Golgi in large vesicles before being exocytosed at the basolateral membrane. The mechanism of post-Golgi transport of CMs is largely unknown. Recently, it has been reported that loss of DENN domain containing protein 5B (Dennd5b) in mice impairs post-Golgi trafficking of CMs in secretory vesicles, as CM secretory vesicles accumulate and fail to fuse with the basolateral membrane despite normal biosynthesis and trafficking of PCTVs (51).

During active lipid absorption, the basement membrane beneath the enterocytes may become leaky to allow CMs to enter the lamina propria, where CM movement is believed to be through diffusion (123). The majority of CMs then enter the lacteal, a blind-ended lymphatic capillary located in the center of each intestinal villus (9). This process is largely mediated paracellularly via size exclusion, whereby large, CM-sized particles pass through intercellular junctions lining the endothelial wall of the lacteal tip (135). Emerging evidence supports the concept that CM uptake by the lacteal is regulated. For example, in mice deficient of the transcription factor PLAGL2, CMs fail to enter lacteals and accumulate in the lamina propria (126). Permeability of the intercellular

junctions is regulated by signaling between vascular endothelial growth factor A (VEGF-A) and vascular endothelial growth factor receptor 2 (VEGFR2), and by vascular endothelial cadherin (VE-cadherin) signaling, both of which influence CM uptake (145). VEGF-C via its main receptor VEGFR3 regulates lacteal maintenance and function; therefore, inactivating VEGFR3 in mice decreases CM secretion (109).

3. CLD FORMATION IN ENTEROCYTES: AN ALTERNATIVE FATE OF RESYNTHESIZED TAG

In addition to packaging and secreting dietary fat in CMs after a high-fat meal, enterocytes can also package and store dietary fat in CLDs (30). CLDs in enterocytes are highly dynamic, and their formation depends on the quantity of dietary fat consumed, time after consumption, and region of the small intestine (147). Generally, CLDs appear in enterocytes after exposure to large quantities of dietary fat, suggesting enterocyte CLDs serve as an overflow pool of lipid that exceeds the CM synthesis and secretion capacity of the enterocyte (78), while also preventing the cellular lipotoxicity that can result from a large influx of dietary FAs. Lipid stored in enterocyte CLDs is thought to contribute to CM secretion hours after a high-fat meal (133), providing a sustained supply of substrates in both postprandial and fasted periods. Therefore, the dynamic nature of enterocyte CLDs allows for a more regulated lipid delivery to circulation and attenuated postprandial lipemia.

The molecular mechanisms of CLD formation in enterocytes have not been directly studied; however, they are expected to follow the general principles of CLD formation that have been defined in other cell types (90, 101). CLD formation consists of TAG accumulation and lens formation in the ER bilayer and eventual budding into the cytosol. This process is mediated by proteins and nonprotein components. After their formation, CLDs can remain attached to or separate from the ER and acquire various proteins that regulate both CLD and cellular metabolism. Recent studies investigating each of these steps have uncovered novel regulators and factors controlling CLD formation that contribute to the dynamic nature of CLDs (90, 101).

3.1. New Insights on CLD Formation: Proteins and Nonprotein Components

Recent discoveries on the molecular mechanisms of CLD formation have uncovered novel proteins and nonprotein components responsible for stimulating and facilitating CLD formation and budding from the ER membrane (49, 90). The major protein implicated in CLD formation is seipin, which moves within the ER and senses sites of neutral lipid accumulation and packing defects where it then stabilizes growing CLDs and facilitates their lipidation (105). To our knowledge, descriptions of the molecular mechanisms of seipin-mediated CLD formation in enterocytes have not been published. Recently, however, seipin in the intestine of *Caenorhabditis elegans* was shown to regulate CLD size and form perilipid droplet cages around certain pools of nascent CLDs for their subsequent growth (17). Although seipin itself appears to be a main factor in CLD formation, the discovery of many interacting partners for seipin reflects the importance of protein complexes in establishing sites and facilitating CLD formation (90). Other proteins have also recently been shown to be responsible for maintaining CLD-ER contacts during CLD formation (reviewed in 104).

In contrast, nonprotein components (i.e., specific lipid species) have recently been shown to play a prominent role in CLD biogenesis by influencing ER membrane curvature and surface tension (49). A determining factor of ER membrane curvature and CLD budding is the phospholipid composition of the ER, as lipids with a positive membrane curvature such as lysophosphatidylcholine favor CLD budding (49). In support of this finding, seipin preferentially localizes to ER tubules, which have higher membrane curvature than other regions of the ER that have a relatively

flat curvature (106). In addition to phospholipid composition, an adequate amount of phospholipids on the cytosolic face of the ER membrane is also required to minimize ER surface tension and facilitate proper budding of the CLD toward the cytosol (24). Therefore, the ER membrane appears to be a determining factor for proper CLD formation.

CIDE: cell death–inducing DNA fragmentation factor alpha-like effector

The membrane composition of the ER may be regulated by specific proteins, which in turn can influence CLD formation and budding. For example, fat storage–inducing transmembrane protein 2 (FIT2), a putative lipid phosphatase, may facilitate CLD budding by regulating DAG concentrations in the ER at sites of CLD biogenesis (26). Interestingly, FIT2 is required for intestinal health and CLD formation in enterocytes, as mice with whole-body knockout of *Fit2* present with enteropathy and defective CLD budding from the ER despite normal TAG absorption (50). More intriguingly, however, *Fit2* deletion specifically in the intestine does not influence CLD budding or accumulation, and this result suggests that the role of FIT2 in enterocyte CLD formation may be extracellular.

After CLDs form and bud from the ER membrane, they can grow by local TAG synthesis, coalescence, or lipid transfer between CLDs. Local TAG synthesis is catalyzed by TAG synthesis enzymes that associate with CLDs. For example, it was previously shown that DGAT2, glycerol-3-phosphate acyltransferase 4 (GPAT4), and 1-acylglycerol-3-phosphate *O*-acyltransferase 3 (AGPAT3) of the glycerol-3-phosphate pathway of TAG synthesis relocate to growing CLDs from the ER membrane through membrane bridges in *Drosophila* and human fibroblast COS7 cells (132). DGAT2 plays an important role in mediating CLD expansion. Recently, McFie et al. (86) identified a C-terminal CLD-targeting domain of DGAT2 that allows it to interact with CLDs while remaining embedded in the ER during CLD growth. In enterocytes, the role of DGAT2 in CLD growth is apparent in *Dgat1*$^{-/-}$ mice, where massive CLDs accumulate due to the partitioning of TAG synthesized by DGAT2 primarily for CLD formation instead of CM secretion (60).

CLDs can also grow by lipid transfer between CLDs, catalyzed by the cell death–inducing DNA fragmentation factor alpha-like effector (CIDE) family of proteins (19). CIDE proteins mediate CLD-CLD contacts to facilitate the movement of TAG from small to large CLDs through potential membrane pores. There are three CIDE proteins: CIDEA, CIDEB, and CIDEC/FSP27, which have different tissue distributions and important roles in various aspects of metabolism (19). In the intestine, CIDEB regulates TAG storage and secretion, as mice lacking CIDEB had a lower TAG secretion rate with smaller secreted CMs and TAG accumulation in enterocyte CLDs (146). Further, in Caco-2 cells, CIDEB localizes to CLDs upon oleate loading and interacts with APOB-48 (146). These results suggest that CIDEB may participate in the lipidation of APOB-48 to promote CM synthesis and secretion, possibly by using TAG stored in CLDs. This participation would be similar to its role in hepatocytes (139) and suggests that CIDEB is an important factor for lipid transfer not only between CLDs but also to nascent lipoproteins.

An increase in CLD size requires an increase in phospholipids to surround the growing neutral lipid core in order to prevent CLD coalescence and abnormal CLD morphology. The rate-limiting enzyme in phosphatidylcholine synthesis, CTP:phosphocholine cytidylyltransferase (CCT), maintains adequate amounts of phospholipids required for CLD expansion (28). For example, CCT translocates from the nuclear membrane to the growing CLD surface, where it is activated for phospholipid synthesis upon oleate loading in *Drosophila* S2 cells (69). In enterocytes, CCT regulates both CLD size and CM secretion. Loss of *PCYT1α*, the gene encoding CCT, in the Caco-2 cell model of enterocytes results in decreased de novo phosphatidylcholine synthesis and increased CLD size (73), consistent with other studies. However, CCT also synthesizes phosphatidylcholine needed for CM secretion, as lack of CCT decreases TAG and APOB-48 secretion upon oleate loading (73). These results demonstrate the importance of CCT for

maintaining adequate amounts of phosphatidylcholine for growing CLDs and also for CM synthesis and secretion in enterocytes.

PLIN: perilipin

3.2. CLD Proteins: Association and Functions

The proteins that associate with CLDs differ in their ability to target to CLDs and are involved in numerous different functions maintaining both CLD and cellular metabolism (144). For example, proteins involved in lipid synthesis and lipolysis regulate the storage and utilization of TAG in CLDs. However, CLD proteins also have roles beyond lipid metabolism, including mediating connections with other organelles, regulating gene expression, and initiating lipid-mediated signaling pathways (144). As proteins often associate with CLDs depending on the metabolic state of the cell, the proteome of CLDs may reflect how lipid storage and utilization are regulated. In enterocytes, proteomic studies of CLDs isolated from mouse enterocytes and differentiated Caco-2 cells have revealed a diverse proteome with potentially novel roles regulating the balance between TAG storage in CLDs and secretion in CMs (7, 12, 31, 32, 60). Future studies investigating the functional significance of these proteins at the CLD surface is necessary to understand how CLD metabolism is regulated in enterocytes.

3.2.1. New discoveries on the association of proteins with CLDs. Two classes of CLD proteins have been defined, including those originating from the ER membrane (class I) and those originating from the cytosol (class II) (10). Class I and class II proteins differ in their characteristics and mechanisms of CLD localization. Class I proteins typically have hydrophobic domains that form a hairpin structure, allowing the protein to insert into membranes (10). These proteins can traffic from the ER to CLDs during CLD formation or can traffic to CLDs through membrane bridges after CLDs have separated from the ER (131, 132). Class II proteins have amphipathic helices that can reversibly associate with CLDs, in part depending on the degree of molecular crowding at the CLD surface (68). Recent studies have identified specific chemical and architectural features of CLDs that have elucidated mechanisms regulating class II protein targeting to the CLD surface monolayer. For example, although the degree of phospholipid packing defects in the CLD surface has been implicated in the targeting of class II proteins, it was recently discovered that packing defects expose the neutral lipid core of CLDs, and this exposure then determines protein binding due to the affinity of the protein to certain neutral lipids (25). Although the exact mechanisms regulating the specificity for protein targeting to the phospholipid monolayer of CLDs instead of other organelles are still unclear, these recent developments have brought us closer to understanding how the proteome of CLDs may be regulated.

3.2.2. Functions of CLD proteins in enterocytes. The most prominent CLD-associated proteins are those controlling CLD physiology and metabolism, for example, the perilipin (PLIN) family of proteins, and those involved in TAG synthesis and lipolysis (144). PLIN proteins are generally thought to protect CLDs from lipolysis (119), although their exact function at the CLD surface has not yet been determined. However, different tissue distributions of each of the five identified PLIN species and apparent specificity for certain pools of CLDs suggest they may have specific functions. For example, although both PLIN2 and PLIN3 are expressed in enterocytes, they differentially associate with CLDs depending on state and cellular location in mice. First, PLIN3 surrounds CLDs that form after an acute dietary fat challenge early in the process of dietary fat absorption, while PLIN2 surrounds CLDs present after chronic high-fat feeding in mice (72). In addition, PLIN2 and PLIN3 also differentially associate with enterocyte CLDs based on the cellular location of CLDs in obese mice (32). For example, after a dietary fat challenge in obese

mice, PLIN3 localizes to CLDs on the apical side of enterocytes while PLIN2 localizes to CLDs on both the apical and basolateral sides. These results support the initial observation that PLIN3 associates with new CLDs synthesized from incoming dietary fat at the apical side of enterocytes, while PLIN2 may stabilize mature CLDs that have been present for longer periods of time.

The differential localization of PLIN2 and PLIN3 to CLDs in enterocytes of obese mice may contribute to a larger CLD size and a decreased rate of TAG secretion in obese mice compared with lean mice (32, 42); however, future studies are required to test this hypothesis. Interestingly, whole-body *Plin2*$^{-/-}$ mice are protected from diet-induced obesity (87). In fact, short-term high-fat feeding in *Plin2*$^{-/-}$ mice results in decreased lipid storage in enterocyte CLDs, increased fecal TAG, and changes in the gut microbiome (46) that may contribute to the effects of PLIN2 on obesity. The multiple factors that influence the localization of PLIN2 and PLIN3 to CLDs suggest distinct roles for each PLIN species in TAG storage and CLD maintenance in enterocytes.

In addition to maintaining CLD integrity, CLD proteins in enterocytes are also thought to regulate the balance between TAG storage and secretion. Although functional studies of CLD proteins in enterocytes are lacking, the localization of many proteins involved in lipid metabolism identified by proteomic studies have been confirmed at the CLD surface by imaging methods, suggesting they may play an active role at the CLD surface. For example, APOA-IV has been visualized at the CLD surface in mouse enterocytes by immunofluorescence microscopy (31) and in differentiated Caco-2 cells by immunoelectron and immunofluorescence microscopy (12). APOA-IV is an apolipoprotein synthesized in the small intestine and is implicated in intestinal TAG secretion and CM metabolism (98); however, the exact role of APOA-IV in these processes remains elusive. Other proteins that have been validated at the CLD surface in enterocytes include acyl-CoA synthetase enzymes ACSL3 and ACSL5: ACSL3 in differentiated Caco-2 cells by immunofluorescence (7) and ACSL5 in mouse enterocytes by immunofluorescence and immunoelectron microscopy (31). Both ACSL3 and ACSL5 have been shown to play a role in dietary fat absorption. Whole-body *Acsl5*$^{-/-}$ mice have a decreased intestinal TAG secretion rate after an oral oil bolus, in addition to lower fat mass, increased insulin sensitivity, and lower blood TAG and glucose levels compared with control mice (13). On the other hand, overexpression of *Acsl3a* in zebrafish delays lipid secretion (29). It is possible that the localization of these proteins on CLDs in enterocytes contributes to their role in regulating intestinal lipid metabolism; however, further studies are required to determine functional mechanisms.

ATGL: adipose triglyceride lipase

HSL: hormone-sensitive lipase

MGL: monoacylglycerol lipase

LAL: lysosomal acid lipase

3.3. CLD Breakdown

The enzymes and mechanisms involved in CLD breakdown have been well studied in cell types such as adipocytes and hepatocytes; however, the enzyme(s) primarily responsible for CLD breakdown in enterocytes remains elusive. CLD breakdown is mediated by either cytoplasmic or lysosomal TAG lipolysis (143), both of which release FA that can be used in different cellular metabolic pathways. Cytoplasmic TAG lipolysis is catalyzed by the action of three sequential enzymes: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL). Lysosomal TAG lipolysis, or lipophagy, is the autophagic breakdown of CLDs and is catalyzed by lysosomal acid lipase (LAL). Both lipolytic pathways are active in enterocytes, and studies investigating the individual functions of enzymes in these pathways have uncovered their unique roles in intestinal lipid homeostasis.

3.3.1. Cytoplasmic TAG lipolysis. The first step in cytoplasmic TAG lipolysis is the hydrolysis of TAG into FA and DAG by ATGL (143). ATGL is activated by CGI-58. Mutations in the

CGI-58 gene cause Chanarin-Dorfman syndrome, also known as neutral lipid storage disease (2). Loss of ATGL or both ATGL and CGI-58 specifically in the intestine of mice results in decreased TAG hydrolase activity and enterocyte CLD accumulation (67, 93). However, intestinal TAG secretion in these mice is unaffected, a result that suggests ATGL/CGI-58 does not release FA used for CM synthesis. Instead, FA released by ATGL regulates peroxisome proliferator-activated receptor α (PPARα) activity and FA oxidation (FAO) (67, 93). Notably, ATGL has recently been identified as the enzyme responsible for hydrolyzing lipid taken up by enterocytes from the circulation (67). Interestingly, although loss of both ATGL and CGI-58 in enterocytes does not influence intestinal TAG secretion, loss of only CGI-58 decreases TAG secretion (136). This result suggests a unique dichotomy between CGI-58 and ATGL and a potential novel role for CGI-58 in TAG lipolysis in enterocytes.

PPARα: peroxisome proliferator-activated receptor α

FAO: fatty acid oxidation

The second step in cytoplasmic TAG lipolysis is the hydrolysis of DAG into FA and MAG by HSL (143). Although one action of HSL is DAG hydrolysis, HSL is also a cholesteryl esterase and has been shown to regulate enterocyte cholesterol homeostasis. Loss of HSL in murine enterocytes eliminates almost all cholesteryl esterase activity leading to cholesteryl ester accumulation after cholesterol-containing oil gavage (53, 92). However, despite increased cholesterol absorption in these mice, loss of HSL in enterocytes does not affect intestinal TAG secretion or intracellular TAG or DAG concentrations (92). These results demonstrate that HSL contributes to cholesterol metabolism in enterocytes but may not contribute significantly to TAG lipolysis.

The final enzyme in the cytoplasmic TAG lipolysis pathway is MGL, which acts on MAG to release FA and glycerol (143). However, MGL acts on a variety of substrates including endocannabinoids. MGL activity in mouse enterocytes regulates energy balance potentially through endocannabinoid signaling. Mice overexpressing intestinal MGL gain significantly more weight than wild-type mice after high-fat feeding despite no change in fat absorption (23). These weight differences were attributed to increased food intake and decreased energy expenditure in mice overexpressing MGL. Interestingly, mice overexpressing MGL had decreased levels of the endocannabinoid 2-arachidonoyl-glycerol in intestinal mucosa, suggesting altered endocannabinoid signaling may play a role in regulating food intake. On the other hand, systemic loss of MGL prevents body weight gain and reduces intestinal TAG secretion (43). Loss of MGL also improves multiple metabolic parameters in mice fed a high-fat diet, including lowering blood lipid concentrations and increasing insulin sensitivity; however, whether these changes are due to loss of intestinal MGL is not clear.

3.3.2. Lysosomal TAG lipolysis. In addition to cytoplasmic TAG lipolysis, CLDs can also be broken down by lysosomal TAG lipolysis, or lipophagy (143). Lipophagy is initiated by the whole or partial engulfment of CLDs by autophagosomes, which fuse with lysosomes containing LAL. LAL then catalyzes TAG breakdown to release FA. Lipophagy regulates TAG mobilization and CLD breakdown in enterocytes. Exposure to dietary fat or lipid micelles in mouse enterocytes and Caco-2 cells induces a rapid autophagic response and targeting of CLDs to lysosomes (64). Consistently, inhibiting either autophagosomal fusion with lysosomes or LAL itself in Caco-2 cells results in considerable CLD accumulation after lipid micelle treatment, confirming a role for lipophagy in CLD breakdown. In support of this, CLD accumulation resulting from indomethacin treatment in IEC-6 cultured enterocytes initiates lipophagy, and inhibiting lipophagy increases CLD accumulation (88). Further, defects in lipophagy and CLD mobilization influence dietary fat absorption, and this relationship is apparent in *Dgat1*$^{-/-}$ mice (59). Mice lacking DGAT1 display a transient defect in lysosomal acidification and lipophagy in enterocytes, resulting in a slower rate of CLD turnover that contributes to reduced intestinal TAG secretion and accumulation

of large CLDs. These results provide evidence that lipophagy plays a role in CLD breakdown and TAG mobilization in enterocytes. Future studies are required to determine the quantitative contribution of lipophagy in mobilizing CLDs for use as a substrate for CM assembly.

CES: carboxylesterase

3.3.3. Emerging players regulating CLD breakdown in enterocytes. Interestingly, loss of ATGL, HSL, or MGL in enterocytes does not completely abolish TAG hydrolase activity, suggesting that other lipolytic enzymes exist. In fact, activity-based proteomics was recently applied to identify the most active lipases in the small intestine (108), and other recent studies have identified novel factors regulating CLD lipolysis in enterocytes. One potential family of enzymes that may catalyze CLD breakdown in enterocytes is the carboxylesterase (CES) family. CES enzymes have lipolytic activity and have been shown to regulate lipid metabolism in multiple cell types, one example being the hydrolysis of ER lumenal lipid droplets for lipoprotein secretion in hepatocytes (74). In enterocytes, CES enzymes are highly expressed (108), and CES2C has been shown to regulate CM size and secretion (82). Overexpression of CES2C in mouse enterocytes increases TAG and DAG hydrolase activity, respiratory capacity, and the expression of PPARα-responsive genes. In fact, mice overexpressing CES2C in enterocytes have reduced weight gain on a high-fat diet and are protected from hepatosteatosis. However, although intestinal TAG secretion was relatively normal in mice overexpressing CES2C, the CMs secreted were larger and cleared faster than those of wild-type mice. Therefore, overexpression of CES2C in enterocytes may increase overall lipolytic activity, releasing more FA substrate to be used for the lipidation of large CMs (82). Despite this hypothesis, the exact lipid pool catalyzed by CES2C in enterocytes is not clear.

Another factor regulating CLD breakdown in enterocytes is ADP ribosylation factor related protein 1 (ARFRP1) (129). ARFRP1 is a GTPase involved in membrane trafficking and protein sorting and has been previously shown to regulate the lipidation of lipoproteins in the intestine (61). Recently, ARFRP1 was also found to regulate CM secretion by regulating the association of lipolytic enzymes with enterocyte CLDs (129). Loss of ARFRP1 in Caco-2 cells increases the association of PLIN2, fatty acid synthase associated factor 2 (FAF2), and transitional endoplasmic reticulum ATPase/valosin containing protein (TERA/VCP) with CLDs, resulting in decreased lipid secretion (129). FAF2-mediated VCP localization to CLDs was previously shown to prevent ATGL-mediated CLD breakdown (94). How ARFRP1 mediates these effects is not clear. However, this brings to light the idea of other factors regulating lipolytic enzyme activity or access to the CLD surface, contributing to cellular mechanisms regulating lipolysis. In fact, ingested nanocellulose, an engineered nanomaterial made from natural wood fiber, binds CLDs and interferes with lipase binding when administered with a high-fat meal (38). This interference may in turn prevent CLD breakdown and the release of FA substrate for CM assembly, as serum TAG in rats administered a high-fat gavage containing nanocellulose was lower than that of rats receiving no nanocellulose (38). Therefore, regulatory factors controlling lipolysis should be considered in future studies investigating CLD catabolism in enterocytes.

4. NEW CONCEPTS AND NOVEL FACTORS REGULATING CLD METABOLISM AND DIETARY FAT ABSORPTION

The identification of novel intracellular and extracellular factors that regulate CLD metabolism and dietary fat absorption in recent years has brought new appreciation to the complexity of small intestinal lipid metabolism and integration with whole-body physiology (**Figure 2**). For example, both dietary and endocrine factors stimulate CM secretion, unveiling the role of the lymphatic system in intestinal TAG storage. In addition, lipid stored in enterocyte CLDs may

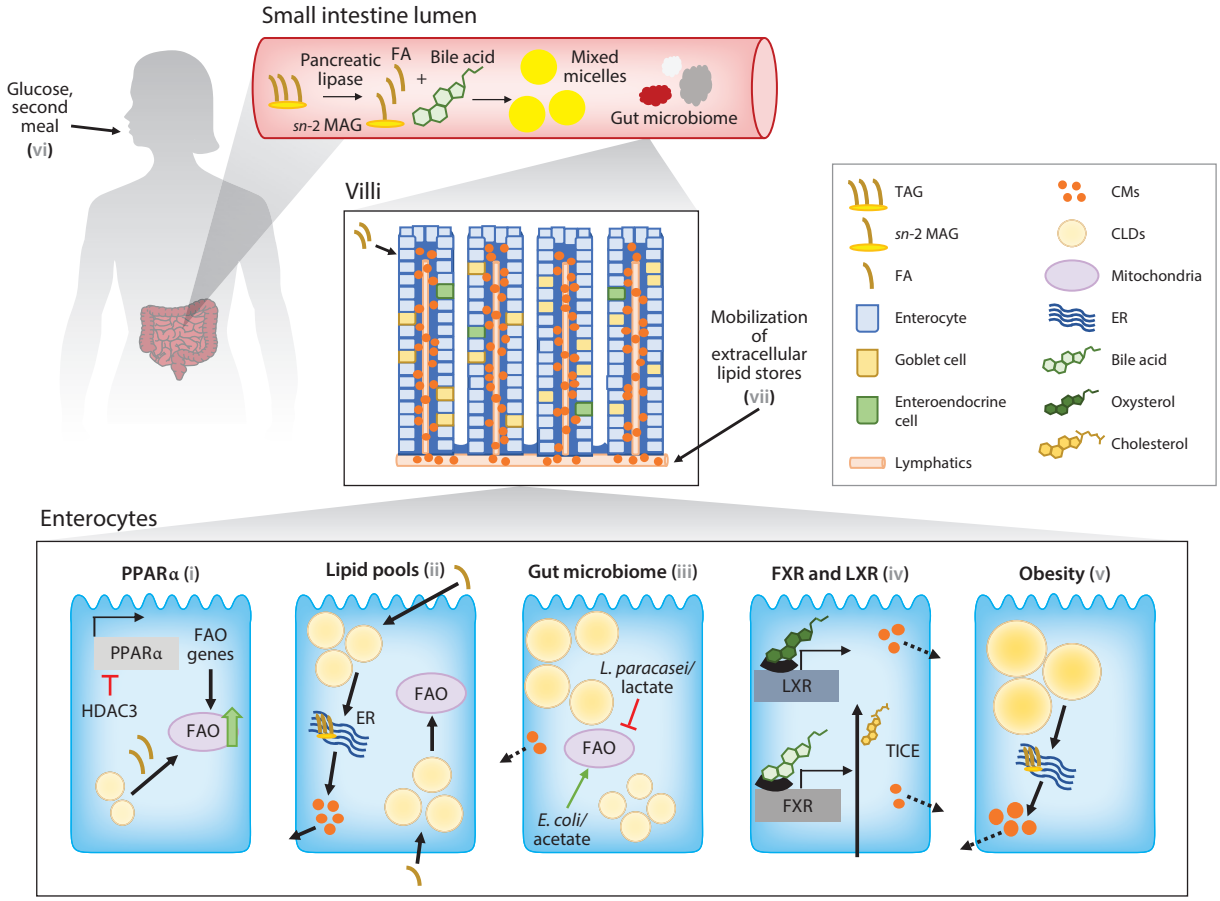

Figure 2

New concepts and novel factors regulating CLD metabolism and dietary fat absorption. CLD metabolism in enterocytes is influenced by extracellular, intracellular, and physiological factors. Specific factors or concepts discussed in Section 4 are numbered in parentheses next to or below each factor or concept. (*i*) PPARα activation regulates lipid storage in CLDs by stimulating the expression of genes involved in FAO. (*ii*) Different enterocyte lipid pools serve specific functions, as apically delivered FAs are preferentially used for CM synthesis and secretion, while basolaterally delivered FAs are preferentially used for FAO. (*iii*) The gut microbiome regulates lipid storage in CLDs and secretion in CMs in part due to differential activation of FAO in enterocytes. (*iv*) LXR and FXR activation by oxysterol and bile acid binding, respectively, decreases CM secretion; FXR activation additionally regulates TICE. (*v*) During obesity, CLD size is increased, while CM secretion is reduced and the CMs secreted are larger. (*vi*) Physiological factors such as glucose and a second meal stimulate the mobilization of CLDs from enterocytes. (*vii*) Other physiological factors such as GLP-2 may stimulate the mobilization of extracellular lipid stores in the intestinal lymphatics. Abbreviations: CLD, cytoplasmic lipid droplet; CM, chylomicron; ER, endoplasmic reticulum; FA, fatty acid; FAO, fatty acid oxidation; FXR, farnesoid X receptor; GLP-2, glucagon-like peptide-2; HDAC3, histone deacetylase 3; LXR, liver X receptor; MAG, monoacylglycerol; PPARα, peroxisome proliferator-activated receptor α; TAG, triacylglycerol; TICE, transintestinal cholesterol excretion.

be compartmentalized into distinct pools and differentially used for certain cellular processes. Further, the microbiome plays an important role in the normal process of dietary fat absorption and when altered influences multiple aspects of enterocyte metabolism. Understanding how such factors regulate enterocyte lipid metabolism is key in targeting the absorption of dietary fat in the prevention or treatment of metabolic disorders.

4.1. PPARα Activation

Although FAO in enterocytes does not contribute greatly to cellular energy metabolism, as glutamine is the primary fuel source (3), FAO in enterocytes regulates other cellular functions including lipid storage. The transcriptional regulator of FAO is PPARα, a ligand-activated transcription factor that regulates the expression of genes involved in FA catabolism (11). Because of the ability of PPARα to transcriptionally activate lipid catabolism, PPARα agonists are used to treat conditions associated with hyperlipidemia (11). Part of these effects may be due to changes in enterocyte lipid metabolism. For example, administering fenofibrate to mice that were fed a high-fat diet results in fat malabsorption and reduced intestinal TAG secretion, in part due to increased enterocyte FAO and decreased TAG storage in CLDs (124). More recent evidence supports these results and confirms an important function of PPARα activation in enterocytes.

HDAC: Histone deacetylase

Recent studies have demonstrated that PPARα activation in enterocytes regulates lipid storage in CLDs. For example, supplementation of tetradecylthioacetic acid, a synthetic FA and pan-PPAR agonist, prevents CLD accumulation in enterocytes of mice consuming a high-fat diet (77). Multiple genes involved in FAO were upregulated in enterocytes of these mice, a result that suggests increased FAO may contribute to the lack of lipid accumulation. Similarly, supplementation of omega-3 FAs containing phospholipids in mice fed a high-fat diet stimulates both the expression of genes involved in FAO and FAO itself in the proximal ileum (70). Therefore, PPARα activation in enterocytes may contribute to the beneficial effects of PPARα agonists on systemic metabolism by upregulating lipid catabolism in enterocytes. However, not all PPARα agonists are the same. For example, oleoylethanolamide, a bioactive FA signaling molecule synthesized from oleic acid in the small intestine, stimulates satiety through its interaction with PPARα and stimulates PPARα-mediated FAO in several tissues (96). However, treating Caco-2 cells with oleoylethanolamide did not stimulate FAO but instead increased TAG synthesis, lipoprotein secretion, and MTTP activity (96). These results suggest that PPARα may have distinct ligands in enterocytes, highlighting unique characteristics of the intestine.

PPARα regulates the expression of genes involved in FA catabolism; however, PPARα itself is also regulated, which in turn influences its ability to stimulate gene expression. One mechanism to regulate PPARα activity is through transcriptional corepressor complexes, which contain histone deacetylases (HDACs) that remove acetyl groups from histone proteins as a mechanism to repress transcription. An example is HDAC3, which regulates *Pparα* and alters expression of target genes and lipid metabolism in enterocytes (34, 130). Deletion of HDAC3 specifically in the small intestine protects mice from diet-induced obesity and attenuates the postprandial TAG response. Further, while wild-type mice fed a high-fat diet demonstrate massive accumulation of CLDs in enterocytes, mice lacking HDAC3 in the intestine did not accumulate enterocyte CLDs (34). Interestingly, a different study observed the opposite effect, in that mice lacking HDAC3 in the intestine did accumulate CLDs (130). This difference may be attributable to the researchers' use of germ-free mice. Since HDAC3 is a transcriptional repressor, the observed effects are likely due to changes in enterocyte gene expression. Consistently, the expression of PPARα-regulated genes including those involved in FAO was upregulated in enterocytes of mice lacking HDAC3 in the intestine (34). This change in gene expression was reflected by an increase in FAO in enterocytes of mice lacking HDAC3. These results suggest that HDAC3 regulates PPARα activity in enterocytes, in turn controlling FA catabolism and regulating TAG storage in CLDs. An interesting observation is that butyrate, a short-chain FA produced by intestinal microbiota, decreases HDAC3 activity in enterocytes (130). This result suggests microbiota may regulate intestinal gene expression, contributing to effects on enterocyte lipid metabolism.

Another factor that regulates PPARα is the transcription factor histone-lysine N-methyltransferase PRDM16 (PRDM16), which plays an important role in intestinal maintenance through activating FAO in intestinal stem cells. PRDM16 associates with PPARs, including PPARα, and enhances their function, therefore regulating genes involved in FAO. PRDM16-mediated regulation of FAO in murine intestinal stem cells is required for progenitor cell function (114). Indeed, loss of PRDM16 in intestinal stem cells alters intestinal morphology and inhibits enteroid budding due to a decrease in PPARα activation and FAO. This phenomenon had regional specificity, as the reliance on FAO for stem cell function was most prominent in the proximal intestine and decreased toward the distal intestine (114). Therefore, intestinal FAO not only regulates lipid storage in enterocytes but also maintains intestinal integrity and renewal capacity that allows for the diversity of cells within intestinal villi.

Although these studies suggest elevated FAO in enterocytes promotes favorable metabolic changes, additional underlying factors may exist. For example, expression of a constitutively active mutant of carnitine palmitoyltransferase 1, the rate-limiting step of FAO, in the small intestine did not prevent weight gain in mice fed either a chow diet or a high-fat diet despite an increase in enterocyte FAO and respiratory capacity (99). Therefore, FAO alone may not be the sole contributor to the observed metabolic changes; instead, other cellular effects or gene targets induced by PPARα activation may play a more prominent role. In fact, inhibiting PPARα in enteroids of intestinal stem cells results in total loss of cell viability (114), a result that suggests an important role for PPARα in regulating intestinal cell metabolism. Future studies are required to determine other cellular functions of PPARα.

4.2. Different Enterocyte Lipid Pools Serve Specific Functions

Early research supports the existence of different lipid pools in enterocytes that arise due to alternate mechanisms of lipid entry into the cell. Lipid stored in enterocytes can originate from three sources. The primary source is dietary lipid from the intestinal lumen, which is taken up on the apical side of enterocytes. As 95% of dietary fat consumed is absorbed, the uptake, synthesis, and transport of dietary fat in CMs constitute an extremely efficient process (81). A second source is lipid from circulation including lipoproteins and nonesterified FAs, which are absorbed at the basolateral side of enterocytes. The third source is de novo synthesized FAs made endogenously. De novo synthesized FAs contribute to TAG secreted from the intestine (110), although their contribution to TAG synthesis and storage in CLDs is not clear. However, de novo FA synthesis is upregulated in enterocytes during insulin-resistant states and may contribute to the elevated CM secretion that occurs in this condition (56). The unique metabolism of lipid originating from different sources suggests enterocytes use a compartmentalization mechanism to regulate their lipid stores.

The mechanism of lipid entry into enterocytes determines its metabolic fate. For example, in 1975, Gangl & Ockner (47) observed that apically delivered FAs were preferentially used for TAG synthesis, while basolaterally delivered FAs were used for phospholipid synthesis in rats. Later studies confirmed this observation in humans (48), mice (116), and the Caco-2 cell model of enterocytes (57). In addition to phospholipid synthesis, basolaterally delivered FAs are preferentially oxidized compared with apically delivered FAs, and oxidation is elevated during the fasting state (116). These results suggest a purposeful compartmentalization of lipid by enterocytes to more efficiently shuttle incoming substrates to specific metabolic pathways. For example, the preferential packaging of lipid from the intestinal lumen at the apical side of enterocytes into TAG may allow for rapid synthesis and secretion of CMs after a meal (80). Lipid originating from the circulation

is generally not utilized for CM synthesis and secretion (79) but instead provides a substrate for alternative metabolic pathways.

As discussed in Section 4.1, FAO in enterocytes is a mechanism to regulate lipid storage in CLDs; however, it also regulates lipid secretion. In fact, the oxidation of basolaterally delivered lipid has recently been shown to be a novel mechanism regulating CLD metabolism and CM secretion (76). For example, lipoproteins delivered basolaterally to cultured mouse enteroids provide a substrate for FAO, sparing apically delivered lipid for secretion in CM. Consistently, enterocytes of mice overexpressing ApoC-III, an apolipoprotein that inhibits the low-density lipoprotein receptor and therefore basolateral lipoprotein uptake, have fewer and smaller CLDs compared with those of wild-type mice (76). This result suggests that apically delivered lipid stored in CLDs acts as a substrate for FAO when basolateral lipid sources are unavailable. Since oxidation of apically delivered lipid comes at the expense of CM secretion, oxidation of basolateral lipid may maintain efficient CM secretion of apically delivered (i.e., dietary) lipid sources. Therefore, basolateral lipid sources play an important role in regulating lipid substrate utilization in enterocytes.

Basolateral lipid sources are stored in CLDs before they are utilized. Although the major enzyme(s) responsible for CLD breakdown in enterocytes remains elusive, a recent study uncovered a role for ATGL in the breakdown of CLDs derived from basolateral lipid sources in mice (67). Mice lacking ATGL and CGI-58 in the small intestine accumulate CLDs after fasting and during consumption of a fat-free diet in which circulating lipid is endogenously synthesized (67). These results indicate that ATGL is active on CLDs formed from the absorption of lipid originating from the circulation. In addition, FAO was decreased in *Atgl*$^{-/-}$/*Cgi-58*$^{-/-}$ mice, consistent with the relationship between ATGL, PPARα activation, and FAO (93). Overall, these studies support a previously unappreciated role for basolateral lipid sources and FAO in the maintenance of efficient lipid absorption and reflect a compartmentalized system of lipid metabolism in enterocytes.

4.3. Role of the Gut Microbiome in the Regulation of Enterocyte Lipid Metabolism

The gut microbiome is an emerging area of research shown to regulate multiple aspects of whole-body metabolism, including enterocyte lipid metabolism and dietary fat absorption. The gut microbiome spans from the beginning of the small intestine to the end of the colon, although the amount and diversity of microbes are higher in the colon (41). This is due in part to the physiological conditions of the small intestine including an acidic environment, rapid gut transit, and higher oxygen levels, which are conducive to only specific types of bacteria. The major role of the gut microbiota is the fermentation of indigestible carbohydrates, producing metabolites that regulate various aspects of host metabolism (16). One aspect is dietary fat absorption. The presence of intestinal microbes is required for normal fat absorption, as germ-free mice, which lack a gut microbiome, have a lower TAG and cholesterol secretion rate from the intestine after oil gavage compared with control mice (83). In support of this, depleting the intestinal microbiome of rats with antibiotics decreases lymphatic TAG and phospholipid output after duodenal lipid infusion compared with rats not treated with antibiotics (107). These results provide evidence that the intestinal microbiome is required for proper dietary fat absorption; however, the mechanisms of this phenomenon are not known.

The composition of the gut microbiome influences the absorption of dietary fat. Although it remains unclear what constitutes a normal microbiome, changes in microbiome composition are associated with multiple diseases such as obesity, irritable bowel diseases, and neurological diseases (102). Interestingly, microbiota isolated from obese mice and humans are shown to have pro-obesogenic properties (36), and consumption of a high-fat diet in humans results in rapid

changes in the composition of the gut microbiome (35). These compositional changes may influence host metabolism and contribute to obesity, potentially by increasing the absorption of dietary fat. For example, germ-free mice conventionalized with microbes originating from the intestine of mice fed a short-term high-fat diet have increased TAG and cholesterol secretion from the small intestine compared with mice colonized with microbes originating from the intestine of mice fed a low-fat diet (83). The increase in lipid secretion from the intestine of mice with high-fat diet microbes may be due to changes in the expression of genes involved in FA uptake and transport and TAG synthesis in enterocytes. For example, the mRNA levels of *Fabp2*, *Cd36*, *Mogat2*, *Dgat1*, and *Dgat2* were increased in murine enteroids and intestinal tissue upon exposure to specific bacterial strains (83). This result suggests that the microbial adaptations that occur with consumption of a high-fat diet affect enterocyte lipid metabolism potentially by influencing intestinal gene expression (130). Whether this effect is direct or indirect is an open question.

EEC: enteroendocrine cell

The gut microbiome also influences intestinal lipid metabolism by modulating the balance between lipid storage in CLDs and secretion in CMs. Specifically, two species of fermenting gut microbes, *Lactobacillus paracasei* and *Escherichia coli*, and their metabolites (lactate and acetate, respectively) differentially influence CLD metabolism (4, 120). For example, treating m-ICcl2 cells, a murine enterocyte cell line, with *L. paracasei* or its fermentation product lactate and *E. coli* or its fermentation product acetate decreases lipid secretion after micelle treatment. Although both bacteria influence lipid secretion, they have opposite effects on CLD size and number inside enterocytes. Treating m-ICcl2 cells with *L. paracasei* or lactate increases CLD size, number, and cellular TAG concentration, while treating cells with *E. coli* or acetate decreases CLD size and cellular TAG concentration but increases CLD number (4, 120). These contrasting observations are due to the differential activation of FAO and presumably CLD catabolism in enterocytes, as *E. coli* and acetate stimulate, and *L. paracasei* and lactate inhibit, FAO (4). Therefore, the novel role of intestinal microbiota in regulating enterocyte lipid metabolism provides one potential mechanism for the contribution of dysbiosis to metabolic disease.

In addition to enterocyte metabolism, the gut microbiota also influence the nutrient sensing capacity of intestinal enteroendocrine cells (EECs) (140). EECs are distributed within small intestinal villi along the length of the intestine and play an important role in detecting and responding to dietary nutrients present in the intestinal lumen (52). Upon exposure to dietary nutrients, EECs secrete specific hormones depending on cellular subtype that act in a paracrine fashion to regulate different aspects of metabolism. In fact, recent studies have unveiled important roles for EECs in regulating nutrient absorption (84). For example, peptide YY potentiates glucose and peptide absorption in human intestinal organoids and mouse small intestine by regulating ion transport (85). Therefore, the activation of EECs produces important signals notifying the body of its intestinal contents in order to prepare and adjust systemic metabolism accordingly.

EECs can assume either an open or closed conformation in the intestinal epithelium (58), which determines their ability to sense nutrients and signal appropriately. For example, an open conformation exposes EECs to the intestinal lumen, where they can interact with lumenal nutrients and respond with hormone secretion. On the other hand, EECs with a closed conformation are not exposed to the intestinal lumen and therefore are unable to sense lumenal nutrients (58). A recent study investigated the influence of dietary components on the conformation of EECs and found that chronic exposure to dietary fat alters EEC conformation in zebrafish (140). For example, 6-h high-fat feeding in zebrafish temporarily induces a closed EEC conformation in the proximal intestine, thereby reducing the ability of EECs to respond to palmitate stimulation (140). This phenomenon is in part due to high-fat diet–mediated changes in the gut microbiome. First, 6-h high-fat feeding in germ-free zebrafish, which do not have a microbiome, did not induce a closed EEC conformation. Second, high-fat feeding in wild-type zebrafish increased the intestine's

microbial density and diversity (140). These results suggest that the gut microbiota greatly contribute to the EEC response to dietary nutrients. Therefore, the ability of the gut microbiota to alter EEC conformation is another novel mechanism regulating small intestinal metabolism and responses to dietary fat. Future studies involving gut microbiome–mediated changes in intestinal lipid metabolism will certainly bring new insight and mechanisms into this interesting aspect of gut metabolism.

LXR: liver X receptor

FXR: farnesoid X receptor

4.4. Role of LXR, FXR, and Bile Acid Signaling in the Regulation of Lipid Absorption

Similar to PPARα, the liver X receptor (LXR) and farnesoid X receptor (FXR) are ligand-activated transcription factors that regulate the expression of genes in certain metabolic pathways (39). Activation of LXR and FXR by steroid-containing molecules in enterocytes regulates lipid absorption and cholesterol metabolism by regulating gene expression. More specifically, LXR responds to the binding of oxysterols and regulates the expression of genes involved in cholesterol and lipid metabolism, and FXR responds to the binding of bile acids and regulates the expression of genes involved in bile acid homeostasis, lipid, and glucose metabolism (21).

Consistent with their role in regulating lipid metabolism, activation of LXR and FXR in enterocytes has been shown to regulate TAG secretion and storage. First, zebrafish overexpressing *Lxrα* have decreased TAG secretion into the vasculature following a lipid gavage compared with wild-type zebrafish or those lacking *Lxrα* (8). Consistently, wild-type zebrafish pretreated with a LXR agonist also had decreased TAG secretion. The decrease in lipid secretion was previously shown to be mediated through LXR-mediated induction of *Acsl3* expression and promotion of lipid storage in CLDs (29). Another potential mechanism by which LXR decreases intestinal lipid secretion is through regulating the cellular localization of scavenger receptor class B member 1 (SR-B1) in enterocytes. Although the primary role of SR-B1 in other cell types is to mediate high-density lipoprotein and cholesterol uptake, SR-B1 in enterocytes is involved in the assembly and secretion of CMs through lipid sensing at the brush-border membrane (6). Therefore, an absence of SR-B1 at the brush-border membrane may prevent the initiation of CM assembly and secretion. In support of this, treating mice or Caco-2 cells with an LXR agonist initiates the relocalization of SR-B1 from the brush-border membrane of enterocytes to an intracellular distribution, in turn removing its lipid-sensing and signaling mechanisms and decreasing CM secretion (15). This phenomenon was shown to be due to the LXR-mediated induction of microRNA targeting SR-B1. Therefore, the activation of LXR in enterocytes may serve as a potential therapeutic target to decrease intestinal TAG secretion.

Similarly, FXR activation in murine enterocytes also decreases TAG secretion but additionally regulates transintestinal cholesterol excretion (TICE), a pathway that contributes to fecal neutral sterol excretion (100). First, FXR activation decreases postprandial lipemia through bile acid signaling (45). Intraduodenal infusion of taurocholic acid, an FXR agonist, administered with an olive oil oral gavage in mice decreases intestinal TAG secretion compared with mice receiving vehicle. Consistently, mice lacking FXR did not respond to taurocholic acid, a result that suggests FXR is required for this effect. The ability of taurocholic acid to decrease intestinal TAG secretion through FXR activation was in part due to decreased gastric emptying; other contributing factors include decreased MTTP activity and increased expression of genes involved in FAO (45). The factors induced by FXR activation that mediate these effects, however, are unclear.

FXR activation in enterocytes stimulates TICE in part by altering the composition of the bile acid pool. TICE involves the removal of cholesterol from the plasma by enterocytes and its subsequent secretion into the intestinal lumen by the apically located cholesterol transporters

ATP-binding cassette subfamily G members 5 and 8 (ABCG5/G8) (100). Since cholesterol secreted into the intestinal lumen is eventually excreted in feces, TICE serves as a second mechanism of reverse cholesterol transport. FXR activation in mice increases TICE through ABCG5/G8-mediated transport (37). The effect of FXR on TICE was facilitated by induction of fibroblast growth factor 19 (FGF19), a signaling product of FXR, and alterations in the hydrophilicity of the bile acid pool. FXR is required for this effect, as TICE was essentially lost in *Fxr*$^{-/-}$ mice (37). Interestingly, another study also demonstrated that FGF19, a signaling molecule induced by FXR activation, meditates cholesterol absorption through small heterodimer partner signaling (66). These results suggest that FXR activation or changes in the bile acid pool composition may be potential therapeutic targets to increase cholesterol elimination.

RYGB: Roux-en-Y gastric bypass

4.5. Influence of Obesity on Dietary Fat Absorption and CLDs

Obesity is associated with multiple metabolic abnormalities in several organs of the body and also affects dietary fat absorption and CLD metabolism in enterocytes. The influence of obesity on intestinal lipid metabolism was determined in two complementary mouse studies (42, 125). Both studies demonstrated a reduced postprandial intestinal TAG secretion rate in diet-induced obese (125) and genetically obese (42, 125) mice compared with lean mice after olive oil gavage. In addition, the CMs secreted from the intestine of obese mice were larger (125). Whether these changes influence the clearance or metabolism of CMs from circulation is not clear. The decrease in TAG secretion rate may be due to alterations in the expression of genes involved in lipid metabolism in enterocytes and/or the accumulation of lipid in CLDs (42, 125). In fact, CLDs in enterocytes of diet-induced obese mice are significantly larger and have an altered proteome compared with CLDs in enterocytes of lean mice (32). How the accumulation of CLDs or changes in their proteome in enterocytes of obese mice influences lipid metabolism is an outstanding question.

One of the major successful treatments for obesity is Roux-en-Y gastric bypass (RYGB) surgery. RYGB results in rapid changes in systemic metabolism and improvements in circulating blood glucose and lipid profiles (71). One mechanism of these effects is through modulation of intestinal lipid metabolism. For example, lymphatic TAG output in RYGB rats was significantly lower than that of sham rats after consumption of a high-fat test meal (63). Interestingly, RYGB differentially influences intestinal lipid metabolism depending on feeding status. In the fasted state, RYGB in rats decreased enterocyte FA uptake and FAO, but increased the expression of genes involved in TAG synthesis. In addition, CLD accumulation tended to be higher. The opposite effect occurred after feeding a high-fat meal, as FA uptake and FAO were increased in enterocytes of RYGB rats (63). These results suggest that RYGB-mediated changes in enterocyte lipid metabolism may contribute to systemic metabolic improvements with bypass surgery.

4.6. Mobilization of CLDs by Physiological Factors

It has been documented that an enteral storage of lipids exists, which appears in circulation as CMs long after ingestion of a fat-rich meal upon receiving certain stimulatory cues (135). For example, hours after a high-fat meal, ingestion of glucose results in an early peak in plasma CM TAG (103, 134). In fact, dietary TAG may be secreted in CMs up to 18 h after the ingestion of a fat-rich meal in humans (18). Further evidence for enteral lipid storage is the presence of CLDs up to 12 h after fat ingestion in mouse jejunum (147) and up to 10 h in human duodenum (103, 134).

Oral glucose has been shown to mobilize intracellular CLDs. In humans, ingestion of a glucose drink 5 h after a fat-rich meal depleted CLDs in duodenal enterocytes, corresponding to the appearance of CM TAG in plasma (103, 134). In addition, glucose ingestion resulted in fewer and

THE MESENTERIC LYMPHATIC SYSTEM AND MOBILIZATION OF INTESTINAL LIPID STORES

The mesenteric lymphatic system is emerging as an active regulator of intestinal lipid absorption, and defective lymphatic function is implicated in impaired dietary fat absorption (9, 40). Regulatory mechanisms that govern lymphatic function influence CM secretion and may underlie mobilization of intestinal lipid stores. For example, VEGF signaling has been shown to play a role in the development and maintenance of the lymphatic vasculature (62). In addition, VEGF-C secreted from villus macrophages influences lacteal integrity via intestinal microbiota (117). Further, lymphatic pumping activities are also acutely modulated via VEGF signaling (14). Contraction of lacteals is controlled by the autonomic nervous system (22) and the σ_1 receptor (122). In addition, smooth muscle cells surrounding intestinal lacteals also express β-adrenergic receptors and muscarinic receptors (5). This evidence supports a mediating role of the vagal nerves that provides dense innervation for intestinal lymphatics and surrounding muscles. In light of the expression of the GLP-2 receptor in enteric neurons, it is plausible that GLP-2 mobilizes lipid stores in the lymphatics via neural signal–mediated contractions of smooth muscle cells along the lymphatic vessel wall. Despite this hypothesis, a link between GLP-2, neural circuity, and VEGF signaling has not been established.

smaller-sized CLDs in enterocytes. The exact underlying molecular mechanism remains poorly understood. However, changes in the proteome of duodenal tissue suggest several candidate proteins may play a role (134). For example, with glucose ingestion, syntaxin-binding protein 5 was upregulated, while ethanolaminephosphotransferase 1 was downregulated. Although the functional roles of syntaxin-binding protein 5 in intestinal lipoprotein metabolism are unknown, it may play a role in CM assembly through mediating the SNARE complex responsible for PCTV docking and fusion with the Golgi membrane. Ethanolaminephosphotransferase 1 catalyzes the last step of the Kennedy pathway in phospholipid synthesis. Whether glucose ingestion mobilizes CLDs by modulating phospholipid homeostasis of organelle membranes via ethanolaminephosphotransferase 1 is unknown.

Besides mobilization of enterocyte CLDs, mobilization of TAG stored in other locations is also possible. Glucagon-like peptide-2 (GLP-2) has been shown to promote the secretion of CMs. However, the mechanisms and TAG storage pool that GLP-2 targets may be distinct from those of glucose. For example, GLP-2 given 7 h after a fat-rich meal in humans mobilizes the release of preformed rather than newly synthesized CMs (33). Further, in rats, glucose and GLP-2 administration displayed different patterns of gut lipid release, a result pointing to the targeting of an intracellular pool for glucose and an extracellular pool for GLP-2 (112). The notion that GLP-2 targets TAG stored outside of enterocytes is supported by the expression of GLP-2 receptors. The GLP-2 receptor is not expressed in the enterocyte (97). Instead, it is expressed on cell types that are rich in the subepithelial regions of the intestine including the lamina propria, such as enteric neurons, myofibroblasts, and stromal cells (54, 95, 97, 142). Therefore, it is likely that GLP-2 triggers the release of CMs in postenterocyte locations, such as intercellular spaces, the lamina propria, and the mesenteric lymphatic vasculature (see the sidebar titled The Mesenteric Lymphatic System and Mobilization of Intestinal Lipid Stores).

5. CONCLUSIONS AND FUTURE DIRECTIONS

GLP-2: glucagon-like peptide-2

Although new discoveries on the factors regulating CLD metabolism in enterocytes have provided a better understanding of the small intestine's complex regulatory circuits during the process of

dietary fat absorption, several questions in the field remain unanswered. For example, what are the molecular mechanisms of CLD mobilization and enzymes responsible for CLD lipolysis in enterocytes? In addition, although multiple studies have proposed a role for FAO in regulating multiple aspects of enterocyte lipid metabolism, the quantitative contribution of FAO to enterocyte energy metabolism or the molecular mechanisms of its effects have not yet been defined. Lastly, and of special importance, the factors determining CM secretion versus CLD formation have not yet been identified. The identification of these factors is imperative to the development of therapeutic targets modulating intestinal lipid storage and dietary fat absorption, which ultimately may help in the prevention or treatment of metabolic disease.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

C.X. was supported by a Start-Up Fund from the College of Medicine, University of Saskatchewan. A.S.Z. was supported by a Bilsland Dissertation Fellowship from Purdue University.

LITERATURE CITED

1. Abumrad NA, Davidson NO. 2012. Role of the gut in lipid homeostasis. *Physiol. Rev.* 92:1061–85
2. Al-Hage J, Abbas O, Nemer G, Kurban M. 2020. Chanarin-Dorfman syndrome: a novel homozygous mutation in the ABHD5 gene. *Clin. Exp. Dermatol.* 45:257–59
3. Alpers DH. 2000. Is glutamine a unique fuel for small intestinal cells? *Curr. Opin. Gastroenterol.* 16(2):155
4. Araújo JR, Tazi A, Burlen-Defranoux O, Vichier-Guerre S, Nigro G, et al. 2020. Fermentation products of commensal bacteria alter enterocyte lipid metabolism. *Cell Host Microbe* 27:358–75.e7
5. Bachmann SB, Gsponer D, Montoya-Zegarra JA, Schneider M, Scholkmann F, et al. 2019. A distinct role of the autonomic nervous system in modulating the function of lymphatic vessels under physiological and tumor-draining conditions. *Cell Rep.* 27:3305–14.e13
6. Béaslas O, Cueille C, Delers F, Chateau D, Chambaz J, et al. 2009. Sensing of dietary lipids by enterocytes: a new role for SR-BI/CLA-1. *PLOS ONE* 4:e4278
7. Beilstein F, Bouchoux J, Rousset M, Demignot S. 2013. Proteomic analysis of lipid droplets from Caco-2/TC7 enterocytes identifies novel modulators of lipid secretion. *PLOS ONE* 8(1):e53017
8. Benítez-Santana T, Hugo SE, Schlegel A. 2017. Role of intestinal LXRα in regulating post-prandial lipid excursion and diet-induced hypercholesterolemia and hepatic lipid accumulation. *Front. Physiol.* 8:280
9. Bernier-Latmani J, Petrova TV. 2017. Intestinal lymphatic vasculature: structure, mechanisms and functions. *Nat. Rev. Gastroenterol. Hepatol.* 14:510–26
10. Bersuker K, Olzmann JA. 2017. Establishing the lipid droplet proteome: mechanisms of lipid droplet protein targeting and degradation. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862:1166–77
11. Botta M, Audano M, Sahebkar A, Sirtori CR, Mitro N, Ruscica M. 2018. PPAR agonists and metabolic syndrome: an established role? *Int. J. Mol. Sci.* 19(4):1197
12. Bouchoux J, Beilstein F, Pauquai T, Guerrera IC, Chateau D, et al. 2011. The proteome of cytosolic lipid droplets isolated from differentiated Caco-2/TC7 enterocytes reveals cell-specific characteristics. *Biol. Cell* 103:499–517
13. Bowman TA, O'Keeffe KR, D'Aquila T, Yan QW, Griffin JD, et al. 2016. Acyl CoA synthetase 5 (ACSL5) ablation in mice increases energy expenditure and insulin sensitivity and delays fat absorption. *Mol. Metab.* 5:210–20

14. Breslin JW, Gaudreault N, Watson KD, Reynoso R, Yuan SY, Wu MH. 2007. Vascular endothelial growth factor-C stimulates the lymphatic pump by a VEGF receptor-3-dependent mechanism. *Am. J. Physiol. Heart Circ. Physiol.* 293:H709–18
15. Briand O, Touche V, Colin S, Brufau G, Davalos A, et al. 2016. Liver X receptor regulates triglyceride absorption through intestinal down-regulation of scavenger receptor class B, type 1. *Gastroenterology* 150:650–58
16. Cani PD. 2018. Human gut microbiome: hopes, threats and promises. *Gut* 67:1716–25
17. Cao Z, Hao Y, Fung CW, Lee YY, Wang P, et al. 2019. Dietary fatty acids promote lipid droplet diversity through seipin enrichment in an ER subdomain. *Nat. Commun.* 10:2902
18. Chavez-Jauregui RN, Mattes RD, Parks EJ. 2010. Dynamics of fat absorption and effect of sham feeding on postprandial lipema. *Gastroenterology* 139:1538–48
19. Chen FJ, Yin Y, Chua BT, Li P. 2020. CIDE family proteins control lipid homeostasis and the development of metabolic diseases. *Traffic* 21:94–105
20. Cheng D, Iqbal J, Devenny J, Chu CH, Chen L, et al. 2008. Acylation of acylglycerols by acyl coenzyme A:diacylglycerol acyltransferase 1 (DGAT1). Functional importance of DGAT1 in the intestinal fat absorption. *J. Biol. Chem.* 283:29802–11
21. Chiang JYL, Ferrell JM. 2019. Bile acids as metabolic regulators and nutrient sensors. *Annu. Rev. Nutr.* 39:175–200
22. Choe K, Jang JY, Park I, Kim Y, Ahn S, et al. 2015. Intravital imaging of intestinal lacteals unveils lipid drainage through contractility. *J. Clin. Investig.* 125:4042–52
23. Chon SH, Douglass JD, Zhou YX, Malik N, Dixon JL, et al. 2012. Over-expression of monoacylglycerol lipase (MGL) in small intestine alters endocannabinoid levels and whole body energy balance, resulting in obesity. *PLOS ONE* 7:e43962
24. Chorlay A, Monticelli L, Veríssimo Ferreira J, Ben M'barek K, Ajjaji D, et al. 2019. Membrane asymmetry imposes directionality on lipid droplet emergence from the ER. *Dev. Cell* 50:25–42.e7
25. Chorlay A, Thiam AR. 2020. Neutral lipids regulate amphipathic helix affinity for model lipid droplets. *J. Cell Biol.* 219(4):e201907099
26. Choudhary V, Golani G, Joshi AS, Cottier S, Schneiter R, et al. 2018. Architecture of lipid droplets in endoplasmic reticulum is determined by phospholipid intrinsic curvature. *Curr. Biol.* 28:915–26.e9
27. Cifarelli V, Abumrad NA. 2018. Intestinal CD36 and other key proteins of lipid utilization: role in absorption and gut homeostasis. *Compr. Physiol.* 8:493–507
28. Cornell RB. 2016. Membrane lipid compositional sensing by the inducible amphipathic helix of CCT. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1861:847–61
29. Cruz-Garcia L, Schlegel A. 2014. Lxr-driven enterocyte lipid droplet formation delays transport of ingested lipids. *J. Lipid Res.* 55:1944–58
30. D'Aquila T, Hung YH, Carreiro A, Buhman KK. 2016. Recent discoveries on absorption of dietary fat: presence, synthesis, and metabolism of cytoplasmic lipid droplets within enterocytes. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1861:730–47
31. D'Aquila T, Sirohi D, Grabowski JM, Hedrick VE, Paul LN, et al. 2015. Characterization of the proteome of cytoplasmic lipid droplets in mouse enterocytes after a dietary fat challenge. *PLOS ONE* 10:e0126823
32. D'Aquila T, Zembroski AS, Buhman KK. 2019. Diet induced obesity alters intestinal cytoplasmic lipid droplet morphology and proteome in the postprandial response to dietary fat. *Front. Physiol.* 10:180
33. Dash S, Xiao C, Morgantini C, Connelly PW, Patterson BW, Lewis GF. 2014. Glucagon-like peptide-2 regulates release of chylomicrons from the intestine. *Gastroenterology* 147:1275–84.e4
34. Dávalos-Salas M, Montgomery MK, Reehorst CM, Nightingale R, Ng I, et al. 2019. Deletion of intestinal Hdac3 remodels the lipidome of enterocytes and protects mice from diet-induced obesity. *Nat. Commun.* 10:5291
35. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, et al. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505:559–63
36. Davis CD. 2016. The gut microbiome and its role in obesity. *Nutr. Today* 51:167–74
37. de Boer JF, Schonewille M, Boesjes M, Wolters H, Bloks VW, et al. 2017. Intestinal farnesoid X receptor controls transintestinal cholesterol excretion in mice. *Gastroenterology* 152:1126–38.e6

38. DeLoid GM, Sohal IS, Lorente LR, Molina RM, Pyrgiotakis G, et al. 2018. Reducing intestinal digestion and absorption of fat using a nature-derived biopolymer: interference of triglyceride hydrolysis by nanocellulose. *ACS Nano* 12:6469–79
39. Ding L, Pang S, Sun Y, Tian Y, Yu L, Dang N. 2014. Coordinated actions of FXR and LXR in metabolism: from pathogenesis to pharmacological targets for type 2 diabetes. *Int. J. Endocrinol.* 2014:751859
40. Dixon JB. 2010. Mechanisms of chylomicron uptake into lacteals. *Ann. N. Y. Acad. Sci.* 1207(Suppl. 1):E52–57
41. Donaldson GP, Lee SM, Mazmanian SK. 2016. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* 14:20–32
42. Douglass JD, Malik N, Chon SH, Wells K, Zhou YX, et al. 2012. Intestinal mucosal triacylglycerol accumulation secondary to decreased lipid secretion in obese and high fat fed mice. *Front. Physiol.* 3:25
43. Douglass JD, Zhou YX, Wu A, Zadroga JA, Gajda AM, et al. 2015. Global deletion of MGL in mice delays lipid absorption and alters energy homeostasis and diet-induced obesity. *J. Lipid Res.* 56:1153–71
44. Drover VA, Ajmal M, Nassir F, Davidson NO, Nauli AM, et al. 2005. CD36 deficiency impairs intestinal lipid secretion and clearance of chylomicrons from the blood. *J. Clin. Investig.* 115:1290–97
45. Farr S, Stankovic B, Hoffman S, Masoudpoor H, Baker C, et al. 2020. Bile acid treatment and FXR agonism lower postprandial lipemia in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 318:G682–93
46. Frank DN, Bales ES, Monks J, Jackman MJ, MacLean PS, et al. 2015. Perilipin-2 modulates lipid absorption and microbiome responses in the mouse intestine. *PLOS ONE* 10:e0131944
47. Gangl A, Ockner RK. 1975. Intestinal metabolism of plasma free fatty acids. Intracellular compartmentation and mechanisms of control. *J. Clin. Investig.* 55:803–13
48. Gangl A, Renner F. 1978. In vivo metabolism of plasma free fatty acids by intestinal mucosa of man. *Gastroenterology* 74:847–50
49. Gao M, Huang X, Song BL, Yang H. 2019. The biogenesis of lipid droplets: Lipids take center stage. *Prog. Lipid Res.* 75:100989
50. Goh VJ, Tan JS, Tan BC, Seow C, Ong WY, et al. 2015. Postnatal deletion of fat storage-inducing transmembrane protein 2 (FIT2/FITM2) causes lethal enteropathy. *J. Biol. Chem.* 290:25686–99
51. Gordon SM, Neufeld EB, Yang Z, Pryor M, Freeman LA, et al. 2019. DENND5B regulates intestinal triglyceride absorption and body mass. *Sci. Rep.* 9:3597
52. Gribble FM, Reimann F. 2016. Enteroendocrine cells: chemosensors in the intestinal epithelium. *Annu. Rev. Physiol.* 78:277–99
53. Grober J, Lucas S, Sorhede-Winzell M, Zaghini I, Mairal A, et al. 2003. Hormone-sensitive lipase is a cholesterol esterase of the intestinal mucosa. *J. Biol. Chem.* 278:6510–15
54. Guan X, Karpen HE, Stephens J, Bukowski JT, Niu S, et al. 2006. GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive peptides and mediates increased blood flow. *Gastroenterology* 130:150–64
55. Haas JT, Winter HS, Lim E, Kirby A, Blumenstiel B, et al. 2012. DGAT1 mutation is linked to a congenital diarrheal disorder. *J. Clin. Investig.* 122:4680–84
56. Haidari M, Leung N, Mahbub F, Uffelman KD, Kohen-Avramoglu R, et al. 2002. Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance. Evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal de novo lipogenesis and ApoB48-containing lipoprotein overproduction. *J. Biol. Chem.* 277:31646–55
57. Ho SY, Delgado L, Storch J. 2002. Monoacylglycerol metabolism in human intestinal Caco-2 cells: evidence for metabolic compartmentation and hydrolysis. *J. Biol. Chem.* 277:1816–23
58. Höfer D, Asan E, Drenckhahn D. 1999. Chemosensory perception in the gut. *News Physiol. Sci.* 14:18–23
59. Hung YH, Buhman KK. 2019. DGAT1 deficiency disrupts lysosome function in enterocytes during dietary fat absorption. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1864:587–95
60. Hung YH, Carreiro AL, Buhman KK. 2017. Dgat1 and Dgat2 regulate enterocyte triacylglycerol distribution and alter proteins associated with cytoplasmic lipid droplets in response to dietary fat. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862:600–14
61. Jaschke A, Chung B, Hesse D, Kluge R, Zahn C, et al. 2012. The GTPase ARFRP1 controls the lipidation of chylomicrons in the Golgi of the intestinal epithelium. *Hum. Mol. Genet.* 21:3128–42

62. Karaman S, Leppänen VM, Alitalo K. 2018. Vascular endothelial growth factor signaling in development and disease. *Development* 145:dev151019
63. Kaufman S, Arnold M, Diaz AA, Neubauer H, Wolfrum S, et al. 2019. Roux-en-Y gastric bypass surgery reprograms enterocyte triglyceride metabolism and postprandial secretion in rats. *Mol. Metab.* 23:51–59
64. Khaldoun SA, Emond-Boisjoly MA, Chateau D, Carrière V, Lacasa M, et al. 2014. Autophagosomes contribute to intracellular lipid distribution in enterocytes. *Mol. Biol. Cell* 25:118–32
65. Khatun I, Clark RW, Vera NB, Kou K, Erion DM, et al. 2016. Characterization of a novel intestinal glycerol-3-phosphate acyltransferase pathway and its role in lipid homeostasis. *J. Biol. Chem.* 291:2602–15
66. Kim YC, Byun S, Seok S, Guo G, Xu HE, et al. 2019. Small heterodimer partner and fibroblast growth factor 19 inhibit expression of NPC1L1 in mouse intestine and cholesterol absorption. *Gastroenterology* 156:1052–65
67. Korbelius M, Vujic N, Sachdev V, Obrowsky S, Rainer S, et al. 2019. ATGL/CGI-58-dependent hydrolysis of a lipid storage pool in murine enterocytes. *Cell Rep.* 28:1923–34.e4
68. Kory N, Thiam AR, Farese RV Jr, Walther TC. 2015. Protein crowding is a determinant of lipid droplet protein composition. *Dev. Cell* 34:351–63
69. Krahmer N, Guo Y, Wilfling F, Hilger M, Lingrell S, et al. 2011. Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP:phosphocholine cytidylyltransferase. *Cell Metab.* 14:504–15
70. Kroupova P, van Schothorst EM, Keijer J, Bunschoten A, Vodicka M, et al. 2020. Omega-3 phospholipids from krill oil enhance intestinal fatty acid oxidation more effectively than omega-3 triacylglycerols in high-fat diet-fed obese mice. *Nutrients* 12(7):2037
71. Laferrère B, Pattou F. 2018. Weight-independent mechanisms of glucose control after Roux-en-Y gastric bypass. *Front. Endocrinol.* 9:530
72. Lee B, Zhu J, Wolins NE, Cheng JX, Buhman KK. 2009. Differential association of adipophilin and TIP47 proteins with cytoplasmic lipid droplets in mouse enterocytes during dietary fat absorption. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1791:1173–80
73. Lee J, Ridgway ND. 2018. Phosphatidylcholine synthesis regulates triglyceride storage and chylomicron secretion by Caco2 cells. *J. Lipid Res.* 59(10):1940–50
74. Lehner R, Lian J, Quiroga AD. 2012. Lumenal lipid metabolism: implications for lipoprotein assembly. *Arterioscler. Thromb. Vasc. Biol.* 32:1087–93
75. Levy E. 2015. Insights from human congenital disorders of intestinal lipid metabolism. *J. Lipid Res.* 56:945–62
76. Li D, Rodia CN, Johnson ZK, Bae M, Muter A, et al. 2019. Intestinal basolateral lipid substrate transport is linked to chylomicron secretion and is regulated by apoC-III. *J. Lipid Res.* 60:1503–15
77. Lundasen T, Pedrelli M, Bjorndal B, Rozell B, Kuiper RV, et al. 2020. The PPAR pan-agonist tetradecylthioacetic acid promotes redistribution of plasma cholesterol towards large HDL. *PLOS ONE* 15:e0229322
78. Mansbach CM, Dowell R. 2000. Effect of increasing lipid loads on the ability of the endoplasmic reticulum to transport lipid to the Golgi. *J. Lipid Res.* 41:605–12
79. Mansbach CM 2nd, Dowell RF. 1992. Uptake and metabolism of circulating fatty acids by rat intestine. *Am. J. Physiol.* 263:G927–33
80. Mansbach CM 2nd, Parthasarathy S. 1982. A re-examination of the fate of glyceride-glycerol in neutral lipid absorption and transport. *J. Lipid Res.* 23:1009–19
81. Mansbach CM 2nd, Siddiqi S. 2016. Control of chylomicron export from the intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 310:G659–68
82. Maresch LK, Benedikt P, Feiler U, Eder S, Zierler KA, et al. 2019. Intestine-specific overexpression of carboxylesterase 2c protects mice from diet-induced liver steatosis and obesity. *Hepatol. Commun.* 3:227–45
83. Martinez-Guryn K, Hubert N, Frazier K, Urlass S, Musch MW, et al. 2018. Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. *Cell Host Microbe* 23:458–69.e5
84. McCauley HA. 2020. Enteroendocrine regulation of nutrient absorption. *J. Nutr.* 150:10–21

85. McCauley HA, Matthis AL, Enriquez JR, Nichol JT, Sanchez JG, et al. 2020. Enteroendocrine cells couple nutrient sensing to nutrient absorption by regulating ion transport. *Nat. Commun.* 11:4791
86. McFie PJ, Banman SL, Stone SJ. 2018. Diacylglycerol acyltransferase-2 contains a c-terminal sequence that interacts with lipid droplets. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1863:1068–81
87. McManaman JL, Bales ES, Orlicky DJ, Jackman M, MacLean PS, et al. 2013. Perilipin-2-null mice are protected against diet-induced obesity, adipose inflammation, and fatty liver disease. *J. Lipid Res.* 54:1346–59
88. Narabayashi K, Ito Y, Eid N, Maemura K, Inoue T, et al. 2015. Indomethacin suppresses LAMP-2 expression and induces lipophagy and lipoapoptosis in rat enterocytes via the ER stress pathway. *J. Gastroenterol.* 50:541–54
89. Nauli AM, Nassir F, Zheng S, Yang Q, Lo CM, et al. 2006. CD36 is important for chylomicron formation and secretion and may mediate cholesterol uptake in the proximal intestine. *Gastroenterology* 131:1197–207
90. Nettebrock NT, Bohnert M. 2020. Born this way—biogenesis of lipid droplets from specialized ER subdomains. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1865:158448
91. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. 2007. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 298:299–308
92. Obrowsky S, Chandak PG, Patankar JV, Pfeifer T, Povoden S, et al. 2012. Cholesteryl ester accumulation and accelerated cholesterol absorption in intestine-specific hormone sensitive lipase-null mice. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1821:1406–14
93. Obrowsky S, Chandak PG, Patankar JV, Povoden S, Schlager S, et al. 2013. Adipose triglyceride lipase is a TG hydrolase of the small intestine and regulates intestinal PPARα signaling. *J. Lipid Res.* 54:425–35
94. Olzmann JA, Richter CM, Kopito RR. 2013. Spatial regulation of UBXD8 and p97/VCP controls ATGL-mediated lipid droplet turnover. *PNAS* 110:1345–50
95. Ørskov C, Hartmann B, Poulsen SS, Thulesen J, Hare KJ, Holst JJ. 2005. GLP-2 stimulates colonic growth via KGF, released by subepithelial myofibroblasts with GLP-2 receptors. *Regul. Pept.* 124:105–12
96. Pan X, Schwartz GJ, Hussain MM. 2018. Oleoylethanolamide differentially regulates glycerolipid synthesis and lipoprotein secretion in intestine and liver. *J. Lipid Res.* 59:2349–59
97. Pedersen J, Pedersen NB, Brix SW, Grunddal KV, Rosenkilde MM, et al. 2015. The glucagon-like peptide 2 receptor is expressed in enteric neurons and not in the epithelium of the intestine. *Peptides* 67:20–28
98. Qu J, Ko CW, Tso P, Bhargava A. 2019. Apolipoprotein A-IV: a multifunctional protein involved in protection against atherosclerosis and diabetes. *Cells* 8(4):319
99. Ramachandran D, Clara R, Fedele S, Michel L, Burkard J, et al. 2018. Enhancing enterocyte fatty acid oxidation in mice affects glycemic control depending on dietary fat. *Sci. Rep.* 8:10818
100. Reeskamp LF, Meessen ECE, Groen AK. 2018. Transintestinal cholesterol excretion in humans. *Curr. Opin. Lipidol.* 29:10–17
101. Renne MF, Klug YA, Carvalho P. 2020. Lipid droplet biogenesis: a mystery "unmixing"? *Semin. Cell Dev. Biol.* 108:14–23
102. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, et al. 2019. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* 7(1):14
103. Robertson MD, Parkes M, Warren BF, Ferguson DJ, Jackson KG, et al. 2003. Mobilisation of enterocyte fat stores by oral glucose in humans. *Gut* 52:834–39
104. Salo VT, Ikonen E. 2019. Moving out but keeping in touch: contacts between endoplasmic reticulum and lipid droplets. *Curr. Opin. Cell Biol.* 57:64–70
105. Salo VT, Li S, Vihinen H, Holtta-Vuori M, Szkalisity A, et al. 2019. Seipin facilitates triglyceride flow to lipid droplet and counteracts droplet ripening via endoplasmic reticulum contact. *Dev. Cell* 50(4):478–93.e9
106. Santinho A, Salo VT, Chorlay A, Li S, Zhou X, et al. 2020. Membrane curvature catalyzes lipid droplet assembly. *Curr. Biol.* 30(13):2481–94.e6

107. Sato H, Zhang LS, Martinez K, Chang EB, Yang Q, et al. 2016. Antibiotics suppress activation of intestinal mucosal mast cells and reduce dietary lipid absorption in Sprague-Dawley rats. *Gastroenterology* 151:923–32
108. Schittmayer M, Vujic N, Darnhofer B, Korbelius M, Honeder S, et al. 2020. Spatially resolved activity-based proteomic profiles of the murine small intestinal lipases. *Mol. Cell. Proteom.* 19(12):2104–15
109. Shew T, Wolins NE, Cifarelli V. 2018. VEGFR-3 signaling regulates triglyceride retention and absorption in the intestine. *Front. Physiol.* 9:1783
110. Shiau YF, Popper DA, Reed M, Umstetter C, Capuzzi D, Levine GM. 1985. Intestinal triglycerides are derived from both endogenous and exogenous sources. *Am. J. Physiol.* 248:G164–69
111. Siddiqi SA, Siddiqi S, Mahan J, Peggs K, Gorelick FS, Mansbach CM 2nd. 2006. The identification of a novel endoplasmic reticulum to Golgi SNARE complex used by the prechylomicron transport vesicle. *J. Biol. Chem.* 281:20974–82
112. Stahel P, Xiao C, Davis X, Tso P, Lewis GF. 2019. Glucose and GLP-2 (glucagon-like peptide-2) mobilize intestinal triglyceride by distinct mechanisms. *Arterioscler. Thromb. Vasc. Biol.* 39:1565–73
113. Stahel P, Xiao C, Nahmias A, Lewis GF. 2020. Role of the gut in diabetic dyslipidemia. *Front. Endocrinol.* 11:116
114. Stine RR, Sakers AP, TeSlaa T, Kissig M, Stine ZE, et al. 2019. PRDM16 maintains homeostasis of the intestinal epithelium by controlling region-specific metabolism. *Cell Stem Cell* 25:830–45.e8
115. Stone SJ, Myers HM, Watkins SM, Brown BE, Feingold KR, et al. 2004. Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. *J. Biol. Chem.* 279:11767–76
116. Storch J, Zhou YX, Lagakos WS. 2008. Metabolism of apical versus basolateral sn-2-monoacylglycerol and fatty acids in rodent small intestine. *J. Lipid Res.* 49:1762–69
117. Suh SH, Choe K, Hong SP, Jeong SH, Mäkinen T, et al. 2019. Gut microbiota regulates lacteal integrity by inducing VEGF-C in intestinal villus macrophages. *EMBO Rep.* 20:e46927
118. Sui X, Wang K, Gluchowski NL, Elliott SD, Liao M, et al. 2020. Structure and catalytic mechanism of a human triacylglycerol-synthesis enzyme. *Nature* 581:323–28
119. Sztalryd C, Brasaemle DL. 2017. The perilipin family of lipid droplet proteins: gatekeepers of intracellular lipolysis. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862:1221–32
120. Tazi A, Araujo JR, Mulet C, Arena ET, Nigro G, et al. 2018. Disentangling host-microbiota regulation of lipid secretion by enterocytes: insights from commensals *Lactobacillus paracasei* and *Escherichia coli*. *mBio* 9(5):e01493-18
121. Tran TT, Poirier H, Clément L, Nassir F, Pelsers MM, et al. 2011. Luminal lipid regulates CD36 levels and downstream signaling to stimulate chylomicron synthesis. *J. Biol. Chem.* 286:25201–10
122. Trujillo AN, Katnik C, Cuevas J, Cha BJ, Taylor-Clark TE, Breslin JW. 2017. Modulation of mesenteric collecting lymphatic contractions by σ_1-receptor activation and nitric oxide production. *Am. J. Physiol. Heart Circ. Physiol.* 313:H839–53
123. Tso P, Balint JA. 1986. Formation and transport of chylomicrons by enterocytes to the lymphatics. *Am. J. Physiol.* 250:G715–26
124. Uchida A, Slipchenko MN, Cheng JX, Buhman KK. 2011. Fenofibrate, a peroxisome proliferator-activated receptor α agonist, alters triglyceride metabolism in enterocytes of mice. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1811:170–76
125. Uchida A, Whitsitt MC, Eustaquio T, Slipchenko MN, Leary JF, et al. 2012. Reduced triglyceride secretion in response to an acute dietary fat challenge in obese compared to lean mice. *Front. Physiol.* 3:26
126. Van Dyck F, Braem CV, Chen Z, Declercq J, Deckers R, et al. 2007. Loss of the PlagL2 transcription factor affects lacteal uptake of chylomicrons. *Cell Metab.* 6:406–13
127. van Rijn JM, Ardy RC, Kuloğlu Z, Härter B, van Haaften-Visser DY, et al. 2018. Intestinal failure and aberrant lipid metabolism in patients with DGAT1 deficiency. *Gastroenterology* 155:130–43.e15
128. Wang L, Qian H, Nian Y, Han Y, Ren Z, et al. 2020. Structure and mechanism of human diacylglycerol *O*-acyltransferase 1. *Nature* 581:329–32
129. Werno MW, Wilhelmi I, Kuropka B, Ebert F, Freund C, Schurmann A. 2018. The GTPase ARFRP1 affects lipid droplet protein composition and triglyceride release from intracellular storage of intestinal Caco-2 cells. *Biochem. Biophys. Res. Commun.* 506:259–65

130. Whitt J, Woo V, Lee P, Moncivaiz J, Haberman Y, et al. 2018. Disruption of epithelial HDAC3 in intestine prevents diet-induced obesity in mice. *Gastroenterology* 155:501–13
131. Wilfling F, Thiam AR, Olarte MJ, Wang J, Beck R, et al. 2014. Arf1/COPI machinery acts directly on lipid droplets and enables their connection to the ER for protein targeting. *eLife* 3:e01607
132. Wilfling F, Wang H, Haas JT, Krahmer N, Gould TJ, et al. 2013. Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. *Dev. Cell* 24:384–99
133. Xiao C, Stahel P, Carreiro AL, Buhman KK, Lewis GF. 2018. Recent advances in triacylglycerol mobilization by the gut. *Trends Endocrinol. Metab.* 29(3):151–63
134. Xiao C, Stahel P, Carreiro AL, Hung YH, Dash S, et al. 2019. Oral glucose mobilizes triglyceride stores from the human intestine. *Cell. Mol. Gastroenterol. Hepatol.* 7:313–37
135. Xiao C, Stahel P, Lewis GF. 2019. Regulation of chylomicron secretion: focus on post-assembly mechanisms. *Cell. Mol. Gastroenterol. Hepatol.* 7:487–501
136. Xie P, Guo F, Ma Y, Zhu H, Wang F, et al. 2014. Intestinal Cgi-58 deficiency reduces postprandial lipid absorption. *PLOS ONE* 9:e91652
137. Xie Y, Matsumoto H, Kennedy S, Newberry EP, Moritz W, et al. 2019. Impaired chylomicron assembly modifies hepatic metabolism through bile acid-dependent and transmissible microbial adaptations. *Hepatology* 70:1168–84
138. Xu H, Diolintzi A, Storch J. 2019. Fatty acid-binding proteins: functional understanding and diagnostic implications. *Curr. Opin. Clin. Nutr. Metab. Care* 22:407–12
139. Ye J, Li JZ, Liu Y, Li X, Yang T, et al. 2009. Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. *Cell Metab*. 9:177–90
140. Ye L, Mueller O, Bagwell J, Bagnat M, Liddle RA, Rawls JF. 2019. High fat diet induces microbiota-dependent silencing of enteroendocrine cells. *eLife* 8:e48479
141. Yen CL, Stone SJ, Koliwad S, Harris C, Farese RV Jr. 2008. Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *J. Lipid Res.* 49:2283–301
142. Yusta B, Matthews D, Koehler JA, Pujadas G, Kaur KD, Drucker DJ. 2019. Localization of glucagon-like peptide-2 receptor expression in the mouse. *Endocrinology* 160:1950–63
143. Zechner R, Madeo F, Kratky D. 2017. Cytosolic lipolysis and lipophagy: two sides of the same coin. *Nat. Rev. Mol. Cell Biol.* 18:671–84
144. Zhang C, Liu P. 2019. The new face of the lipid droplet: lipid droplet proteins. *Proteomics* 19:e1700223
145. Zhang F, Zarkada G, Han J, Li J, Dubrac A, et al. 2018. Lacteal junction zippering protects against diet-induced obesity. *Science* 361:599–603
146. Zhang LJ, Wang C, Yuan Y, Wang H, Wu J, et al. 2014. Cideb facilitates the lipidation of chylomicrons in the small intestine. *J. Lipid Res.* 55:1279–87
147. Zhu J, Lee B, Buhman KK, Cheng JX. 2009. A dynamic, cytoplasmic triacylglycerol pool in enterocytes revealed by ex vivo and in vivo coherent anti-Stokes Raman scattering imaging. *J. Lipid Res.* 50:1080–89