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Gastrointestinal factors regulating lipid droplet formation in the intestine

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ABSTRACT

Cytoplasmic lipid droplets (CLD) are considered as neutral lipid reservoirs, which protect cells from lipotoxicity. It became clear that these fascinating dynamic organelles play a role not only in energy storage and metabolism, but also in cellular lipid and protein handling, inter-organelle communication, and signaling among diverse functions. Their dysregulation is associated with multiple disorders, including obesity, liver steatosis and cardiovascular diseases. The central aim of this review is to highlight the link between intra-enterocyte CLD dynamics and the formation of chylomicrons, the main intestinal dietary lipid vehicle, after overviewing the morphology, molecular composition, biogenesis and functions of CLD.

1. Introduction

Cytosolic lipid droplets (CLD) are organelles found in most tissues, which are mainly composed of triacylglycerol (TG) [1]. They were primarily studied in the adipose tissue and liver where their accumulation was associated with obesity-related disorders such as insulin resistance (IR), atherosclerosis and non-alcoholic fatty liver steatosis [2]. The small intestine is par excellence the organ responsible for the absorption of dietary fat via the obligatory formation of chylomicrons (CM) [3]. These vehicles are essential not only for the transport of alimentary lipid but also for the release of fat-soluble vitamins into the bloodstream via the lymphatic system [4]. Thus, non-esterified fatty acids (FAs) become readily available for the different cell types of the organism and eventually for their organelles in terms of structural and signaling purposes, as well as for the regulation of energy homeostasis. The mechanisms controlling CM assembly and output are critical since the up-regulation of these processes in the postprandial state is proposed to cause cardiometabolic disorders [5]. Growing evidence shows that FAs, present in excess after dietary fat consumption, can be esterified and incorporated into CLD within enterocytes [3]. Hence, the modulation of the enterocyte storage in the form of CLD is extremely important since it protects from FA toxicity and exaggerated postprandial TG levels, which constitute a strong risk for cardiovascular disorders [6]. However, despite significant advances, the pathways and key factors coordinating CLD dynamics and CM handling in the gut are incompletely defined. Furthermore, the role of gastrointestinal (GI) peptides on these specific interactions between droplets and CM is far from established. In the present review, the challenging issue relative to the involvement of CLD in CM processing under physiological and pathophysiological conditions will be underlined. The importance of the GI peptides in their interconnection will also be emphasized. Finally, various gaps in this area of research and investigation requirements to further our knowledge will be highlighted.

2. From dietary lipid to chylomicron production

2.1. Fat digestion step

In the intestine, lipid transport and CM formation are regulated through a complex processes [3,7]. In the digestive phase, dietary TGs are partially hydrolyzed by gastric lipase (20–30%) and subsequently by pancreatic lipase (70–80%), resulting in 2 molecules of FA and sn-2 monoacylglycerol [8]. The pancreatic phospholipid (PL)-hydrolyzing enzymes include phospholipase A_2 that hydrolyzes phosphatidylcholine

Abbreviations: ACC, Acetyl-CoA carboxylase; ACSL, Long-chain acyl-CoA synthetase; AMPK, AMP-activated protein kinase; Apo, Apolipoprotein; ATGL, Adipose triglyceride lipase; CD36, Cluster of differentiation 36; CGI-58, Comparative gene identification-58; CIDE, Cell death-inducing DNA fragmentation factor 45-like effector family proteins; CM, Chylomicron; CE, Cholesteryl Ester; CLD, Cytoplasmic lipid droplet; DGAT, Diacylglycerol transferase; ER, Endoplasmic reticulum; ERLLD, ER luminal LD; FA, Fatty acids; FABP, Fatty acid binding protein; FAS, Fatty acid synthase; FOXO3, Forkhead box O3; GHSR1a, Growth hormone secretagogue receptor of type 1a; GLP-1, Glucagon-like peptide 1; GLP-2, Glucagon-like peptide 2; GI, Gastrointestinal; IR, Insulin resistance; MFGE8, Milk fat globule epidermal growth factor–like 8; MTTP, Microsomal triglyceride transfer protein; PC, Phosphatidylcholine; PL, Phospholipids; PLIN, Perilipin; PYY, Peptide tyrosine-tyrosine; TG, Triacylglycerol

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(PC), the preponderant dietary PL, to form lyso-PC and FA. The third digestive enzyme is cholesterol esterase that decomposes cholesteryl ester (CE) into free cholesterol and FA [9–11]. Concomitantly, lipolytic products are solubilized by bile acids to generate micelles in the lumen of the duodenum and the proximal jejunum [12,13]. Following the formation of micelles, in the presence of low concentrations of long-chain FA, the lipolytic uptake is achieved by passive diffusion [14]. By contrast, in the presence of higher levels of long-chain FA, a carrier membrane fatty acid binding protein (FABP) is fully required [15,16]. Later, many additional lipid transporters were discovered in the small intestine. For example, the carrier protein cluster of differentiation 36 (CD36) transports mainly long chain FAs into enterocytes and plays a major role in the regulation of food intake, perception of fat taste and lipid absorption [17,18]. CD36 not only promotes CM formation, but also its protein ubiquitination and degradation by the proteasome [17,18]. Other cholesterol transporters are found in the enterocytes such as scavenger Receptor class B type I, Nieman Pick C1-Like, and ATP Binding Cassette ABCG5/G8 that cooperates to limit sterol intestinal absorption [19–23]. Further, ABCA1 is also capable of cholesterol efflux to basolateral apolipoprotein (Apo) A-I to form high-density lipoproteins (HDL) [24].

2.2. Intracellular lipid transport step

After crossing the brush border membrane, the lipolytic products are processed by cytosolic proteins for their intracellular trafficking towards various compartments, including the endoplasmic reticulum (ER), where their re-esterification takes place [25–28]. Two cytosolic FABP have been identified: intestinal-FABP (I-FABP) and liver-FABP (L-FABP) [29–31]. I- and L-FABP bind differently according to lipid classes, but both proteins with a greater affinity for unsaturated than saturated FAs [32]. It is suggested that L-FABP mostly transports FA to mitochondria for β-oxidation while I-FABP promotes TG synthesis in the ER [33]. In this organelle, the lipolytic products are re-esterified to form TG, PL and CE through specific enzymatic pathways. Monoacylglycerol acyltransferases (MGAT) 2 and 3 catalyze the esterification of acyl-CoA and monoacylglycerol (MG) to form diacylglycerol (DG) while diacylglycerol transferase (DGAT) 1 and 2 esterify DG into TG [25–28]. TG are either stored in lipid droplets (LD) or used for CM formation [34].

Following their esterification, hydrophobic lipids are assembled with apolipoproteins (Apo) to form complexes known as CM in order to allow their transport in the blood circulation [35]. Three types of proteins are critical for CM assembly and secretion: Apo B-48, microsomal triglyceride transfer protein (MTTP) and Sar1b GTPase [3,36,37]. Apo B-48 is an essential structural protein facilitating the formation of CM within the ER while MTTP enhances the lipoprotein biogenesis by shuttling neutral lipid to Apo B acceptor molecules. In fact, MTTP can interact physically with Apo B-48, allowing an optimal structural configuration of the latter in order to accept more lipids. The pre-CM vesicle is transported to the Golgi apparatus with the crucial presence of the small Sar1b GTPase that initiates the vesicular coat protein complex II-dependent transport of cargo from the ER to the Golgi apparatus [38,39]. Genetic defects of *Apo B-48*, *MTTP* and *SAR1B* lead to hypobetalipoproteinemia, abetalipoproteinemia and CM retention disease, respectively [40].

3. Cytosolic lipid droplets in the intestine

CLDs are composed of a hydrophobic TG and CE core surrounded by a PL monolayer with very few cholesterol molecules and proteins [41,42]. CLD can accumulate in the intestine, liver, skeletal muscle, adrenal cortex, macrophage, mammary glands and adipose tissues [43]. In the intestinal tract, they are mainly detected in the jejunum but are also present in decreasing numbers and sizes in the distal part of the small intestine [44]. Dietary TGs are directly related to the FA composition of CLD in enterocytes [45]. Two distinctive pathologies are associated with excessive CLD accumulation in humans: abetalipoproteinemia and CM retention disease [46,47]. As mentioned before, these two conditions are characterized by defective lipid absorption and especially CM secretion.

3.1. Lipid droplet formation

As mentioned above, CLDs store excess energy in the form of TG and releases FFA by TG degradation, a process highly dynamic in cells. In fact, their lipid storage capacity is determined by their “size” and is controlled by the balance between TG synthesis and hydrolysis.

3.1.1. The importance of DGAT

Approximately 95% of all TG absorbed by the small intestine are used for CLD and CM formation [48,49]. Currently, it is expected that CLD formation results from an initial clustering of neutral lipid within the hydrophobic region of the ER membrane [50]. It is within this ER region that FAs are esterified into TG by DGAT1 and DGAT2 [49,51]. Interestingly, TG produced by DGAT1 are directly packaged into CM while those generated by DGAT2 are mostly stored in CLD [49,51]. Previous studies reported that DGAT1$^{-/-}$ mice have reduced concentrations of TG in most tissues while an accumulation of abnormal lipid levels was observed in the intestine [52]. It seems that upon reaching a certain size, sequestered TG bud off from the ER and form nascent LDs that are either transported to the cytosol or the lumen [53–55]. However, the mechanisms governing this process remain largely uncharacterized.

3.1.2. Role of endoplasmic luminal lipid droplet

As previously proposed, ER luminal LDs (ERLLDs) that do not carry an Apo B-48 molecule are stabilized as pre-CM following the addition of PL monolayer in the ER lumen [7,56]. In response to ERLLD hydrolysis, lipids are re-esterified and used for CM core expansion and assembly [57]. Interestingly, LDs accumulate in the absence of Apo B-48 but not in response to MTTP inactivation when LDs are completely absent in the ER lumen [58,59]. Previous observations indicate that MTTP may associate to ERLLD and fulfill a crucial function in the formation and stabilization of luminal LDs.

3.1.3. Protein composition of CLD

A total of 181 different proteins were recently shown to be associated with CLD [60]. Among them, only 19% are expected to influence lipid metabolism and transport. The other proteins are also present in other organelles, such as ER, mitochondria and Golgi, and have various roles in carbohydrate metabolism, localization and transport of proteins, and cytoskeleton integrity, which underlines the necessity to better characterize their physiological relevance moieties in order to improve our knowledge relative to the regulatory functions of CLD. CLD protein composition is proposed to differ depending on its location in the tissues [60]. For instance, proteins such as long-chain acyl-CoA synthetase (ACSL) 5, MTTP and Apo A-IV are only reported to be expressed in cultured human Caco-2 epithelial cells whereas CLDs are mostly coated by perilipins (PLIN) 2 and 3 in the intestine, which is not the case in other organs such as the adipose tissues and liver [60–63]. PLIN proteins are the most abundant in CLDs and they are mainly involved in their formation, stabilization and hydrolysis. They translocate from the cytosol to nascent LDs and are later submitted to ubiquitin-proteasome degradation in the cytosol [64,65]. The ingestion of a chronic high fat meal is reported to increase PLIN-2 concentration while an acute lipid challenge is also shown to upregulate PLIN-3 at the level of the CLD membrane [66].

3.2. Lipid droplet hypertrophy

Various ER resident proteins are critical for CLD formation and