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Commentary

Lipins play important roles in adipogenesis, insulin sensitivity, and gene regulation, and mutations in these genes cause lipodystrophy, myoglobinuria, and inflammatory disorders. While all lipins (lipin 1, 2, and 3) act as phosphatidic acid phosphatase (PAP) enzymes, which are required for triacylglycerol (TAG) synthesis from glycerol 3-phosphate, lipin 1 has been the focus of most of the lipin-related research. In the current issue of the *JCI*, Zhang et al. show that while lipin 2 and 3 are expendable for the incorporation of dietary fatty acids into triglycerides, lipin 2/3 PAP activity has a critical role in phospholipid homeostasis and chylomicron assembly in enterocytes.

To absorb fat – supersize my lipid droplets

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Lipins play important roles in adipogenesis, insulin sensitivity, and gene regulation, and mutations in these genes cause lipodystrophy, myoglobinuria, and inflammatory disorders. While all lipins (lipin 1, 2, and 3) act as phosphatidic acid phosphatase (PAP) enzymes, which are required for triacylglycerol (TAG) synthesis from glycerol 3-phosphate, lipin 1 has been the focus of most of the lipin-related research. In the current issue of the *JCI*, Zhang et al. show that while lipin 2 and 3 are expendable for the incorporation of dietary fatty acids into triglycerides, lipin 2/3 PAP activity has a critical role in phospholipid homeostasis and chylomicron assembly in enterocytes.

Triacylgycerol-rich chylomicrons in the intestine

Triacylgycerols (TAGs) are major sources of stored substrates that are metabolized to provide energy. Composed of 3 fatty acid chains attached to a glycerol backbone, these nonpolar molecules cannot cross cell membranes. This is overcome by a seemingly futile cycle in which most dietary TAGs are dissociated into nonesterified fatty acids (NEFAs) by a series of parallel enzymatic steps that ultimately lead to cellular NEFA uptake, its resynthesis in the endoplasmic reticulum (ER) membrane, and storage within cytosolic lipid droplets (LDs). The newly synthesized TAGs can also be stored as lipid droplets in the lumen of the enterocyte and hepatocyte ER and assembled into TAG-rich lipoproteins, chylomicrons, or very low-density lipoproteins, and secreted. In the intestine, lack of tight junctions between endothelial cells is needed to allow the large chylomicrons to cross the endothelial barrier and enter the lymphatics (1).

While TAG synthesis within enterocytes involves two pathways, the glycerol phosphate pathway and monoacylglycerol pathway, the monoacyglycerol pathway is the predominant pathway of TAG synthesis in the intestine. In the current issue of the *JCI*, the studies by Zhang et al. provide evidence that intermediates in the glycerol phosphate pathway can regulate assembly of TAG-rich chylomicrons (2).

Due to their limited solubility in the membrane bilayer, newly synthesized TAGs become part of three types of micelles: cytosolic LDs, ER lumenal LDs, and apoB-containing lipoproteins. TAGs bud off as LDs to the cytosol (the default pathway) (3, 4) or to the ER lumen, a specialized active pathway that involves microsomal TAG transfer protein (MTP) (5–7) and possibly other proteins. Besides formation of these LDs, the ER lumen is also the site for the assembly of TAG-rich chylomicrons. In this process, newly synthesized apolipoprotein B48 (apoB48) interacts with the ER membrane and forms nucleation sites for lipoprotein assembly (6, 7). MTP helps in further lipidation of apoB and in the formation of primordial lipoprotein particles. These primordial lipoproteins presumably fuse with ER lumenal LDs, resulting in the formation of mature larger lipoproteins. TAGs from cytosolic LDs are also substrates for the assembly of chylomicrons. This involves hydrolysis of TAGs in the cytosolic LDs, transfer of fatty acids to the ER membrane, resynthesis of TAGs, and incorporation into chylomicrons or ER lumenal LDs. Some proteins, e.g., perilipin 2 and CGI-58, involved in cytosolic LD formation, negatively impact TAG secretion, suggesting competition for the formation of cytosolic LD formation and lipoprotein secretion (3, 4). Thus, LDs are transient storage sites for TAGs that are ultimately secreted by enterocytes as lipoproteins.

How are TAGs within cytosolic LDs incorporated into lipoproteins? As discussed above, TAGs in cytosolic and ER lumenal LDs are a major source of TAGs in secreted lipoproteins. Do the composition, cellular location, or associated surface molecules (proteins and phospholipids) regulate the movement of the LDs between the cytosol and ER lumen? Or must the cytosolic LD increase to an appropriate size to allow its interaction with hydrolases involved in their hydrolysis and transfer of fatty acids across the ER lumen? These questions remain unanswered.

Lipin 2 and 3 regulate TAG secretion by the small intestine

Lipins are a family of three proteins (lipin 1, 2, and 3) that have enzymatic (8) and transcriptional actions (9). In most tissues, they participate in the synthesis of TAGs and phospholipids by converting phosphatidic acid (PA) to diacylglycerol. While most metabolic tissues predominantly express lipin 1, the small intestine almost exclusively expresses lipin 2 and 3. To understand the role of these lipins in the gut, Zhang et al. created lipin 2 and 3 double-knockout mice. Those mice were underweight on a chow diet due to reduced fat mass. On high-fat, high-carbohydrate diet, they lost significant body weight within 6 days. The mice were defective in lipid absorption and showed no postprandial response after a fat tolerance test, indicating defective lipid absorption and chylomicron production. ApoB and MTP expression was not affected by lipin 2 and 3 deficiency. Therefore, the defect in chylomicron assembly was determined to be proximal to apoB and MTP.

Electron microscopic studies showed accumulation of LDs in the cytosol and absence of LDs in the secretory pathway. Further, there were increased lipid stacks,

▶ Related Article: p. 281

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presumably expanded ER, in enterocytes. The cytosolic LDs were smaller and homogeneous in size, had greater surface-to-core ratio, and a higher phospholipid/TAG ratio. Lipid analyses revealed that lipin 2 and 3 deficiency had no effect on cellular TAG levels but phosphatidylcholine (PC) levels were increased. Further, PA levels were also increased. These changes in phospholipids were associated with increased phospholipid synthesis and increased expression of the synthetic enzyme CTP:phosphocholine cytidylyltransferase α (CCTα). It has previously been shown that PA, acting via mTORC1, increases the protein levels of CCTα, leading to increased PC levels (10). In the current study, inhibition of CCTα reduced phospholipid synthesis, increased LD size, and rescued the defective chylomicron synthesis. Thus, these studies have identified a novel regulatory pathway of chylomicron assembly and secretion, and have highlighted the importance of proper phospholipid synthesis in this process. In this regulatory pathway, lipin 2 and 3 avoid accumulation of PA by hydrolyzing it and facilitate TAG synthesis and transport into the ER lumen for the formation of chylomicrons.

It is unclear why accumulation of PA inhibits transport of TAG across the ER membrane, why cytosolic LDs cannot be hydrolyzed, and why free fatty acids are not mobilized to the ER lumen. Must cytosolic LDs obtain an optimal size and diameter to allow them to interact with lipases involved in their hydrolysis? Other lipid-interacting enzymes — such as lipoprotein lipase, hepatic lipase, and endothelial lipase — act on lipids within TAG-rich lipoproteins, LDL, and HDL, respectively. This differential interaction is thought to depend on the size of the particles that affects the interaction of the enzymes with the lipoprotein surface (11). Besides the well-known stimulated lipolysis pathways involving phosphorylation of LD proteins, a similar biology might affect the ability of enzymes required for the hydrolysis of cytosolic LDs (12).

TAG-rich lipoproteins in physiology and pathology

Why is this important for normal physiology and pathology? TAG-rich lipoproteins have again generated greater interest for their relationship to atherosclerotic cardiovascular disease (CVD), as GWAS studies have implicated genes associated with defective TAG lipolysis (13, 14). This validates the observations in 1973 by Goldstein et al. that increased TAGs as well as cholesterol levels associate with CVD (15). These circulating TAG levels could affect arterial biology either by serving as a source of toxic vascular lipids via their partial degradation to remnant particles, or toxic effects of locally released lipolysis products (16). Creation of chylomicrons, an obligatory step in intestinal fat absorption, is still mysterious. Understanding various regulatory steps may help identify targets to reduce lipid absorption and reduce heart disease and obesity. Thus, there is a need to unravel different molecules, proteins, and pathways involved in chylomicron assembly and secretion. This study provides an indication that phospholipids are not only necessary for surface stabilization of different LDs and lipoproteins, but they also play a regulatory role in their biogenesis.

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