Lipid Droplet-Associated Proteins in Cardiomyopathy

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Keywords

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Abstract

Background: The heart requires a high rate of fatty-acid oxidation (FAO) to meet its energy needs. Neutral lipids are the main source of energy for the heart and are stored in lipid droplets (LDs), which are cytosolic organelles that primarily serve to store neutral lipids and regulate cellular lipid metabolism. LD-associated proteins (LDAPs) are proteins either located on the surface of the LDs or reside in the cytosol and contribute to lipid metabolism. Therefore, abnormal cardiac lipid accumulation or FAO can alter the redox state of the heart, resulting in cardiomyopathy, a group of diseases that negatively affect the myocardial function, thereby leading to heart failure and even cardiac death. **Summary:** LDs, along with LDAPs, are pivotal for modulating heart lipid homeostasis. The proper cardiac development and the maintenance of its normal function depend largely on lipid homeostasis regulated by LDs and LDAPs. Overexpression or deletion of specific LDAPs can trigger myocardial dysfunction and may contribute to the development of cardiomyopathy. Extensive connections and interactions may also exist between

LDAPs. **Key Message:** In this review, the various mechanisms involved in LDAP-mediated regulation of lipid metabolism, the association between cardiac development and lipid metabolism, as well as the role of LDAPs in cardiomyopathy progression are discussed.

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Introduction

Lipids are biological macromolecules that dissolve in nonpolar solvents including triglycerides (TAGs), phospholipids, glycolipids, cholesterol, and cholesterol esters [1, 2]. Although different lipids perform different roles in regulating cellular function, their main functions are related to energy storage and signal transduction and serve as one of the primary components of the cell membrane. Additionally, lipids also play a non-negligible role in the process of individual growth and development along with homeostasis regulation. Therefore, it is pivotal for the body to maintain lipid regulation at a normal stage and within a feasible range.

The heart is the “engine” of the human body. It generally functions as a pump, promoting blood circulation via myocardial contraction and relaxation to provide nutri-

ents and oxygen to the brain and other tissues to satisfy metabolic needs, and to remove the final metabolites from the tissues and organs to perpetuate normal cellular functions. Therefore, the heart requires the highest amount of energy supply and also possesses the strongest fatty-acid oxidation (FAO) capacity among other organs and tissues to meet its energy need. A large amount of energy used to maintain sustained cardiac contraction is provided primarily by mitochondrial β -oxidation fatty acids (FAs) [3]. Since healthy adult cardiomyocytes use FAs as their main energy substrate, lipid regulation is vital in cardiac metabolism [4].

Lipid Metabolism in the Heart

The heart requires large amounts of FAs for its energy supply. However, the heart does not actively use glucose or amino acids as a substrate for the *de novo* synthesis of FAs like the liver [5]. Therefore, the heart needs to continuously obtain FAs from the circulation [3, 6–8]. Plasma FAs can be ingested into the heart by 2 major pathways: (1) the lipolysis of plasma lipoprotein TAGs by lipoprotein lipases which are synthesized, processed, and abundantly expressed primarily in cardiomyocytes, or (2) the ingestion of albumin-bound serum-free FAs (FFAs) that are released from adipose tissue [9–11]. The heart can also obtain FAs by internalizing whole lipoproteins or by catabolizing the cardiac storage of endogenous TAGs [9]. FAs are esterified and converted to TAGs via acyl-CoA synthetase and FA transport protein, which are then stored in the form of lipid droplets (LDs) and are used for the subsequent hydrolysis to provide FAs for oxidation and can also serve as peroxisome proliferator-activated receptor α (PPAR α) ligands [12–14]. The heart has relatively low TAG stock, which indicates its high lipolytic turnover rate compared to other tissues [15].

Lipid Droplets

LDs are crucial for regulating lipid metabolism. They are intracellular organelles that are expressed ubiquitously in all eukaryotic cells. Structurally, LDs consist of a phospholipid monolayer surrounding its lipid core [16, 17]. Lipidomics shows that the core of LDs can store >160 different types of neutral lipids, primarily TAGs, sterol esters, retinol esters, and waxes [16, 18–20]. The specific content of neutral lipids in the LDs of different cells varies with cell functions [21, 22].

Recent studies have provided substantial evidence on LD formation. The mainstream perspective is that the LDs

are originated from the perilipin (PLIN) 2-rich domain of the endoplasmic reticulum (ER) [23]. This model of LD biogenesis is comprised of at least 4 major steps, including (1) synthesis of TAGs on the ER by diglyceride acyltransferase (DGAT), mainly DGAT2 [24, 25]; (2) formation of oil mirrors on the ER membrane; (3) generation of nascent LDs through budding; and (4) interaction of LDs with specific proteins for growth and expansion. Moreover, the plasma membrane is also participating by providing TAGs for LDs, and the Golgi apparatus has a close interaction with LDs [26–29]. In addition to neutral lipid and phospholipid synthesis enzymes, proteins such as seipins, PLINs, FIT proteins, and ER-shaping proteins also serve critical roles in LD formation. However, the exact mechanism of LD formation still needs further investigation.

The size of an LD is increased mainly via 3 different ways: fusion, caveolae conversion, and local synthesis [26, 30–35]. The fusion model states that fat-specific protein 27 is capable of interacting with PLIN1 to form a channel-like junction between 2 LDs and in this way, promote the trafficking of neutral lipids from one LD to the other [34]. The caveolae model suggests that exogenously administered oleic acid can be taken up by primary adipocytes and converted into TAGs directly in PLIN1-localized plasma membrane caveolae. It was also observed that human lysophosphatidylcholine acyltransferases 1 and 2 were localized to LDs with their expression levels correlated with the ability of LDs to synthesize phosphatidylcholine locally. This provides evidence for the local synthesis model [32]. LDs can be broken down by 2 pathways: (1) lipolysis by lipases such as adipose TAG lipase (ATGL) and hormone-sensitive lipase (HSL) and (2) delivery to autolysosomes to be broken down by lysosomal acid lipases through the autophagic process [32, 36]. In the starvation state, TAG hydrolysis is upregulated to supply adequate FFA for β -oxidation to fulfill cellular energy requirements. Concurrently, the breakdown of LDs by lipophagy serves as an additional energy source. The suppression of lysosomal activity was associated with increased cellular TAG and cholesterol content as well as LD accumulation [37]. LDs were seen to co-localize with lysosomal-associated membrane protein type 1 in oleate or methionine- and choline-deficient medium (MCDM), and increased under lysosomal inhibition. Further research also showed that autophagy-related LD breakdown was probably relevant to LD-associated proteins (LDAPs) such as ATGL, the PLIN family, and the Rab family [38–44]. After LD degradation, the FAs stored in LDs are released, followed by β -oxidation in mitochondria and peroxisomes for its conversion to energy [45].

Fig. 1. Schematic diagram of LDAPs on the surface of the LD. Different LDAPs have different lipid-regulating abilities. LDAP, lipid droplet-associated proteins; LD, lipid droplet; PLIN, perilipin; ATGL, adipose triglyceride lipase; BSCL2, Berardinelli-Seip congenital lipodystrophy type 2; HILPDA, hypoxia-inducible lipid droplet-associated protein; HSL, hormone-sensitive lipase; FA, fatty acid; TAG, triglyceride; DAG, diacylglycerols; FSP27, fat-specific protein 27; DGAT, diglyceride acyltransferase.

LDs were initially considered fat deposits, which were specifically used to store neutral lipids for energy use. Recent studies have shown that LDs are more than just fat reservoirs. LD functions in numerous biological processes, including the storage of various substances such as vitamins [46, 47], signaling precursors [48–50], proteins [51–53], membrane synthesis [16], cell signaling [32, 54, 55], protein handling, and management of cellular stress [16]. LDs can also protect cells from lipotoxicity by sequestering toxic lipids, for example, ceramides and diacylglycerols (DAGs), in the cytoplasm [16].

LD-Associated Proteins

LDAPs are proteins which predominantly regulate the extremely high LD turnover rate of the heart [56]. LDAPs are also fundamental to the formation and degradation of LDs. These proteins can be localized to the surface of the LD through 2 major pathways: the class I pathway and the class II pathway. In the class I pathway, proteins are inserted into a particular ER membrane domain, potentially a hairpin loop. They are subsequently repositioned from the ER to the LD surface as membrane-embedded proteins using membrane bridges. In the class II pathway,

proteins are bound to the LD surface via an amphiphilic helix or through multiple amphiphilic and hydrophobic helices [57, 58].

Studies have discovered numerous variations of LDAPs. LDAPs, including the PLIN family, the cell death-inducing DNA fragmentation factor-like (CIDE) family, abhydrolase domain containing 5 (ABHD5/CGI-58), ATGL, Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2), and hypoxia-inducible LDAP (HILPDA) have been extensively studied. Within the human body, the PLIN family comprises of 5 members, including PLIN1, PLIN2 (PLIN2/adipophilin/adipose differentiation-related protein/ADFP), PLIN3 (PLIN3/Tip47), PLIN4 (PLIN4/S3-12), and PLIN5 (PLIN5/lipid storage droplet protein 5/myocardial LD protein/OXPAT/PAT1). The CIDE family includes 3 members: CIDEA, CIDEB, and CIDEC (termed fat-specific protein 27 in mice) [59–63]. Although all cells collect LDs to a variable extent, the expression of LDAPs in LDs of different cells depends on the function of cell types and tissues. Since the composition of LDAPs in each cell type and tissue should fulfill its functional demands, different LDAPs have different lipid-regulating abilities (summarized in Fig. 1).

The first recognized LDAP was PLIN1, which was primarily located in the white adipose tissue (WAT) and brown adipose tissue (BAT) [64]. PLIN2 and PLIN3 are commonly expressed in all cell types. Specifically, PLIN2 is strongly activated by lipid overload and is massively expressed in macrophages, atherosclerotic plaques, the liver, and muscles. PLIN3 is mainly located on the surface of LDs but can be found in the cytoplasm when not associated with LDs. PLIN4 is predominantly found in WAT but is also detectable in tissues, such as the heart and the muscles. PLIN5, on the other hand, is chiefly expressed within oxidative tissues, such as the heart, BAT, the liver, and skeletal muscles. Both ABHD5 and PLIN5 can be phosphorylated by protein kinase A (PKA), leading to the activation of ATGL. ATGL is another well-known LDAP, which is found in all cell types that contain neutral lipids. ATGL is generally found on the surface of LDs. BSCL2 is a protein found in the ER that can interact with nascent LDs directly and facilitate the maturation of LDs from the ER [65]. Also, high levels of HILPDA have been identified in the WAT, BAT, heart, and lungs of mice [66].

Generally, the PLIN family is known to be involved in modulating lipase activity. Under primary conditions, PLINs tend to limit the hydrolysis of LDs and, subsequently, the release of FAs. PLIN1 interacts with comparative gene identification (CGI)-58 (CGI-58) to interrupt the passage of hydrolases into LDs, thus protecting the TAGs in LDs from hydrolysis [67, 68]. Also, PLIN5 attenuates the myocardial ischemia/reperfusion injury by suppressing LD lipolysis to lessen oxidative stress [69]. However, upon stimulation, PLIN1 and PLIN5 have been observed to promote hydrolysis to provide tissues with FAs for oxidation and energy [12]. Additionally, LDs combined with PLIN2 are protected from breakdown by the proteasome pathway [70, 71]. LDAPs are also engaged in the process of intracellular cargo transportation. PLIN3 binds to the cytoplasmic domain of mannose 6-phosphate receptor, facilitating the translocation of mannose 6-phosphate receptor from endosomes to the trans-Golgi network [72]. Meanwhile, PLIN5 was observed to bind to LD-derived monounsaturated FAs and transport them to the nucleus, following the activation of cAMP/PKA-mediated lipolysis [73].

Cardiac Dysfunction Associated with Lipid Metabolic Disorders

Lipids act as the substrates of mitochondrial β -oxidation; play vital roles in signal transduction, membrane structure, and molecular transport; and are essen-

tial regulators of cardiac function [74–77]. Lipid homeostasis is a crucial part of the normal development of the heart. During the early stages of heart development, glycolysis is the primary energy source for the heart. As cardiomyocytes mature and terminate their differentiation, the oxidative capacity of mitochondria enhances, and FAO progresses to become the major source of energy for the heart. If the adult heart is subjected to excessive stress that cannot be fully compensated by cardiac hypertrophy, the energy metabolism of the heart would convert to a fetal-like type. Glycolysis is enhanced, whereas mitochondrial FAO diminishes. Hence, alterations in cardiac energy metabolism throughout the development of the heart have critical implications for the maturation ability of cardiomyocytes [78]. Abnormal accumulation of cardiac lipids is typically associated with 2 forms of cardiomyopathy: obesity-related cardiomyopathy, which might lead to sudden death, and diabetic cardiomyopathy, which usually has a failing cardiac function [79, 80]. In addition, visceral fat accumulation is also associated with excess epicardial and pericardial fat [79, 81, 82]. Therefore, lipid metabolic disorders are closely related to progressive cardiac dysfunction.

Certain lipids can be directly toxic to the heart. Earlier studies have considered TAGs to be potentially toxic. A study that directly compared the progression of cardiac dysfunction in ob/ob and db/db mouse models identified that metabolic dysfunction and myocardial TAG accumulation preceded the onset of contractile dysfunction, and myocardial TAG accumulation preceded the occurrence of contractile dysfunction and hyperglycemia [83]. Clinical studies also found elevated intramyocardial TAGs in patients with heart failure and diabetes with or without obesity, whose gene expression profiles resemble the Zucker diabetic fat rodent model, an animal model of lipotoxicity. Consistently, the same pattern of altered mRNA expression was undetected in heart failure hearts without lipid accumulation [84]. Further findings also suggested that TAGs may not be directly cytotoxic but may serve as markers of lipotoxicity. FA metabolites that are indeed cardioliipotoxic include ceramides, DAGs, acyl-coenzyme synthase 1 (ACSL1), acylcarnitines, and lysophospholipids, where ceramides and DAGs are the major toxic lipids [85]. Mice with overexpressed long-chain ACSL1 accumulate FFA, DAG, and ceramide and exhibit mitochondrial and cardiac dysfunction. At the same time, the overexpression of DGAT1, a TAG-synthesizing enzyme which utilizes FFA to convert DAG to TAG, improves ACSL1-induced cardiac dysfunction by reducing cardiomyocyte apoptosis as well as attenuating DAG and

ceramide levels. Thus, cardiac-specific DGAT1 transgenic mice show massive amounts of TAG accumulation but reduced cardiac ceramide, DAG, and FA levels without an overt cardiac phenotype [86]. In human studies, patients implanted with a left-ventricular assist device, which promotes the use of FAs by the myocardium, exhibit decreased ceramide and DAG levels but similar TAG and FA levels. This indicates that myocardial lipotoxicity might be caused by ceramide and DAG rather than TAG [87].

Several mechanisms have been proposed to explain how this occurs, including direct toxic effects of neutral droplets or FAs on myocardial myofiber function, FA-induced apoptosis, generation of reactive oxygen species (ROS) as toxic byproducts of lipid oxidation, and activation of signaling pathways via protein kinase C (PKC) activation or ceramide-mediated processes [88]. Although DAGs are thought to be more efficient lipid second messengers that activate classical and novel isoforms of PKC, both DAGs and ceramides bind to PKC, thereby mediating myocardial contractility, gene expression, and cell growth [75]. PKC $\alpha^{-/-}$ mice showed enhanced cardiac contractile ability and were less susceptible to heart failure [89]. The inhibition of PKC β preserved the heart from diabetic cardiac diastolic dysfunction and cardiomyocyte hypertrophy [90], and mice with PKC β_2 overexpression exhibited left-ventricular hypertrophy as well as cardiomyocyte necrosis [91]. Novel PKC isoforms such as PKC θ and PKC ϵ exert regulatory effects through the insulin signaling pathway [85]. Furthermore, the structure of DAGs can affect their PKC activation capabilities. It is suggested that cellular DAGs are characterized by 3 different stereoisomers.

The insulin-signaling defect might be another pathway used by DAGs and ceramides to produce lipotoxicity. Studies have suggested that both ceramide and DAGs can negatively regulate insulin action. Ceramide interrupts insulin-mediated activation of the Akt/PKB pathway either by the direct inhibition of Akt/PKB phosphorylation or indirectly by the activation of the protein phosphatase 2A to dephosphorylate Akt/PKB, whereas DAG acts by promoting the phosphorylation of IRS-1, which can contribute to the development of cardiomyopathy [92].

Cardiac dysfunction could also occur due to enhanced FA oxidation and subsequent changes in the substrates of energy metabolism. Normally, the heart relies primarily on mitochondrial β -oxidation of FAs for energy. However, the heart also acts as a metabolic omnivore with the ability to metabolize glucose, amino acids, and ketone bodies. This metabolic diversity and flexibility

have contributed to the maintenance of normal contractile function of the heart under various physiological and pathophysiological conditions. Thus, excess FAs in the obese heart may result in enhanced mitochondrial FA β -oxidation, reducing glucose utilization by causing substrate competition and triggering insulin resistance. There are at least 2 possible explanations for this shift in oxidative substrates to impair cardiac function: (1) FA intake exceeds the capacity of LDs to be stored safely and (2) the heart produces energy less efficiently with greater oxygen consumption as mitochondrial β -oxidation of FAs is more oxygen-intensive than glucose oxidation [82, 93, 94]. Leptin is a protein hormone synthesized and secreted by WAT and is known for its ability to inhibit food intake, reduce energy intake, and increase energy expenditure. Generally, leptin mRNA is ubiquitously expressed in all myocardium and has higher expression in the atria than the ventricles [95]. Felines with hypertrophic cardiomyopathy have been shown to exhibit elevated cardiac leptin transcription compared with those without heart diseases, indicating enhanced FA oxidation under pathological conditions [95] (summarized in Fig. 2).

LDAPs and Cardiomyopathy

As mentioned, the dysregulation of cardiac lipids affects the myocardial systolic and diastolic functions, leading to the occurrence of cardiomyopathies. Therefore, maintaining cardiac lipid homeostasis is especially important. LDs are organelles that serve as mediators of lipid metabolism. LDAPs are functional proteins of LDs [63, 96]. Dysfunction of cardiac LDAPs may affect cardiac function, resulting in cardiomyopathies.

Within the heart, the major LDAPs include PLIN2, PLIN3, PLIN4, and PLIN5 [75]. Recent studies have also detected the protein expression of ABHD5, ATGL, BSCL2, and HILPDA in the heart. Among these LDAPs, PLIN2, PLIN5, ABHD5, and ATGL are relatively well-characterized, while there is a scarcity of reports on HILPDA, and its function remains unclear. Although PLIN1 is undetected in the heart, PLIN1 deficiency has been shown to impact the function of the myocardium. Several studies have tried to illustrate the relationship between different LDAPs and cardiomyopathy (shown in Table 1). We will provide a detailed introduction to the role of 4 main LDAPs in cardiac lipid metabolism and cardiomyopathies.

Fig. 2. Schematic diagram of energy metabolism in the heart. The metabolic diversity and flexibility have contributed to the maintenance of normal contractile function of the heart under various physiological and pathophysiological conditions. FA, fatty acid; TAG, triglyceride; DGAT, diglyceride acyltransferase; ATGL, adipose triglyceride lipase; FAO, fatty-acid oxidation; PKC, protein kinase C.

Perilipin 2

PLIN2 was identified in LDs of preadipocytes and was found to be replaced by PLIN1 after the differentiation and maturation of preadipocytes [97]. Previous studies demonstrated that both overexpression and deficiency of PLIN2 can contribute to LD accumulation. PLIN2 has also been proven to promote LD formation, FA-induced insulin secretion, as well as LD stabilization [97, 98].

Transgenic mice with heart-specific overexpression of PLIN2 showed an accumulation of small LDs around the mitochondria with massive TAG deposition in the atria. Still, notably, the heart weights of WT and Tg mice were comparable, with their hearts being analogous in terms of mitochondrial size as well as shape. They suffered from tolerance-induced myocardial infarction. The overexpression of HSL can overcome the tendency of steatosis of PLIN2 [98]. PLIN2-induced LDs were sensitive to lipolytic stimuli after fasting, and hence, the accumulation of LDs would be reduced despite the high concentrations of FFA in the circulating plasma [97]. Cardiac-specific expression of PLIN2 resulted in slower conduction propagation and induced a higher incidence of atrial fibrillation in aged mice [99].

PLIN2 deficiency could result in TAG accumulation in cardiomyocytes, which was seen exclusively in cardiomyocytes but not in cardiac fibroblasts, demonstrating that the effects of PLIN2 deficiency can be cardiomyocyte-specific. Reductions in stroke volume and cardiac output were also observed in PLIN2^{-/-} mice. However, there were no significant differences in cardiac function between PLIN2^{-/-} and wild-type mice [98].

Perilipin 5

PLIN5 is identified predominantly in oxidative tissues, particularly in the heart which is crucial to the cardiac lipid regulation process [100, 101]. Studies have suggested that PLIN5 functions as a lipolysis inhibitor. PLIN5 protects LDs from lipolysis by forming a shield on the surface of LDs, hence preventing ATGL and HSL from reaching the substrate inside the LDs [102]. The hearts of PLIN5^{-/-} mice are detected with relatively small LDs that lack neutral lipids compared with typical LDs [102]. Consistently, myocardial TAG levels and FA uptake were also markedly reduced, with an increase in glucose uptake and ROS production [103]. The mouse model of cardiomyocyte-specific PLIN5 overexpression (CM-PLIN5) was

Table 1. Cardiac phenotype in mice with disruption of LDAP genes expression

LDAPs	Cardiac phenotype in mice with disruption of LDAP genes expression
<i>The PLIN family</i>	
PLIN1	Global knockout of PLIN1 exhibits enhanced lipolysis which leads to ectopic lipid accumulation with enhanced FA transportation and oxidation and eventually develop hypertrophic cardiomyopathy along with left-ventricular diastolic dysfunction at 20 weeks of age.
PLIN2	Transgenic mice with cardiac-specific overexpression of PLIN2 showed accumulation of small LDs around mitochondria and massive deposition of TAG in the atrium, and they suffered tolerating induced myocardial infarction. PLIN2 ^{-/-} mice did not show any reduction in myocardial lipid storage but increased TAG accumulation, with normal cardiac function under baseline conditions or under mild stress.
PLIN4	Mice with PLIN4 deficiency exhibit a marked attenuation in the TAG content along with a concomitant reduction of cardiac PLIN5 expression but maintained normal cardiac function as WT mice.
PLIN5	PLIN5 ^{-/-} mice showed a marked decrease in the content of TAG and FA, along with the generation of ROS significantly promoted. Aged PLIN5 ^{-/-} mice showed worse cardiac function than their WT littermates. Cardiomyocyte-specific PLIN5-deficient mice exhibit cardiac steatosis, increased heart weight, left-ventricular hypertrophy, and mild cardiac dysfunction.
<i>Other LDAPs</i>	
ABHD5	Cardiac-specific ABHD5 knockout mice exhibit cardiomyopathy that is clearly associated with NLSDs and show cardiac hypertrophy and fibrosis and ventricular dysfunction with massive lipid accumulation in cardiomyocytes. Cardiac-specific overexpression of ABHD5 blunted cardiomyopathy but did not cause observable detrimental effect under physiological conditions.
BSCL2	Global BSCL2 ^{-/-} mice display dramatic loss of fat mass and develop insulin resistance and hypertrophic cardiomyopathy.
ATGL	ATGL knockout mice suffer from massive accumulation of TAG in the myocardium and exhibit hypertrophic cardiomyopathy and interstitial fibrosis, which may eventually lead to heart failure and cardiac death.
LDAP, lipid droplet-associated proteins; PLIN, perilipin; LD, lipid droplet; TAG, triglyceride; FA, fatty acid; ROS, reactive oxygen species; NLSD, neutral lipid storage disease; BSCL2, Berardinelli-Seip congenital lipodystrophy type 2; ATGL, adipose triglyceride lipase.	

found to have a 3.5-fold increase in TAG content in the hearts and exhibited restricted ATGL- and HSL-mediated TAG mobilization [104, 105]. Also, chronic cardiac LD overload may lead to mild cardiac dysfunction with reduced FA oxidation gene expression and compromised mitochondrial function and manifest a phenotype of left-ventricular centripetal hypertrophy [104]. In terms of the phenotype, mice with CM-PLIN5 exhibited cardiac steatosis, cardiac weight gain, and mild cardiac malfunction [100, 104, 105].

PLIN5 deficiency was also closely related to abnormal mitochondrial oxidative capacity. Studies revealed intense targeting of PLIN5 to the interface of cardiac LDs and peridroplet-mitochondria (PDM) but not at the mitochondrial matrix. Treatment with CL316, 243 (CL), the β 3-adrenergic receptor, elevated this PLIN5 target [106]. PLIN5 contains a mitochondrial recruitment domain [107, 108]. The LDs of PLIN5-deficient mice tend to dissociate from the mitochondria [103]. In the hearts of

PLIN5-deficient mice, mitochondria exhibited a marked impairment in oxidative capacity by modifying the fatty-acyl composition of phospholipids within the mitochondrial membrane, consequently leading to impaired mitochondrial membrane depolarization [108]. In compensation for this, PLIN5 deficiency is able to stimulate mitochondrial proliferation, which was observed in both animal and cellular experiments [109].

Subsequent studies have focused on the role of PLIN5 in different pathophysiological conditions. PLIN5^{-/-} hearts exhibited postischemic metabolic dysregulation, including a decrease in C₂ and C₄ acylcarnitine levels, but there was no rise in glycogen levels compared to WT littermates. Moreover, after inducing myocardial infarction by either the β -adrenergic agonist dobutamine or myocardial stress, the cardiac function of PLIN5^{-/-} mice was significantly impaired, accompanied by a reduced survival rate [103]. Zheng et al. [69] later reported that the absence of PLIN5 was character-

ized by enlarged myocardial infarct size, accelerated impairment of myocardial systolic and diastolic function, reduced cardiac lipid stores, elevated FFAs in the myocardium during myocardial ischemia-reperfusion, severe impairment of the mitochondrial structure and function, elevated levels of ROS and malondialdehyde, and reduced superoxide dismutase activity. A human cohort study on coronary artery disease found that rs884164, a common noncoding polymorphism known to lower the cardiac expression of PLIN5, leads to reduced cardiac function following myocardial ischemia [103]. More recently, Wang et al. [109] treated PLIN5^{-/-} mice with transverse aortic constriction (TAC) and identified that PLIN5 deficiency aggravated myocardial hypertrophy and caused TAC-induced heart failure. These findings indicate the protective effect of PLIN5 under stress conditions, indicating its role as a treatment solution for cardiomyopathy.

However, one study has observed some contrasting results. In this study, PLIN5^{-/-} mice had greater heart weight and showed elevated mRNA expression of heart failure-associated gene myosin heavy-chain beta (Myh7). However, mRNA expression of other heart failure-associated genes such as myosin heavy-chain alpha (Myh6), Nppa, and Nppb did not differ significantly between PLIN5^{-/-} and PLIN5^{+/+} mice. Besides, PLIN5^{-/-} hearts exhibited slight alterations in gene expression related to FA and glucose metabolism compared with PLIN5^{+/+} hearts. Also, myocardial infarct sizes were similar between isolated, perfused PLIN5^{+/+} and PLIN5^{-/-} mouse hearts. The researchers also found that PLIN5^{-/-} cardiomyocytes retained higher glycogen levels and exhibited enhanced tolerance to hypoxia after preincubation with oleic acid [110]. These findings challenged some of the previous outcomes.

Since most studies have employed global PLIN5 knockout mouse models, cardiomyocyte-specific knockout mice are needed to exclude the effects of systemic lipid dysregulation on the heart in order to investigate the role of PLIN5 on the maintenance of cardiac function. Studies are also needed to further elucidate the function of PLIN5 in the heart and the potential relationship between LDs, PLIN5, and mitochondria.

ABHD5/CGI-58

Recent studies have characterized another LDAP, ABHD5, also known as CGI-58. ABHD5 localized at cytosolic LDs [111]. PKA phosphorylates both ABHD5 and PLIN5, subsequently dissociating ABHD5 from PLIN5, resulting in the activation of ATGL [112, 113]. After the

activation of ATGL, the initial lipolysis starts, transforming TAG to DAG, contributing to lipotoxicity.

In humans, mutations in genes encoding ABHD5 can lead to a rare and fatal syndrome called the Chanarin-Dorfman syndrome (CDS), also neutral lipid storage disease (NLSD) [114, 115]. CDS is characterized by the accumulation of TAG-rich cytoplasmic LDs in most cell types including the cardiomyocytes, and the patients often manifest cardiomyopathy [111]. Although both ABHD5 and ATGL are associated with TAG accumulation in tissues, studies have revealed that their functions are not entirely bound together. On the one hand, patients with impaired ATGL function develop more severe myopathy, whereas mutations in the genes encoding ABHD5 are more associated with the development of ichthyosis, which was unobservable in patients with ATGL deficiency [116]. On the other hand, ABHD5 is found to interact with numerous other lipolysis-regulatory proteins, including PLIN1, PLIN2, PLIN3, and PLIN5 in the PAT family and the FA-binding protein [111]. Patients with NLSD exhibit severe cardiomyopathy, which requires heart transplantation. This clinical phenotype caused by ABHD5 and ATGL mutations indicates that these LDAPs are essential for maintaining normal cardiac function [117].

Jebessa et al. [112] recently explored the specific pathways through which ABHD5 regulated cardiac lipid homeostasis. They found that ABHD5 functions as a serine protease both in vitro and in vivo and cleaves class IIa histone deacetylase 4 (HDAC4) in response to catecholamine stimulation, producing an N-terminal polypeptide labeled HDAC4-NT [112]. However, the activation of ABHD5 for the cleavage of HDAC4 and its association with PKA phosphorylation is still unclear. Studies also found that cardiac-specific ABHD5 knockout mice developed cardiomyopathy related to abnormalities in neutral lipid storage capacity, with massive lipid accumulation in their cardiomyocytes, and predominantly exhibited cardiac hypertrophy and myocardial fibrosis with apparent ventricular dysfunction [112]. Similarly, another muscle ABHD5 knockout mouse model exhibited intracellular deposition of neutral lipids in both cardiac and oxidative skeletal muscles and showed myocardial fibrosis and cardiac remodeling, which may finally result in heart failure. These 2 murine models indicated that cardiac lipid deposition in human CDS patients is probably the result of localized ABHD5 deficiency in the myocardium [118]. Although intracellular lipid deposition was not significantly altered, restoration of HDAT4-NT expression using adeno-associated viruses could attenuate these patho-

logical myocardial changes by inhibiting the hexosamine biosynthesis pathway [112]. These findings challenge the current knowledge of cardiometabolic diseases by suggesting that lipotoxicity is probably not the only cause of cardiac dysfunction in the development of cardiometabolic diseases. Additionally, a preclinical study showed that cardiac-specific overexpression of ABHD5 slowed the progression of cardiomyopathy with no observable adverse effects under general conditions [119]. Further studies are required to convert this new possible approach to practical application.

Adipose TAG Lipase

ATGL, also termed patatin-like phospholipase domain containing 2, is the most researched LDAP. Although ATGLs are primarily detected in the adipose tissue and the breast, they exist in almost all tissues at the protein level [120–123]. ATGL has been reported to localize in the cytoplasm, on the surface of LDs, and in membranes [124–127]. ATGL mainly functions to mediate TAG hydrolysis by converting TAG to a molecule of FFA and another molecule of DAG [128]. ATGL also possesses acylglycerol transacylase activity which allows the transfer of FAs from TAGs to retinols, the hydrolysis of retinyl esters, and acts on TAGs to produce 1,3-DAGs [129]. The truncated mutant ATGL variant lacking the C-terminal protein region of approximately 220 amino acids was unable to localize to LDs and exhibited a marked enhancement in TAG hydrolase activity in vitro, which also suggests that the catalytic activity of ATGL is present in the N-terminal portion [124, 130].

Comparative proteomic analysis of both healthy and dysfunctional rat hearts revealed that ATGL expression on the LD surface was significantly reduced in the dysfunctional hearts, whereas overall ATGL expression levels in the heart remained similar, pointing out that ATGL is associated with the maintenance of normal heart function and that ATGL localization rather than expression in the heart contributes significantly to the maintenance of a healthy heart [32, 131]. Patients with mutations in the gene encoding ATGL would develop NLSD with myopathy, which is characterized by the accumulation of systemic TAG in multiple organs and tissues as well as cardiomyopathy, as described above [124]. Consistently, global ATGL knockout mice showed massive myocardial TAG accumulation along with hypertrophic cardiomyopathy and interstitial fibrosis, which could culminate in heart failure and cardiac death [132]. To exclude the effects of systemic lipid metabolism on the heart, follow-up studies established a cardiac-specific ATGL overexpres-

sion mouse model. They revealed that cardiac-specific ATGL overexpression shielded both diabetic and diet-induced obese mice from developing lipotoxic cardiomyopathy and cardiac steatosis with increased expression of PPAR α [32, 133, 134]. Cardiac-specific ATGL overexpression could also protect against stress-induced myocardial contractile dysfunction and contribute to cardiovascular structural remodeling after TAC [135]. Diabetic cardiomyopathy is currently a common secondary cardiomyopathy in clinical practice which has been recently related to lipotoxicity. Both short-term and prolonged hyperglycemic states can induce LD deposition in the myocardium [136–138], which may be attributed to ATGL inhibition in obese type 2 diabetes mellitus individuals [139]. The specific role of LDAPs in the regulation of lipids in diabetic cardiomyopathy needs further characterization.

Further studies investigated the specific underlying mechanisms by which ATGL inhibition causes disturbances in lipid metabolism and ultimately myocardial hypertrophy. Both the pressure overload-induced cardiac hypertrophy mice model induced by abdominal aortic constriction surgery and PE-induced cardiomyocyte hypertrophy in vitro showed decreased ATGL expression and TAG hydrolase activity [128].

ATGL also interacts with many other LDAPs to exert biological effects. PLIN5 and ABHD5 interact with ATGL independently but exhibit opposite effects. ABHD5 is the coactivator of ATGL whose interaction promotes lipolysis. Conversely, the binding between PLIN5 and ATGL results in reduced lipolysis in cardiomyocytes. However, further studies are required to understand whether PLIN5 explicitly inhibits the functioning of ATGL or suppresses all lipases [140, 141]. PLIN2 also mediates the connection between ATGL and LDs. PLIN2 has been shown to decelerate TAG turnover by cutting the interaction between ATGL and LDs [142]. The deletion of BSCL2 is known to increase ATGL protein stability [143]. Also, the hydrophobic domain of G0S2 interacts exclusively with the patatin-like domain of ATGL, thereby inhibiting ATGL from hydrolyzing TAG to DAG and FFA [144].

The researches examined cardiac lipid metabolism in global ATGL knockout mice under pathological conditions to find that downregulation of ATGL did not attenuate postburn cardio-lipid accumulation and cardiac injuries. However, cardiac-specific overexpression of ATGL upregulated glucose transporter 1 expression as well as reduced cardio-lipid accumulation, thus alleviating burn-induced cardiac lipid dysregulation and heart damages [145], suggesting the therapeutic potential of ATGL in the future.

Conclusion

Despite the current attempts to describe the interaction between lipids, LDs, LDAPs, and cardiomyopathy, related research is still in its infancy. The current research on LDs and LDAPs mainly concentrates on their function in adipose tissue, the liver, and macrophages. This review focuses on the role of LDs and LDAPs in cardiac lipid regulation and their association with cardiomyopathy. The results from cell culture, preclinical, and clinical studies indicate that the predominant function of the PLIN family is to isolate lipids from lipases, contributing to cardiac lipid accumulation. Some other LDAPs, such as BSCL2, promote the accumulation of cardiac lipids, while others, including ATGL, promote the lipolysis process. The role of ATGL in cardiac lipid regulation is probably the most critical. Several LDAPs interact with ATGL to exert their regulatory function on cardiac lipids. These results on the interaction between LDs, LDAPs, cardiac lipid regulation, and cardiomyopathy in cell and animal experiments need to be further applied to human cardiomyopathy. Do changes in LDAP genes and cardiac lipid regulation disorder really result in cardiomyopathy? Can we combat the development of cardiomyopathy or even

reverse its progression by regulating the expression of relevant genes to achieve therapeutic success? Further studies are required to answer these questions.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

W. Huang wrote the first draft; F. Gao and Y. Zhang participated in discussing the manuscript; C. Tianhui participated in polishing the manuscript; C. Xu revised and finalized the manuscript.

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