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Commentary

Pharmacological intervention of liver triacylglycerol lipolysis: The good, the bad and the ugly

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ABSTRACT

Excessive triacylglycerol (TG) accumulation is the distinctive feature of obesity. In the liver, sustained TG accretion leads to nonalcoholic fatty liver disease (NAFLD), eventually progressing to non-alcoholic steatohepatitis (NASH) and cirrhosis, which is associated with complications including hepatic failure, hepatocellular carcinoma and death. Pharmacological interventions are actively pursued to prevent lipid accumulation in hepatocytes and, therefore, to ameliorate the associated pathophysiological conditions. Here, we sought to provide an overview of the pharmacological approaches to up- or downregulate the expression and activities of the enzymes involved in hepatic TG hydrolysis. Fatty acids (FA) released by hydrolysis of hepatic TG can be used for β-oxidation, signaling, and for very low-density lipoprotein (VLDL)-TG synthesis. Originally, lipolysis was believed to be centered in the adipose and to be catalyzed by only two lipases, hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MAGL). However, genetic ablation of HSL expression in mice failed to erase TG hydrolysis in adipocytes leading to the identification of a third lipase termed adipose triglyceride lipase (ATGL). Although these three enzymes are considered to be the main players governing lipolysis in the adipocyte, other lipolytic enzymes have been described to contribute to hepatic TG metabolism. These include adiponutrin/patatin-like phospholipase domain containing 3 (PNPLA3), some members of the carboxylesterase family (CES/Ces), arylacetamide deacetylase (AADAC), lysosomal acid lipase (LAL) and hepatic lipase (HL). This review highlights the consequences of pharmacological interventions of liver lipases that degrade TG in cytosolic lipid droplets, in the endoplasmic reticulum, in the late endosomes/lysosomes and along the secretory route.

1. Introduction

Excessive accumulation of triacylglycerol (TG) in the cytosol of hepatocytes leads to nonalcoholic fatty liver disease (NAFLD), the most common form of chronic liver disease. NAFLD embraces a wide range of diseases from hepatic steatosis to nonalcoholic steatohepatitis, the more aggressive form of fatty liver disease, which can progress to cirrhosis and its complications including hepatic failure and hepatocellular carcinoma [1]. Therefore, a tight regulation between TG synthesis, hydrolysis, secretion and fatty acid (FA) oxidation is required to prevent lipid accretion as well as lipid depletion from cells. The aforementioned regulation may be achieved by the use of drugs modulating or even altering the activities or expression of the key enzymes involved in TG metabolism. There has been significant interest to target these enzymes in order to ameliorate the pathological conditions associated with their down- or over-expressions and/or activities. Although most of the approaches to date have been discouraging, the development of new specific compounds targeting lipid hydrolases is desirable and together with changes in patients' life style would provide a path to treat aberrant metabolism related illnesses. This review focuses on the pharmacological aspects of these interventions. Table 1 shows a summary of the compounds described as modulators of liver lipase activities. TG catabolism in the liver is achieved by lipases localized on cytosolic lipid droplets (LDs), in the late endosome/lysosome compartment or

Abbreviations: 2-AG, 2-arachidonoylglycerol; AADAC, arylacetamide deacetylase; ABHD5/CGI-58, alpha-beta-hydrolase domain containing 5/comparative gene identification-58; ATGL/ PNPLA2, adipose triglyceride lipase; CE, cholesteryl esters; CES/Ces, carboxylesterase; DG, diacylglycerol; ER, endoplasmic reticulum; FA, fatty acids; G0S2, G0/G1 switch gene 2; HFD, high fat diet; HILPDA, hypoxia-inducible LD-associated protein; HL, hepatic lipase; HSL, hormone-sensitive lipase; LAL, lysosomal acid lipase; LD, lipid droplets; LDL, low density lipoprotein; MAGL, monoacylglycerol lipase; MG, monoacylglycerol; NAFLD, nonalcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, adiponutrin/patatin-like phospholipase domain containing 3; RE, retinyl esters; TG, triacylglycerol; VLDL, very low-density lipoprotein

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Table 1
Summary of the compounds described as modulators of liver lipase activities.

Lipase	Substrates	Compound	Action	Ref.
ATGL	TG	Atglistatin	Inhibitor	[17]
	DG (weak)	Peptide derived from G0S2	Inhibitor	[21]
		Hydrazine-based compounds	Inhibitor	[22]
		Atglistatin analogs	Inhibitors	[24]
		ABHD5 compound	Activator	[27]
HSL	DG, CE	Carbamate-based pseudosubstrates	Inhibitors	[36]
	TG, MG, RE			
		Cay10499	Inhibitor	[20]
		Unnamed	Inhibitor	[32]
		Boronic acid derivates	Inhibitors	[42,43]
		24b	Inhibitor	[41]
		BAY 59-9435 (BAY)	Inhibitor	[37]
MAGL	MG	Multiple compounds	Inhibitors	[51]
		JZL184	Inhibitor	[52]
		MJN110	Inhibitor	[53]
		Reversible inhibitors	Inhibitors	[50]
PNPLA3	TG	No specific modulators have been described yet		
Carboxylesterases	TG, DG, MG	Paraoxon (E600)	Inhibitor	[73,82,83]
	CE, RE	Bisbenzene sulphonamides	Inhibitor	[86]
		1,2-Diones	Inhibitor	[86]
		Benzils, trifluoroketones	Inhibitors	[86]
		Isatins, flavonoids	Inhibitors	[87]
		27-Hydroxycholesterol	Inhibitor	[88]
		Lovastatin and Simvastatin	Inhibitors	[90]
		Ezetimibe	Inhibitor	[90]
		GW4064	Inhibitor	[90]
		JW480	Inhibitor	[90]
		WWL113	inhibitor	[91]
		WWL229	Inhibitor	[91]
		GR148672X	Inhibitor	[73]
AADAC	TG, DG, CE	No specific compounds have been described yet		
LAL	CE, TG	HEP14	Activator	[103]
		Lalistat	Inhibitor	[105]
HL	TG, phospholipids	No specific modulators have been described yet		

associated with the endoplasmic reticulum membranes or lumenal LDs (Fig. 1).

2. Pharmacological intervention of cytosolic lipid droplet lipases involved in hepatic TG metabolism

Several lipases are involved in hydrolysis of TG stored in cytosolic LDs. These lipases can be found both in the soluble cytosolic fraction and on the surface of cytosolic LDs [2]. There are both similarities and also substantial differences between hydrolysis of lipids stored in hepatic cytosolic LDs and the well-characterized mobilization of adipose tissue cytosolic LDs where hydrolysis of TG to glycerol and three FA molecules are catalyzed by sequential reactions of adipose triglyceride lipase (ATGL, annotated as patatin-like phospholipase domain containing protein 2, PNPLA2 or desnutrin) hydrolyzing TG to diacylglycerol (DG) and FA [3], hormone-sensitive lipase (HSL) breaking down DG to monoacylglycerol (MG) and FA and monoacylglycerol lipase (MAGL) completing the lipolytic reaction of MG to glycerol and FA [4]. The hepatic parenchymal cells also contain the complete machinery to undergo complete hydrolysis of TG to glycerol and FA. Both ATGL and MAGL are expressed in the parenchymal cells, however, HSL only appears to be expressed in rodent liver, but not in human liver [5]. In addition, specific ablation of HSL expression in mouse liver had no effect on liver fat content [6], suggesting that additional lipases might compensate for the absence of HSL.

2.1. Adipose triglyceride lipase (ATGL)

ATGL is expressed in virtually all tissues with highest expression in white and brown adipose tissues; however, it is also present at much lower levels in other tissues, including the liver [3]. ATGL localizes to the surface of cytosolic LDs and other intracellular membranes [7]. ATGL displays high hydrolytic activity toward TG, very weak activity against DG and no activity against cholesteryl esters (CE) [3]. ATGL becomes 20 times more active than in its basal condition after binding a cofactor alpha-beta-hydrolase domain containing 5/comparative gene identification-58 (ABHD5/CGI-58) [8]. ATGL activity can be inhibited by interaction with G0/G1 switch gene 2 (G0S2) [9] and by hypoxia-inducible LD-associated protein (HILPDA) [10]. In the low lipolysis state (eg. during the fed state) ABHD5/CGI-58 is sequestered away from ATGL by binding to perilipin 1 (adipose tissue) or perilipin 5 (the liver and other tissues expressing perilipin 5). Fasting leads to increased cAMP levels, activation of protein kinase A and subsequent phosphorylation of perilipins leading to release of ABHD5/CGI-58 that becomes available for activation of ATGL [11].

Two possible scenarios could be envisioned when ATGL activity is either downregulated or upregulated: a) downregulation of ATGL would lead to decreased plasma FA concentration and amelioration of FA induced lipotoxicity; b) overexpression of ATGL would lead to decreased lipid accumulation in tissues with released FA directed to β-oxidation. Unexpectedly, global ATGL deficiency in mice did not protect against lipotoxicity but caused multi-organ TG accumulation, with a massive preference for the heart [12] leading to impaired heart function and premature death from cardiac malfunction. ATGL deficiency results in impaired release of FA from adipose tissue during fasting, causing decreased provision of substrates for hepatic very low-density lipoprotein (VLDL) assembly and consequently low plasma lipid levels. Thus, global ATGL deficient mice depend on glucose to fulfill

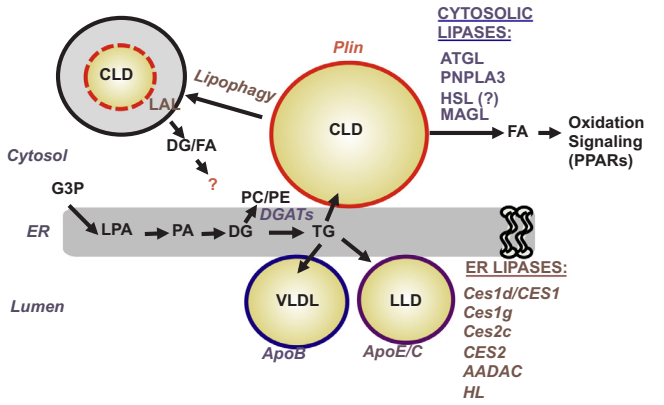

Fig. 1. The liver generates three distinct types of LDs each with it's a unique set of associated proteins and metabolic fate. Cytosolic LDs (CLDs) lipids are hydrolyzed by a set of lipases including adipose triglyceride lipase (ATGL), patatin-like phospholipase domain containing 3 (PNPLA3), hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MAGL) to liberate fatty acids (FA) used for energy production through beta-oxidation and for signaling. During prolonged starvation CLDs can undergo lipophagic process and lipids are hydrolyzed in the lysosomal/late endosomal compartment by lysosomal acid lipase (LAL). Liberated FA re principally used for energy production (beta-oxidation). Two types of LDs are formed in the endoplasmic reticulum (ER), apoB-containing lipoprotein particles (VLDL) that are secreted into the circulation and lumenal LDs (LLDs) that have been proposed to provide substrates for lipidation of apoB. ER-associated lipids can be hydrolyzed by several members of the carboxylesterase (CES/Ces) family of enzymes, arylacetamide deacetylase (AADAC) and hepatic lipase (HL), which can affect both VLDL assembly and storage of lipids in CLDs.

their energy demands and, as expected, these mice are highly insulin sensitive despite steatosis. In humans, mutations in the *PNPLA2* gene encoding ATGL results in the development of neutral lipid storage disease associated with myopathy [13]. On the other hand, selective hepatocyte ablation of *Pnpla2* expression in mice results in 4-fold increase of hepatic TG storage accompanied by decreased FA oxidation without affecting plasma glucose or lipid concentrations, glucose tolerance and insulin sensitivity [14]. The phenotype of global ATGL deficiency in mice suggests that the benefit of developing of ATGL inhibitors to increase glucose sensitivity and decrease hyperlipidemia would be offset by cardiac lipotoxicity and steatosis. Importantly, cardiac ATGL expression completely rescued the lethality of global ATGL deficient mice [15]. Furthermore, cardiac-exclusive ATGL expression reversed cardiac hypertrophy and the massive TG accumulation observed in hearts from whole-body ATGL-deficient mice, together with improved insulin sensitivity and glucose tolerance. Adipose-specific ATGL deficient mice showed improved systemic glucose and insulin tolerance, due to augmented hepatic insulin signaling, which was accompanied by marked reduction in diet-induced hepatic steatosis as well as hepatic immune cell infiltration and activation [16]. Considering the role of ATGL in the first and rate-limiting step in TG hydrolysis, this enzyme becomes an interesting target for the design of new pharmacological inhibitors with therapeutic potential to treat metabolic disorders, with the caveat that inhibitors would need to avoid inactivation of cardiac ATGL activity.

Mayer et al. [17] have developed a specific inhibitor of ATGL named Atglistatin. This compound inhibits the activity of mouse ATGL (IC_{50} = 0.7 μM) in a competitive and reversible manner [17]. Atglistatin-mediated inhibition of TG hydrolysis was found to be associated with a beneficial metabolic phenotype. In recent very promising studies, Schweiger et al. [18] demonstrated that wild-type mice fed a HFD and treated with Atglistatin both acutely and chronically were leaner, highly insulin sensitive and resistant to the development of NAFLD compared to untreated HFD-fed mice. The most important property of Atglistatin from a therapeutic point of view was lack of systemic TG

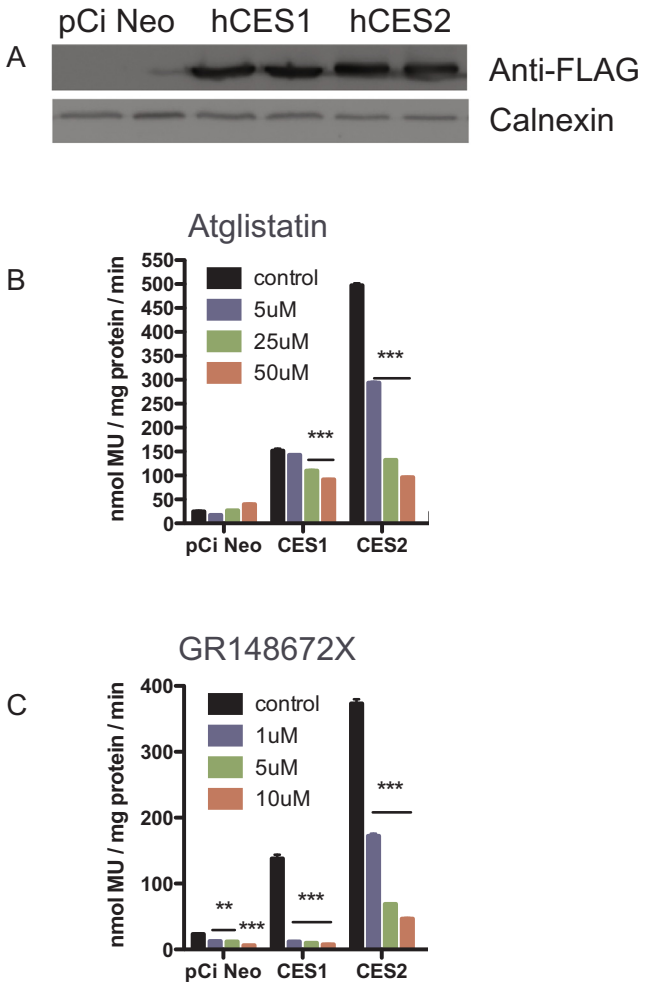

Fig. 2. Atglistatin and GR148672X inhibit human carboxylesterases. (A) Rat hepatoma cells McArdle RH7777 stably transfected with empty pCI Neo vector or with pCI Neo vector encoding FLAG-tagged human CES1 and CES2. Calnexin is an ER protein used as a loading control. McArdle RH7777 microsomal fractions were incubated in the presence of various concentrations of either Atglistatin (B) or GR148672 (C) and analyzed for carboxylesterase activity using MU-heptanoate.

accumulation in tissues such as skeletal muscle, cardiac muscle or liver that was seen in global ATGL deficient mice [12,13,19]. Atglistatin delivered to mice did not accumulate in cardiac or skeletal muscle and therefore did not inhibit ATGL in these tissues [18]. A downside to these findings is that Atglistatin was unable to inhibit lipolysis in human adipocytes, thus the search for new inhibitors targeting human ATGL in adipocytes is ongoing. In addition, while Atglistatin does not inhibit human ATGL or human/mouse HSL or human MAGL at 5 μM concentration [20], it can inactivate human carboxylesterases CES1 [20] and CES2 (Fig. 2A and B).

Other ATGL-mediated lipolysis inhibitors have been developed in the last few years, including a peptide derived from G0S2 protein [21] and a hydrazine base compound [22]. G0S2 acts as a physiological inhibitor of ATGL [23], and the hydrazine compound resulted to act not only on ATGL, but also on HSL, but the compound turned out to be more toxic than Atglistatin. Other approaches were recently evaluated through testing several Atglistatin derivatives including carbamate, carbamodithioate, thiourea, carbamothioate, hydrazinecarboxamide, and guanidine from the urea chemotype. These derivatives were assessed for their inhibition of lipolysis in cultured adipocytes as an indicator of their potential to inhibit ATGL in adipose tissue [24]. Among

the 29 tested Atglistatin analogs, one of the thiourea compounds showed potent ATGL inhibitory activity *in vitro*, which was more potent than Atglistatin, and its inhibitory activity *in vivo* was similar to that of Atglistatin *in vivo* [24]. However this compound remains to be validated since thiourea carries severe toxic effects which makes it unsuitable for drug design [25].

On the other hand, liver ATGL induction was also thought to represent a pharmacological therapeutic target for ameliorating NAFLD. In 2008, Reid and collaborators [26] reported a crucial role of hepatic ATGL in lipid partitioning and fate based on the evidence that ATGL deficient mice accumulated 2.3-fold more TG than normal mice in the liver. Adenovirus-mediated overexpression of ATGL, as well as HSL, contributed to increased lipolysis promoting FA oxidation, stimulating direct release of FA, and ameliorating hepatic steatosis without increasing hepatic apoB or TG secretion. Rondini and colleagues [27] characterized compounds that activate lipolysis by directly dissociating ABHD5/CGI-58 from perilipin 1 and perilipin 5. Fibric acids fenofibrate, bezafibrate and clofibric acid induce ATGL expression in a dose dependent manner and in a similar extent [28]; however, the mechanisms of action of the fibrates were clearly different but not evaluated.

2.2. Hormone-sensitive lipase (HSL)

Although historically considered as a TG lipase, HSL has been demonstrated to act as a DG lipase rather than TG lipase (10 to 20 fold greater preference for DG over TG and MG) [29,30]. It is now accepted that HSL has a broad spectrum of substrate specificity and is capable of hydrolyzing TG, DG, MG, CE, and retinyl esters (RE) [31]. HSL (gene nomenclature *Lipe* for mouse and *LIPE* for human) expression is the highest in adipose tissue; however it is also found in adrenal, ovary, testis, and to a lesser extent in muscles (skeletal and cardiac) and mouse macrophages. Decreased HSL abundance has been described in obesity [32] and absence of HSL has marked effects on fat metabolism, glucose homeostasis, and cell signaling in humans [33]. HSL deficiency in mice does not increase adipose TG storage, which could be partially due to downregulation of lipogenesis [34]. Most if not all of the studies on inhibition of HSL were centered on the action of the inhibitors in the adipose tissue. The reason for this might be that although HSL is present in mouse liver, it is still not clear which lipase would serve for the corresponding function in human liver where HSL expression has not been detected. Hepatic overexpression of HSL in mice reduces hepatic steatosis by promoting FA oxidation; however, since human liver does not express HSL, the development of potential liver-targeted activators of HSL activity does not appear to be a viable strategy. On the other hand, specific ablation of adipose HSL expression in mice was shown to cause fatty liver [6], similarly to human subjects with hereditary HSL deficiency [33].

Only a few HSL inhibitors have been designed and evaluated so far. One of the main issues to consider when inhibiting HSL is the possible accumulation of intracellular DG, which has been implicated in the activation of a serine/threonine kinase cascade leading to attenuation of insulin signaling, but also because HSL inhibitors act as pseudo-substrates. These pseudo-substrates undergo a hydrolytic process releasing a toxic subproduct, which is of considerable concern since the chemical nature of the subproduct is variable: it could be a toxic product itself or it could also be a product that accumulates in tissues following glutathionylation or sulfonylation [35]. Chemical inhibition of HSL activity in wild-type and ATGL-deficient mice suggested that HSL accounts for about 10% of cytosolic TG lipase activity; however, up-regulation of other lipases usually occurs to compensate for the loss of hepatic HSL activity [26]. Potent (IC_{50} < 50 nM) and selective carbamate-based reversible pseudo-substrate HSL inhibitors have been developed by Novo Nordisk [36]. These inhibitors did not show any activity toward other tested esterases and lipases (hepatic, pancreatic and lipoprotein lipases, butyryl and acetylcholine esterases). An HSL inhibitor 4-isopropyl-3-methyl-2-{1-[3-(*S*)-methyl-piperidin-1-yl]-methanoyl}-2H-isoxalo-5-one (BAY 59-9435) [37] did not show any activity toward ATGL and did not have any effect on lipolysis in HSL deficient mice [38] suggesting specificity. On the other hand, the commonly used HSL inhibitor Cay10499 shows similar inhibitory potency toward ATGL and MAGL and 10-fold increased potency toward CES1 [20]. It has been shown that HSL haploinsufficient mice [39] or mice treated with an unnamed HSL inhibitor [32] presented with improved insulin tolerance without impact on body weight, fat mass, and adipose tissue inflammation in mice subjected to a HFD, suggesting that a long-term moderate inhibition of adipose tissue lipolysis may be beneficial in the treatment of obesity-related insulin resistance; however, these studies did not analyze the impact of decreased HSL activity in the liver or any other tissues.

Finally, Ogiyama et al. have developed several compounds that function as reversible HSL inhibitors [40–42]. They first developed boronic acid derivatives based on the reported feature as reversible HSL inhibitors [43]. Gavage of rats with one of these HSL inhibitors (IC_{50} = 7 nM) at 3 mg/kg body weight led to decreased plasma glycerol concentration, demonstrating its antilipolytic action [42]. However, despite the striking *in vitro* and *in vivo* inhibition of HSL-catalyzed lipolysis, subsequent studies revealed that boronic acid-derived compounds had the potential to form reactive metabolites. This, as stated above, is an undesirable effect that could cause organ toxicity and carcinogenesis, since these metabolites form covalent adducts with biological macromolecules. Thus, a new compound (24b) was developed with a potent HSL inhibitory activity (IC_{50} = 2 nM). Oral administration of 24b at 3 mg/kg body weight significantly decreased plasma glycerol concentration in rats but no other physiological parameters such as adipose, plasma and hepatic lipid concentrations have been evaluated [41]. Additionally, while 24b inhibited recombinant human HSL activity, the selectivity of this compound is not clear, that is, activity toward ATGL, MAGL or carboxylesterases has not been tested/reported.

2.3. Diacylglycerol lipase (DAGL)

In addition to HSL, two DG hydrolyzing enzymes DAGLα and DAGLβ have been characterized. These enzymes primary function appears to be the synthesis of the endocannabinoid signaling lipid 2-arachidonoylglycerol (2-AG) since DAGLα and DAGLβ deficient mice show a 50% and 90% reduction of 2-AG in the liver, respectively [44]. Physiological roles of the two DAGLs aside from the documented effect on generation of 2-AG is unclear and liver lipid metabolism in these mice has not been studied. Lower 2-AG, arachidonic acid and inflammatory eicosanoids in macrophages from DAGLβ mice [45] suggested that inhibition of this enzyme could be beneficial. Several individual (KT109 and KT172) and dual (LE1105) DAGL inhibitors have been developed and tested for lowering 2-AG, arachidonic acid and pro-inflammatory eicosanoid concentrations in mice (reviewed in [46]). In addition, another DAGL inhibitor RHC20867 is widely used in biomedical research. However, recently it has been shown that in addition to DAGLs, all of these inhibitors potently inhibit another monoacylglycerol lipase ABHD6 and human carboxylesterase CES1 [20], therefore, their utility in studying the role of DAGLs in hepatic lipid metabolism remains questionable.

2.4. Monoacylglycerol lipase (MAGL)

MAGL hydrolyzes MG to glycerol and FA and therefore regulates several physiological and pathophysiological processes since both the substrate and the product can act as signaling lipids. MAGL has no activity against DG, TG, CE or RE. Mouse, rat and human MAGLs share ~84% protein sequence identity. The enzyme is ubiquitously expressed, and is found in the cytosol, particulate (membrane) fraction, and on the surface of the LDs [47]. MAGL was demonstrated to possess

high specific activity toward both medium-chain and long-chain MG, and together with its presence in many tissues, it appears that the final step in TG hydrolysis is not highly regulated. MAGL deficient mice have been generated and analyzed [4]. MG hydrolase activity in the cytoplasmic fraction from liver, white adipose tissue and brain prepared from MAGL deficient mice was decreased by 30%, 50% and 100%, respectively; indicating that liver and adipose tissue possess other MG hydrolytic enzymes, while in the brain MAGL appears to be the sole enzyme responsible for MG hydrolytic activity. Correspondingly, MG concentrations were increased 4-fold in adipose tissue and brain and 2–3-fold in the liver. Absence of MAGL in the adipose tissue of MAGL deficient mice reduced glycerol generation and release during stimulated hydrolysis by 25%, which is in agreement with the presence of additional MG lipolytic activity. Hepatic TG and VLDL secretion was decreased by 50% in MAGL deficient mice despite comparable plasma FA concentration that serve as substrates for both processes [4]. Importantly, MAGL deficiency was shown to prevent HFD-induced insulin resistance [4].

One of the most important functions of MAGL is diminishing cannabinoid signaling in the brain through catalyzing 2-AG hydrolysis [48]. 2-AG is the most abundant endogenous agonist of cannabinoid receptors in the body. Furthermore, 2-AG represents an important source for arachidonic acid, the precursor of prostaglandins, leukotrienes, lipoxins and other (pro)inflammatory signaling molecules [49].

MAGL participates in the regulation of lipid and glucose metabolism and energy homeostasis in a prominent and differential way depending on the context, since hyperphagy is observed upon acute inhibition of MAGL, whereas reduced HFD-induced obesity is attenuated after a long-term inhibition of MAGL (reviewed in [50]).

In the last few years substantial experimental evidence has accumulated, supporting the many benefits of blocking MAGL for modulating multiple lipid signaling pathways, and therefore, modulating disease etiology. Despite the obvious benefits, given the importance of the endocannabinoid system in the multiple physiological processes, MAGL inhibition requires cautious evaluation [50]. Several MAGL inhibitors have been developed; as reviewed by Granchi et al. [51] approximately 20 patents on MAGL inhibitors were deposited in the last 5 years. Cravatt's group has pioneered the development of and characterization of MAGL inhibitors. Initial efforts led to discovery of potent irreversible MAGL inhibitor JZL184 [52]; however, this inhibitor also blocks activities of other hydrolases, including fatty acid amide hydrolase and carboxylesterases. More recent efforts yielded a potent MAGL inhibitor MJN110 (IC_{50} = 0.4 nM using 2-AG as substrate), which did not show inhibitory activity against fatty acid amide hydrolase and has been shown to significantly attenuate mechanical allodynia in a rat model of diabetic neuropathy [53]. Many other compounds have been designed [50]. Schlosburg et al. [54] reported that chronic MAGL ablation produces functional antagonism of the endocannabinoid system and a mild physical dependence, together with an impaired endocannabinoid-dependent synaptic plasticity. In this sense, blockade of the CB1 receptor with rimonabant is of interest. The drug ameliorated hepatic steatosis and dyslipidemia in obese mice and rats [55], and promoted weight loss and improved cardiovascular risk factors in obese and diabetic patients [56,57]. Nevertheless, rimonabant was withdrawn from clinical use for the treatment of obesity, due to increased anxiety, depression, and suicidal tendencies. In 2011, Busquets-Garcia and collaborators provided evidence that the functional antagonism associated with chronic MAGL blockade may be circumvented by only partial blockage of MAGL [58] without the undesirable associated effects. This might be achieved by the use of reversible MAGL inhibitors that could temporarily inhibit the enzyme, while leaving the endocannabinoid system intact [54,59].

Finally, one of the most important features of MAGL inhibition is the protection of the effects associated with the use of COX-1/COX-2 inhibitors, since MAGL inhibitors do not affect arachidonic acid and prostaglandin pathways in the gastrointestinal tract [49].

In summary, 2-AG degradation by MAGL affects lipid signaling by cannabinoid receptor-dependent and independent mechanisms, the reason why MAGL is considered to be a promising drug target for several disorders including pain and inflammation, metabolic disorders such as obesity and diabetes, neurodegenerative illnesses such as Alzheimer's disease, anxiety and epilepsy, cell migration, invasiveness, and tumorigenicity in various aggressive cancer cells [50].

2.5. Patatin-like phospholipase domain containing 3 (PNPLA3)

PNPLA3 (also called adiponutrin) shares significant sequence identity with ATGL (PNPLA2, desnutrin) and similarly resides on LDs. PNPLA3 expression is highest in the liver, but the enzyme is also expressed in other tissues including adipose [60,61]. PNPLA3 is upregulated by several nutritional and metabolic factors including insulin, glucose and hepatic lipid status [62] and its expression is altered in several models of obesity and metabolic diseases [63]. A genetic variant of PNPLA3 with I48M substitution is associated with reduced *in vitro* TG lipase activity [64] and with liver fat accumulation, hepatic inflammation and susceptibility to steatohepatitis [65]. However, genetic deficiency of PNPLA3 in mice does not lead to hepatic steatosis, suggesting that the lipid accumulation in PNPLA3 I48M is not due to loss-of-function [62,66], but rather PNPLA3 I48M might be inducing *de novo* lipogenesis [67]. All pharmacological efforts in subjects with PNPLA3 I48M substitutions could be focused on the development of therapies centered on increasing lipase (ATGL?) activity or decreasing lipogenesis. PNPLA3 activity appears to be beneficial for lowering liver lipid concentration and overexpression of the wild-type PNPLA3 is protective against liver fibrosis [68].

3. Pharmacological intervention of endoplasmic reticulum (ER) lipases

Much less known and studied, is the impact on lipid metabolism by pharmacological intervention of lipases localized in the ER. These lipases include several members of the carboxylesterase gene family, arylacetamide deacetylase and hepatic lipase that has transient presence in the ER before being secreted from the hepatocyte. Over the past decade studies on mouse carboxylesterases 1d (Ces1d/Ces3/Tgh), 1g (Ces1g/Ces1/Es-x) and Ces2c as well as human carboxylesterases CES1 and CES2 have demonstrated important roles for these enzymes in hepatic lipid metabolism ([69–75] reviewed in [76]). Carboxylesterases and arylacetamide deacetylase have been extensively investigated for their ability to hydrolyze a wide variety of ester and amide (pro)drugs (for recent reviews see [77–79]).

3.1. Carboxylesterases

Hepatic VLDL assembly relies on obtaining lipid substrates from TG storage pools (pre-existing LDs) through lipolysis followed by reesterification [80]. As stated above, HSL and ATGL expression in the liver are very low and their activities do not appear to contribute substrates for hepatic VLDL secretion, indicating that other lipases may be playing this important role in the liver. Rat/mouse carboxylesterase Ces1d and its functional human ortholog CES1 were demonstrated to participate in the VLDL assembly process [71,73,75,81]. Ablation of Ces1d activity through genetic approaches resulted in decreased plasma TG [70,71]. Surprisingly, this was not accompanied by increased hepatic TG storage but rather increased FA oxidation and protection against HFD-induced steatosis/steatohepatitis and led to improved glucose metabolism [81]. These data suggested that CES1 could be a pharmacological target for treatment of metabolic syndrome, steatosis and hyperlipidemia. Interestingly, ablation of closely related family member Ces1g resulted in mild obesity accompanied by hepatic steatosis, and hyperlipidemia [69]. Similar to Ces1g, ablation of Ces2c induced fatty liver and inflammation, while overexpression of Ces2c in obese mice reduced

steatosis [72]. The human functional ortholog(s) of Ces1g and Ces2c have not yet been defined. Recent elegant studies [74] demonstrated that decreased human CES2 is associated obesity, which plays a causative role in the pathogenesis of obesity-related metabolic disorders, and overexpression of CES2 in mice reversed HFD-induced steatosis. Thus, CES2 and Ces1g/Ces2c appear to have similar effect on lipid metabolism.

CES1 appears to be an attractive inhibition target for mitigating metabolic syndrome complications, however, because of the reported role of this enzyme in (pro)drug metabolism, potential drug-drug interactions need to be taken into consideration. Carboxylesterases are potently inhibited by organophosphates, such as paraoxon (diethy-4-nitrophenylphosphate, E-600) [73,82,83]. To date, no drug has been designed to selectively and specifically inhibit CES1 activity; however, a few inhibitors had been developed that show preference for CES1 over CES2 [84–86]. These include bisbenzene sulphonamides, 1,2-diones, benzils, isatins and trifluoroketones (reviewed in [86]). CES enzymes can also be inhibited *in vitro* by a number of natural compounds [87] including (poly)unsaturated fatty acids, flavonoids and tanshinones. Importantly, CES1 shows significant selectivity for 27-hydroxycholesterol (IC_{50} < 50 nM) compared with CES2 (IC_{50} > 100 nM) [88]. Recently, it has been shown that CES1 inactivation reduces 27-hydroxycholesterol levels and attenuates LXR-mediated transcription of genes involved in cholesterol metabolism in macrophages [89]. Interestingly, several statin molecules that are used clinically to lower hyperlipidemia through inhibition of a rate-limiting enzyme in cholesterol synthesis HMG-CoA reductase potently inhibit carboxylesterase (both CES1 and CES2) activity. These include Lovastatin and Simvastatin [90]. A cholesterol absorption lowering drug Ezetimibe was also found to significantly inactivate both CES1 and CES2 as was the FXR agonist GW4064 and a 2-acetylmonoalkylglycerol ether (MAGE) hydrolase KIAA1363 inhibitor JW480 [90]. Cravatt and Saez groups have synthesized and tested a number of carboxylesterase inhibitors [91]. One of these inhibitors WWL113 was found to inactivate human CES1 and mouse Ces1d, Ces1g, Ces1c and Ces1f, but also exhibited activity toward monoacylglycerol lipase ABHD6, while another inhibitor WWL229 appeared to be selective for Ces1d [91]. Treatment of obese diabetic mice with WWL113 (30 mg/kg body weight) for nine weeks improved dyslipidemia and glucose metabolism, reduced expression of hepatic lipogenic genes and induced FA oxidation genes [91]. These studies provided another proof-in-principle that pharmacological inhibition of CES1 might improve obesity-driven metabolic dysregulation. Glaxo-Welcome developed a CES1 inhibitor 4,4,4-trifluoro-2-[2-(3-methylphenyl)hydrazono]-1-(2-thienyl)butane-1,3-dione, GR148672X, (IC_{50} = 5 nM) that was tested for modulation of lipid metabolism. Treatment of hepatocytes [73] hepatocytes with GR148672X significantly decreased TG secretion. However, while GR148672X did not show any activity against mouse Ces1g [92] it does inhibit human CES2 (Fig. 2C), albeit with reduced efficacy.

3.2. Arylacetamide deacetylase (AADAC)

Another quantitatively important ER lipase is arylacetamide deacetylase (AADAC) [2]. AADAC shares protein sequence homology with HSL in the active site [93]. In humans, AADAC protein is present in the liver and the intestine with much lower AADAC expression in adrenal gland and pancreas [93]. Studies on substrate specificity of AADAC demonstrated that AADAC has preference for DG instead of TG [94] and can hydrolyze cholesteryl esters [95]. Expression of AADAC in rat hepatoma McArdle RH-7777 cells that lack endogenous AADAC resulted in decreased TG accumulation and increased FA oxidation [94]. HCV infection of Huh7.5 cells results in downregulation of *AADAC* expression and defective lipolysis [96]. *In vivo* evidence for the role of AADAC in lipid metabolism is still missing, however, hepatic AADAC activity is decreased in human obesity [74]. These studies suggest that inhibition of AADAC activity might not be beneficial. AADAC has been shown to hydrolyze several therapeutic drugs, including an anti-androgen drug flutamide used for the treatment of prostate cancer (reviewed in [78]). Shimizu et al. [90] screened 542 chemicals for inhibitors against human CES1, CES2, and AADAC. A specific AADAC inhibitor that would not show some activity either against CES1 or CES2 was not identified; however, several compounds exhibited AADAC preference over CES1 or CES2, including a natural flavonoid kaempferol, an angiotensin II antagonist Irbesartan and resveratrol. From lipid metabolism point of view it seems that increasing AADAC activity rather than inhibition would be beneficial. Positive regulators of *AADAC/Aadac* expression are currently unknown. On the other hand, mouse hepatic *Aadac* expression appears to be suppressed by activation of nuclear hormone receptors AhR, CAR, PXR and Nrf2 [97].

4. Lysosomal acid lipase (LAL)

As its name suggests, LAL is localized to the acidic organelle lysosome/late endosome. Historically, LAL is known to be crucial for hydrolysis of CE (and TG) present in LDL and chylomicron remnants taken up into cells through endocytosis. Absence or near-complete deficiency in LAL activity results in severe metabolic diseases, Wolman's disease and cholesteryl ester storage disease, respectively. More recently, LAL has also been reported to hydrolyze TG and CE in cytosolic LDs that are delivered to late endosomes/lysosomes by a process termed lipophagy [98]. Many additional studies have shown the importance if lipophagy in hepatic lipid metabolism, including signaling crosstalk between ATGL and lysosomal lipolysis and these findings have been recently documented in several excellent reviews [96,99–102] Inhibition lipophagy was shown to increases intracellular lipid storage with a concomitant decrease in FA oxidation. Therefore, activation of lipophagy (and LAL) might be beneficial for treatment of NAFLD. Lipophagy is activated during prolonged fasting/starvation, by treatment with resveratrol or by inhibitors of mTORC1. Much less is known about direct activators of LAL. Expression of the gene encoding LAL (*Lipa*) is dependent on activation (nuclear translocation) of master transcriptional regulators of lysosome biogenesis TFEB and TFE3. Li et al. recently identified a natural small-molecule compound named HEP14 that dramatically increased TFEB nuclear localization [103]. Treatment of HepG2 hepatoma cells with HEP14 reduced the number of LDs demonstrating the therapeutic potential of increasing LAL expression [103]. Helquist's group have synthesized a number of LAL inhibitors [104], one of which (Lalistat2) has been useful in studying lipid metabolism after acute inhibition of LAL [105].

5. Hepatic lipase (HL)

Hepatic lipase is synthesized by hepatocytes (and macrophages) and secreted out of the cells where it hydrolyzes TG and phospholipids in high-density and intermediate-density lipoproteins. HL acquires its lipase activity intracellularly [106], and therefore it was not unexpected to find that HL exerts some influence on the formation of lumenal LDs and secretion of VLDL. Increased presence of active HL in the ER compartment in hepatoma cells depleted TG stores, attenuated VLDL secretion and increased FA oxidation [107]. These findings suggest that activation of HL could be beneficial for mitigating liver TG accumulation, however, HL activation would also increase hepatic cholesterol uptake due to augmented lipoprotein uptake, and therefore it is not clear whether HL is suitable for pharmacologic intervention. HL activity can be augmented by androgens [108] and fibrates [109]. There are no reported HL inhibitors.

6. Conclusion

To conclude, liver lipases play crucial roles in health and disease and represent attractive targets for pharmacological intervention. Many inhibitors targeting hepatic lipases have been developed and used in

various studies. Unfortunately, most of the inhibitors show lack of specificity and this should be taken into account while interpreting data using these compounds without appropriate controls (such as treatment of cells/animals lacking the lipase for which the particular small molecule was developed).

While initial results from ATGL deficient mice and from humans with ATGL deficiency suggested that inhibition of this enzyme would be undesirable, recent inhibitor studies indicate potential benefits, especially if the inhibitors can specifically target the liver and adipose tissue. HSL and MAGL might not be good pharmacological targets for improving liver lipids given their unclear role in hepatic lipid metabolism (HSL) and potential interference with endocannabinoid signaling (MAGL). Developing specific CES1 inhibitors might be beneficial to mitigate NAFLD/NASH and hyperlipidemia although there is a risk of adverse drug-drug interactions. Compounds that would increase LAL or CES2 activities could be potentially useful for treatment of metabolic dysregulation.

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Conflict of interest statement

The authors declare no conflict of interest.

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