

## REVIEW ARTICLE

# Regulation and function of triacylglycerol lipases in cellular metabolism

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The ability to store energy in the form of energy-dense TAG (triacylglycerol) and to mobilize these stores rapidly during times of low carbohydrate availability (fasting or famine) or during heightened metabolic demand (exercise or cold-stress) is a highly conserved process essential for survival. Today, in the presence of nutrient excess and sedentary lifestyles, the regulation of this pathway is viewed as an important therapeutic target for disease prevention, as elevated circulating fatty acids in obesity contribute to many aspects of the metabolic syndrome including hepatic

steatosis, atherosclerosis and insulin resistance. In the present review, we discuss the metabolic regulation and function of TAG lipases with a focus on HSL (hormone-sensitive lipase), ATGL (adipose triacylglycerol lipase) and newly identified members of the lipolytic proteome.

**Key words:** adipose tissue, adipose triacylglycerol lipase (ATGL), fatty acid metabolism, hormone-sensitive lipase (HSL), obesity, skeletal muscle, Type 2 diabetes.

## INTRODUCTION

Intracellular lipid pools are in a constant state of flux, with overall lipid content ultimately dependent on the balance between the rates of FA (fatty acid) uptake, esterification, hydrolysis and oxidation [1–3]. FAs are used in the cell for membrane biosynthesis, as signalling intermediates and for ATP production from  $\beta$ -oxidation and flux of acetyl-CoA through the tricarboxylic acid cycle. Although FAs can be derived from both intracellular and extracellular sources, with the exception of adipose tissue, the latter is most prevalent due to limited intracellular stores. Under postprandial conditions, ~30% of total energy expenditure is reliant on FAs derived from adipose TAG (triacylglycerol) hydrolysis, and this becomes quantitatively more important with extended fasting or exercise. In cases of caloric surplus leading to obesity, elevated circulating FAs contribute to the accumulation of intramuscular and hepatic lipids which indirectly inhibit insulin sensitivity [1]. Therefore the liberation of FAs from stored TAG and release into the systemic circulation is, under most conditions, the first point of control in the regulation of FA metabolism and, accordingly, the precise control of lipolysis is an important event in the regulation of whole-body metabolic homeostasis.

The sequential hydrolysis of TAG is regulated by specific enzymes and results in the liberation of FA at each step with the generation of DAG (diacylglycerol), MAG (monoacylglycerol) and glycerol (Figure 1). For many years, the rate-limiting enzyme controlling this process was believed to be HSL (hormone-sensitive lipase). HSL exhibits broad substrate specificity towards acylglycerols and it also hydrolyses cholesteryl, retinyl and lipoidal esters [4–6]. Studies over the last two decades have demonstrated the important role of phosphorylation by stress-activated protein kinases in controlling HSL intracellular localization, enzyme activity and interaction with accessory proteins, such as perilipin, to ultimately control lipolysis (Figure 2).

However, HSL-null mice accumulated DAG and were able to maintain basal and some degree of  $\beta$ -adrenergic-stimulated lipolysis, suggesting the requirement of alternative enzymes for TAG hydrolysis. In 2004, three laboratories identified this enzyme as ATGL (adipose triacylglycerol lipase; also known as desnutrin and phospholipase A<sub>2</sub>- $\zeta$ ) [7–9]. ATGL exhibits high substrate specificity for TAG [7], and its activity appears to be largely dependent on association with CGI-58 (comparative gene identification 58) [10]. These new findings have led to the current view that ATGL and HSL work hierarchically to regulate TAG hydrolysis (Figure 1): ATGL initiates lipolysis by specifically removing the first FA from TAG to produce DAG substrate, which is then hydrolysed by HSL to generate an additional FA and MAG substrate. MAGs are converted into FA and glycerol by monoacylglycerol lipase in the final step of lipolysis [11]. However, owing to differences in expression of HSL and ATGL across tissues, there may also be a role for other members of the recently identified lipolytic proteome (see Tables 1 and 2) [12]. In the present review, we discuss the physiological function and metabolic role of the TAG lipases HSL, ATGL and newly discovered lipolytic proteins beginning with HSL, since it was the first TAG lipase to be identified.

## HORMONE-SENSITIVE LIPASE

For nearly half a century, researchers have known that adipose tissue lipolysis is sensitive to catabolic stimuli such as adrenaline (epinephrine), noradrenaline (norepinephrine), corticotropin and glucagon [13–15], but it was several decades later before the protein responsible for much of this lipolytic activity, HSL, was purified from rat epididymal fat pads [11]. In initial studies, semi-purified preparations of HSL were found to have lipolytic activity towards not only TAG, but also DAG, MAG and cholesteryl esters,

Abbreviations used: ADRP, adipocyte differentiation-related protein; AICAR, 5-amino-4-imidazolecarboxamide-1- $\beta$ -D-ribofuranoside; AMPK, AMP-activated protein kinase; ATGL, adipose triacylglycerol lipase; CGI-58, comparative gene identification 58; DAG, diacylglycerol; ERK, extracellular-signal-regulated kinase; FA, fatty acid; FABP, fatty acid-binding protein; HFD, high-fat diet; HSL, hormone-sensitive lipase; LD, lipid droplet; MAG, monoacylglycerol; PAT, perilipin, adipose differentiation-related protein and tail-interacting protein, 47 kDa; PEDF, pigment epithelium-derived factor; PEDF-R, pigment epithelium-derived factor receptor; PKA, protein kinase A; PNPLA, patatin-like phospholipase domain-containing protein; PPAR, peroxisome-proliferator-activated receptor; TAG, triacylglycerol; TGH, TAG hydrolase; TNF $\alpha$ , tumour necrosis factor  $\alpha$ ; VLDL, very-low-density lipoprotein.

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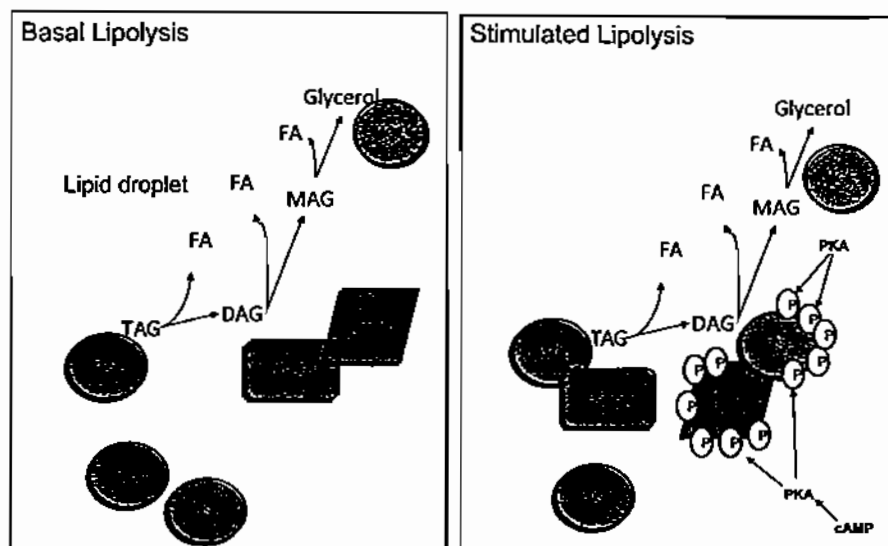


Figure 1 The emerging view of regulated lipolysis

Basal lipolysis: perilipin A (Peri A) and CGI-58 form a complex on the LD. ATGL is localized partially to the LD and HSL mostly in the cytoplasm. The rate of lipolysis (i.e. the production of FA and glycerol) is very low under these conditions, with released fatty acids being resynthesized back into lipid (futile cycling) or directed towards oxidation in mitochondria. Stimulated lipolysis: PKA activation results in phosphorylation of HSL at Ser<sup>663</sup>, Ser<sup>669</sup> and Ser<sup>670</sup> and perilipin at six serine residues (denoted by P). Phosphorylation of perilipin releases CGI-58, which binds ATGL to initiate lipolysis and the breakdown of TAG. HSL translocates to the LD, associates with phosphorylated perilipin A and degrades DAG. MAG lipase (MGL) cleaves the final FA to produce glycerol. The production of FA and glycerol is much higher than in the basal state. An animated version of this Figure can be seen at <http://www.BiochemJ.org/bj/414/0313/bj4140313add.htm>.

although it should be noted that the relative hydrolase activity *in vitro* is 11-fold greater against DAG than TAG [11]. In addition, HSL also shows a preference for activity against FAs in the *sn*-1 or *sn*-3 position [16]. The cloning of HSL in 1988 revealed a protein of ~84 kDa [17] which was expressed in many tissues other than adipose tissue, including skeletal muscle, pancreas, macrophages and testis [18]. HSL contains three domains [19,20]: a catalytic domain, a regulatory domain containing several phosphorylation sites and an N-terminal domain involved in protein–protein and protein–lipid interactions, each of which is discussed below.

#### Catalytic triad

HSL, like other lipases and esterases, contains an  $\alpha/\beta$  hydrolase fold which is composed of a central, mostly parallel,  $\beta$ -sheet surrounded by a number of  $\alpha$ -helices. Within this fold is the catalytic triad, which is a mirror image of that found in serine proteases and is composed of a GXSGX (Gly-Xaa-Ser-Xaa-Gly) motif [19,20]. Through both site-directed mutagenesis and modelling against known lipase structures, Ser<sup>423</sup>, Asp<sup>703</sup> and His<sup>733</sup> were demonstrated to make up the catalytic triad and were shown to be critical for mediating HSL activity (Figure 2) [21,22].

#### Regulatory domain: HSL regulation via phosphorylation

Although it was known for several years that PKA (protein kinase A) increased HSL activity through a phosphorylation-dependent mechanism [23–26], mutagenesis experiments demonstrated an absolute dependence for this effect on phosphorylation at Ser<sup>663</sup>, Ser<sup>669</sup> and Ser<sup>670</sup> (Ser<sup>532</sup>, Ser<sup>649</sup> and Ser<sup>650</sup> in humans) [27,28], although it should be noted that there is some doubt as to the importance of Ser<sup>663</sup> [27] (Figure 2). The PKA phosphorylation of HSL results in modest activation of the enzyme (2–3-fold); however, more importantly, it also promotes the translocation of HSL to the LD (lipid droplet) [29], an effect which is critical for enhancing lipolytic capacity as a result of  $\beta$ -adrenergic stimulation (as discussed below and reviewed in [30]).

The ERK (extracellular-signal-regulated kinase) has also been shown to increase HSL activity in 3T3-L1 adipocytes through phosphorylation of the enzyme at Ser<sup>660</sup> (Ser<sup>589</sup> in humans) [31].

The inhibition of HSL activity is primarily regulated following feeding by insulin and is controlled via both cAMP-dependent and -independent pathways. The cAMP-dependent pathway is believed to involve insulin stimulation of PDE3B (phosphodiesterase 3B) which degrades cAMP, thereby reducing PKA activity [32,33] and resulting in dephosphorylation of HSL by protein phosphatases [34]. The cAMP-independent pathway involves enhanced HSL dephosphorylation by protein phosphatase-1 which is activated by insulin [35].

HSL activity is also negatively regulated through phosphorylation of HSL at Ser<sup>663</sup> (Figure 2) by AMPK (AMP-activated protein kinase) [25], calcium/calmodulin-dependent protein kinase II [25] and glycogen synthase kinase IV [36]. The inhibitory effects of AMPK towards basal and PKA-stimulated lipolysis have been confirmed in 3T3-L1 adipocytes infected with adenovirus expressing constitutively active or dominant-negative AMPK mutations or in mice deficient in AMPK  $\alpha$ 1 [37]. The recent findings that many cytokines regulate metabolism through either the inhibition [e.g. TNF $\alpha$  (tumour necrosis factor  $\alpha$ ) or resistin] or activation [e.g. IL-6 (interleukin 6), leptin, CNTF (ciliary neurotrophic factor)] of AMPK signalling [38] implicates inflammatory signalling as a potentially important mechanism in lipolytic control.

#### HSL translocation, protein–protein and protein–lipid interactions

Although phosphorylation of HSL results in a 2–3-fold increase in catalytic activity *in vitro*, this cannot account for the 30–100-fold increase in lipolysis in intact cells upon PKA stimulation. Recent developments have revealed that HSL-mediated lipolysis is more complex, involving control by translocation of HSL to the LD and a series of protein–protein interactions. HSL translocates from the cytosol to the LD upon PKA stimulation [29], an effect

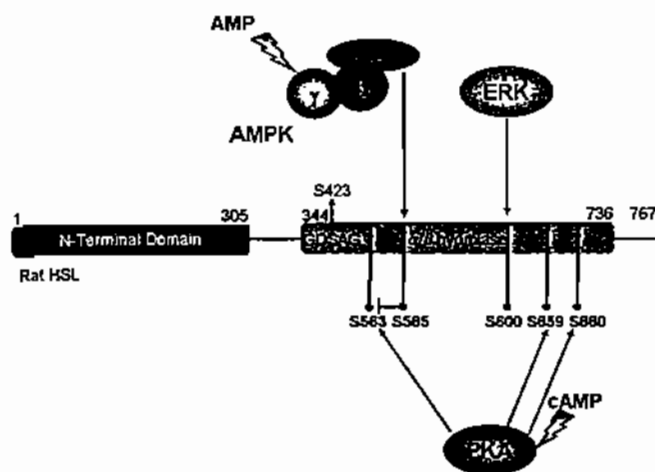


Figure 2 Hormone-sensitive lipase

Rat sequence of HSL (accession number P15304), showing recognized N-terminal and  $\alpha/\beta$  hydrolase domains. S423 indicates the active site within HSL with the motif shown below. Phosphorylation sites within the regulatory unit are indicated along with the corresponding kinases, with green arrows indicating activating phosphorylation and a red arrow indicating inhibitory phosphorylation.

which is dependent on phosphorylation of HSL at either Ser<sup>659</sup> or Ser<sup>660</sup> [39].

LDs are bound by a family of proteins which contain PAT (perilipin, adipose differentiation-related protein and tail-interacting protein, 47 kDa) domains. Of these proteins, perilipin A appears to be the most critical for regulating HSL activity. A detailed review of perilipin is beyond the scope of this review and readers are directed to several excellent reviews [40,41]. Perilipin was discovered by Greenberg et al. [42] as a heavily PKA-phosphorylated protein found exclusively in adipocytes. Its localization surrounding LDs in adipocytes led to the view that it forms a regulated barrier between cytosolic lipases and the TAGs within [43], an idea supported by functional studies. Perilipin A expression in pre-adipocytes suppressed lipolysis, particularly in the basal state [44], and phosphorylation of perilipin A by PKA enhances lipolysis, even in the absence of HSL [45]. These features of perilipin function were confirmed in perilipin A-null mice that were lean and protected from obesity due to increased basal lipolysis and TAG breakdown [46,47]. A surprising finding was that  $\beta$ -adrenergic lipolysis was reduced dramatically in perilipin A-null mice, indicating that perilipin co-ordinates the recruitment and/or activation of lipases under lipolytic conditions. It is now known that perilipin plays a central role in HSL action at the LD. Indeed, PKA phosphorylates perilipin directly on six residues, and, although this phosphorylation is not required for the translocation and docking of HSL to LDs, it is critical for HSL lipolytic action at the LD [48]. Furthermore, a recent report examining the trafficking of lipolytic proteins showed translocation to be rapid and HSL and perilipin A to be complexed tightly, although the exact residues mediating this interaction have yet to be determined [49]. Taken together, these results suggest that the phosphorylation of perilipin A enhances the activity of HSL by factors independent of translocation alone, perhaps by enhancing LD remodelling which may allow better access for HSL or indirectly by increasing co-localization with ATGL, which would be anticipated to enhance the supply of substrate (DAG) for HSL. The process does not appear to involve interactions with the pseudo- $\alpha/\beta$  hydrolase family member, CGI-58, as recombinant expression stimulates lipolysis in adipose from HSL-null mice,

indicating that CGI-58 does not interact directly with HSL as it does ATGL (discussed in detail below) to enhance lipolysis [50].

#### N-terminal domain

The N-terminal residues of HSL (192–200) interact directly with FABP (fatty acid-binding protein) 4 in a process dependent on the presence of FAs [51–53]. FABPs are an abundantly expressed family of proteins that are believed to act as intracellular chaperones for FAs, although their exact intracellular functions are still largely unknown [54]. In support of a physiological role of this interaction, FABP-null mice have suppressed rates of adipose tissue lipolysis and are protected from diet-induced insulin resistance [55], an effect that is recapitulated using a specific FABP inhibitor [56]. The use of this inhibitor in HSL-null mice will be interesting to evaluate the importance of this interaction in regulating insulin sensitivity and metabolism.

#### HSL transcription

Although HSL activity is primarily dependent on post-translational control involving phosphorylation, cellular localization and protein–protein interactions, the transcriptional regulation of HSL has also been reported to be important under some conditions. In both mouse [57] and human [58] adipocytes, glucose increases HSL expression. This effect is indirect as non-metabolizable forms of glucose such as 3-O-methylglucose and 2-deoxyglucose have no effect on HSL expression, suggesting the involvement of a glycolytic metabolite downstream of glucose 6-phosphate [58]. The physiological importance of this regulation is not clear, since, *in vivo*, high levels of glucose would be expected to stimulate insulin production which would suppress HSL phosphorylation and, in turn, lipolysis. Another positive regulator of HSL expression is PPAR (peroxisome-proliferator-activated receptor)  $\gamma$ , a critical transcription factor that regulates adipogenesis. The promoter region of HSL contains a GC-box which is acted on by PPAR $\gamma$  to enhance SP1 (specificity protein 1) binding and, in turn, HSL promoter activity [59,60]. Interestingly, *in vivo* treatment with the synthetic PPAR $\gamma$  ligand, rosiglitazone, increased HSL expression only in skeletal muscle and liver, consistent with the positive effects of rosiglitazone on improving insulin sensitivity in these tissues [60]. These insulin-sensitizing effects may be related to reduced DAG and protein kinase C  $\theta$  and  $\delta$  activation, which are known inhibitors of insulin receptor substrate signalling [61,62].

#### HSL regulation in skeletal muscle

The regulation of intramyocellular TAG hydrolysis is important in response to physical stress such as exercise [63,64] and in regulating skeletal muscle insulin sensitivity [1]. HSL accounts for ~60% of total neutral lipase activity in skeletal muscle at rest and almost all activity during contractions and adrenergic stimulation [65–68]. Adrenaline is elevated during exercise and increases HSL activity in resting and contracting muscle [67,69,70] via  $\beta$ -adrenergic receptor stimulation, resulting in PKA activation and the phosphorylation of Ser<sup>563</sup> and Ser<sup>660</sup> (see below) [71]. In addition, HSL translocates to LDs within myocytes and associates with ADRP (adipocyte differentiation-related protein) in response to adrenaline and contraction [72]. The association with ADRP may be essential for lipolytic control in skeletal muscle given the absence of perilipin A.

AMPK appears to negatively regulate HSL activity during muscle contractions. In both resting [73] and contracting [74]

muscle, TAG hydrolysis is suppressed in response to AMPK activation by the adenosine analogue AICAR (5-amino-4-imidazolecarboxamide-1- $\beta$ -D-ribofuranoside). Consistent with reductions in TAG hydrolysis, PKA-stimulated HSL activity is reduced in L6 myotubes treated with AICAR [65] or following the overexpression of a constitutively active AMPK [71]. *In vivo* evidence supporting a role of AMPK inhibition of HSL stems from findings in human skeletal muscle that enhanced activation of AMPK, owing to glycogen depletion before exercise, prevents exercise-induced increases in HSL activity [65]. Similarly, prolonged endurance exercise (90 min) that results in significant activation of AMPK corresponds with reduced HSL activity and increased Ser<sup>66</sup> phosphorylation [71]. It should be noted that this inhibitory effect of AMPK on HSL activity has not been observed in all studies [68]; however, the majority of studies indicate that increased activation of AMPK is capable of overriding elevations in adrenaline and PKA stimulation of HSL, a concept which is consistent with a reduction in FA oxidation during intense exercise.

#### HSL-null mice

Four independent laboratories have generated and characterized HSL-null mice in the last decade [75–78]. These findings have provided important insights into the functional significance of HSL, and a detailed analysis of these results has recently been completed [79]. HSL-null males are infertile because of reduced spermatogenesis [80] and sperm motility [81], an effect which is rescued following the transgenic overexpression of human HSL in the testes [82,83] and is related to the absence of neutral cholesterol esterase activity in the testis of HSL-null mice [76,83,84]. Although HSL-null mice appear to be otherwise phenotypically normal, total fat mass is surprisingly reduced [76,78], with marked heterogeneity in white adipose tissue cell size [76], although others have reported a modest reduction in cell diameter in adipose explants [78]. Measurement of plasma concentrations of glycerol and FA showed a modest reduction in circulating levels under basal conditions [76,78], a difference that was, for the most part, eliminated following  $\beta$ -adrenergic stimulation [76]. Consistent with these findings, *in vitro* analysis of adipocytes revealed that, in the absence of HSL, there is still residual TAG lipase activity (40–50%) [75,76,78] and adipocytes are able to maintain some degree of catecholamine-stimulated lipolysis [78], supporting the idea that there are alternative lipases that may substitute for HSL activity at least during unstimulated conditions. One of the most enlightening findings was that alternative lipases were probably responsible for the breakdown of TAG to DAG, since there was considerable accumulation of DAG in tissues of HSL-null mice [75]. This is consistent with the previously identified substrate specificity of HSL for DAG compared with that of TAG.

A second major phenotype observed was that HSL ablation had a significant protective effect against the development of obesity when fed on an HFD (high-fat diet) [76,85]; however, this is not observed after a relatively short time on the diet (3 weeks) [86]. Reduced adiposity in animals fed on an HFD for an extended time occurs even though there was a higher food intake per unit of body mass in HSL-null mice, which did not appear to be due to lipid malabsorption [85]. Instead, the reduced body mass could be accounted for by a much higher core body temperature that reduced adipose tissue mass both on an HFD [85] or when HSL-null mice were crossed on to an *ob/ob* background [87]. This effect may be related to an increase in brown adipose tissue and uncoupling protein 2 expression which would be expected to increase mitochondrial uncoupling [88]. In addition to the

increased energy expenditure in HSL-null mice, reduced adiposity was associated with a compensatory decrease in the re-esterification of FA secondary to marked down-regulation of genes associated with FA and TAG metabolism, such as adipocyte FABP, lipoprotein lipase, GPAT (glycerophosphate acetyltransferase), ACC (acetyl-CoA carboxylase), FA synthase, acyl-CoA synthetase, and DAG acyltransferase 1 and 2 [88]. The down-regulation of esterification genes in the absence of HSL is believed to be a compensatory mechanism to prevent futile cycling so as to enhance the export of FAs in the presence of diminished lipolytic capacity.

Despite the consistent findings demonstrating protection from weight gain on an HFD, the effect of HSL deficiency on glucose tolerance and insulin sensitivity has been equivocal. Glucose tolerance in HSL-null mice has been reported to be decreased [77] or normal [89], findings which may be related to differences in insulin secretion which has been reported to be normal [77,90] or modestly impaired [91]. Similarly, the effect of HSL deletion on insulin sensitivity also varies between studies: three independent studies using a hyperinsulinaemic-euglycaemic clamp reported either impaired [77] or enhanced [86,89] hepatic insulin sensitivity, with conflicting findings also being reported with regard to adipose tissue and muscle insulin sensitivity [77,86]. The basis for these differences may be related to different genetic backgrounds of the mice which can markedly alter glucose metabolism and susceptibility to weight gain on an HFD [92]. Given that a consistent finding is that HSL mice accumulate DAG, which activates protein kinase C, a known negative regulator of insulin signalling [61,62], it is somewhat surprising that insulin sensitivity is not impaired. In addition, other studies have reported increased TNF $\alpha$  and macrophage infiltration into white adipose tissue which would also be anticipated to compromise glucose and FA metabolism [93]. Future studies in HSL tissue-specific null mice will be important for assessing in which tissues HSL activity is important for regulating glucose and lipid homeostasis.

In conclusion, the findings in HSL-null mice have been critical for confirming the substrate specificity of HSL for the breakdown of DAG and for demonstrating the absolute reliance on HSL for the hydrolysis of neutral cholesteryl esters. The most important and unexpected finding was the maintenance of basal and, to a lesser degree, adrenergic-stimulated TAG lipase activity and lipolysis in the absence of HSL, thereby demonstrating the presence of alternative lipase(s). These findings led several groups on a quest to identify alternative TAG lipases which is the focus of the remainder of this review.

#### ATGL: A NEW LIPASE IN TAG METABOLISM

Three laboratories simultaneously identified ATGL [7], which was also termed desnutrin [8], phospholipase A<sub>2</sub>- $\zeta$  [9] and, later, PEDF-R (pigment epithelium-derived factor receptor) [94]. Murine ATGL is a 486-amino-acid protein with a molecular mass of 54 kDa, whereas the human gene encodes a 504-amino-acid protein that shares 86% homology with the murine protein [7]. Surprisingly, ATGL and classical lipases have no clear sequence similarity and differ with regard to fold and catalytic mechanism [95]. The N-terminal region of ATGL encompasses ~260 amino acids and contains a 'predicted esterase of the  $\alpha/\beta$ -hydrolase' fold domain and a 'patatin domain' (Pfam01734), which was first identified as a potato storage protein with lipid acyl hydrolase activity [96] and is conserved in eukaryotes and prokaryotes. The patatin domain contains a GXSGX consensus sequence for serine lipases that includes the predicted active Ser<sup>47</sup>. The active serine residue is conserved in *Drosophila* (Ser<sup>38</sup>) and yeast (Ser<sup>115</sup>) and substitution of alanine for serine severely attenuates lipase

activity [97–100]. Although the crystal structure for ATGL is presently unknown, both patatin [101] and cytosolic phospholipase A<sub>2</sub> [102] contain a Ser-Asp catalytic dyad in the active site, indicating that ATGL could function in a similar manner. This would represent a critical difference from the regulation of traditional lipases that contain a catalytic triad. The C-terminal region of ATGL contains a putative lipid-binding domain (amino acids 309–391) that is rich in hydrophobic residues [103]. Studies using C-terminal truncation mutants demonstrate that the C-terminal region is critical for ATGL localization to LDs [104,105]. This mutation provides the mechanistic basis of the recessively inherited disorder neutral lipid storage disease with myopathy, which is characterized by ATGL dysfunction and excessive TAG deposition [105]. It also appears that an undefined region of the C-terminus interferes with CGI-58 interaction with ATGL and subsequent enzyme activation [104].

### ATGL expression and function

ATGL is expressed in most tissues examined with high expression noted in white and brown adipose tissue and with lower levels observed in the testis, cardiac and skeletal muscle [7,8,100,106]. ATGL expression is progressively increased during adipocyte differentiation [7–9,107], and the temporal pattern of expression is similar to that of HSL [106], but not of other members of the PNPLA (patatin-like phospholipase domain-containing protein) family [106,107]. ATGL exhibits high substrate specificity for TAG, very weak activity against DAG and no activity against cholesterol or retinyl ester bonds [7], findings that are supported by studies across multiple species and systems. For example, in yeast, the ATGL orthologue Tgl4 exhibits substrate specificity for TAG, and a second lipase, Tgl3, hydrolyses both TAG and DAG substrates [98]. In *Drosophila*, the chronic overexpression of the ATGL orthologue, Brummer lipase, depletes fat stores, whereas the loss of Brummer lipase causes obesity. This is consistent with the biology of lipid mobilization in insects where DAG, and not FA, is utilized predominately as a substrate [97]. Functional analyses of ATGL in murine adipocytes have demonstrated that siRNA (short interfering RNA) or adenoviral-mediated knockdown of ATGL drastically impairs, whereas retroviral and adenoviral overexpression increases both basal and  $\beta$ -adrenergic-stimulated lipolysis [7,106]. Consistent with this notion in muscle and COS-7 cells, overexpression of ATGL decreased LD size and TAG content, whereas ATGL depletion increased LD size and TAG accumulation, demonstrating an important role of ATGL in also regulating TAG metabolism in cells other than adipocytes [99,108]. These findings are supported by the phenotype of ATGL-null mice, which exhibit reduced basal and catecholamine-stimulated lipolysis and display an expanded adipose tissue mass and increased TAG deposition in non-adipose tissue, including the heart, liver, pancreas, kidney and skeletal muscle [109].

Aside from its role in TAG degradation, Jenkins et al. [9] also reported acylglycerol transacylase activity of ATGL using an oleate donor, which, in the presence of mono-olein or di-olein, produced DAG and TAG respectively. This is exciting in the context of TAG futile cycling, which is proposed to provide a continuous pool of FAs to facilitate an immediate response to an organism's metabolic demands [110]. Regulation of ATGL transacylation and lipase activities by undefined mechanisms could thereby alter the metabolic balance within adipocytes, from anabolic (i.e. high transacylase activity) to catabolic (i.e. high lipase activity) situations and thereby alter substrate delivery to peripheral tissues. Further studies are required to address the post-translational regulation and the metabolic relevance of these opposing functions.

Although the evidence from *in vitro* studies utilizing constructs harbouring murine ATGL [7,50,106,111] and from *in vivo* animal studies [109] demonstrates a critical role for ATGL in basal and stimulated lipolysis, results from human studies are less clear. Langin et al. [112] indirectly tested the role of ATGL in human adipocytes by incubating cells with a selective HSL inhibitor. They reported complete ablation of catecholamine- and natriuretic peptide-stimulated lipolysis and a 50% reduction in basal lipolysis, suggesting that HSL entirely accounts for stimulated lipolysis, whereas ATGL (or other undefined lipases) plays a role in basal lipolysis. Some [9,100], but not all [113], groups have also demonstrated significant TAG hydrolase activity of partially purified human ATGL obtained from cellular extracts. Supporting further a central role of ATGL in human adipocyte lipolysis are the findings of decreased TAG storage in human embryonic kidney cells with human ATGL overexpression [100], whereas immunoinhibition of ATGL in subcutaneous and visceral human adipose tissue lysates reduced total TAG lipase activity by 70–83% [114]. Further studies, perhaps using a specific ATGL inhibitor, are required to ascertain the role of ATGL in human lipolysis.

### Transcriptional regulation of ATGL

ATGL mRNA content is strongly controlled by nutritional factors, being transiently increased during fasting and decreased by re-feeding in adipose tissue [8,106] and liver [100]. Although glucocorticoids, but not glucagon, have been implicated in ATGL mRNA induction [8], insulin appears to be the most potent regulator of ATGL. Insulin dose-dependently inhibits ATGL transcription *in vitro* and enhances ATGL mRNA expression in murine models of insulin deficiency (streptozotocin-induced diabetes) and insulin resistance (fat-specific insulin receptor knockout) [106,115,116]. TNF $\alpha$  also represses ATGL mRNA expression at low concentrations [106,115,116], whereas the adrenergic agonist isoprenaline (isoproterenol) inhibits ATGL mRNA at high concentrations [116]. Conversely, neither cAMP analogues nor phosphodiesterase inhibitors affected ATGL expression in pre-adipocytes, questioning the role of adrenergic activation as an ATGL transcriptional regulator [8]. Although these studies provide some information on nutritional and hormonal regulation, there are few detailed studies evaluating ATGL transcription. ATGL is transactivated by PPAR $\gamma$  [115], which is consistent with the enhanced ATGL mRNA expression observed during adipocyte differentiation and during treatment with the anti-diabetic drug rosiglitazone [106,117,118].

### Post-translational regulation of ATGL

Relatively little is known about the post-translational regulation of ATGL. Unlike HSL, ATGL activity does not appear to be regulated directly by PKA phosphorylation, although preliminary evidence in mammalian cells indicates that ATGL is phosphorylated by other unknown kinases [7]. A phosphoproteomic analysis of purified LDs reported phosphorylation at Ser<sup>404</sup> and Ser<sup>428</sup> of ATGL, the former being a predicted ERK consensus sequence [119]. Biochemical evaluation of cell lysates and high-resolution imaging of fluorescence reporters in living cells indicate that ATGL resides in the cytoplasm and on LDs in the basal state [7,49] and PKA activation elicits only minor translocation of ATGL to LDs (Figure 1) [49].

In addition to possible regulation of activity by phosphorylation, ATGL activity is highly dependent on several biochemical events for efficient lipolysis: first by association with an activating protein, CGI-58 and secondly by phosphorylation of perilipin A (Figure 1).

CGI-58 is a member of the esterase/thioesterase/lipase subfamily of proteins characterized by the presence of  $\alpha/\beta$  hydrolase folds; however, unlike most lipases, the serine residue within the GXSG motif is replaced by an asparagine residue that prevents lipase activity [10,120]. Mutations in CGI-58 underpin a rare human disorder termed Chanarin–Dorfman syndrome (neutral lipid storage disease) that is characterized by ichthyosis and excessive TAG deposition in most tissues, except adipose tissue [121]. A major advance in understanding lipolysis was the discovery that ATGL requires CGI-58 for full activation. Lass et al. [10] demonstrated that, upon interaction with CGI-58, ATGL TAG hydrolase activity increased 20-fold, although others have since reported less dramatic increases in cultured cells (30–70% increase [108,120,122]). Overexpression of either ATGL or CGI-58 alone in COS-7 cells did not affect TAG storage, whereas overexpression of both proteins markedly reduced TAG deposition, indicating that the interaction between ATGL and CGI-58 is required for efficient lipolysis [10]. The dependency of the double overexpression in COS7 cells is not observed in myotubes [108] or hepatoma cells [122], and the differences between these studies probably reflects very low endogenous ATGL and CGI-58 protein in COS7 cells (M.J. Watt, unpublished work).

Perilipin serves a critical role in regulating basal and stimulated lipolysis by co-ordinating the recruitment of proteins to the LD, as discussed in [40]. CGI-58 resides on the surface of LDs and interacts with perilipin A under basal conditions [120,123–125]. After  $\beta$ -adrenergic stimulation, CGI-58 disperses into the cytoplasm within minutes [120,124–126], which appears to be due to phosphorylation of perilipin A [125]. Independent studies using perilipin-null MEFs (mouse embryonic fibroblasts) with adenoviral expression of perilipin A PKA site mutants indicate that phosphorylation at Ser<sup>317</sup> of perilipin A is mandatory for stimulated lipolysis [111], which may mediate the dissociation of perilipin and CGI-58. It appears that, after dissociation from perilipin, CGI-58 associates with ATGL predominantly at micro (fragmented) LDs [120,125] and sites lacking perilipin [125].

Collectively, the current literature indicate that ATGL resides predominantly on the LD, but is largely inactive because CGI-58 associates with perilipin A (Figure 1). HSL is located almost exclusively in the cytosol and is thereby removed from TAG substrate. This could explain the primary role of ATGL in basal lipolysis. Upon  $\beta$ -adrenergic stimulation, PKA phosphorylates perilipin A which then dissociates from CGI-58, thereby permitting binding of CGI-58 with ATGL at micro LDs. PKA also promotes the rapid translocation of HSL from the cytosol to the LD and hence is the major contributor to maximal lipolysis. Although the understanding of TAG lipase regulation and lipolytic protein trafficking has evolved in recent years, several key questions remain. For example, ATGL co-localizes with the mannose 6-phosphate receptor, tail-interacting protein of 47 kDa and adipocyte differentiation-related protein [99,127]; however, the nature of these interactions and the involvement of other PAT proteins are unknown. Also, what post-translational events influence the ATGL co-activator CGI-58? And, finally, are these events conserved in non-adipose tissues that lack perilipin, such as skeletal muscle and liver?

## ROLE OF ATGL IN CELLULAR METABOLISM

### ATGL-null mice

The generation and phenotypic evaluation of ATGL-null mice has extended the understanding of ATGL's role in metabolism [109]. The most striking phenotype in ATGL is that, as a consequence

of the cardiac TAG accumulation, ATGL-null mice developed heart failure due to myocardial fibrosis and a mechanical contraction defect, thereby reducing life span (50% died by 16–20 weeks). ATGL-null mice displayed defective cold adaptation, suggesting that ATGL is required to provide FA substrate to fuel thermogenesis. The reduction in plasma FA availability also resulted in greater glucose utilization, which was suggested to explain the enhanced glucose and insulin tolerance in these mice. This finding was surprising given that TAG accumulates in the muscle and liver of obese individuals and Type 2 diabetes patients and is positively associated with insulin resistance [128]. Conversely, this dissociation supports a prevailing hypothesis that increasing TAG deposition does not cause insulin resistance [129,130] and may act as a reservoir to prevent the accumulation of bioactive lipid species known to interfere with insulin signal transduction, such as DAG, ceramides and long-chain fatty acyl-CoAs. The apparent insulin-sensitizing effects in ATGL-null mice may have also resulted from reduced plasma FA delivery, secondary to decreased adipose tissue lipolysis, the release of an undefined hormone/adipokine or due to direct effects of ATGL in non-adipose tissues. Studies examining tissue-specific deletion/overexpression of ATGL are evidently required to resolve these questions.

### Skeletal muscle

Skeletal muscle ATGL protein content and TAG hydrolase activity are reduced in obesity [108], suggesting that ATGL may play an important biochemical role in this tissue. ATGL is a TAG hydrolase in skeletal muscle that alters fat metabolism/partitioning both *in vitro* and *in vivo*. Retroviral-mediated ATGL overexpression increased the oxidation of TAG-derived Fas, while concomitantly reducing oxidation of extracellularly derived FA [108]. Consequently, DAG and ceramide accumulate in myotubes and negatively feed back on the insulin signalling cascade to induce insulin resistance. This supports the hypothesis that TAG may protect against insulin resistance and is consistent with the insulin sensitization in ATGL-null mice [109]. The DAG accumulation indicates that endogenous HSL is insufficient to compensate for the increased TAG turnover when ATGL activity is elevated. *In vivo* overexpression of ATGL by DNA electrotransfer mildly enhanced ATGL protein expression and TAG hydrolase activity, halved TAG content and promoted DAG and ceramide accumulation, but did not induce insulin resistance as assessed by hyperinsulinaemic–euglycaemic clamp [108].

### Liver

ATGL is expressed in the liver and its ablation leads to steatosis [7,100]. CGI-58 also appears to play an important role in hepatic lipid secretion by facilitating the mobilization of cytoplasmic TAG for lipoprotein secretion [122]. Using a yeast two-hybrid screen and human PEDF (pigment epithelium-derived factor) cDNA fragments as bait, ATGL was recently cloned as the receptor (termed PEDF-R in) for the anti-angiogenic factor PEDF [94]. PEDF is a non-inhibitory member of the serine protease inhibitor superfamily most widely recognized for preventing angiogenesis in the retina and promoting neuronal survival and differentiation. Notari et al. [94] showed that binding of PEDF to its receptor (ATGL) increased phospholipase A<sub>2</sub> activity and that ATGL is membrane-bound. The localization of PEDF-R (ATGL) in this study [94] is consistent with the prediction of up to four transmembrane domains in PEDF-R, but is at odds with other studies demonstrating localization at the LD and cytoplasm [7,8,49,119]. Others have supported the premise that PEDF binding is important

**Table 1 Protein characteristics of the lipolytic proteome**

Bold underlined residues in the Active site column indicate the active serine residue.

Gene name	Protein name	Other names	Accession number	Molecular mass (Da)	Number of amino acids	Palatin domain	$\alpha/\beta$ hydrolase fold	Active site
<i>LIPE</i>	Hormone-sensitive lipase (HSL)	HU-45	O05469 NP_005348	84 000	775	No	Yes, residues 345–726	G <sup>415</sup> D <sup>548</sup> SAG
<i>MGLL</i>	Monoglycerol lipase (MGL)	Lysophospholipase homologue Lysophospholipase-like Monoglycerol lipase	O99685 NP_001003794	33 261	303	No	Yes, residues 70–285	G <sup>124</sup> H <sup>248</sup> SAGG
<i>CEST</i>	Liver carboxylesterase 1	Triacylglycerol hydrolase (TGH) Acyl-CoA:cholesterol acyltransferase (ACAT) Monocyte/macrophage serine esterase (HMS) Serine esterase 1 Brain carboxylesterase (hBrt) Egagyn relinyl ester hydrolase (REH) Lysophospholipase I (LPL-I) Lysophospholipase II (LPL-II)	P23141 NP_001020365	62 521	567	No	No	G <sup>34</sup> INIKQEFG G <sup>468</sup> DHGELEFS
<i>LYPLA1</i>	Acyl-protein thioesterase 1	Adipose triacylglycerol lipase (ATGL)	O75608 NP_006321	24 670	230	No	Yes, residues 10–226	G <sup>197</sup> F <sup>206</sup> SGG
<i>LYPLA2</i>	Acyl-protein thioesterase 2	Deshulin	O95372 NP_009191	24 737	231	No	Yes, residues 11–228	G <sup>124</sup> GF <sup>206</sup> SGG
<i>PNPLA1</i>	Palatin-like phospholipase domain-containing protein 1	Transport-secretion protein 2 (TTS2) Transport-secretion protein 2.2 (TTS2.2)	O6N8W4 NP_775947	57 915	532	Yes, residues 16–185	No	G <sup>71</sup> T <sup>185</sup> SAG <sup>35</sup>
<i>PNPLA2</i>	Palatin-like phospholipase domain-containing protein 2	Calcium-independent phospholipase A <sub>2</sub> (iPLA2- $\zeta$ ) Pigment epithelium-derived factor receptor (PEDF-R)	O96405 NP_065109	55 316	504	Yes, residues 10–179	No	G <sup>4</sup> A <sup>5</sup> SAG <sup>49</sup>
<i>PNPLA3</i>	Adiponutrin	Acylglycerol O-acyltransferase Calcium-independent phospholipase A <sub>2</sub> - $\epsilon$ (iPLA2- $\epsilon$ ) Palatin-like phospholipase domain-containing protein 3	O9NST1 NP_079501	52 865	481	Yes, residues 10–179	No	G <sup>4</sup> A <sup>5</sup> SAG <sup>49</sup>
<i>PNPLA4</i>	Palatin-like phospholipase domain-containing protein 4	GSZ	P41247 NP_004641	27 980	253	Yes, residues 6–176	No	G <sup>4</sup> A <sup>5</sup> SAG <sup>49</sup>
<i>PNPLA5</i>	Palatin-like phospholipase domain-containing protein 5	GSZ-like	O72626 NP_620169	47 912	429	Yes, residues 12–181	No	G <sup>4</sup> T <sup>181</sup> SSG <sup>31</sup>
<i>PNPLA6</i>	Calcium-independent phospholipase A <sub>2</sub> - $\gamma$	Intracellular membrane-associated calcium-independent phospholipase A <sub>2</sub> - $\gamma$ (iPLA2- $\gamma$ ) Palatin-like phospholipase domain-containing protein 6	O9NP80 NP_056538	68 477	782	Yes, residues 445–640	No	G <sup>4</sup> Y <sup>5</sup> STG <sup>448</sup>
<i>ABHD5</i>	Abhydrolase domain-containing protein 5	Comparative gene identification 58 (CGI-58)	O8WTS1 NP_057090	39 096	349	No	Yes, residues 102–343	

Table 2 Tissue specificity, subcellular localization and known function of TAG lipases

Name	Tissue specificity	Subcellular localization	Known function
Hormone-sensitive lipase (HSL)	Widely expressed in all tissues except liver	Translocates from the cytoplasm to lipid droplet and co-localizes with perilipin following PKA stimulation	TAG and DAG lipase In steroidogenic tissues, it principally converts cholesteryl esters into free cholesterol for steroid hormone production
Monacylglycerol lipase	Detected in adipose tissue, lung, liver, kidney, brain and heart	Lipid droplet	MAG lipase
Liver carboxylesterase 1 (TGH)	Expressed predominantly in liver with lower levels in heart and lung	Endoplasmic reticulum lumen	TAG hydrolase Activation of ester and amide prodrugs Hydrolyses aromatic and aliphatic esters Lipid hydrolase (by similarity)
Palatin-like phospholipase domain-containing protein 1	Digestive system	Unknown	
Palatin-like phospholipase domain-containing protein 2 (ATGL)	Highest expression in adipose tissue  Also detected in heart, skeletal muscle and portions of the gastrointestinal tract Detected in normal retina and retinoblastoma cells Detected in retinal pigment epithelium and, at lower intensity, in the inner segments of photoreceptors and in the ganglion cell layer of the neural retina (at protein level)	Lipid droplet membrane  Single-pass type II membrane protein	Catalyses the initial step in TAG hydrolysis in adipocyte and non-adipocyte LDs Also has acylglycerol transacylase activity
Adiponutrin	Adipose tissue	Membrane Single-pass membrane protein	TAG lipase and acylglycerol O-acyltransferase activities Lipid hydrolase
Palatin-like phospholipase domain-containing protein 4	Expressed in all tissues examined, including heart, brain, placenta, lung, liver, muscle, kidney, pancreas and spleen	Unknown	
Palatin-like phospholipase domain-containing protein 5	Unknown	Unknown	Lipid hydrolase (by similarity)
Calcium-independent phospholipase A <sub>2</sub> - $\gamma$	Expressed in parenchymal tissues including heart, skeletal muscle, placenta, brain, liver and pancreas Also expressed in bronchial epithelial cells and kidney Highest expression is observed in skeletal muscle and heart	Possible single-pass membrane protein	Calcium-independent phospholipase A <sub>2</sub> , which catalyses the hydrolysis of the sn-2 position of glycerophospholipids Cleaves membrane phospholipids
Abhydrolase domain-containing protein 5 (CGI-58)	Widely expressed in various tissues, including adipose tissue, skin, lymphocytes, liver, skeletal muscle and brain	Cytoplasm and lipid droplet Co-localized with perilipin, ADRP and ATGL on the surface of lipid droplets	No hydrolase activity Interacts with ATGL to activate ATGL

for ATGL regulation of lipid metabolism in the liver [131]. PEDF-null hepatocytes accumulated neutral lipids and PEDF-null mice developed steatosis when challenged with an HFD. When recombinant PEDF was provided to hepatocytes, TAG content was rapidly decreased. It appears as though PEDF directly binds ATGL at the LD to mediate TAG degradation. These intriguing results suggest that endogenous levels of ATGL are not sufficient to prevent hepatic steatosis and that activation by PEDF is mandatory for efficient ATGL function. Further studies are required to clarify how PEDF enhances ATGL function and the tissue specificity of the PEDF-ATGL interaction.

#### OTHER MEMBERS OF THE LIPOLYTIC PROTEOME

In addition to HSL and ATGL, there are several lipases that have been reported to be involved in lipolysis. Functional proteomic screening from mouse adipose tissue revealed that the lipolytic/esterolytic proteome contains a total of 23 proteins [12]. The regulation of many of these lipolytic proteins is largely undefined, but a summary of gene and protein names with predicted molecular masses and known  $\alpha/\beta$  hydrolase folds or patatin domains

is listed in Table 1, whereas protein functions and tissue distributions are summarized in Table 2.

The PNPLA family consists of ten family members (denoted PNPLA1–PNPLA10). These proteins, with the exception of PNPLA2 (ATGL), PNPLA3 (adiponutrin) and PNPLA4 (GS2), have not been well defined and in many cases are poorly expressed [107,114]. Adiponutrin has known TAG lipase activity and is mostly highly expressed in brown and white adipose tissue, but is also expressed to a lesser degree in most metabolically active tissues including liver [100]. During adipocyte differentiation, adiponutrin expression is strongly induced; however, in adipose tissue of obese mouse models, its expression is either reduced [100] or increased [132], this difference possibly being dependent on the degree of fasting which strongly suppresses expression [100,132,133]. In fasted obese humans, adiponutrin is up-regulated in both subcutaneous and visceral adipose tissue in parallel with changes in ATGL expression [114]. Adiponutrin expression is increased in liver with obesity, although its role in this and other tissues has not been defined.

Two newly identified PNPLA family members that exhibit TAG lipase activity are PNPLA4 (GS2) and PNPLA5 (GS2-like protein) [100]. GS2 is expressed in most metabolically active tissues in a similar fashion to adiponutrin; however, GS2-like

protein is expressed at very low levels in all tissues examined and is undetectable via Northern blot analysis, suggesting that it may be of minor functional significance [100].

TGH (TAG hydrolase) [also named liver carboxylesterase 1 and CEH (cholesterol ester hydrolase)] is expressed in adipocytes [134] and hydrolyses TAG. TGH is reported to account for non-HSL lipase activity in adipose tissue *in vitro* and to mediate up to 50% of basal HSL-independent lipolysis in adipocytes [135,136]. TGH-2 exhibits high sequence homology with and similar tissue distribution to TGH and is also capable of hydrolysing TAG [137]. The *in vivo* relevance of TGH and TGH-2 is unknown, since the chemical inhibition of HSL derived from ATGL-null mice results in a greater than 95% reduction of TAG hydrolase activity compared with activities in wild-type lysates [50], suggesting that the contribution of alternative lipases to TAG hydrolysis is likely to be minimal, at least in white adipose tissue. However, TGH is reported to be an important lipase in the liver [138]. TGH overexpression increased TAG hydrolysis which enabled re-esterification of the released products into VLDL (very-low-density lipoprotein) TAG [138,139]. Attenuating TGH activity decreased TAG mobilization for VLDL assembly [140]. HSL is not expressed in mammalian liver [18], and the role of ATGL is poorly understood (see above), suggesting that TGH may be important in regulating lipid homeostasis in this tissue.

In summary, although there are many newly identified proteins that have predicted and demonstrated TAG lipase activity, it is not clear what their physiological significance is since the use of a specific HSL inhibitor in ATGL-null adipocytes results in a greater than 95% reduction in TAG hydrolase activity [50]. However, it should also be noted that, in other tissues, the contributions of ATGL and HSL have not been well characterized, leading to the possibility that alternative lipases may still have a significant role in regulating lipolysis.

#### THE ROLE OF TAG LIPASES IN THE PATHOPHYSIOLOGY OF OBESITY

Dysregulation of lipolysis and elevated postprandial FAs are a hallmark of obesity [141], and contribute to metabolic disturbances, including insulin resistance in skeletal muscle and liver [71,142], pancreatic  $\beta$ -cell dysfunction [143] and increased VLDL TAG production [144]. Because of their role in TAG mobilization, TAG lipases have been suggested as a target for anti-obesity therapies [113]. Although some studies have demonstrated reduced HSL expression in obesity [113,145–147], others have shown that *in vitro* HSL activity is not affected in non-obese individuals with the metabolic syndrome [148] and that HSL is not related to Type 2 diabetes [149] or familial combined hyperlipidaemia [150]. The inconsistency between reports suggests that HSL may not be critically important for the development of obesity or Type 2 diabetes. There are consistent findings between obesity and increased rates of basal lipolysis [151,152], an effect that appears to be mediated by a reduction in perilipin A levels [147]. In obesity, TNF $\alpha$  is elevated and down-regulates the transcription of perilipin A [152,153] via an NF- $\kappa$ B (nuclear factor  $\kappa$ B)-mediated pathway [154], in a similar manner to the increase in basal lipolysis observed in the perilipin-null mouse [46,47].

Since the phosphorylation state of HSL and perilipin plays a more critical role in the regulation of lipolysis than total expression levels, studies examining the phosphorylation state of these proteins may be more critical when determining the impact of obesity. Insulin blunts PKA-stimulated phosphorylation of HSL and reduces its activity [32]; it might be anticipated that insulin resistance in obesity may be the major factor contributing

to enhanced lipolysis. HSL phosphorylation in adipose tissue of obese and Type 2 diabetes patients has not been examined to date. Whether or not obesity is a cause of the increased plasma FA in obesity, or whether this is simply a consequence of the development of insulin resistance will be important for understanding the causes of increased FA in obesity.

Two recent reports suggest that ATGL mRNA is not regulated in human obesity [112,113], whereas other reports indicate that ATGL mRNA [155,156] and protein expression [114] are negatively associated with insulin resistance and not obesity itself. Despite the uncertainty regarding ATGL in obesity, single nucleotide polymorphisms within the ATGL gene are associated with free FA levels and increased risk of Type 2 diabetes [157]. Furthermore, a premature truncation mutation in the highly conserved patatin domain [158] or in the C-terminal region [159] of ATGL causes neutral lipid storage disease and myopathy. Mutations in the lipase family member CGI-58 cause TAG accumulation in several tissues, resulting in serious pathologies including cardiomyopathy and liver steatosis [121]. Adiponutrin has been reported to be either increased [160] or unchanged [133] with obesity, whereas results from our laboratory demonstrate up-regulation of several PNPLA family members in subcutaneous and visceral adipose tissue with obesity [114]. Taken together, these data suggest that the dysregulation of ATGL/CGI-58 may be important in the development of obesity and secondary complications. Evidently, more detailed studies are required to clarify this position.

#### SUMMARY AND FUTURE DIRECTIONS

Our understanding of the regulation of TAG hydrolysis and the metabolic function of TAG lipases has expanded rapidly over the last decade. The role of HSL as an important TAG and DAG lipase has been well defined and its regulation largely characterized. More recent studies demonstrate that ATGL and its interacting partner CGI-58 are major players in TAG lipolysis and whole-body metabolic control. In this regard, whole-body HSL- and ATGL-knockout mice have provided critical information with respect to lipase regulation and function in several metabolic processes, but have also raised important questions with respect to the tissue specificity of lipases in metabolic control and whole-body energy homeostasis. Most recently, we have also begun to appreciate the importance of lipase trafficking and the complexity of lipase interactions with other scaffold-like proteins such as perilipin A; however, there is a paucity of information with respect to the molecular and cellular pathways controlling these events. Another important issue is the need to address the *in vivo* relevance and regulation of other TAG lipases, including members of the PNPLA family and TGH. Understanding these relationships may provide important information to develop novel pharmaceutical/dietary interventions for the treatment of obesity and associated disorders.

M. J. W. is supported by an R. Douglas Wright Fellowship, and G. R. S. is supported by a Senior Research Fellowship from the National Health and Medical Research Council of Australia.

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