Lipolysis: pathway under construction

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Purpose of review

The lipolytic catabolism of stored fat in adipose tissue supplies tissues with fatty acids as metabolites and energy substrates during times of food deprivation. This review focuses on the function of recently discovered enzymes in adipose tissue lipolysis and fatty acid mobilization.

Recent findings

The characterization of hormone-sensitive lipase-deficient mice provided compelling evidence that hormone-sensitive lipase is not uniquely responsible for the hydrolysis of triacylglycerols and diacylglycerols of stored fat. Recently, three different laboratories independently discovered a novel enzyme that also acts in this capacity. We named the enzyme 'adipose triglyceride lipase' in accordance with its predominant expression in adipose tissue, its high substrate specificity for triacylglycerols, and its function in the lipolytic mobilization of fatty acids. Two other research groups showed that adipose triglyceride lipase (named desnutrin and Ca-independent phospholipase A2ζ, respectively) is regulated by the nutritional status and that it might exert acyl-transacylase activity in addition to its activity as triacylglycerol hydrolase. Adipose triglyceride lipase represents a novel type of 'patatin domain-containing' triacylglycerol hydrolase that is more closely related to plant lipases than to other known mammalian metabolic triacylglycerol hydrolases.

Summary

Although the regulation of adipose triglyceride lipase and its physiological function remain to be determined in mouse lines that lack or overexpress the enzyme, present data permit the conclusion that adipose triglyceride lipase is involved in the cellular mobilization of fatty acids, and they require a revision of the concept that hormone-sensitive lipase is the only enzyme involved in the lipolysis of adipose tissue triglycerides.

Keywords

adipose tissue, fatty acid mobilization, lipases, lipolysis

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Abbreviations

ATGL	adipose triglyceride lipase
FFA	free fatty acid
HSL	hormone-sensitive lipase
PKA	protein kinase A
PNPLA	patatin-like phospholipase domain containing protein A
WAT	white adipose tissue

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Introduction

Obesity in mammals and humans occurs when energy substrate intake exceeds energy expenditure, and is characterized by the pathological accumulation of fat and white adipose tissue (WAT). Although adipose tissue homeostasis is regulated by a vast number of neural and hormonal signals [1,2$^{\bullet}$–4$^{\bullet}$] they, in a simplified view, all funnel into a metabolic equilibrium between triacylglycerol synthesis and triacylglycerol degradation. Investigation of these processes has been so extensive that until recently most of the lipolytic and lipogenic pathways were thought to be completely described. However, with the generation and characterization of induced mutant mouse lines that lacked known enzymes for lipid synthesis and catabolism, it became evident that important aspects have been missed. In this review, we summarize and discuss recent progress in the field of fat cell lipolysis.

Lipolysis: new players on the team

Triacylglycerols in WAT are continuously turned over by lipolysis and re-esterification. Under fasting conditions or periods of increased energy demand, triacylglycerol-associated free fatty acids (FFAs) are released into the circulation and transported to other tissues. The mobilization of triacylglycerol stores is tightly regulated by hormones, and requires the activation of lipolytic enzymes. Until recently, hormone-sensitive lipase (HSL) was the only known and therefore presumed rate-limiting enzyme for the initial steps of fat catabolism, namely the hydrolysis of triacylglycerols and diacylglycerols. However, three important observations have cast doubt on the view that HSL initiates the lipolytic process. First, mice lacking HSL (HSL-knockout mice) exhibited normal body weight and decreased fat mass [5–8]. Second, these animals retained a marked basal and isoproterenol-stimulated lipolytic capacity in adipose tissue [5–9]. Third, lipolysis in the absence of HSL led to the accumulation of diacylglycerol in fat cells [10]. Taken

together these results suggested that: (1) at least one unidentified lipase must exist and is enzymatically active when HSL is absent; (2) the unknown lipase exhibits a preference for the hydrolysis of the first ester bond of the triacylglycerol molecule; and (3) HSL is rate-limiting for diacylglycerol hydrolysis rather than triacylglycerol hydrolysis.

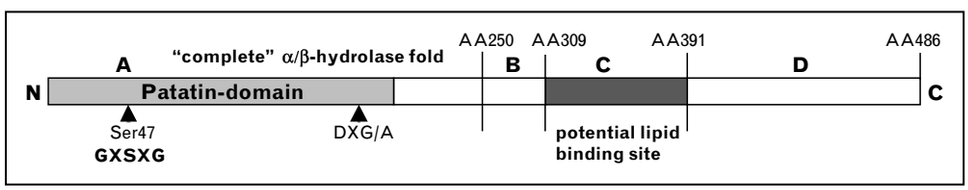
Very recently, a novel triacylglycerol lipase was discovered that indeed exhibited essentially all predicted properties [11•]. The enzyme, named 'adipose triglyceride lipase' (ATGL), is expressed predominantly in WAT, is localized to the adipocyte lipid droplet, and specifically initiates triacylglycerol hydrolysis resulting in the generation of diacylglycerols and FFAs. Several important findings strongly support a role for ATGL in the mobilization of FFAs from mammalian triacylglycerol stores: (1) the overexpression of ATGL enhanced basal and isoproterenol-stimulated lipolysis in 3T3-L1 adipocytes; (2) the inhibition of ATGL by antisense technologies reduced basal and isoproterenol-stimulated lipolysis in 3T3-L1 adipocytes; (3) the antibody-directed inhibition of ATGL in murine fat pads decreased triacylglycerol lipase activity in murine adipose tissue of wild-type mice as much as 70%, and led to an almost complete loss of triacylglycerol hydrolase activity in WAT of HSL-knockout mice.

More or less simultaneously with the publication on ATGL, two additional publications added important insights. Villena *et al.* [12•] found that the level of messenger RNA for a protein they named desnutrin, which is identical to ATGL, exhibited a nutritional response expected for a lipolytic enzyme, namely it is highly upregulated in fasted mice and reduced again when the animals are refed. Although the enzymatic function of desnutrin was not investigated in that study, the authors found that the transient overexpression of desnutrin caused decreased triacylglycerol accumulation in transfected COS-7 cells, and thus speculated that the protein could be a triacylglycerol hydrolase. Most interestingly, desnutrin mRNA levels were found to be induced by glucocorticoid treatment of differentiated 3T3-L1 cells and reduced in adipose tissue of genetically obese ob/ob and db/db mice. As part of a general analysis of patatin domain-containing proteins, Jenkins *et al.* [13•] measured triacylglycerol-hydrolase activity for a protein they called calcium-independent phospholipase A2ζ (iPLA2ζ; identical to ATGL and desnutrin). Taken together, these results suggested that ATGL is the missing triglyceride lipase responsible for most of the lipolytic activity in HSL-deficient adipose tissue. These results do not exclude the existence of additional triacylglycerol hydrolases in adipocytes such as the recently described triacylglycerol hydrolase [14,15]. However, the quantitative contribution of these factors to fat cell lipolysis is currently unknown and remains to be determined.

Adipose triglyceride lipase: a novel type of metabolic triacylglycerol lipase containing a 'patatin' domain

The mouse ATGL gene (chromosome 7F5) is approximately 6 kb in length and contains nine exons. The 2.0 kb mRNA codes for a 54 000 M_r protein of 486 amino acids. The human ATGL ortholog (chromosome 11p15.5) exhibits 87% amino acid identity with the mouse enzyme. Interestingly, a 'patatin' domain (Pfam01734) can be detected in the N-terminal region of ATGL (Fig. 1). Patatin domain-containing proteins comprise a large gene family across eukarya and microorganisms [16,17]. They are commonly found in plant storage proteins such as the prototype patatin, an abundant protein of the potato tuber [18]. These proteins have been shown to have acyl-hydrolase activity on phospholipid, monoacylglycerol and diacylglycerol substrates [18]. In the human genome, 10 putative, patatin domain-containing proteins are found in databases. Four of them are closely related to ATGL, comprising a gene family of 'patatin-like phospholipase domain containing proteins A1–5' (PNPLA1–5) (Table 1). The pairwise homology among the PNPLAs ranges between 25 and 45% amino acid identity within the patatin domain. The nearest phylogenetic neighbor of ATGL within the gene family is adiponutrin (PNPLA3). Patatin domains are also

Figure 1. A provisional assignment of functional domains in adipose triglyceride lipase based on sequence conservation and the presence of structural motifs in adipose triglyceride lipase, adiponutrin, and GS-2-like protein



Stretch A is assumed to contain the lipolytic domain including the putative active serine at position 47. A 'patatin' domain (Pfam01734) can be detected in the same region. Stretch C is possibly membrane or lipid associated because of the elevated number of hydrophobic amino acid residues. The sequences of stretches B, and D have no deducible functional domains.

Table 1. Adipose triglyceride lipase-related sequences in the human and the mouse

Human sequence	Mouse ortholog	Enzymatic function
PNPLA1	PNPLA1	Unknown
ATGL (TTS2.2, iPLA2ζ, PNPLA2)	ATGL (TTS2.2, desnutrin PNPLA2)	Triacylglycerol-hydrolase [11•,13•] Diacylglycerol-transacetylase [13•]
Adiponutrin (iPLA$_2\varepsilon$, PNPLA3)	Adiponutrin (PNPLA3)	Triacylglycerol-hydrolase [13•] Diacylglycerol-transacetylase [13•]
GS-2 (iPLA$_2\eta$, PNPLA4)	Unknown	Triacylglycerol-hydrolase [13•] Diacylglycerol-transacetylase [13•]
GS-2-like (PNPLA5)	PNPLA5	Unknown

ATGL, Adipose triglyceride lipase; iPLA2, calcium-independent phospholipase A2; PNPLA, patatin-like phospholipase domain containing protein A; TTS2.2, transport-secretion protein 2.2.

present in TGL3, a triacylglycerol lipase of *Saccharomyces cerevisiae* [19] and in human cytosolic phospholipase A2 [20]. The crystal structure of both patatin and cytosolic phospholipase A2 revealed a novel topology for lipases with an unusual Ser-Asp catalytic dyad in the active site [20,21]. Accordingly, it is possible that ATGL functions by a similar molecular mechanism.

In addition to the patatin domain, the N-terminal region (Fig. 1, stretch A) also harbours a 'predicted esterase of the α/β hydrolase fold' domain (COG1752) as well as a GXSXG-consensus sequence for Ser-lipases containing a putative active serine at position 47 of ATGL, which are also present in four out of the five PNPLA family members. The function of sequence stretches B, C, and D in ATGL is less well defined. The elevated amount of hydrophobic residues in stretch C suggests a potential lipid/membrane-binding site in ATGL. The sequences of stretches B and D are diverse with no deducible functional domains.

Enzymatic function of adipose triglyceride lipase and other members of the PNPLA gene family

The described structural features such as the patatin domain, the α/β hydrolase fold and the GXSXG lipase/esterase consensus sequence present in several PNPLA family members, as well as the observation of an enzymatic activity as triacylglycerol hydrolase, implied that ATGL and perhaps other family members contribute to the lipolytic pathway.

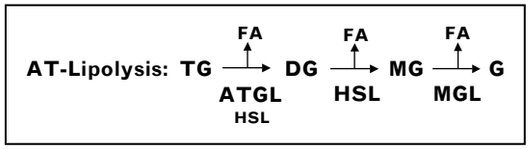
Adipose triglyceride lipase (PNPLA2)

In mice and humans, ATGL is predominantly expressed in white and brown adipose tissue, with progressively decreasing amounts found in the testis, cardiac muscle, and skeletal muscle [11•,12•]. The enzyme exhibits high substrate specificity for the hydrolysis of triacylglycerol, whereas little or no activity is measured against cholesteryl oleate, retinyl palmitate or phosphatidylcholine substrates [11•,13•]. ATGL catalysed hydrolysis of triacylglycerol substrates leads to the accumulation of diacylglycerol in assay mixtures, indicating the low substrate specificity of ATGL for diacylglycerol. In contrast, HSL has been shown to hydrolyse diacylglycerol and cholesteryl-oleate much better than triacylglycerol [22,23]. In adipose tissue of HSL-knockout mice, the observed diacylglycerol accumulation is indicative of a rate-limiting role of HSL in the catabolism of diacylglycerol [10]. ATGL and HSL thus possess distinctly different substrate specificities, and it has been suggested [11•] that the hydrolysis of the first ester bond in triacylglycerol is predominantly catalysed by ATGL, whereas the resulting diacylglycerols are efficiently hydrolysed by HSL (Fig. 2). The hydrolysis of monoacylglycerol is performed by monoglyceride lipase [24]. These results imply that every step within the lipolytic cascade of triacylglycerol hydrolysis employs a distinct lipase, and raises the possibility that each point may be subject to both independent and coordinate mechanisms of regulation. This independent regulation could be important for the ATGL-mediated synthesis of diacylglycerol, which at low HSL activity (basal lipolysis), could be utilized for re-esterification or remodeling into glycerophospholipids. In hormone-stimulated adipocytes, the drastic induction of HSL would prevent diacylglycerol accumulation and result in efficient glycerol and FFA release from the cells (for a postulated model see Fig. 3).

Adiponutrin (PNPLA3)

Adiponutrin was originally identified by differential display techniques during the differentiation of 3T3-L1 cells [25]. The human adiponutrin gene consists of nine exons, is located on chromosome 22q13.31 and codes for a 3.2 kb mRNA. The gene is expressed exclusively in white and brown adipose tissue and the adiponutrin protein is 413 amino acids long. Adiponutrin exhibits high sequence homology with ATGL (approximately 40%) and shares many structural domains including the

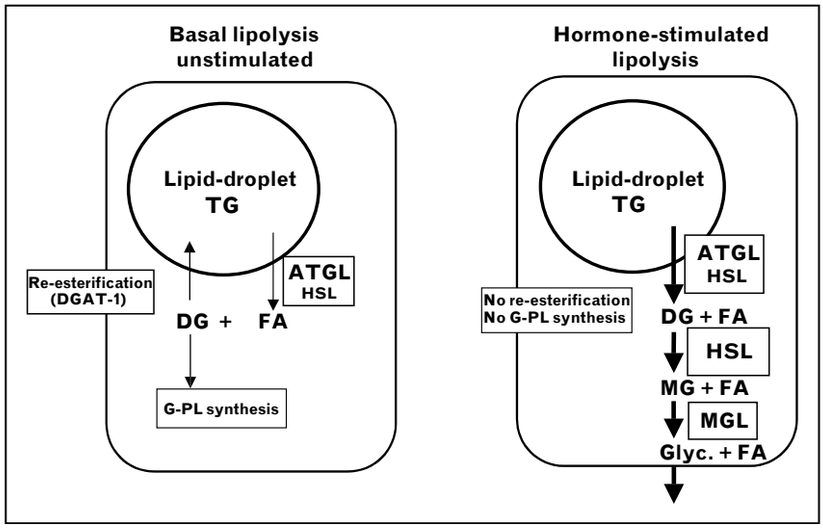
Figure 2. Proposed function of adipose triglyceride lipase, hormone-sensitive lipase and monoglyceride lipase within the hydrolysis cascade of triacylglycerol



Adipose triglyceride lipase (ATGL) predominantly performs the initial step in triacylglycerol (TG) hydrolysis resulting in the formation of diacylglycerols (DG) and free fatty acids (FA). Hormone-sensitive lipase (HSL) hydrolyses triacylglycerols, diacylglycerols and monoacylgycerols (MG) at a ratio of 1 : 10 : 1. Monoglyceride lipase (MGL) is believed to represent the rate-limiting enzyme for monoacylgycerol hydrolysis to form glycerol (G) and fatty acids. AT, adipose triglyceride.

Figure 3. Model of hormonally unstimulated or stimulated lipolysis in adipocytes

In unstimulated cells, adipose triglyceride lipase (ATGL) hydrolyses triacylglycerols (TG) to diacylglycerols (DG). Low, basal hormone-sensitive lipase (HSL) activities permit diacylglycerol re-esterification or their use as substrates for glycerophospholipid (G-PL) synthesis. Hormonal stimulation and the recruitment of HSL to the lipid droplet causes efficient diacylglycerol hydrolysis and glycerol ((Glyc.) and free fatty acids (FA) are released from the cells. MG, monoacyglycerol; MGL, monoglyceride lipase.



patatin domain, the α/β hydrolase fold, the GXSXG lipase consensus domain, and several hydrophobic, possibly membrane-binding domains. However, major differences between ATGL and adiponutrin have been reported with respect to their regulation and cellular localization. In contrast to ATGL, adiponutrin mRNA levels are dramatically reduced in WAT when mice or humans are fasted [26,27,28•]. Furthermore, adiponutrin mRNA concentrations are upregulated in genetically obese fa/fa rats, whereas ATGL mRNA levels are down-regulated in genetically obese mice [12•,25]. Finally, adiponutrin was reported to be bound to membranes, whereas ATGL is cytosolic or associated with the lipid droplet in adipocytes [11•,12•,25]. The expression profile of adiponutrin and its cellular localization would thus seem to exclude a function for the protein as a metabolic lipase involved in the hydrolysis of stored fat during fasting. Therefore, it was quite unexpected when in contrast to our findings [11•], Jenkins *et al.* [13•] reported human adiponutrin (named Ca-independent phospholipase A2ε in their paper) to be enzymatically active against a triacylglycerol substrate. The contradicting observations may be explained by species-specific differences between the human and mouse adiponutrin or differences between the substrates tested (triacylglycerol stabilized in deoxytaurocholate micelles [13•] versus phosphatidylcholine-stabilized triacylglycerol emulsions [11•]). However, additional experiments will be necessary to determine whether adiponutrin indeed acts as a triacylglycerol lipase in fat cells.

Gene sequence 2 (PNPLA4)

The gene sequence 2 (GS2) gene was first isolated by Lee *et al.* [29] from a CpG island, and is located on human chromosome Xp22.3 between the genes for steroid sulfatase and the Kallmann syndrome gene. The GS2 gene has seven exons, is expressed in essentially all human tissues, and codes for a protein of 253 amino acids. Although GS2 exhibits only approximately half the size of ATGL, the polypeptide harbours a complete patatin domain as well as the α/β-hydrolase domain including the GXSXG lipase-consensus sequence. To date, no murine ortholog of GS2 has been identified. For human GS2 (named Ca-independent phospholipase A2η) Jenkins *et al.* [13•] demonstrated triacylglycerol-hydrolase activity when the protein was overexpressed from a baculovirus expression system in SF9 insect cells and triacylglycerol/deoxytaurocholate micelles were used as substrate. Most interestingly, the authors also showed that all three members of the gene family, ATGL, adiponutrin, and GS2 can catalyse a transesterification of fatty acids from monoglycerides or diacylglycerols to triacylglycerols [13•]. Such an activity has not been described before in adipose tissue and would provide an acyl-coenzyme A independent pathway for the synthesis of triacylglycerol in WAT that might be crucially involved in the re-esterification process of incompletely lipolysed acylglycerides.

The other putative members of the PNPLA gene family, GS2-like protein (PNPLA5, human chromosome 22q13.31) and PNPLA1 (human chromosome 6p21.31) have not been cloned or studied for function.

Regulation of adipocyte lipolysis and the participation of adipose triglyceride lipase

Changing physiological conditions tightly control adipocyte lipolysis. Regulation is mediated by the direct or indirect action of numerous lipolytic and antilipolytic hormones and (adipo)cytokines such as growth hormone,

glucocorticoids, atrial natriuretic peptide, leptin, resistin, TNF-α, IL-6, and adiponectin [30,31]. Other adipokines, such as the newly discovered visfatin [32•], have not been analysed for their effects on lipolysis. Unfortunately, the signal transduction pathways and molecular mechanisms that regulate lipolysis in response to these agonists/antagonists are unknown or incompletely understood. Well-characterized exceptions are the regulatory circuits responding to the most prominent stimulators of lipolysis, catecholamines, and the most potent antilipolytic hormone, insulin. The action of catecholamines and insulin has been extensively studied with regard to HSL [33,34, 35•]. The binding of catecholamines to β-adrenergic receptors stimulates adenylate cyclase via a stimulatory G protein, leading to increased cellular cyclic adenosine monophosphate levels and the activation of protein kinase A (PKA), which induces lipolysis by the phosphorylation of HSL and perilipin A. PKA phosphorylates HSL at three serine residues (563, 569, 660) resulting in moderately heightened activity against triglyceride and cholesteryl ester substrates. Full HSL activation relies on its translocation from the cytosol to the lipid droplet. This process involves the PKA-mediated phosphorylation of perilipin A [36–38], an abundant structural protein bound at the surface of adipocyte lipid droplets by hydrophobic interaction [39]. In the non-phosphorylated state, perilipin protects the lipid store from hydrolysis. Interestingly, the absence of perilipin, as observed in perilipin knockout mouse models, leads to increased basal lipolysis and a drastic reduction of the lipid mass [40,41]. The opposing effect of insulin is caused by the stimulation of phosphodiesterase 3B resulting in decreased cyclic adenosine monophosphate levels and suppressed activation of PKA [42].

Insufficient time has passed since the discovery of ATGL to understand the nature of its regulation. However, from the limited data available, it appears that ATGL is regulated differently than HSL. For example, various effectors including fasting/feeding, glucocorticoids, or the absence of leptin expression affect ATGL mRNA concentrations [12•], whereas HSL is predominantly regulated by post-translational mechanisms. Whether changes in ATGL mRNA levels in response to the above effectors are also reflected in changes of ATGL enzyme activity, however, is presently not known and needs to be determined.

On the protein level, both HSL and ATGL are phosphorylated, but ATGL phosphorylation has been shown to be independent of PKA [11•], whereas this kinase is crucial to the activation of HSL. It will be important to establish whether ATGL phosphorylation affects enzyme activity and to identify the kinases involved. Conceivable candidates already known to participate in the regulation of lipolysis include extracellular signal-regulated kinase [43–45], cyclic guanosine monophosphate-dependent protein kinase I [46], and adenosine 5′-monophosphate-activated kinase [47–49].

Another important regulatory difference between HSL and ATGL refers to enzyme recruitment to the lipid droplet in response to lipolytic effectors. Whereas lipolytic agonists, such as catecholamines, initiate a translocation of HSL from the cytoplasm to the surface of the lipid droplet, such reversible enzyme recruitment has not been observed with ATGL. From the preliminary data available on ATGL, the enzyme appears to be lipid associated under stimulated and unstimulated conditions [11•]. The constitutive presence of ATGL on lipid droplets implies that a translocation-based activation pathway is unlikely. Alternative mechanisms that regulate ATGL activity in response to hormones and cytokines will have to be considered. One option would be that the interaction with co-factors (in)activates the enzyme. Well-known examples among other metabolic lipases exist that interact with co-factors before hydrolysing triacylglycerol in large fat globules. HSL interacts with adipocyte lipid binding protein [50,51•], and possibly lipotransin [52], thereby affecting HSL enzyme activity. Other examples include lipoprotein lipase, which requires apolipoprotein CII for optimal activity against chylomicron and VLDL associated triacylglycerols [53,54] and pancreatic lipase(s) [55,56], which need colipase during the intestinal digestion of alimentary fat. These co-factors are believed to increase the hydrophobicity of the enzyme–co-factor complex and promote substrate binding and enzymatic activity at the water–lipid interphase. It is conceivable that ATGL also requires such 'interphase activation', and potential candidates could include already known lipid droplet-associated proteins [57•] such as PAT protein family members (perilipin, adipophilin, TIP47) [58•,59,60], other established lipid globule binding proteins (S3-12, CGI-58, adipophilin) [61,62•–64•], or presently unknown proteins.

Adipose triglyceride lipase: a potential drug target?

Given that ATGL is a critical lipase for the degradation of stored lipids, this might have important implications for the pathogenesis of type 2 diabetes, and render ATGL a potential drug target. The mechanisms involved in the development of insulin-resistance and type 2 diabetes are multifactorial and are only partly understood. However, elevated concentrations of circulating FFAs in plasma are considered a causative factor for the impaired uptake of glucose in muscle and liver causing insulin resistance, hyperinsulinaemia, hyperglycaemia, and dyslipidaemia [65–67]. As the lipolytic process critically affects the concentration of circulating FFAs, inhibiting lipases to decrease FFA release is considered a potential target for

the treatment of insulin resistance in type 2 diabetes. Accordingly, specific inhibitors of ATGL (and HSL) offer novel therapeutic approaches for the treatment of these conditions.

The evidence supporting the premise that the pharmacological regulation of metabolic lipases to control FFA release from WAT will affect carbohydrate and energy metabolism predominantly originates from HSL-knockout mice. HSL deficiency in mice results in decreased plasma fatty acid concentrations, low plasma triacylglycerol and VLDL levels, and increased HDL-cholesterol concentrations [6]. In addition, HSL-knockout animals exhibit decreased adipose tissue mass and resistance to obesity induced by high-fat diets or leptin deficiency [68,69•]. The negative effect of male infertility [5], the accumulation of diglycerides in many tissues including WAT [10], and insulin resistance in WAT (unpublished observation) compromise the preferential lipid and lipoprotein phenotype in HSL-deficient animals. The implications of HSL deficiency on carbohydrate metabolism have been controversial. Voshol *et al*. [70] found that HSL deficiency resulted in low FFA, decreased insulin, and increased glucose levels in plasma, which were associated with decreased hepatic triacylglycerol stores and increased hepatic insulin sensitivity. Using an independently established HSL-knockout mouse line, Park *et al*. [71] found similar results in chow-fed mice, but also demonstrated that HSL-knockout mice on a high-fat diet were resistant to muscular lipid accumulation that caused decreased insulin resistance. In contrast, Roduit *et al*. [72] and Mulder *et al*. [73] concluded from their studies that HSL-deficient mice were insulin resistant because of a blunted response of plasma glucose levels and an impaired suppression of hepatic insulin production in response to insulin. Whether changes in ATGL activity will affect energy metabolism in a similar way to HSL deficiency remains to be determined.

Conclusion

The discovery of ATGL and the demonstration that it is involved in the hydrolysis of triacylglycerols to form diacylglycerols supports the concept that each step of the lipolytic cascade is subject to independent regulation by various agonists and antagonists. To date, however, numerous questions need to be addressed to understand the regulation of lipolytic enzymes, their coordinate interaction, and the metabolic fate of their products. The generation and characterization of mouse lines that overexpress or lack ATGL will apparently provide important animal models to answer some of these questions.

Acknowledgement

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- • of special interest
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