Lipolysis and lipases in white adipose tissue – An update

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ABSTRACT
Lipolysis is defined as the sequential hydrolysis of triacylglycerol (TAG) stored in cell lipid droplets. For many years, it was believed that hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MGL) were the main enzymes catalyzing lipolysis in the white adipose tissue. Since the discovery of adipose triglyceride lipase (ATGL) in 2004, many studies were performed to investigate and characterize the actions of this lipase, as well as of other proteins and possible regulatory mechanisms involved, which reformulated the concept of lipolysis. Novel findings from these studies include the identification of lipolytic products as signaling molecules regulating important metabolic processes in many non-adipose tissues, unveiling a previously underestimated aspect of lipolysis. Thus, we present here an updated review of concepts and regulation of white adipocyte lipolysis with a special emphasis in its role in metabolism homeostasis and as a source of important signaling molecules.

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Adipose triglyceride lipase; hormone-sensitive lipase; monoacylglycerol lipase; perilipin; triacylglycerol hydrolysis

INTRODUCTION
Pre-formed lipids that come from the diet or even those that are synthetized are stored mainly in the white adipose tissue (WAT) as triacylglycerols (TAG), neutral lipids made up of three fatty acids esterified to the carbon backbone of a glycerol molecule (1-3). The WAT, described as the main energy reservoir in mammals, has a mesenchymal origin and is made up by a matrix of connective tissue, immune system cells, blood vessels, sympathetic innervation, stem cells and pre-adipocytes, as well as adipocytes, cells specialized in the intake, storage, and mobilization of fat (4).

Adipocytes store TAG in a single, large lipid droplet that takes up 85-90% of the cytoplasm and pushes the nucleus and a fine cytosol layer to the periphery of the cell. The lipid droplet is recognized as a dynamic cell organelle, surrounded by a monolayer of phospholipids, structural proteins, and enzymes, which protects cell organelles from the cytotoxic effects of fatty acids (5-7). Among the several proteins that make up this monolayer, perilipin 1A may be emphasized, as it is the main perilipin found in the adipocyte. Depending on its activation status, perilipin 1A may protect and delimit the lipid droplet, or make lipid hydrolysis easier (7,8).

The amount of TAG stored in the adipocytes, which are in a constant flow in the TAG-fatty acid cycle, depends on the balance between lipogenesis (the biosynthesis, incorporation, and storage of TAG in the lipid droplet in the cytoplasm), and lipolysis (the sequential hydrolysis of TAG, fatty acids, and glycerol) (1,9,10). In prolonged periods of positive energy balance, which result from excessive consumption of high-calorie foods added to the lack of physical activity, adiposity increases. This chronic process may lead to the development of obesity (11,12). On the other hand, in prolonged periods of fasting or of high demands of energy, lipolysis is essential to supply fatty acids and glycerol as energy substrates to tissues. However, changes in this process have frequently been associated with lipodystrophy, hyperlipidemia, insulin resistance, and obesity (12,13).

For many years, it was believed that hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MGL) were the main enzymes involved in WAT lipolysis. However, after the discovery of the adipose triglyceride lipase (ATGL) in 2004, many studies were performed to investigate and characterize the actions of this lipase, as well as of other proteins and possible regulatory mechanisms involved, which reformulated the concept of lipolysis. Novel findings from these studies include the identification of lipolytic products as signaling molecules regulating important metabolic processes in many non-adipose tissues, unveiling a previously underestimated aspect of lipolysis. Thus, we present here an updated review of concepts and regulation of white adipocyte lipolysis with a special emphasis in its role in metabolism homeostasis and as a source of important signaling molecules.
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lipase (ATGL) in 2004 (14-16) and the later characterization of its actions and regulation mechanism, a new concept of lipolysis was proposed. During this period, evidence was found that indicated that, besides providing energy, final and intermediate products generated in the lipolysis process may act as signaling molecules that regulate important metabolic process in many non-adipose tissues, unveiling previously underestimated aspects of lipolysis. Therefore, we present here an updated review of lipolysis concepts and regulation in white adipocytes, with special emphasis in its role as a source of important signaling molecules in the control of metabolic homeostasis.

Lipolysis in white adipocytes

Lipolysis consists of the sequential hydrolysis of TAG to its constituent molecules glycerol and three fatty acids, catalyzed by three different enzymes. In the first stage of this process, TAG is hydrolyzed to diacylglycerol (DAG) and one molecule of fatty acid, in a reaction mainly catalyzed by the enzyme ATGL. After that, DAG is converted to monoacylglycerol (MAG) and a second fatty acid by the action of HSL. In the end, MGL hydrolyses MAG, producing glycerol and a last fatty acid (9,13) (Figure 1).

![Figure 1. Sequential hydrolysis of triacylglycerol.](image)

FA: fatty acid; ATGL: adipose triglyceride lipase; DAG: diacylglycerol; HSL: hormone-sensitive lipase; MGL: monoacylglycerol lipase; MAG: monoacylglycerol; TAG: triacylglycerol.

Fatty acids derived from lipolysis are, most of the times, released in the bloodstream and bound to albumin to be used as energy substrates by other tissues. However, part of these molecules is kept inside adipocytes, acting as intracellular signaling mediators and working as substrates for the synthesis of other fatty acids and lipids (including membrane lipids and other lipids involved in cell metabolism), as gene transcription regulators, oxidation substrates or undergoing reesterification to make up TAG again. Approximately 30-40% of these fatty acids undergo this reesterification, a process that is dependent on the intracellular generation of glycerol 3-phosphate (17).

In relation to glycerol produced in the lipolytic process, most of it is released into the bloodstream and mainly functions as substrates for glucose production in liver gluconeogenesis (3). Recently, with the discovery of the presence glycerol kinase, an enzyme the converts glycerol into glycerol 3-phosphate, and its activity in adipocytes raised the hypothesis that part of the glycerol produced by lipolysis may be recycled into TAG still inside of this cell (18).

Lipolysis is a process that occurs under non-stimulated (basal conditions) or in conditions stimulated by hormones (19,20). In basal conditions, HSL predominantly remains in the cytosol, whereas perilipin, ATGL and its co-activator, protein Abhd5 (abhydrolase domain-containing 5), also called CGI-58 (comparative gene identification 58), are found on the surface of the lipid droplet (7). ATGL is highly specific for TAG hydrolysis, and it is responsible for large part of the lipolytic activity found both in basal and stimulated conditions. However, in basal conditions, the co-activator CGI-58 remains bound to perilipin (forming an inactive complex), that limits the hydrolytic activity of lipase (9,19).

In periods of fasting or high energy demands, the adipocyte is stimulate by hormones, mainly by the action of catecholamines, leading to a series of intracellular reactions that culminates in the activation of protein kinase A and G (PKA/PKG), which promotes the phosphorylation of HSL and perilipin (19,21,22). After phosphorylation, HSL is translocated into the lipid droplet and acts together with ATGL, accelerating the lipolytic process. Together, ATGL and HSL are responsible for about 95% of the hydrolysis of TAG (23). Similar, after the phosphorylation of perilipin, the position of this compound on the lipid droplet changes, making TAG hydrolysis by the lipases easier. With the phosphorylation of perilipin, CGI-58 is released and binds to ATGL, forming an active complex and steeply increasing the action of this lipase (13,19,22).

HSL is more specific for DAG hydrolysis, and may also hydrolyze TAG and MAG, with lower efficiency, though. MGL, on the other hand, is located both in the cytosol of the cell and on the surface of the lipid droplet, and shows specificity for MAG hydrolysis, catalyzing the final step of lipolysis, that is, the conversion of MAG into glycerol and a free fatty acid (13,19) (Figure 2).

ATGL shows strong specificity and/or selectivity for the hydrolysis of the long-chain fatty acid esters (16
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or 18 carbons) found in position sn2 of the glycerol backbone, which mainly includes the hydrolysis of palmitoleic acid followed by palmitic and stearic acid. The main product of this reaction is 1,3-DAG, the preferential substrate of the lipogenic enzyme diacylglycerol acyltransferase 2 (DGAT2). This suggests that the enzymes ATGL and DGAT act in a coordinated fashion during the TAG hydrolysis/reesterification cycle. On the other hand, after binding to CGI-58, ATGL starts to hydrolyze fatty acids also found in position sn1, releasing 2,3-DAG, which is the preferential substrate of HSL (24).

The main phenotypical consequence of ATGL reduction is the massive accumulation of TAG in adipocytes and other tissues, favoring obesity development and other metabolic complications (19,25). Still, this reduced TAG hydrolysis negatively affects thermoregulation in these animals, due to the reduced offer of free fatty acids for mitochondrial respiration. Therefore, mice with lower expression of ATGL show severe hypothermia when exposed to cold environments for five hours (25).

In the cardiac muscle, for example, the lack of ATGL leads to an increase of up to 20 times TAG content, causing cardiac failure followed by premature death. Reestablishment of ATGL expression only in the heart reverses the accumulation of TAG and associated cardiac failure (27). Besides, ATGL-deficient mice develop micro- and macro-endothelial dysfunctions that are reverted after treatment with agonists of nuclear peroxisome proliferator-activated receptors (PPAR) type (α) (28).

As for the lipid profile, ATGL-deficient animals show reduced plasma concentration of free fatty acids.
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TAG, β-hydroxybutyrate, total cholesterol, very low density lipoprotein (VLDL) and high density lipoprotein (HDL) (19). In pancreatic islets, lipolysis promoted by ATGL is essential to generate signaling lipid molecules that modulate positively glucose-stimulated insulin secretion (GSIS), an effect that depends on PPAR type delta (δ) (29). Different from the deficiency, ATGL overexpression is associated with increased basal and stimulated lipolysis (19), fatty acid oxidation, and reduced TAG deposition and adipocyte size (30).

Several factors have been identified in the regulation of gene expression and ATGL hydrolytic activity. The levels of messenger RNA (RNAm) of this enzyme are elevated, for example, by the action of PPAR agonists type α and γ, glucocorticoids, AMP-activated protein kinase (AMPK), and fasting; and are reduced by insulin, activation of mTOR complex 1, and food intake (17,31-35).

Eight ATGL phosphorylation sites, including serine 406 and 430 have been recently described (32,36,37). Although the importance of these phosphorylations for efficient ATGL activity has not been completely elucidated, it was already demonstrated that AMPK phosphorylates ATGL at serine 406, increasing its activity (32). Pagnon and cols. observed that phosphorylation at serine 406 of ATGL is also a PKA target, confirming that β-adrenergic/catecholamine activation is capable of positive modulation of this lipase, accelerating lipolysis (36), as previously demonstrated for HSL. On the other hand, Xie and cols., in 2014, identified that ATGL phosphorylation at threonine 372 modulates the activity of the lipase negatively, hindering its localization in the lipid droplet of the adipocytes (37).

Evidence also indicates that ATGL activity is modified by its interaction with other proteins, such as CGI-58, perilipin (previously described) and G0/G1 switch gene 2 (G0S2). G0S2, a protein that acts in the transition from G0 to G1 in the cell cycle, is located in adipocyte lipid droplet, cytoplasm, endoplasmic reticule and mitochondria. In the lipid droplet, G0S2 inhibits ATGL action by physical interaction of its N-terminal with the N-terminal patatine domain of ATGL (38).

Additionally, fat-specific Protein 27 (FSP27), also found in the lipid droplet, interacts with ATGL reducing its activity and, consequently, decreasing lipolysis (39). Another important ATGL regulation mechanism involves the expression and control of the proteins that transport ATGL from the endoplasmic reticule to the lipid droplet, such as ADP-ribosylation factor 1, GTB-binding protein 1 (SAR1), and golgi-brefeldin A resistance factor (GBF-1) (40).

All these findings point out to ATGL as the main enzyme responsible for TAG hydrolysis. Still, many studies are being carried out in order to clarify the mechanisms that regulate the activity of this lipase, as well as its role in physiological process in different tissues.

HORMONE-SENSITIVE LIPASE (HSL) – FUNCTION AND REGULATION

In the beginning of the 1960s, it was observed that lipolytic activity in the adipose tissue was induced by hormone stimulation. A classical study was published by Dr. Steimberg’s group (41) describing HSL and MGL. At that moment, HSL, a protein of 81 kDa, was considered the main lipase of adipocytes, and the only one involved in TAG hydrolysis, as ATGL had not been discovered yet. HSL has at least five phosphorylation sites, from which serine 660 and 663 seem to be particularly important for hydrolytic activity (42). Haemmerle and cols., using HSL-deficient mice, observed that animals did not accumulate TAG in adipose and other tissues. Differently, they accumulated large amounts of DAG; determining, therefore, the high specificity of HSL in DAG hydrolysis (43).

HSL-deficient mice showed reduced hormone-stimulated lipolysis (43). Similarly, mice that overexpress this lipase show normal basal lipolytic activity, and increased stimulated lipolysis (44).

The regulation of this lipase activity is highly influenced by hormones. Among them, catecholamines (released by the adrenal medulla or by direct sympathetic innervation of the adipose tissue), the atrial natriuretic peptide (ANP), and growth hormone (GH) are important activators. Insulin is an important inhibitor of HSL (17). Catecholamines bind to β-adrenergic receptors that bind to stimulatory protein G, activating adenylate cyclase, which increases the levels of AMPc and, consequently, activates PKA. On its turn, PKA phosphorylates HSL and perilipin. ANP stimulates lipolysis by PKG activation (9,45). On the other hand, the antilipolytic effect of insulin is mediated by phosphodies-terase 3B activation, which decreases AMPc and PKA activity, reducing HSL and perilipin phosphorylation (45). Insulin promotes an increase in protein FSP27 in the lipid droplet and increases adipocyte-specific phospholipase A2 (AdPLA), reducing adenylate cyclase ac-
tivity and, consequently, reducing cytoplasmic levels of AMPc and PKA activity (46,47).

Another known form of regulation of this enzyme is via FABP (fatty acid binding protein). FABP4 binds to HSL when it is phosphorylated, making it easier for fatty acid to be transported inside of the cell after lipolysis. However, FABP4 regulates its action as it promotes a negative feedback for HSL by transporting fatty acids and/or by affecting the dimerization of the enzyme in the lipid droplet (48).

**MONOACYLGlycerol Lipase (MGL) – Function and Regulation**

MGL catalyzes the final step of TAG hydrolysis, that is, MAG hydrolysis into glycerol and fatty acid. Its molecular weight is 33 kDa and it belongs to the serine hydrolase superfamily (41). Evidence shows that MGL hydrolyses specifically MAG, and affects TAG or DAG (49). At the cell level, MGL is located in the cytoplasm, in the plasma membrane and in the lipid droplet. It is widely expressed in the WAT, lungs, liver, kidneys, testicles, brain, and heart (49). Animals deficient in MGL showed to be protected from the development of glucose intolerance and insulin resistance; reduced MGL activity is partially reverted by HSL (50). The MGL-deficient phenotype in animals is partially influenced by its effect on the central nervous system and in the endocannabinoid system (50). It is still unclear if MGL activity is influenced by cell energy status or hormones. In spite of this, it was demonstrated that treating rats with rosiglitazone, a PPARγ agonist (15 mg/kg for 7 days), positively regulates lipolysis and RNAm transcription of this lipase in WAT (51).

**Proteins Associated with the Lipid Droplet**

Proteins associated with the lipid droplet, besides protecting cell organelles from the cytotoxicity caused by the lipids, have a fundamental role in lipolysis regulation (8,52). These proteins belong the PAT family, which includes perilipins 1 to 5. Perilipin 1 has three isoforms: perilipin 1A, perilipin 1B, and perilipin 1C, with perilipin 1A as the main isoform found in WAT. Perilipin 5, or OXPAT, is mainly expressed in tissues of high oxidative capacity, such a brown adipose tissue and skeletal muscle, whereas perilipin 2 and perilipin 3 are found in the adipose tissue and liver (7,53). As described throughout the text, when it is phosphorylated by PKA or PKG, perilipin undergoes a change in position on the lipid droplet, making HSL interaction with the droplet easier. Besides, it releases CGI-58, which binds to ATGL. A reduction in the expression of perilipin 1 leads to increased lipolysis, attributed to greater HSL activity and to the ATGL/CGI-58 interaction (54). On the other hand, perilipin 1 overexpression leads to reduced stimulated lipolysis without changes in basal lipolysis (55).

Recently, new proteins associated with the lipid droplet have been described, such as the CIDE family (cell death-inducing DNA fragmentation factor-a-like effector). The CIDE family has three members: Cidea, Cideb, and Cidec/Fsp27 (7). Cidea and Cideb are found in brown adipose tissue and the liver, but are not expressed in the WAT. On the other hand, Cidec is highly expressed in white and brown adipose tissue. Its deficiency leads to increased basal lipolysis and does not change stimulated lipolysis levels (39,56).

**Peroxisome Proliferator-Activated Receptors (PPARs) and Lipolysis**

PPARs α, γ, and δ are members of the large family of nuclear receptors that act as transcription factors, controlling genes that have fundamental roles in the regulation of lipid metabolism, inflammation, and cell growth and differentiation (57). These nuclear receptors are known as lipid sensors, and have their production and activity highly influenced by nutritional status. Among their important effects in the adipocytes, PPARγ and α have also been described to positively modulate lipolysis (51,58).

PPARγ activation with rosiglitazone, for example, stimulates lipolysis and increases RNAm expression of ATGL e MGL in vivo (51). Corroborating this study, Kershaw and cols. demonstrated that PPARγ positively regulates RNAm and the protein content of ATGL in mature adipocytes, in vitro and in vivo, suggesting that ATGL is a mediator of the PPARγ effects in lipid metabolism (33).

Similarly, treatment of rats with a PPARα agonist, fenofibrate, induces a significant increase of 30% in lipolysis speed in the presence of insulin in WAT (59). Besides, an endogenous elevation of the lipid oleoyl ethanolamine in the small intestine stimulates lipolysis and fatty acid oxidation in epididymis adipose tissue by mechanisms that involve the activation of PPARα (34). In this context, our group has recently demonstrated...
that adipocyte treatment in vivo and in vitro with palmitoleic acid increases lipolysis, under basal conditions and after stimulation with isoproterenol, and the protein content of ATGL and HSL phosphorylated at serine 660 by a PPARα-dependent mechanism (58).

Overall, these results determine PPARγ and PPARα as important regulators of the lipolysis in the white adipose tissue, besides their known roles as positive modulators of adipogenesis and β-oxidation/mitochondrial function, respectively (57).

On the other hand, fatty acids derived from lipolysis are important PPAR activators. According to Zechner and cols., plasma fatty acids, when entering cells, do not bind directly to PPAR (17). A fatty acid esterification and hydrolysis cycle is necessary for these molecules to act as PPAR ligands (17,60). This hypothesis was proven by the reduced lipolytic activity found in ATGL-deficient animals, which causes important reduction in PPARα signaling. Consequently, oxidation of substrates of several tissues and cell types, such as liver, macrophages, and brown adipose tissue, is reduced (17,32,61,62). HSL deficiency also moderately reduces PPARα signaling (60). Besides, ATGL deficiency leads to reduced PPARα activation in the heart and vascular endothelium, and PPARδ in the pancreas, deeply compromising the physiological function of these tissues (28,29,60).

AMP-ACTIVATED PROTEIN KINASE (AMPK) AND LIPOLYSIS

AMPK is an important molecule in the regulation of cell energy balance. It is sensitive to nutritional status and responds to reduction in the ATP/AMP ratio. This molecule is able to phosphorylate and regulate proteins related to energy metabolism, decreasing the anabolic pathways (ATP consumption) and increasing catabolic ones (ATP generation – such as the increase in glucose capture and oxidation of fatty acids and glucose) (32,35,63,64).

Studies revealed important effects of AMPK in the WAT metabolism, including lipolysis regulation (32,63,64). A previous study found that acute AMPK activation with AICAR (5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide), an AMP analogue, promotes inhibitory phosphorylation of HSL at serine 565 (65). However, as demonstrated by Ahmadian and cols., AMPK activation with AICAR leads to phosphorylation of ATGL at serine 406, expressly increasing the activity of this lipase and, therefore, lipolysis in HEK293 cells and 3T3-L1 adipocytes (32). In another study, prolonged activation of AMPK induced by the treatment of epididymis adipocytes in vitro with AICAR (0.5 mM) for 15 hours, or rats treated with a single intraperitoneal injection of AICAR (0.7 g/kg) showed increased lipolysis, ATGL protein content, and gene expression of PPARα, PPARδ, and PGC-1α (64). Additionally, chronic activation of AMPK in the adipose tissue induced remodeling of the metabolism of these cells, potentiating catabolic pathways of ATP production instead of routes of energy storage and ATP consumption (64).

AMPK effect in the activation of lipolysis in WAT, increasing the offer of fatty acids and glycerol to be used as energy substrates in different tissues may be another mechanism by which this kinase favors the increased production of ATP to reestablish energy homeostasis. This phenomenon is the proof of the important contribution of lipolytic activity and lipases in the control of the energy metabolism.

FINAL REMARKS

Since the discovery of ATGL in 2004, a large number of studies have been carried out leading to wide progress in lipolysis characterization, besides revealing a growing number of enzymes, kinases, and regulatory proteins, recently.

It is clear, nowadays, that lipolysis is not only a process that supplies energy substrates for ATP generation in different tissues, but that lipid products (fatty acids and their derivatives) have essential roles in multiple systemic and intracellular signaling pathways, including, for example, PPARs (17,52). These roles of lipolysis are not restricted to the WAT, as proven by severe damage found in the pancreas, liver, heart and vascular endothelium associated with reduced lipolysis. Normal lipolytic activity seems to be crucial for energy homeostasis in different tissues.

On the other hand, excessive mobilization of fatty acids found in lipolysis was previously associated with high plasma concentrations of fatty acids, dyslipidemias, and insulin resistance (12). Because of that, prescription of drugs that reduce lipolytic activity has been a therapeutic strategy in the control of these diseases, although there are objections to the efficacy of these drugs, such as nicotinic acid and its analogue, Acipimox® (13,66).
Curiously, studies have demonstrated that animals that show increased lipolytic activity have reduced adipose mass without, however, elevation of fatty acids serum levels. This fact is due to the fact that these animals show greater β-oxidation and reesterification of fatty acids into TAG (TAG-fatty acid cycle or futile cycle) inside the adipocytes (58,67-69). These data reveal that drugs/substances that are capable of activating lipolysis in white adipocytes are promising therapeutic strategies for the treatment of obesity.

Evidently, there are ongoing studies to increase the knowledge on this lipolytic machinery and its regulation, as well as on the importance of lipolysis on multiple signaling pathways of body tissues.

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