Triacylglycerol lipases and metabolic control: implications for health and disease

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Watt MJ, Spriet LL. Triacylglycerol lipases and metabolic control: implications for health and disease. Am J Physiol Endocrinol Metab 299: E162–E168, 2010. First published January 12, 2010; doi:10.1152/ajpendo.00698.2009.—Fatty acids derived from the hydrolysis of adipose tissue and skeletal muscle triacylglycerol (TG) are an important energy substrate at rest and during physical activity. This review outlines the identification of the new TG lipase, adipose triglyceride lipase, the current understanding of how cellular TG lipases are regulated, and the implications for understanding the integrated control of TG lipolysis. Furthermore, this review outlines recent advances that propose a “revised” role for TG lipases in cellular function, metabolic homeostasis, and disease prevention.

Fatty acid; metabolism; adipose; skeletal muscle

Triacylglycerols (TG) are stored within discrete cytosolic lipid droplets within most tissues. Contrary to the common misconception in the literature that the TG pool is “inert,” these droplets are highly dynamic, and the TG within is highly labile. The overall TG content within these droplets is ultimately dependent on the balance between the rates of TG hydrolysis and fatty acid uptake, oxidation, and storage. The fatty acids derived from TG breakdown participate in a variety of cellular processes, including membrane biosynthesis, signal transduction, and, most critically for this review, ATP production after β-oxidation. From a systemic viewpoint, fatty acids can be derived from both intracellular and extracellular sources, and 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 fatty acids derived from adipose TG hydrolysis, and this becomes quantitatively more important with extended fasting or exercise. In cases of caloric surplus leading to obesity, elevated circulating fatty acids may contribute to the accumulation of intramyocellular and hepatic lipids, which are associated with secondary metabolic complications such as insulin resistance (77). Therefore, the liberation of fatty acids from adipocyte TG and release into the systemic circulation is under most conditions the first point of control in the regulation of fatty acid metabolism, and accordingly, tight control of adipocyte lipolysis is a critical mediator of whole body metabolic homeostasis. Similarly, an emerging body of evidence indicates that altering TG metabolism within ectopic tissues, such as liver and skeletal muscle, can influence local fatty acid metabolism with implications for obesity-related metabolic disorders.

This review will first focus on the regulation of TG lipolysis and how the understanding of TG regulation has evolved since the discovery and partial characterization of adipose triglyceride lipase (ATGL) only 5 years ago. The review will then outline how changes in TG metabolism can influence adipocyte and skeletal muscle metabolism and summarize the recent evidence suggesting that altering flux through the TG pool may mediate local changes in mitochondrial capacity and an enhanced capacity to dispose of “excess” fatty acids through oxidative pathways.

Triacylglycerol Lipases Act in Series to Regulate Lipolysis

The sequential hydrolysis of TG by specific enzymes results in the liberation of a fatty acid (FA) at each step with the generation of diacylglycerol (DG), monoacylglycerol (MG), and glycerol. For many years hormone-sensitive lipase (HSL) was presumed to be the rate-limiting enzyme controlling this process. Studies over the past 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 A, to ultimately control lipolysis (83). However, HSL-null mice accumulated DG and were able to maintain basal and some degree of β-adrenergic stimulated lipolysis (32, 58, 75). In 2004, three laboratories identified these enzymes as desnutrin, phospholipase A2-ζ, and ATGL (39, 74, 84). ATGL exhibits high substrate specificity for TG (84), and its activity is increased markedly by association with HSL (51). Combined with the knowledge that HSL exhibits markedly greater activity against DG vs. TG substrate (23), these findings have led to the current view that ATGL and HSL work hierarchically to regulate complete TG hydrolysis. ATGL initiates lipolysis by specifically removing the first FA from TG to produce DG substrate, which is then hydrolyzed by HSL to generate an additional FA and MG substrate. MGs are converted to FA and glycerol by MG lipase in the final step of lipolysis (23).
Because MGs do not accumulate in adipocytes, even under conditions of maximally activated lipolysis, MG lipase is not considered to be rate limiting for lipolysis and will not be discussed herein. Due to differences in the tissue-specific expression of ATGL and HSL, there may also be a role for other members of the recently identified lipolytic proteome (6).

**Regulation of TG Lipases**

**ATGL.** ATGL is expressed in most tissues examined, with the highest expression in white and brown adipose tissue, lower levels in the testis and cardiac and skeletal muscle, and very low expression in the liver (32, 42, 48, 58, 74, 75, 84). Unless stated otherwise, the following sections discuss ATGL in the adipocyte. ATGL exhibits high substrate specificity for TG, very weak activity against DG, and no activity against cholesterol or retinyl ester bonds (84). It is now unequivocal that ATGL mediates basal and β-adrenergic TG lipolysis in adipocytes (1, 5, 42, 49, 84) and other cell types, including skeletal muscle (80), hepatocytes (61), embryonic kidney (48), and cancer cells (69). These cell-based studies are supported by interventions in several lower-order species (e.g., *S. cerevisiae* and *C. elegans*) (30, 47) and mice (1, 31), showing that ATGL deletion induces obesity and that ATGL overexpression confers a lean phenotype. Similarly, in vivo ATGL overexpression in skeletal muscle (80) or liver (61) reduced TG mass. Finally, immunoinhibition of ATGL in human adipose tissue lysates reduced total TG lipase activity by 70–83%, indicating an important role for ATGL in human adipocyte lipolysis (71). Aside from its role in TG degradation, Jenkins et al. (39) reported acylglycerol transacylase activity of ATGL using an oleate donor, which in the presence of monoolein or diolein produced DG and TG, respectively. This unusual finding is relevant for TG futile cycling, which in the presence of monoolein or diolein is essential for lipid binding (20). 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 (84). A phospho-proteomic analysis of purified LDs reported phosphorylation at Ser404 and Ser428 of human ATGL (4); however, site-directed mutagenesis of these sites did not affect TG breakdown under basal conditions (20). Hence, the reason for ATGL phosphorylation in cells is currently unresolved, as are the upstream kinases. Interestingly, AMP-activated protein kinase (AMPK) phosphorylation of ATGL (Ser307) in *C. elegans* is required to suppress the rapid depletion of lipid stores during dauer (fasting) (54); however, the relevance to mammals is uncertain because of the low-sequence homology with *C. elegans*.

A major advance in understanding lipolysis was the discovery that ATGL requires CGI-58 for full activation. Lass et al.

**Fig. 1.** Representation of the structural domains and phosphorylation sites of human adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). The important structural components for enzyme function are indicated. For ATGL, note the patatin domain containing the α/β hydrolase region, active site serine (Ser47), and aspartic acid (D166) (blue region), the putative lipid-binding domain (pink region), and 2 phosphorylation sites (Ser404 and Ser428). For HSL, note the recognized NH2-terminal (N) domain (blue shading) thought to be important for lipid binding and protein-protein interactions and the COOH-terminal (C) catalytic domain containing the α/β hydrolase fold and regulatory subunit. Ser454 indicates the active site within HSL. Phosphorylation sites within the regulatory unit are indicated along with the corresponding kinases below. AMPK, AMP-activated protein kinase.
(51) demonstrated that CGI-58 interaction with ATGL increased adipose tissue TG hydrolyase activity 20-fold, although others have since reported less dramatic increases (30–70%) in cultured nonadipose cells (9, 80, 82), possibly reflecting the low abundance of ATGL in these cells. Perilipin A is also implicated in ATGL activation. Perilipin A serves a critical role in regulating basal and stimulated lipolysis by coordinating the recruitment of proteins to the lipid droplet (7). CGI-58 resides on the surface of lipid droplets and interacts with perilipin A under basal conditions, and after β-adrenergic stimulation, CGI-58 rapidly disperses into the cytoplasm (8, 28, 82). Phosphorylation at Ser517 of perilipin A is mandatory for stimulated lipolysis (53) and may mediate the dissociation of perilipin A and CGI-58. It appears that, after dissociation from perilipin A, CGI-58 associates with ATGL predominantly at micro (fragmented) lipid droplets and sites lacking perilipin A (28, 82). Such regulation is unlikely to occur in other cell types that do not express perilipin A, and potential regulation by other members of the perilipin family, including PLIN2 (adipocyte differentiation-related protein), PLIN3 (tail-interacting protein of 47 kDa), PLIN4 (S3–12), and PLIN5 (LSDP5), requires more study. Finally, recent work shows that the adipokine pigment epithelium-derived factor, which is elevated in obesity (15), associates with ATGL (13) and enhances adipocyte lipolysis (15).

**HSL.** Since the discovery of ATGL, HSL has taken the “back seat” with respect to research focus of lipolytic regulation. HSL remains a critical enzyme for complete TG lipolysis, and its characterization and function have been extensively reviewed elsewhere (35, 78, 83). HSL is expressed in adipocytes and less so in many other tissues, including skeletal muscle, pancreas, macrophages, and testis (34). HSL exerts lipolytic activity toward not only TG but also DG, MG, and cholesteryl esters. The relative hydrolase activity in vitro is 11-fold greater against DG than TG (23), and this substrate preference was supported by studies in HSL-null mice that reported marked DG accumulation (32). HSL contains three domains: a catalytic domain, a regulatory domain containing an NH2-terminal domain involved in protein-protein and protein-lipid interactions (Fig. 1). Although it was known for several years that PKA increased HSL activity through a phosphorylation-dependent mechanism, mutagenesis experiments demonstrated dependence for this effect on phosphorylation at Ser563, Ser569, and Ser660 (2, 67). However, it should be noted that there is some doubt as to the importance of Ser563 (2). The PKA phosphorylation of HSL results in modest activation of the enzyme (2- to 3-fold) and promotes the translocation of HSL to the LD (21), an effect that is critical for enhancing lipolytic capacity as a result of β-adrenergic stimulation. The extracellular signal-regulated kinase phosphorylates HSL at Ser660, which increases HSL activity (29). HSL activity is negatively regulated by insulin (22, 55) and via phosphorylation of HSL Ser565 by AMPK (18, 26). LDs are bound by a family of proteins that contain PAT domains (for perilipin, adipose differentiation-related protein, and tail-interacting protein 47 kDa). Perilipin A appears to be the most critical for regulating HSL activity in adipocytes, whereas other PAT proteins are likely to be important for lipolytic regulation in most other tissues that do not express perilipin A. Readers are directed to an excellent recent review of this topic (7). PKA phosphorylation of perilipin A is critical for HSL lipolytic action at the LD (53), which is mediated by direct binding, although the exact residues have not been determined (28, 66). This regulation is not relevant for other key tissues of FA metabolism that do not express perilipin A.

**ATGL and Mitochondrial Biogenesis**

Aside from their role in energy metabolism, FAs are involved in a myriad of cellular process, one of which is serving as PPAR ligands. PPARs belong to the nuclear hormone receptor superfamily that functions as FA-activated transcription factors. The PPAR family consists of several isomorphs that display tissue specificity and discrete physiological functions. PPARα is expressed predominantly in liver and controls mitochondrial and peroxisomal FA catabolism, whereas PPARγ is abundant in adipose tissue and is a key transcription factor for adipogenesis. Although PPARγ is lowly expressed in skeletal muscle, it nevertheless exerts an important role in metabolism in this tissue (33). PPARδ is highly expressed in skeletal muscle, and its activation induces the expression of genes involved in β-oxidation, energy uncoupling, and cholesterol efflux (19). Indeed, treating mice with PPARδ ligands (GW1516) can reprogram the muscle transcriptome and is sufficient to initiate an oxidative response in skeletal muscle in vivo (55).

ATGL is a TG hydrolase in skeletal muscle that alters fat metabolism. Retroviral-mediated ATGL overexpression in muscle cells increases TG lipase activity (80) and the oxidation of TG derived FAs, suggesting enhanced flux of intracellular FAs. Interestingly, the ATGL target genes PPARγ coactivator-1α (PGC-1α), pyruvate dehydrogenase-4, and carnitine palmitoyltransferase I were all upregulated, as was citrate synthase activity, indicating enhanced oxidative capacity in ATGL-overexpressing myotubes (Watt MJ, unpublished observations). Similar results were observed when ATGL was electroporated into rat skeletal muscle in vivo (Watt MJ, unpublished observations). Others have previously reported reduced PGC-1α and uncoupling protein-1 expression in brown adipose tissue of ATGL−/− mice (31), although our own studies indicate that the gene expression of β-oxidation or TCA cycle proteins is not downregulated in skeletal muscle of ATGL−/− mice (36). The crystal structures of recombinant human PPARδ lipid-binding domain revealed FAs C16:0, C16:1, C18:0, and C18:1 binding in a ratio of ~3:2:1:4 (25). FAs located in the sn-1 position of TG are both saturated (~70%) and unsaturated (~30%), and lipolysis of TG-rich lipoproteins by lipoprotein lipase can provide FA ligand for PPARα activation in endothelial cells (85) and macrophages (11). Furthermore, ATGL overexpression enhances lipolysis and drives PPARα activity in rat hepatocytes (64), and ATGL overexpression in adipocytes increases the expression of PPAR target genes (1). Also, PPARγ agonism increases adipose tissue lipolysis, mitochondrial biogenesis, and FA oxidation (50, 81). Together, these data support a presumptive role for ATGL-mediated TG hydrolysis in providing ligand for PPARα/β/γ activation, which in turn would drive transcription of FA catabolism genes and enhanced fat-burning capacity in several cell types (Fig. 2). In other words, whenever ATGL is activated to release FAs for oxidation, such as during fasting or moderate physical activity, the FAs serve a dual or supplementary role as signals to activate the expression of genes that may be required should
the requirement for increased fat fuel persist (e.g., exercise training, high-fat feeding). Of course, the relevant question in humans is, what type of repetitive “real-world” signal would be needed to cause the translation of this putative regulatory network in vivo?

AMPK may also play a role in the regulation of adipocyte mitochondrial biogenesis. Lipolysis decreases the cellular energy state, presumably due to the ATP hydrolysis required for acylation of the released FAs (27), and lipolysis thereby activates AMPK (27). Futile cycling of FAs is also an energy-consuming process. The reesterification of free FAs (FFA) requires acylation by acyl-CoA synthetase (2 × ATP) and produces LCFA-CoA and AMP. The increase in cellular AMP and LCFA-CoA can allosterically activate AMPK, which in turn can stimulate fatty acid oxidation and enhance the transcriptional coactivator peroxisome proliferator-activated receptor (PPAR) coactivator-1α (PGC-1α). This would further enhance mitochondrial biogenesis. GPAT, glycerol-3-phosphate acyltransferase; DGAT, diacylglycerol acyltransferase. CPT I, carnitine palmitoyltransferase I; OxPhos, oxidative phosphorylation.
plasma-derived FAs may enter the IMTG pool before their eventual oxidation. Kanaley et al. (41) recently combined a “pulse-chase” study design using 13C-FFA tracers in humans with sensitive mass spectrometry determination of muscle lipids and showed that the direct contribution of plasma FFA to muscle FA oxidation under resting conditions is negligible. Instead, the isotopic enrichments are consistent with the interpretation that FFAs must first be diluted into intracellular pools (presumably IMTG). ATGL−/− mice (31) and mice with mutant perilipin that cannot be phosphorylated by PKA (Peri AKOΔ1–6) (70) have defective brown adipose tissue lipolysis, FA oxidation, and thermogenesis, demonstrating that this cycling of FAs occurs in other cell types. These findings suggest that TG lipases are critical in modulating whole body FA metabolism and would place ATGL/HSL at the forefront of potential therapeutic interventions aimed at enhancing FA oxidation, which has been proposed as a therapy for obesity-associated insulin resistance/type 2 diabetes (68).

Indeed, ATGL−/− and HSL−/− mice have impaired TG lipolysis and FA metabolism (36), which supports the model proposed by Dagenais et al. (17) and Kanaley et al. (41); however, these transgenic models are confounded by reduced plasma FA levels. Also, mild short-term ATGL overexpression in skeletal muscle does not enhance insulin action in vivo (80). Future studies using muscle-specific ablation of TG lipases will be important tools to address this issue and also extend this work to the exercise situation.

Adipocytes. An emerging model that is formulated from the metabolic characterization of several genetic mouse models suggests that elevating adipocyte lipolysis promotes FA oxidation and increased energy expenditure, albeit not necessarily through common mechanisms, resulting in a leaner phenotype. For example, adipose-specific ATGL transgenic mice have increased lipolysis, enhanced oxygen consumption, and increased adipocyte FA oxidation that was ascribed to a transcriptional reprogramming of the adipose tissue toward a more “oxidative” phenotype (1). Similar, albeit not completely overlapping, phenotypes have been reported in perilipin-null (52), fat-specific protein of 27-kDa-null (57), and adipocyte phospholipase-A2-null (38) mice. The described changes were restricted mostly to the adipocyte, which is consistent with the notion that enhancing intracellular FFA flux is driving gene transcription (described above). Considering that mitochondrial content and oxygen consumption are reduced in the adipose tissue of genetic and fat-induced obesity (12, 62, 73, 81) and that enhancing adipocyte mitochondrial function promotes leanness (14, 16, 59), enhancing lipolytic flux in adipocytes by regulating TG lipase activity may be a viable approach to prevent obesity or ameliorate existing obesity. “Shifting” white adipose tissue toward a brown adipose phenotype that is characterized by mitochondrial biogenesis and uncoupled respiration is not a novel strategy for obesity treatment (72). The advantage of upregulating TG lipases is that both lipolysis and respiration are concomitantly enhanced. Such an approach would only be advantageous if the FA from TG hydrolysis were oxidized locally, rather than released, which would obviate the requirement to dispose of “excess” FAs in nonadipose tissues and circumvent the potential for lipotoxicity.

Conclusions

Researchers have known for more than a century that FAs are an important metabolic substrate (3) and presumed for about 25 years that HSL was the critical enzyme controlling FA availability (24). The discovery of ATGL has altered the view of lipolytic control and reignited the field of TG metabolism, with several studies revealing novel roles for TG lipases in metabolic control. We now know that ATGL is an essential protein in the lipolytic cascade and in the regulation of body mass. However, there is also much to be examined. How is ATGL regulated in adipocytes and other cell types? Where is ATGL placed in the regulation of intracellular fatty acid oxidation and metabolic control? Can regulating ATGL and FA fluxes influence the capacity of cells to respond to energy stresses by enhancing mitochondrial function? And how can ATGL be targeted for therapeutic benefit for the treatment of obesity and related disorders? Future research will answer these questions and further elucidate the important role for ATGL in cellular function, metabolic homeostasis, and disease prevention.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


