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Regulation of adipose tissue lipolysis revisited

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Human obesity and its complications are an increasing burden in developed and under-developed countries. Adipose tissue mass and the mechanisms that control it are central to elucidating the aetiology of obesity and insulin resistance. Over the past 15 years tremendous progress has been made in several avenues relating to adipose tissue. Knowledge of the lipolytic machinery has grown with the identification of new lipases, cofactors and interactions between proteins and lipids that are central to the regulation of basal and stimulated lipolysis. The dated idea of an inert lipid droplet has been appropriately revamped to that of a dynamic and highly-structured organelle that in itself offers regulatory control over lipolysis. The present review provides an overview of the numerous partners and pathways involved in adipose tissue lipolysis and their interaction under various metabolic states. Integration of these findings into whole adipose tissue metabolism and its systemic effects is also presented in the context of inflammation and insulin resistance.

Adipose tissue: Lipolysis: Lipase: Lipid droplet

Hormonal regulation of adipocyte lipolysis

White adipose tissue (WAT) essentially represents an unlimited pool of energy. In WAT NEFA originating from dietary intake or *de novo* synthesis are stored as TAG in highly-structured hydrophobic lipid droplets (LD). With its storage capacity and ability to hydrolyse TAG (a process termed lipolysis) WAT provides a NEFA buffering system for other organs⁽¹⁾. Lipolysis is the breakdown of one TAG molecule to three energy-rich NEFA and one glycerol molecule, which are released into the bloodstream and are available for uptake by other tissues. NEFA are not only an energy source, they are also signalling molecules. Over-abundance of NEFA can interfere with normal metabolism, as is the case in obesity and type 2 diabetes. Chronically-elevated NEFA alter glucose and lipid metabolism in skeletal muscle and liver and may lead to insulin resistance⁽²⁾.

Tight regulatory control of lipolysis is provided by catecholamines and insulin (Fig. 1). The hormone

adrenaline and neurotransmitter noradrenaline stimulate lipolysis through the activation of β 1- and β 2-adrenergic receptors (AR). Coupling of β 1- and β 2-AR to stimulatory GTP-binding protein receptors activate adenylyl cyclase, increasing cAMP production. A rise in cAMP activates protein kinase (PK) A, which phosphorylates hormone-sensitive lipase (HSL) and LD-coating protein perilipin (PLIN) to stimulate lipolysis. Conversely, catecholamines can inhibit lipolysis via the activation of α 2-AR and their coupling to inhibitory GTP-binding protein receptors. The latter inhibit adenylyl cyclase action and cAMP production. Thus, AR-dependent lipolysis is dictated by the combined effects of pro-lipolytic β -AR and anti-lipolytic α 2-AR. Impairment in PKA-stimulated lipolysis observed in obesity is thought to result from accentuated stimulation of α 2-AR^(3–5). Insulin also regulates lipolysis when binding to its receptor on adipocytes. Insulin binding to insulin receptor substrate 1 leads to phosphodiesterase 3B activation, which degrades cAMP, and consequently reduces PKA activation. Thus, in a postprandial state insulin not

Abbreviations: AMPK, AMP-activated protein kinase; AR, adrenergic receptors; ATGL, adipose TAG lipase; CGI-58, comparative gene identification 58; DAG, diacylglycerols; FABP, fatty acid-binding protein; HSL, hormone-sensitive lipase; LD, lipid droplet; MGL, monoacylglycerol lipase; PK, protein kinase; PLIN, perilipin; WAT, white adipose tissue.

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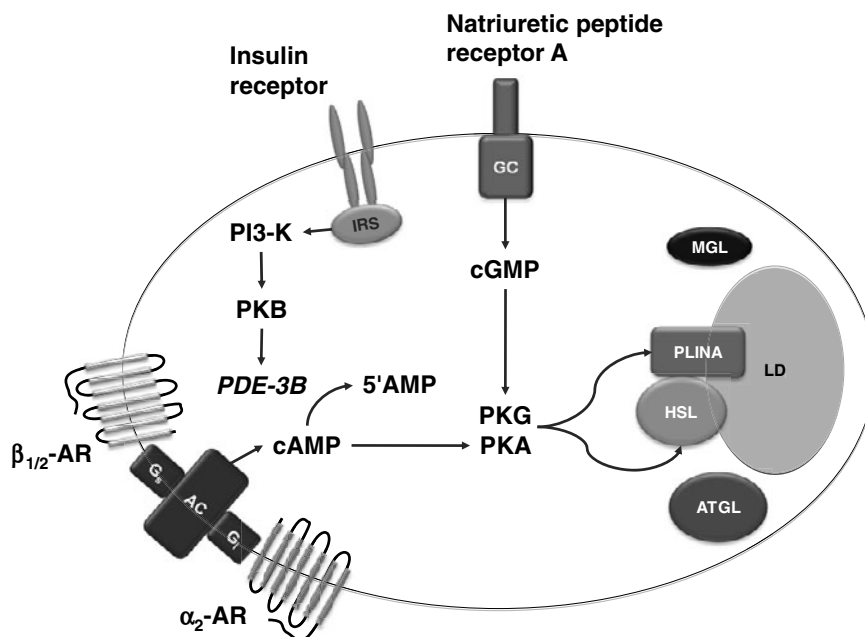


Fig. 1. Signal transduction pathways implicated in hormonal control of human adipocyte lipolysis. Coupling of β_1 and β_2 ($\beta_{1/2}$)- and α_2 -adrenergic receptors (AR) respectively stimulate and inhibit cAMP production by adenylyl cyclase (AC) and protein kinase A (PKA) activation. Insulin favours cAMP degradation through activation of protein kinase B and phosphodiesterase 3B (PDE-3B) activity. Natriuretic peptides promote cGMP accumulation and PKG activation via type A receptor. PKA and PKG phosphorylate hormone-sensitive lipase (HSL) and perilipin A (PLINA). Adipose TAG lipase (ATGL) and monoacylglycerol lipase (MGL) are not thought to be directly hormonally-regulated. Gs, stimulatory GTP-binding protein; Gi, inhibitory GTP-binding protein; IRS, insulin receptor substrate; PI3-K phosphatidylinositol-3 phosphate kinase; GC, guanylyl cyclase; LD, lipid droplet.

only favours substrate uptake and storage but also minimizes TAG breakdown in adipocytes.

In human fat cells an additional signal transduction pathway, independent of catecholamines and insulin, is implicated in pro-lipolytic events. Natriuretic peptides bind type A receptors, which possess intrinsic guanylyl cyclase activity (Fig. 1). Rises in cGMP activate PKG, which similarly to PKA phosphorylates HSL and PLIN⁽⁶⁾. Stimulation of lipolysis by natriuretic peptides is of similar magnitude to that of catecholamines and is particularly pronounced during exercise^(7,8).

Natriuretic peptides, catecholamines and insulin provide the main regulatory control of lipolysis in human adipocytes. Additional hormones and factors such as growth hormone, TNF α , and IL-6 also influence lipolysis by altering the signalling pathways or lipolytic machinery described earlier. There is also a wealth of anti-lipolytic systems activated by catecholamines, adenosine, PG and metabolites for which the physiological relevance is still unknown.

Lipases in lipolysis regulation

Tremendous progress has been made in the regulation of lipolysis over the past 10 years. For approximately three decades HSL was thought to be the rate-limiting step in lipolysis. It is now established that other lipases, cofactors

and lipid-associated proteins each participate in the regulation of lipolysis.

Hormone-sensitive lipase

In the 1960s HSL was characterized as a lipolytic enzyme sensitive to adrenaline^(9,10). For the following 30 years HSL remained the undisputed regulator of lipolysis. HSL is highly expressed in WAT⁽¹¹⁾ and displays *in vitro* hydrolysis activity for TAG, diacylglycerols (DAG), monoacylglycerols⁽¹²⁾, cholesterol and retinyl esters^(13,14). Its relative affinity is ten times greater for DAG than TAG^(12,15) and shows a preference for fatty acids in the sn-1 and sn-3 position of TAG molecules⁽¹⁶⁾.

The cloning of HSL in the rat and human subjects^(17,18) has provided an insight into its gene and protein structure. The carboxy terminal of HSL harbours the active site and regulatory module of the enzyme⁽¹⁹⁾. The amino terminal, although less characterized, appears to be required for protein-protein interaction, notably with fatty acid-binding protein (FABP) 4 (detailed later)⁽²⁰⁾. As alluded to earlier, HSL action is in part regulated by PKA. Three PKA phosphorylation sites have been identified in rat HSL: Ser⁵⁶³; Ser⁶⁵⁹; Ser⁶⁶⁰⁽²¹⁾. The corresponding sites in human HSL are Ser⁵⁵², Ser⁶⁴⁹ and Ser⁶⁵⁰⁽²²⁾. PKA phosphorylation of rat HSL residue Ser⁵⁶³ appears to regulate intrinsic activity⁽²³⁾ while residues Ser⁶⁵⁹ and Ser⁶⁶⁰ favour the translocation of a predominantly cytosolic

HSL to LD^(24–27). In human HSL PKA phosphorylation of residues Ser⁶⁴⁹ and Ser⁶⁵⁰ has been shown to be the most important in increasing enzymic activity⁽²⁸⁾. The pro-lipolytic effect of PKA on HSL is therefore two-pronged: increasing the enzyme's intrinsic activity; promoting its access to TAG molecules in a whole-cell context.

Additional HSL regulatory pathways include the extracellular signal-regulated kinase and AMP-activated PK (AMPK) pathways. HSL is positively regulated by the extracellular signal-regulated kinase pathway via phosphorylation of Ser^{660(29,30)} and negatively regulated by AMPK. AMPK, the cellular energy sensor, is activated by increasing AMP:ATP to restore energy levels⁽³¹⁾. Once activated AMPK phosphorylates HSL on Ser⁵⁶⁵ in human adipocytes^(32,33), resulting in an anti-lipolytic effect⁽³⁴⁾.

Doubt on the lone regulatory role of HSL in lipolysis slowly grew over the years. First, puzzlement revolved around an important mismatch between HSL activity and adipocyte lipolysis in response to PKA activation. PKA-dependent phosphorylation of HSL leads to a two- to three-fold increase in TAG hydrolase activity, while whole-cell lipolysis increases ≤ 100 -fold. These contrasting observations suggested additional, yet unidentified, regulatory steps in lipolysis. The critical role of the LD structural protein PLIN would later shed light on this issue (described later). Additionally, DAG accumulation in adipose tissue of HSL-null mice⁽³⁵⁾ suggested the presence of an alternative lipase targeting TAG molecules, possibly to complement the strong affinity of HSL for DAG. The identification of adipose TAG lipase (ATGL) in 2004 (see later) supports more recent findings obtained from HSL manipulation; for example, residual TAG hydrolase activity and lipolysis despite HSL silencing^(36–38) or specific pharmacological inhibition^(39–41) and the failure of HSL overexpression to promote whole-cell lipolysis^(38,42).

Adipose TAG lipase

In 2004 three groups independently identified an additional lipase with TAG hydrolase activity^(43–45). ATGL (also known as desnutrin or phospholipase A2 ξ) belongs to the family of patatin-like phospholipase domain-containing proteins. It is highly expressed in WAT and brown adipose tissue and to a lower extent in testes, skeletal and cardiac muscle^(36,43,45,46). The carboxy-terminal region of ATGL contains a hydrophobic section permitting protein–lipid interactions⁽⁴⁷⁾. Accordingly, in mouse models and COS-7 cell lines native or ectopic ATGL is mostly associated with LD^(43,47,48). Also within the carboxy-terminal region of ATGL are two phosphorylation sites, Ser⁴⁰⁴ and Ser⁴²⁸⁽⁴⁹⁾. The nature of the PK targeting ATGL and the functional role of such sites are unknown. Last, the enzymic activity of ATGL and its interaction with co-activator comparative gene identification 58 (CGI-58) are dependent on the carboxy-terminal region⁽⁴⁷⁾.

Studies with ATGL-null mice have revealed the importance of ATGL in energy homeostasis⁽⁵⁰⁾. ATGL-null mice display increased WAT mass and ectopic TAG storage in several tissues, including heart tissue, resulting in premature death. A strong body of evidence has further established the central role of ATGL in lipolysis in murine

adipocytes or non-adipocyte cell lines. Overexpression of ATGL increases TAG hydrolysis^(45,46) and basal and isoproterenol-stimulated lipolysis⁽³⁶⁾ while its silencing decreases TAG hydrolase activity^(36,43), TAG storage and LD size⁽³⁷⁾. Unlike HSL, ATGL hydrolysis capacity is mainly targeted towards TAG. In human adipocytes, however, the *in vitro* TAG hydrolase capacity of ATGL is lower than that of HSL⁽⁴¹⁾. Nonetheless, ATGL plays a crucial role in orchestrating lipolysis in human adipocytes⁽³⁸⁾. Modulation of ATGL with adenoviral transduction and gene silencing dictates basal and PKA-stimulated lipolysis⁽³⁸⁾. The latter study has also demonstrated, in response to PKA-stimulation, translocation of ATGL from the cytosol to LD and consequently its enrichment with HSL. Collectively, these findings suggest that ATGL and HSL act sequentially, despite their common capacity for TAG hydrolysis. HSL remains the lone enzyme capable of DAG hydrolysis, but DAG supply by ATGL controls PKA-stimulated lipolysis in human adipocytes.

Monoacylglycerol lipase

Monoacylglycerol lipase (MGL) was purified from rat adipose tissue in the 1970s⁽⁵¹⁾. This enzyme is expressed in WAT, lung, liver, kidney, testes, brain and heart⁽⁵²⁾. Despite the *in vitro* capacity of HSL to hydrolyse monoacylglycerols, the presence of MGL *in vivo* is required for complete lipolysis⁽⁵³⁾. MGL hydrolyses the 1(3) and 2-ester bonds of monoacylglycerols at equal rates but has no affinity for DAG, TAG or cholesteryl esters. Site-directed mutagenesis has confirmed the importance of Ser¹²², Asp²⁵⁹ and His²⁶⁹ in the lipase and esterase activities of MGL⁽⁵²⁾. MGL is not thought to be rate limiting in lipolysis because of its abundance⁽⁵³⁾.

Lipid droplet-associated proteins and lipid-binding proteins

It is now widely accepted that lipases do not act alone in regulating lipolysis. Several proteins interact with LD, lipases and NEFA to offer additional regulatory control of lipolysis and lipid homeostasis.

Perilipins

Over the past 15 years it has become clear that LD are not simple aggregates of lipids but rather dynamic and highly-structured organelles, important for cellular homeostasis and the synthesis of lipid signalling molecules. Providing structure to LD is a family of lipid-coating proteins termed PAT. The PAT family in human adipocytes includes perilipin, adipophilin, tail-interacting protein of 47 kDa, S3-12 and oxidative tissues-enriched PAT protein. The proportion of each lipid-coating protein on LD is altered as LD mature^(54,55). Perilipin, which was discovered in 1991⁽⁵⁶⁾, is highly expressed in WAT and brown adipose tissue^(56–59) and is the most abundant lipid-coating protein on mature LD⁽⁵⁴⁾. Three isoforms arise from alternate splicing of a single mRNA transcript, PLINA being most abundant in WAT LD^(56,57). Ectopic expression of PLINA in 3T3 L1 preadipocytes naturally devoid of PLIN suggests that

PLINA forms a physical barrier around LD to reduce lipase access⁽⁶⁰⁾. Three hydrophobic sequences play a major role in anchoring PLINA to LD⁽⁶¹⁾ yet it is the amino and carboxy terminals that are critical in promoting TAG storage⁽⁶²⁾.

Investigations with PLIN-null mice clearly illustrate the regulatory role of PLIN in lipolysis. PLIN-ablated mice are lean, have smaller adipocytes and are resistant to diet-induced obesity^(58,59,63). In addition, they exhibit elevated basal lipolysis and attenuated stimulated lipolysis. Interestingly, experiments with mouse embryonic fibroblasts from PLIN^{-/-} mice and COS-7 cells lacking PLIN have shown that HSL fails to translocate to LD in response to β -adrenergic stimulation⁽⁶³⁾. Additionally, live culture cell experiments have demonstrated that PKA activation facilitates fluorescence resonance energy transfer between fluorescent probes fused to HSL and PLIN⁽⁶⁴⁾. Together, these results not only highlight the regulatory role of PLIN as a physical barrier to HSL, but also suggest that PLIN may provide an HSL-docking site on LD.

PLINA has six serine phosphorylation sites targeted by PKA^(27,56,65,66). Of those sites, residue Ser⁵¹⁷ has been demonstrated to be essential to ATGL-dependent lipolysis in stimulated conditions⁽⁶⁷⁾. However, specific phosphorylation of Ser⁴⁹² in murine adipocytes is also of importance as it causes a remodelling of large LD into a myriad of microLD, independently of lipolysis⁽⁶⁸⁾. On phosphorylation PLINA remains on the surface of LD but increased LD surface area following fragmentation facilitates lipolysis. Thus, PLINA limits lipase access to LD in the basal state but provides greater access to lipases in stimulated conditions by docking HSL and promoting fragmentation of LD.

Caveolin-1

Caveolae are small invaginations on cell plasma membranes^(69,70). They are common to many cell types but highly expressed in adipocytes⁽⁷¹⁾. Caveolin is the marker protein for these structures such that ectopic expression of caveolin results in the formation of invaginations on cellular membranes⁽⁷²⁾. Caveolae have several putative functions, including participation in signal transduction⁽⁷³⁾, membrane trafficking pathways and NEFA binding and transport⁽⁷⁴⁾. Interestingly, caveolin-1 also associates with LD⁽⁷⁵⁻⁷⁸⁾, hinting at a role for caveolin-1 in lipolysis. Accordingly, caveolin-1-deficient mice display a blunted response to pharmacological and physiological lipolytic stimuli⁽⁷⁹⁾. Surprisingly, PKA activity is not impaired in this genotype, but rather increased⁽⁷⁹⁾ as a result of the absence of aromatic residues within the caveolin scaffolding domain that mediate PKA inhibition⁽⁸⁰⁾. Despite accentuated PKA activity, PLIN phosphorylation is dramatically reduced in the absence of caveolin-1. A likely explanation has arisen from the *in vivo* and *in vitro* evidence that caveolin-1 facilitates the interaction between the catalytic subunit of PKA and PLIN⁽⁸⁰⁾. The heavy representation of caveolae on plasma membranes therefore suggests an important pro-lipolytic function for caveolin-1, via PLIN phosphorylation. Importantly, the contribution of caveolin-1 in the regulation of lipolysis has yet to be

explored in human fat cells but certainly warrants attention.

Fatty acid-binding protein 4

FABP4, also known as adipocyte lipid-binding protein, belongs to the large family of lipid-binding proteins. This low-molecular-mass soluble protein is highly expressed in WAT and displays a high affinity for hydrophobic species such as NEFA and retinoic acids^(81,82). FABP are thought to provide solubility to NEFA and facilitate their intracellular trafficking between metabolic enzymes and membranes^(83,84). FABP4 physically binds to HSL *in vitro* and *in vivo*. The first 300 amino acids of HSL provide a docking domain for FABP4⁽²⁰⁾. HSL and FABP4 bind 1:1 in the cytosol in response to accentuated lipolysis⁽⁸⁵⁾. As demonstrated by fluorescence resonance energy transfer analysis, this complex translocates to LD on PKA activation⁽⁸⁶⁾.

Comparative gene identification 58

CGI-58, also known as α/β -hydrolase domain-containing protein 5, is yet another protein associated with LD. CGI-58 is a α/β -hydrolase fold-containing protein that resembles a lipase⁽⁸⁷⁾. However, the putative catalytic triad of CGI-58 contains an asparagine in place of the usual serine residue. CGI-58 in itself therefore lacks lipase activity. In the mouse CGI-58 is highly expressed in WAT and testes, and to lower levels in liver, skin, kidney, heart, stomach, and lung⁽⁸⁸⁾. CGI-58 stimulates lipolysis by potently and selectively activating ATGL⁽⁸⁹⁾. In mature murine adipocytes CGI-58 is localized to the surface of LD via association with PLINA^(88,90). On β -AR stimulation CGI-58 is rapidly dispersed to the cytosol, an event reversible with the addition of β -AR antagonists. Under these conditions CGI-58 and ATGL co-localization is greatly accentuated and tends to migrate to small LD⁽⁶⁴⁾. Interestingly, CGI-58 has recently been found to exert lysophosphatidic acid acyltransferase activity⁽⁹¹⁾. This activity is independent of its functions as an activator of ATGL. Thus, while CGI-58 overexpression in yeast increases overall phospholipid content, it reduces neutral lipid content.

In human subjects CGI-58 has been identified as a causal gene of the Chanarin-Dorfman syndrome, a disorder characterized by the accumulation of abnormally large amounts of LD in several organs⁽⁹²⁾. In total, nine mutations of CGI-58 have been identified in patients with Chanarin-Dorfman syndrome^(92,93). CGI-58 mutants with Chanarin-Dorfman syndrome point mutations are not recruited to LD as expected and display weak interactions with PLIN⁽⁹⁰⁾. This outcome may be physiologically relevant to basal and PKA-stimulated lipolysis. Recently, the importance of CGI-58 in both basal and PKA-stimulated lipolysis has been shown in human adipocytes. Gene silencing of CGI-58 not only reduces basal lipolysis by half but also completely abrogates PKA-stimulated lipolysis in hMADS adipocytes (a human white adipocyte model)⁽³⁸⁾. The precise whole-cell dynamics involving CGI-58, PLINA and ATGL in basal and PKA-stimulated

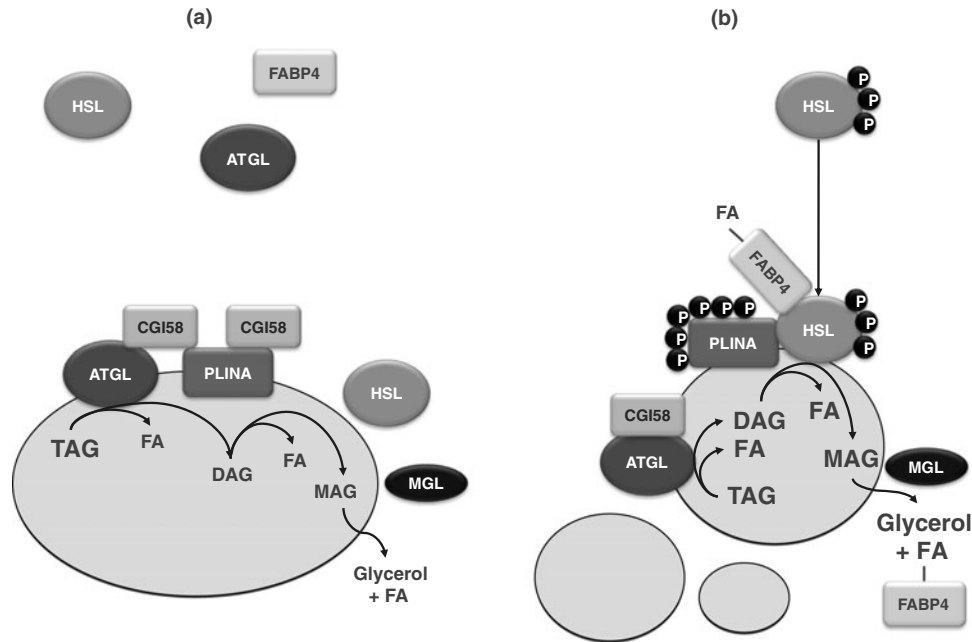


Fig. 2. Hypothetical model of basal and protein kinase A (PKA)-stimulated lipolysis in human adipocytes. In the basal state (a) adipose TAG lipase (ATGL) is found both in the cytosol and on the surface of lipid droplets (LD). On LD ATGL is activated by comparative gene identification 58 (CGI-58), which is also bound to perilipin A (PLINA). During basal lipolysis ATGL and CGI-58 facilitate the hydrolysis of TAG to diacylglycerols (DAG). Hormone-sensitive lipase (HSL) is mainly cytosolic but also is involved in DAG degradation provided by ATGL action. In PKA-stimulated conditions (b) PLINA phosphorylation (P) promotes LD fragmentation and the release of CGI-58. ATGL and CGI-58 form a highly-active complex on small LD where they catalyse TAG degradation. Phosphorylated HSL associates with FABP4 and translocates to LD where it hydrolyses DAG produced by ATGL. Monoacylglycerol (MAG) lipase (MGL) completes lipolysis by hydrolysing DAG to a fatty acid (FA) and glycerol molecule. FABP4 ensures the intracellular trafficking of FA from LD to the plasma membrane.

lipolysis have not been fully elucidated but CGI-58 appears important in both states.

Models of lipolysis activation

The recent identification of an additional lipase and its co-activator, as well as the characterization of novel protein–protein and lipid–protein interactions have drastically changed the working model of basal and PKA-stimulated lipolysis. Fig. 2 presents a hypothetical model of human adipocyte lipolysis.

A model has been proposed that integrates the newly-identified ATGL into lipolysis⁽⁹⁴⁾. It is hypothesized that in the basal state ATGL is mostly located on the surface of LD and exerts little activity because of the association between CGI-58 and PLINA. HSL is mainly found in the cytosol and has limited access to TAG or DAG. Only on phosphorylation of PLINA would CGI-58 dissociate from the latter to bind and activate ATGL on LD and initiate TAG hydrolysis. HSL translocation to the surface of LD via its docking on PLINA would allow the enzyme to participate in PKA-stimulated lipolysis by catalysing DAG hydrolysis. This model supports ATGL- and HSL-dependent lipolysis in PKA-stimulated conditions but offers limited insight into the control of basal lipolysis.

A model has been proposed that addresses more explicitly the role of ATGL in basal lipolysis⁽⁹⁵⁾. It is suggested that in the basal state ATGL is associated with LD in a PLINA-independent manner. It is bound to its co-activator CGI-58 despite the latter's docking on PLINA. Together ATGL and CGI-58 dictate the rate of basal lipolysis by hydrolysing TAG to DAG. HSL is largely cytosolic and has minimal access to TAG or DAG. On PKA activation HSL and PLINA are phosphorylated. HSL translocates to LD via phosphorylated PLINA and hydrolyses DAG. PLINA phosphorylation also leads to the release of CGI-58 in the cytosol. Two scenarios are envisaged for ATGL and CGI-58 in PKA-stimulated conditions; cytosolic CGI-58 is either not involved in stimulated lipolysis or it forms a complex with cytosolic ATGL and migrates to LD in a PLINA-independent manner. Together ATGL and CGI-58 participate in PKA-stimulated TAG hydrolysis. Generated DAG are further hydrolysed by HSL. MGL completes lipolysis by generating NEFA and glycerol.

Results generated from a human adipocyte cell line provide additional information⁽³⁸⁾. First, the data demonstrate a 50% reduction in basal lipolysis following single and dual gene silencing of ATGL and CGI-58, while HSL silencing has no effect. This finding strongly suggests that ATGL and CGI-58 govern basal lipolysis through TAG hydrolysis. Second, immunofluorescence results indicate

important amounts of cytosolic ATGL in the basal state, with translocation to small LD on PKA activation. In this condition a specific HSL inhibitor reduces NEFA release by 60–65%, which suggests that in the whole adipocyte uniquely ATGL hydrolyses TAG (HSL and MGL releasing the second and third NEFA). This notion is further supported by complete abrogation of PKA-stimulated lipolysis with single and dual silencing of ATGL and CGI-58. Thus, it is believed that the increased number of ATGL–CGI-58 complexes formed following PLINA phosphorylation and docked on small LD govern PKA-stimulated lipolysis. Overall, it is the sequential effect of ATGL-accentuated TAG hydrolysis, phosphorylated HSL and MGL action that yields massive increases in NEFA release in response to PKA activation.

A regulatory step is also provided by the association between FABP4 and HSL. NEFA binding to FABP4 and HSL phosphorylation precede the association between FABP4 and HSL⁽⁹⁶⁾. Thus, in addition to supporting NEFA trafficking to the plasma membrane in a reaction that is independent of physical association with HSL, FABP4 bound to fatty acids associates with activated phosphorylated HSL on the surface of LD. Fatty acid–FABP4–HSL association could either limit HSL activity or alter the formation of the complex on LD⁽⁹⁶⁾. However, in the absence of FABP4 lipolysis is decreased and the NEFA content within adipocytes is three times greater than that in wild-type littermates⁽⁹⁷⁾. As NEFA need to be trafficked from the site of hydrolysis (LD) to the plasma membrane, the loss of FABP4 may explain reduced NEFA release.

Integration of lipolysis into adipose tissue biology

Lipolysis and re-esterification

Attention in WAT metabolism thus far has been mainly directed towards catabolic pathways but WAT mass is also dependent on NEFA esterification. Lipolysis and esterification are not limited to fasted and postprandial states respectively, but rather undergo constant cycling in both anabolic and catabolic states⁽⁹⁸⁾. In postprandial states glucose is the main source of the glycerol backbone. The abundance of both NEFA and glucose facilitates esterification. In catabolic states glucose levels cannot support esterification; rather, phosphoenolpyruvate carboxykinase provides glycerol backbones from pyruvate via the glyceroneogenesis pathway (for review, see Forest *et al.*⁽⁹⁹⁾). Accordingly, phosphoenolpyruvate carboxykinase expression and activity are increased with fasting⁽¹⁰⁰⁾ and β -AR agonist treatment⁽¹⁰¹⁾, both highly catabolic states.

Re-esterification is the esterification of NEFA on existing acylglycerol molecules. Similarly to esterification, re-esterification occurs concurrently with lipolysis^(102–104). The regulation of re-esterification is unclear. Strong correlations between re-esterification and lipolysis rates over a wide range of lipolytic flux have been observed in mature adipocytes⁽¹⁰²⁾ and a human adipocyte cell line⁽³⁸⁾. In hMADS adipocytes altering lipase content quantitatively changes lipolysis and re-esterification fluxes, the coupling of the two variables remaining constant and elevated at 86%⁽³⁸⁾. In human subjects re-esterification is estimated at

50–75%^(105,106) but can decrease to 20–35% with fasting and exercise^(107,108).

It was previously thought that re-esterification of NEFA occurs through an extracellular route⁽¹⁰⁹⁾. With current knowledge of LD structure, questions relating to trafficking dynamics extend beyond NEFA. They also apply to acylglycerol species that are synthesized in association with the smooth endoplasmic reticulum but stored and hydrolysed in LD. Preferential hydrolysis or esterification of one acylglycerol species over another is therefore of interest. It has previously been shown that DAG are preferentially hydrolysed over TAG during PKA-stimulated lipolysis⁽¹¹⁰⁾. Despite overall activation of lipolysis, this preferential hydrolysis occurs because of the strong capacity and affinity of HSL for DAG in human WAT^(38,41). Conversely, it has been found that DAG are preferentially re-esterified in the basal state and crucial to the preservation of a fixed fractional re-esterification rate in hMADS adipocytes. While forskolin uncouples re-esterification from lipolysis, inhibition of HSL restores the coupling⁽³⁸⁾. The implication of these findings in human adipocytes could be favoured re-esterification in obese individuals, for whom PKA-activated DAG breakdown by HSL is challenged^(3,40,111).

Lipolysis and adipose tissue inflammation

The past 15 years have provided evidence of the endocrine function of WAT. WAT secretes numerous proteins implicated in the control of energy homeostasis, blood pressure and coagulation, vasculature and the immune system. Immune system proteins are not only intrinsically produced and secreted by adipocytes but also by WAT-resident macrophages. As adiposity increases, so does WAT infiltration of macrophages^(112,113). WAT-resident macrophages express and secrete pro-inflammatory factors and establish the low-grade inflammation state observed in WAT with obesity and believed to be an important mediator of insulin resistance^(113,114). FABP are involved in linking WAT inflammation and systemic effects. Targeting FABP with a small-molecule inhibitor reduces WAT macrophage infiltration and the expression of inflammatory products by macrophages⁽¹¹⁵⁾. Moreover, FABP deficiency in either macrophages or adipocytes improves insulin action and signalling⁽¹¹⁶⁾. This process is thought to occur as a consequence of a unique lipid profile in FABP-null mice⁽¹¹⁷⁾.

A selected group of pro-inflammatory cytokines directly promote lipolysis. The resulting elevated circulating levels of NEFA further aggravate insulin resistance. TNF α is a pro-inflammatory cytokine highly expressed in obesity. Chronic TNF α treatment induces a process termed adipocyte de-differentiation, whereby PPAR γ expression levels are drastically reduced⁽¹¹⁸⁾. Consequently, expression of its target genes is reduced, including HSL⁽¹¹⁹⁾ and ATGL^(120,121). However, TNF α exerts pro-lipolytic effects independently of lipase content. First, TNF α interferes with the anti-lipolytic action of insulin. Specifically, TNF α inhibits insulin receptor substrate 1 activation by promoting its serine phosphorylation through the p42-44 mitogen-activated PK pathway^(122,123). Second, TNF α increases

stimulatory GTP-binding protein-coupled receptors: inhibitory GTP-binding protein-coupled receptors by markedly reducing the protein content of all three inhibitory GTP-binding protein subtypes on fat cells⁽¹²⁴⁾. Although this effect is limited to rodent fat cells⁽¹²⁵⁾, TNF α -induced degradation of inhibitory GTP-binding proteins by the proteasomal pathway⁽¹²⁶⁾ mitigates the anti-lipolytic action of adenosine. Last, TNF α treatment reduces total PLINA content in adipocytes^(30,127) and their phosphorylation by PKA⁽¹²⁸⁾. This effect promotes lipolysis by increasing exposure of lipids to ATGL and HSL.

IL-6 is a pro-inflammatory cytokine heavily secreted from visceral WAT⁽¹²⁹⁾. Its expression is elevated in patients suffering from obesity and type 2 diabetes^(130,131); IL-6 stimulates basal⁽¹³²⁾ and PKA-activated lipolysis⁽¹³³⁾ and induces insulin resistance^(134,135). Stimulation of lipolysis is thought to take place independently of PKA, through the extracellular signal-regulated kinase pathway, resulting in diminished PLINA content⁽¹³⁶⁾. However, IL-6 also promotes fatty acid oxidation^(132,137) via the AMPK pathway^(138,139). Thus, despite the pro-inflammatory status of IL-6, its overall systemic effects have been rather challenging to discern^(140,141). Conversely, the action of IL-1 β is better defined. IL-1 β stimulates lipolysis in cultured adipocytes^(142,143) and inhibits lipogenesis in bone marrow adipocytes⁽¹⁴⁴⁾. These effects are thought to partially occur as a result of impaired phosphorylation of insulin receptor substrate 1⁽¹⁴⁵⁾.

While certain pro-inflammatory cytokines stimulate lipolysis, products of lipolysis have been shown to mediate inflammation in adipose tissue⁽¹⁴⁶⁾. Using co-cultures of adipocytes and macrophages it has been demonstrated that saturated NEFA can activate macrophages and lead to the up-regulation of macrophage-related genes⁽¹⁴⁷⁾. Saturated NEFA can therefore be defined as adipocyte-derived paracrine mediators of WAT inflammation. This response is thought to take place through the mitogen-activated PK and NF- κ B pathways^(147,148). Thus, the presence of a paracrine loop between adipocytes and macrophages probably aggravates adipose tissue inflammation. The existence of a cross talk between adipocyte fat metabolism and macrophage activation is supported by *in vivo* clinical data on the regulation of WAT gene expression during a dietary weight-loss programme⁽¹⁴⁹⁾.

Summary

Knowledge about adipose tissue lipolysis has been considerably expanded in the recent years. The hormonal regulation of lipolysis is no longer limited to HSL. Other key players have been characterized. ATGL, CGI-58 and PLIN each play an important role in the regulation of basal and stimulated lipolysis. Co-activation mechanisms, e.g. CGI-58 action on ATGL, have been identified. Protein-protein interactions such as FABP4-HSL and caveolin-PLIN have been shown to influence cellular lipid stores. Cellular trafficking and distribution of the lipolytic machinery under various physiological conditions is of current interest and should provide an important insight into whole-adipocyte lipolysis. The understanding of the

cross talk within adipose tissue between metabolism and inflammation may constitute a promising avenue for the understanding of obesity- and type 2 diabetes-related complications.

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