Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease

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Abstract

Lipolysis is the process by which triglycerides (TGs) are hydrolyzed to free fatty acids (FFAs) and glycerol. In adipocytes, this is achieved by sequential action of adipose TG lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase. The activity in the lipolytic pathway is tightly regulated by hormonal and nutritional factors. Under conditions of negative energy balance such as fasting and exercise, stimulation of lipolysis results in a profound increase in FFA release from adipose tissue (AT). This response is crucial in order to provide the organism with a sufficient supply of substrate for oxidative metabolism. However, failure to efficiently suppress lipolysis when FFA demands are low can have serious metabolic consequences and is believed to be a key mechanism in the development of type 2 diabetes in obesity. As the discovery of ATGL in 2004, substantial progress has been made in the delineation of the remarkable complexity of the regulatory network controlling adipocyte lipolysis. Notably, regulatory mechanisms have been identified on multiple levels of the lipolytic pathway, including gene transcription and translation, post-translational modifications, intracellular localization, protein–protein interactions, and protein stability/degradation. Here, we provide an overview of the recent advances in the field of AT lipolysis with particular focus on the molecular regulation of the two main lipases, ATGL and HSL, and the intracellular and extracellular signals affecting their activity.

Key Words

- lipolysis
- adipose tissue
- ATGL
- HSL
- free fatty acids
- type 2 diabetes

Introduction

The major energy reserve in mammals consists of fat stored in adipose tissue (AT). In periods of excess energy intake, dietary lipids are taken up by fat cells (adipocytes) in AT and esterified into triglycerides (TGs), which are stored in cytosolic lipid droplets (LDs). In conditions like fasting and exercise, when mobilization of endogenous energy stores is required, TG is hydrolyzed through the process of lipolysis and released to the circulation as free fatty acids (FFAs). These are delivered to peripheral tissues where they can serve as substrate for β-oxidation and ATP production. Only adipocytes have the ability to secrete FFAs into the circulation (Kolditz & Langin 2010). Hence, in the post-absorptive state and during physical exercise, the vast majority of the systemic FFA originates from AT (Jensen 2003). The unique ability of AT to balance storage and release of lipids in response to altered nutrient demands

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provides the organism with an FFA-buffering system of essentially unlimited capacity (Frayn 2002). However, the metabolic consequences of an excessive expansion of AT are considerable. In humans, obesity is closely associated with numerous risk factors that constitute the so-called metabolic syndrome. This includes abdominal obesity, hypertension, dyslipidemia, and glucose intolerance, which are key elements in the pathogenesis of cardiovascular disease and type 2 diabetes (Alberti et al. 2009). The physiological link between obesity and metabolic disease in humans is currently not completely understood, and one of the great enigmas is the remarkable individual differences in the predisposition to obesity-induced metabolic disease. This has led to the proposal of the – AT expandability hypothesis – (Virtue & Vidal-Puig 2010, Hardy et al. 2012), which states that the capacity of AT to expand appropriately when lipid storage is needed is limited for a given individual. Hence, when the limit is exceeded, lipids begin to accumulate in ectopic tissues causing metabolic dysfunction and insulin resistance due to lipotoxic effects. An emerging view is that this lipotoxicity is likely not caused by excess TG in itself, but rather by an excess of lipid intermediates and metabolites released from hypertrophic adipocytes. Several recent reviews have addressed these mechanisms in detail (Boura-Halfon & Zick 2009, Virtue & Vidal-Puig 2010, Copps & White 2012, Hardy et al. 2012, Zechner et al. 2012, Czech et al. 2013). Notably, it is now clear that besides serving as energy-dense metabolic substrates, most, if not all, lipolytic products and intermediates like diacylglycerol (DG), monoacylglycerol (MG), and FFA (and metabolites derived from these) play essential roles in multiple signaling pathways, both at the systemic and at the intracellular level (Zechner et al. 2012). When present in excess, several of these lipid intermediates have been suggested to induce insulin resistance in ectopic tissues by interfering with insulin signaling at the level of the insulin receptor substrate (IRS) proteins (Boura-Halfon & Zick 2009, Copps & White 2012).

Being the major lipid species released from AT, FFA is likely one of the key elements in ectopic lipid accumulation and lipotoxicity. Thus, experimental elevation of plasma FFA levels in human subjects acutely and dose dependently counteracts peripheral insulin-stimulated glucose uptake and oxidation (Belfort et al. 2005, Gormsen et al. 2007, Hoeg et al. 2011). Furthermore, high FFA levels attenuate the insulin-mediated suppression of hepatic glucose production contributing to the impairment of whole-body glucose tolerance (Roden et al. 2000). Consistently, improvements in whole-body insulin sensitivity and oral glucose tolerance can be obtained by pharmacological reductions of chronically elevated plasma FFA levels both in type 2 diabetic patients, obese nondiabetic subjects, and nondiabetic subjects genetically predisposed to type 2 diabetes (Santomauro et al. 1999, Cusi et al. 2007). Accordingly, excessive FFA mobilization from AT is widely conceived as playing a pivotal role in insulin resistance and type 2 diabetes, suggesting that dysregulation of AT lipolysis in the obese state is a contributing factor to the development of metabolic disease.

Major signaling pathways in AT lipolysis

Lipolysis is the sequential hydrolysis of one TG molecule into three FFAs and one glycerol by a class of hydrolytic enzymes commonly known as lipases. In mammalian lipolysis, three lipases act in sequence with the concomitant release of one FFA in each step (Fig. 1): adipose TG lipase (ATGL) converts TG to DG and is the rate-limiting enzyme in the lipolytic pathway (Zimmermann et al. 2004). DG is hydrolyzed to MG by hormone-sensitive lipase (HSL; Haemmerle et al. 2002), and monoglyceride lipase (MGL) cleaves MG into glycerol and FFA (Fredrikson et al. 1986). The major positive regulators of human lipolysis are catecholamines and natriuretic peptides (NPs), while antilipolysis primarily is mediated by insulin and catecholamines.

Catecholamines

The catecholamines, and specifically the stress hormones adrenaline and noradrenaline, are the primary mediators of adrenergic signaling in AT. The manner by which catecholamines regulate lipolysis is unusual as these hormones are able to both stimulate and inhibit lipolysis depending on their relative affinity for different adrenergic receptors (ARs). Thus, stimulation of lipolysis requires the activation of β-ARs on the surface of the adipocyte, while antilipolytic signals are transmitted by the α2-AR.
(Robidoux et al. 2004; Fig. 2). Three different β-AR subtypes exist (β₁, β₂, and β₃), but in humans, only the β₁ and β₂ isoforms are involved in lipolysis (Mauriege et al. 1988, Barbe et al. 1996, Tavernier et al. 1996). Both α₂-AR and β-AR belong to the G-protein-coupled receptor (GPCR) family: the G-protein associated with α₂-AR contain the inhibitory Gᵢ subunit, while β-AR-associated G-proteins contain the stimulating Gₛ subunit (Lafontan & Berlan 1993). The activation of the receptors causes the G-proteins to interact with adenylyl cyclase (AC), which is inhibited by interaction with Gᵢ and activated by interaction with Gₛ (Lafontan & Berlan 1993). Upon activation, AC converts ATP to cAMP, resulting in an increase in intracellular cAMP levels, which activates protein kinase A (PKA, also known as cAMP-dependent protein kinase; Langin 2006). Activated PKA phosphorylates the LD-associated protein PLIN1 (Greenberg et al. 1991) and cytoplasmic HSL (Stralfors et al. 1984, Garton et al. 1988, Anthonsen et al. 1998). Phosphorylation of PLIN1 promotes the release of comparative gene identification-58 (CGI-58), which is a potent co-activator of ATGL (Lass et al. 2006, Granneman et al. 2009). This facilitates the activation of ATGL, thus initiating the stimulated lipolytic cascade. Furthermore, PKA-mediated phosphorylation of HSL causes a rapid activation and translocation of the lipase from the cytosol to the surface of the LDs (Egan et al. 1992). Here, it docks on the phosphorylated PLIN1 and thereby gains access to its DG substrate, which is being generated by ATGL (Shen et al. 2009, Wang et al. 2009).

**Natriuretic peptides**

In addition to catecholamines, the cardiac hormones atrial NP (ANP) and B-type NP (BNP) are important positive regulators of AT lipolysis in humans. NPs, which

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**Figure 2**

Primary signaling pathways in human lipolysis. Black and red lines indicate pro-lipolytic and anti-lipolytic signaling events, respectively. Arrows indicate stimulation and/or translocation and blunt lines indicate inhibition. Stimulation of lipolysis is dependent on PKA- or PKG-mediated phosphorylation of HSL and PLIN1. PKG is activated by cGMP, which is increased in response to activation of the GC-coupled NPR-A. Similarly, stimulation of the Gₛ-protein-coupled β₁/₂-ARs activates AC, which generates cAMP and activates PKA. Conversely, activation of Gᵢ-protein-coupled α₂-ARs inhibits AC and thereby reduces cAMP-dependent signaling to lipolysis. Stimulation of the insulin signaling pathway through the IR increases the activity of PDE3B, which converts cAMP to 5′-AMP, thus decreasing PKA activity and suppressing lipolysis. PKG activity is reduced by PDE5-mediated conversion of cGMP to 5′-GMP, although the upstream signals regulating this process are currently unknown. The dashed line indicates a putative Akt-independent insulin pathway acting selectively on PLIN1. α₂-ARs, α₂-adrenergic receptors; AC, adenylyl cyclase; TG, triglyceride; ATGL, adipose TG lipase; β₁/₂-ARs, β₁- and β₂-adrenergic receptors; CGI-58, comparative gene identification-58; DG, diacylglycerol; FFA, free fatty acid; GC, guanylyl cyclase; HSL, hormone-sensitive lipase; IR, insulin receptor; IRS1/2, IR substrates 1 and 2; MG, monoacylglycerol; MGL, monoglyceride lipase; NPR-A, type-A natriuretic peptide receptor; PDE3B, phosphodiesterase 3B; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKB/Akt, protein kinase B; PLIN1, perilipin 1.
are released from the atrial and ventricular walls of the heart in response to myotube distension (Clerico et al. 2011), stimulate the guanylyl cyclase (GC)-linked type-A NP receptor on the adipocytes (Sengenes et al. 2000) (Fig. 2). Accordingly, upon stimulation of the receptor, GC converts intracellular GTP into cGMP resulting in the activation of PKG (also known as the cGMP-dependent protein kinase), and, just like PKA, this kinase activates the antilipolytic cascade by phosphorylation of PLIN1 and HSL (Sengenes et al. 2003). However, despite the similarities between PKA- and PKG-mediated lipolysis, they are distinct pathways and, unlike the cAMP-dependent pathway, NP-mediated lipolysis is unresponsive to the antilipolytic effects of phosphodiesterase 3B (PDE3B; Sengenes et al. 2000, Moro et al. 2004a). Instead, counter-regulation of the NP pathway is believed to occur by hydrolysis of cGMP by other members of the PDE family of PDEs (Armani et al. 2011). Indeed, PDES expression and activity has been found in isolated human adipocytes from both subcutaneous AT (Moro et al. 2007a) and visceral AT (Aversa et al. 2011); however, this enzyme appears to be insufficient to control ANP-mediated lipolysis (Moro et al. 2007a). Hence, at present, the details of the regulatory pathways counteracting the lipolytic action of NPs in vivo remains poorly understood (Armani et al. 2011).

Insulin

Lipolysis is exceptionally sensitive to the action of insulin (Jensen & Nielsen 2007), which constitutes the major antilipolytic pathway in human lipolysis (Fig. 2). The IR possesses intrinsic tyrosine kinase activity. Thus, binding of insulin induces IR autophosphorylation and subsequent phosphorylation of the IRS1/2 (White 1998). This promotes the activation of phosphatidylinositol 3-kinase (PI3K), which converts phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3) (Whitman et al. 1988, Carpenter et al. 1990). Generation of PIP3 activates the phosphoinositide-dependent kinase causing phosphorylation and activation of Akt (also known as PKB; Alessi et al. 1997, Stokoe et al. 1997). Finally, PKB/Akt activates PDE3B, which degrades cAMP to 5′-AMP (Choi et al. 2006). This inactivates PKA leading to reduced phosphorylation of HSL and PLIN1 and suppression of lipolysis.

Interestingly, in a study predating the elucidation of the canonical insulin signaling pathway, it was demonstrated that besides the inhibitory effect on PKA-mediated signaling, insulin also acts to reduce lipolysis in primary rat adipocytes by a cAMP-independent mechanism (Londos et al. 1985). By carefully measuring PKA activity ratios in response to increasing concentrations of isoprenaline (a β-AR agonist), the authors found that when insulin was added to the cells at submaximal lipolytic stimulation, the insulin-mediated reduction in PKA activity was not sufficient to explain the resulting drop in lipolysis. Conversely, under conditions of maximal lipolysis, insulin-mediated changes in PKA activity could fully account for the resulting change in lipolytic rates. A recent study in 3T3-L1 adipocytes has partially delineated this bimodal insulin effect by showing that insulin-mediated antilipolysis at submaximal β-AR stimulation (but not at maximal stimulation) can proceed through an alternative PI3K-dependent pathway that is independent of Akt (Choi et al. 2010). Acting through as yet unidentified downstream effectors, the pathway was shown to inhibit lipolysis by selectively reducing PLIN1 phosphorylation without affecting the phosphorylation status of HSL (Fig. 2). Considering the key role of PLIN1 in the regulation of ATGL- and HSL-mediated lipolysis such a pathway would indeed be expected to have a substantial impact on overall lipolytic rates.

Besides the inhibitory effects on the lipolytic pathway, insulin also promotes lipid storage by activating a range of pathways involved in the uptake, synthesis, and storage of TG in adipocytes. A comprehensive review of these lipogenic effects of insulin has been published recently (Czech et al. 2013).

Alternative regulatory pathways

Although catecholamines, insulin, and NPs represent the major regulators of human lipolysis, several other factors can modulate lipolysis in AT, either directly by receptor-mediated signaling or indirectly by remodeling of the lipolytic cascade. Figure 3 shows an overview of the different alternative pathways described below.

Agents acting through cAMP-dependent signaling

GPCR pathways affecting the activity of AC are particularly numerous, emphasizing the central role of the cAMP-dependent pathway in the regulation of TG hydrolysis.

Thyroid-stimulating hormone (TSH) and the melanocortins (MCs) adrenocorticotropic hormone (ACTH) and melanocyte-stimulating hormone stimulate AC by activation of the Gc-coupled TSH receptor (Laugwitz et al. 1996, Endo & Kobayashi 2012) and MC receptors (Cho et al. 2005, Rodrigues et al. 2013) respectively (Fig. 3).
TSH-mediated lipolysis has been found to be particularly important in neonates and newborns because physiological levels of TSH, as opposed to adrenaline or noradrenaline, potently stimulate human lipolysis at this developmental stage (Marcus et al. 1988, Janson et al. 1995). By contrast, although a strong lipolytic potential of the MCs has been observed in several animal species, including rodents, hamsters, guinea pigs, and rabbits (Richter & Schwandt 1983, Ng 1990), they seem to have limited effects on human lipolysis (Bousquet-Melou et al. 1995, Kiwaki & Levine 2003).

Neuropeptide Y (NPY) and peptide YY (PYY) are released from sympathetic neurons and inhibit AC by binding to the G\(_1\)-protein-coupled NPY-receptor (NPY-Y\(_1\); Fig. 3) on human adipocytes (Serradeil-Le et al. 2000). Also, in humans, the highest expression of NPY receptors has been found in subcutaneous AT (Castan et al. 1993), suggesting that the impact of NPY/PYY on lipolysis is depot specific. The importance of neuronal regulation of lipolysis has been underscored by elegant studies in rodents demonstrating that isolated stimulation of the hypothalamus with insulin suppresses peripheral AT lipolysis (Scherer et al. 2011).

The fasting-induced circulating factor angiopoietin-like protein 4 (ANGPTL4) is a well-established negative regulator of lipid uptake in rodent adipocytes through inhibition of extracellular lipoprotein lipase activity (Koster et al. 2005, Sukonina et al. 2006, Lafferty et al. 2013). Recently, however, ANGPTL4 has also been found to be intimately involved in the regulation of intracellular cAMP-mediated lipolysis in mice (Gray et al. 2012). By as yet unidentified mechanism, extracellular ANGPTL4 was shown to act independently of \(\beta\)-AR activation to increase intracellular cAMP production via activation of AC (Gray et al. 2012). The identity of the putative ANGPTL4 receptor responsible for this effect in adipocytes is unknown and is currently under investigation (Koliwad et al. 2012).
In rat and human adipocytes, extracellular adenosine efficiently inhibits lipolysis via the G<sub>i</sub>-coupled adenosine receptor (A1-R; Fig. 3; Lonnroth et al. 1989, Liang et al. 2002). However, in humans, the concentrations required in the interstitial fluid for a significant reduction of lipolysis have been found to be at the very high end of the physiological range and therefore of uncertain significance (Lonnroth et al. 1989). The ketone body β-hydroxybutyrate (β-OHB) has been shown to inhibit lipolysis in vitro by activating the human G<sub>i</sub>-coupled receptor HM74a (Fig. 3; Taggart et al. 2005). HM74a, which is the ortholog of the mouse PUMA-G receptor, is also the target of the lipid-lowering drug nicotinic acid (niacin; Tunaru et al. 2003). Importantly, the observed inhibition of lipolysis by β-OHB was obtained at concentrations similar to those seen in humans during fasting, suggesting a feedback mechanism by which β-OHB can regulate its own production in order to prevent ketoadiposis during starvation (Taggart et al. 2005). Similarly, the receptor responsible for the antilipolytic effect of lactate has been identified as GPR81 (Fig. 3; Cai et al. 2008, Liu et al. 2009a), which is a G<sub>i</sub>-coupled receptor highly homologous to HM74a (Cai et al. 2008). Like β-OHB, lactate inhibits lipolysis in adipocytes from several mammalian species including primates, rodents, and humans at concentrations within the normal physiological range (Liu et al. 2009a). Consequently, it has been hypothesized that GPR81 could act as a sensor of hypoxia by suppressing lipolysis in response to increased lactate production (Cai et al. 2008).

**Growth hormone**

Growth hormone (GH) potently and dose dependently stimulates lipolysis in humans (Hansen et al. 2002). In mice, knockout (KO) of the GH receptor renders the animals susceptible to obesity, while GH over-expression results in a lean phenotype (Berryman et al. 2004, 2006). The nature of the molecular pathway involved in GH-mediated lipolysis is not entirely clear. However, results from animal studies indicate that it involves remodeling of the cAMP-dependent regulatory signaling pathways such that the responsiveness toward β-adrenergic signaling is increased (Doris et al. 1994, Yang et al. 2004) while insulin sensitivity is reduced (Chen et al. 2001, Johansen et al. 2003; Fig. 3). Although definitive mechanistic evidence is lacking, this model has been supported by human studies. Thus, GH administration acutely stimulates lipolysis and causes peripheral insulin resistance (Nellemann et al. 2013). Also, the in vivo lipolytic effect of GH is counteracted by the AC inhibitor acipimox (a niacin derivative; Nielsen et al. 2001, 2002). Notably, stimulation with GH does not increase lipolysis in explants of human AT (Fain et al. 2008) or isolated human adipocytes (Marcus et al. 1994), but the sensitivity toward β-adrenergic agonists is enhanced by the presence of GH in the culture medium (Marcus et al. 1994). In line with this, it has recently been demonstrated that GH administration in human subjects increases ANGPTL4 levels in plasma (Clasen et al. 2013). Given the permissive effect of this protein on cAMP-mediated lipolysis, it seems likely that elevations in systemic ANGPTL4 levels could be one of the mechanisms by which GH stimulates AT lipolysis. Interestingly, the responsiveness toward GH stimulation varies among human AT depots, and visceral AT has been shown to be particularly sensitive to the lipolytic effects of GH (Nam et al. 2001, Pasarica et al. 2007, Plockinger & Reuter 2008).

**Glucocorticoids**

In a manner similar to GH, the lipolytic effects of glucocorticoids have been attributed to an increased β-adrenergic responsiveness and a reduction of insulin-mediated antilipolysis (Fig. 3). Thus, in rat adipocytes, dexamethasone treatment has been shown to promote PKA-mediated lipolysis by reducing the mRNA and protein expression levels of PDE3B (Xu et al. 2009). Also, dexamethasone potentiates the response toward β-AR agonists both by inducing an increase in the number of β-ARs and by increasing the catalytic response of AC toward receptor-mediated activation (Lacasa et al. 1988). In agreement with this, elevated cortisol levels in humans have been found to reduce the postprandial suppression of FFA release, suggesting a decreased antilipolytic effect of insulin (Dinneen et al. 1993). As for GH, glucocorticoid-mediated lipolysis in rodents is partially dependent on an increase in ANGPTL4 (Koliwad et al. 2009, Gray et al. 2012), indicating that these hormones share some of the mechanisms by which they stimulate lipolysis. However, acute in vivo studies on humans have also demonstrated additive independent effects of GH and cortisol on lipolysis, suggesting that alternative, and distinct, lipolytic pathways exist for these hormones (Djurhuus et al. 2004).

**Tumor necrosis factor α**

Multiple effects of the pro-inflammatory cytokine tumor necrosis factor-α (TNFα) on the lipolytic pathway have
been described. Acting through TNF receptor I (Fig. 3) in adipocytes from mice (Sethi et al. 2000) and humans (Ryden et al. 2002), TNFα activates the three MAPKs p42/44, JNK, and p38 of which p42/44 and JNK are involved in the induction of lipolysis (Ryden et al. 2002). In human fat cells, PDE3B protein expression is decreased dramatically by TNFα (Zhang et al. 2002) and in rat adipocytes antilipolytic signaling via the 2-AR is blunted by TNFα by specific proteasomal degradation of Gi1 (Gasic et al. 1999, Botion et al. 2001). The combined effect of these alterations of the insulin and 2-AR signaling pathway is an increased intracellular cAMP level and a resulting activation of PKA-mediated lipolysis. In addition to modulation of antilipolytic signaling, exposure to TNFα increases ATGL activity due to remodeling of core components of the lipolytic machinery. This is discussed in the following section.

Physiological regulation of human AT lipolysis

As discussed earlier, the lipolytic rate in human AT is determined by a delicate balance between several regulatory pathways. In healthy subjects, this regulation facilitates a proper lipolytic response to changes in systemic nutrient demand.

Feeding/fasting

Following the ingestion of a meal, the post-prandial increase in plasma insulin efficiently suppresses lipolysis to promote the storage of dietary lipids (Roust & Jensen 1993, Jensen 1995). Conversely, in the fasting state, FFA mobilization is promoted by the combined effects of reduced plasma insulin and increased release of adrenaline and noradrenaline (Gjedsted et al. 2007). Also, it has been demonstrated that lipolysis is further promoted by a combination of increased β-adrenergic sensitivity and decreased insulin sensitivity in AT during fasting (Jensen et al. 1987). Similarly, in obese individuals subjected to a hypocaloric diet (<3 MJ/day) for 28 days, lipolytic stimulation by β-adrenergic agonists as well as by ANP and BNP was enhanced significantly (Sengenes et al. 2002). These fasting-induced changes in hormonal sensitivity are mediated, at least in part, by GH, which is elevated significantly during prolonged fasting (Norrelund et al. 2001, 2003, Vendelbo et al. 2010). Likewise, the diurnal fluctuations in serum FFA levels mirror the pulsatile secretion pattern of GH, and the nocturnal increase in FFA during sleep is virtually absent in GH-deficient patients (Jorgensen et al. 1990).

Exercise

Physical exercise is the other major situation in which lipolysis is stimulated in humans and this is believed to involve the concerted action of several signaling pathways (Frayn 2010). Thus, circulating levels of adrenaline, noradrenaline, ANP, GH, and cortisol increase and insulin decreases in proportion to exercise intensity, and these gradual changes are reflected in the magnitude of the resulting lipolytic response (Moro et al. 2007b). Furthermore, the adrenergic responsiveness of subcutaneous AT is altered with a shift from predominant β-adrenergic suppression during rest toward predominant β-adrenergic stimulation during exercise (Arner et al. 1990), and in the post-exercise recovery phase, β-AR blockade has been shown to dramatically reduce plasma levels of FFA and glycerol (Wijnen et al. 1993). Similar to fasting conditions, GH and cortisol are likely to be some of the hormonal mediators of these exercise-induced alterations in adrenergic responsiveness (Kanaley et al. 2004). The primary adrenergic stimulus of AT during exercise originates from circulating catecholamines, with only a minor contribution from noradrenaline released from sympathetic neurons (Stallknecht et al. 2001, de Glisezinski et al. 2009). Additionally, the NPs have been found to play a prominent role in exercise-induced lipolysis in humans (Moro et al. 2004b, de Glisezinski et al. 2009), and they have been suggested to account for most of the nonadrenergic lipolytic signaling in AT during exercise (Moro et al. 2006, Lafontan et al. 2008).

Depot-specific regulation of lipolysis

AT is not a homogenous organ, and significant regional differences exist between depots in terms of hormonal responsiveness and metabolic activity. Also, the distribution of body fat is gender specific, with men generally having a more central (upper-body) and women a more peripheral (lower-body) fat deposition (Demerath et al. 2007).

Regarding the lipolytic activity of the different depots, visceral and subcutaneous abdominal ATs are generally more responsive toward lipolytic stimuli like catecholamines or prolonged fasting than subcutaneous gluteal and femoral fat (Gjedsted et al. 2007, Manolopoulos et al. 2012). The reduced lipolytic effect of catecholamines in lower-body fat depots is caused by enhanced 2-AR and reduced β-AR responsiveness compared with upper-body depots (Manolopoulos et al. 2012). Additionally, in upper-body obese women, the antilipolytic effect of insulin is blunted in the abdominal depots, which enhances
lipolysis further (Nellemann et al. 2012). Another important difference between upper- and lower-body subcutaneous AT is the primary way by which adipogenesis occurs as obesity develops. Thus, AT can expand either via an increase in the number of fat cells (i.e. hyperplasia) or by enlargement of the existing adipocytes (i.e. hypertrophy), of which the latter has been found to be an independent marker for increased metabolic risk (Weyer et al. 2000, Lundgren et al. 2007). Importantly, irrespective of gender, subcutaneous abdominal AT is more prone to expansion by hypertrophy than subcutaneous femoral AT, which preferentially undergoes hyperplasia (Tchoukalova et al. 2010).

As a consequence of these regional differences in adipogenesis and lipolytic responsiveness, it has been found repeatedly that upper-body obesity, but not lower-body obesity, is associated with elevated systemic FFA levels and metabolic dysfunction (Nielsen et al. 2004, Piche et al. 2008, Lapointe et al. 2009, Amati et al. 2012). In fact, it has been suggested that the preference of gluteofemoral fat for ‘trapping’ lipids serves as a ‘metabolic sink’ providing metabolic and cardiovascular protection from the deleterious effects of an excessive daily influx of dietary lipids (Manolopoulos et al. 2010). The regulation and implications of these gender- and depot-specific differences in terms of AT metabolism and signaling has been covered in great detail in a recent review (White & Tchoukalova 2014).

The lipolytic pathway: enzymes and co-regulators

The core enzymatic machinery for TG hydrolysis in AT consists of ATGL and HSL. Studies of ATGL-KO mice have revealed that the absence of ATGL reduces the lipolytic response of adipocytes to β-AR stimulation by ~70%, and by adding a specific HSL inhibitor lipolysis is reduced by more than 95% (Schweiger et al. 2006). Furthermore, both the basal and stimulated lipolytic capacity of human and mouse adipocytes are increased by ATGL overexpression and deceased by ATGL silencing (Kershaw et al. 2006, Bezaire et al. 2009). By contrast, HSL overexpression or silencing does not affect the basal lipolytic rates in human adipocytes, but the maximal stimulated lipolytic rate is decreased by reduced HSL levels (Bezaire et al. 2009).

Adipose TG lipase

The important function of ATGL as a TG hydrolase was discovered simultaneously in 2004 by three different groups (Jenkins et al. 2004, Villena et al. 2004, Zimmermann et al. 2004). Initially named ATGL (Zimmermann et al. 2004), phospholipase A2ζ (Jenkins et al. 2004), and desnutrin (Villena et al. 2004), the enzyme is now formally annotated as patatin-like phospholipase domain-containing protein 2 (Wilson et al. 2006). Expectedly, the expression of ATGL in mice has been found to be highest in white AT (WAT) and brown AT (BAT), but the transcript has been identified at lower levels in virtually all tissues studied (Villena et al. 2004, Kershaw et al. 2006).

The transcriptional control of ATGL gene expression is complex. A peroxisome proliferator-activated receptor γ (PPARγ)-responsive element has been identified in the promoter sequence of the mouse Atgl gene (Kim et al. 2006), and accordingly thiazolidinediones (PPARγ agonists) like rosiglitazone increase Atgl expression (Kim et al. 2006, Liu et al. 2009b). Furthermore, Atgl mRNA expression in 3T3-L1 adipocytes is negatively regulated by insulin as well as by TNFα-mediated p42/44 MAPK activation (Kim et al. 2006). Several additional studies have addressed the regulation of ATGL expression, and it has been found that in humans, ATGL protein is upregulated by fasting (Nielsen et al. 2011), while in mice, the mRNA is suppressed by feeding (Kershaw et al. 2006), but upregulated by glucocorticoids (Villena et al. 2004), and by SIRT1-mediated activation of the transcription factor Foxo1 (Chakrabarti et al. 2011, Shan et al. 2013). However, numerous studies have found that changes in mammalian ATGL mRNA and protein levels are often reciprocal, suggesting that ATGL is subject to extensive post-transcriptional regulation (Steinberg et al. 2007, Li et al. 2010, Nielsen et al. 2011, 2012).

ATGL is a specific TG hydrolase, and the activity toward other lipid substrates like DG, MG, retinylesters (RE), or cholesterylesters (CE) is very limited (Zimmermann et al. 2004). Although the 3D structure of ATGL has not been reported, studies on mutated and truncated human and murine ATGL have revealed that the N-terminal half of the enzyme contains the catalytic patatin domain (Duncan et al. 2010), while the C-terminal part is believed to be involved in regulation of enzymatic activity and to mediate the interaction between ATGL and LDs (Kobayashi et al. 2008, Schweiger et al. 2008). The two serine residues Ser404 and Ser428 in the C-terminal part of the human ATGL sequence (corresponding to Ser406 and Ser430 in murine ATGL) have been identified as phosphorylation sites (Bartz et al. 2007). However, the role of these sites in the regulation of ATGL activity, and the identity of the upstream kinases is somewhat unclear. Thus, Ser406 has been suggested to be a consensus site for
AMP-activated protein kinase (AMPK), and in murine 3T3-L1 adipocytes, it was shown that pharmacological stimulation of lipolysis with the AMPK agonist AICAR was dependent on ATGL Ser\textsuperscript{406} phosphorylation (Ahmadian et al. 2011). However, another study found that phosphorylation of Ser\textsuperscript{404} in human ATGL was increased by β-adrenergic stimulation, while AICAR treatment had no effect (Pagnon et al. 2012). Additionally, in mouse AT, Ser\textsuperscript{406} phosphorylation was increased with fasting, exercise, and \emph{ex vivo} stimulation of the cAMP-dependent pathway in a PKA-dependent manner, thereby implicating this kinase in the phosphorylation of ATGL (Pagnon et al. 2012). Notably, in human skeletal muscle (SM), Ser\textsuperscript{404} phosphorylation is also associated with PKA signaling and not with AMPK signaling, indicating that PKA is indeed the upstream kinase for this site (Mason et al. 2012). Whether PKA and/or AMPK are responsible for phosphorylation of Ser\textsuperscript{430} is currently unknown, and so far no reports have been published on the functional role of this site.

**ATGL: activation by CGI-58**

The primary way by which ATGL activity is increased under acute lipolytic stimulation is via interaction with the co-activator CGI-58 (also known as α/β hydrolase domain-containing protein 5; Fig. 4 A and B; Lass et al. 2006). Activation depends on the interaction of CGI-58 with the patatin domain of ATGL (Schweiger et al. 2008, Cornaciu et al. 2011) and requires direct protein–protein interactions between ATGL and CGI-58 (Granneman et al. 2007, Cornaciu et al. 2011). Also, mutational studies have shown that additional binding of CGI-58 to the LD is crucial in order to activate ATGL (Gruber et al. 2010). The molecular mechanism by which CGI-58 activates ATGL is unclear, but it could potentially involve induction of conformational changes, presentation of substrate, or removal of reaction products. Interestingly, in addition to its role in regulating lipolysis, both mouse and human CGI-58 have been identified as a CoA-dependent lysophosphatidic acid acyltransferase (LPAAT; Ghosh et al. 2008, Gruber et al. 2010, Montero-Moran et al. 2010), and it has been speculated that it promotes lipolysis by channeling fatty acids released from TG hydrolysis into phospholipids to reduce end product inhibition of ATGL and HSL (Montero-Moran et al. 2010). Although the potential relevance of this LPAAT activity of CGI-58 in lipolytic regulation remains to be investigated, CGI-58-derived phospholipids are essential second messengers in murine liver and AT when exposed to pro-inflammatory cytokines like TNFα, interleukin 1β (IL1β), and IL6 (Lord et al. 2012).

Consequently, as downstream inflammatory stress kinases can impair insulin signaling, CGI-58 seems to be involved in cross talk between insulin sensitivity and inflammation, at least in mice.

**ATGL: inhibition by G0S2**

Recently, the protein product of G0/G1 switch gene 2 (G0S2) was identified as an inhibitor of ATGL (Yang et al. 2010). In mice, G0S2 is primarily expressed in brown and white adipocytes, but a significant expression has also been detected in liver, heart, and SM (Zandbergen et al. 2005). Murine G0S2 mRNA and protein expression has been shown to be induced by insulin and PPARγ and suppressed by lipolytic agents like TNFα and the β-AR agonist isoprenaline (Zandbergen et al. 2005, Yang et al. 2010). Like CGI-58, G0S2 interacts directly with
the catalytic patatin domain of ATGL (Yang et al. 2010, Cornaciu et al. 2011), but the ATGL-G0S2 interaction is independent of the ATGL-CGI-58 interaction, and inhibition by G0S2 appears to be dominant to activation by CGI-58 (Fig. 4B; Yang et al. 2010, 2011, Schweiger et al. 2012). Human G0S2, like the murine ortholog, inhibits ATGL in a dose-dependent manner and is also involved in regulating the intracellular localization of ATGL by recruiting it to LDs (Schweiger et al. 2012).

It has been proposed that G0S2 acts as a long-term regulator of lipolysis, as G0S2 protein levels are gradually reduced during prolonged lipolytic stimulation resulting in increased ATGL activity (Fig. 4C; Yang et al. 2010). Notably, G0S2 protein and mRNA expression is dramatically reduced in human AT by prolonged physiological stimulation of lipolysis with a 72-h fast (Nielsen et al. 2011) and similar effects have been observed in birds (Oh et al. 2011) and pigs (Ahn et al. 2013). However, it is currently not known if the association between ATGL and G0S2 is dynamic and subject to regulation or if G0S2-mediated ATGL inhibition primarily depends on the intracellular levels of G0S2. Recent evidence has suggested the latter although. Thus, in 3T3-L1 adipocytes, stimulation with TNFα for up to 16 h reduces G0S2 levels gradually, while the rate of lipolysis increases nearly proportionally to the G0S2 reduction (Yang et al. 2011). Conversely, overexpression of G0S2 significantly reduces the TNFα mediated lipolytic response. Murine G0S2 is a short-lived protein with a half-life of <1 h, and its stability can be greatly improved by inhibition of the proteasomal pathway (Yang et al. 2011). Hence, it appears that one of the mechanisms by which TNFα promotes adipocyte lipolysis is by suppressing G0S2 mRNA expression leading to cytosolic depletion of G0S2 protein through proteasomal degradation and, consequently, increased ATGL activity.

Animal models of ATGL deficiency

Insight into the crucial role of ATGL in whole-body TG metabolism has been provided from the characterization of ATGL-deficient mice (Haemmerle et al. 2006). These animals exhibit massive ectopic lipid accumulation in virtually all tissues and especially in AT, liver, SM, and heart (Haemmerle et al. 2006). Accordingly, the animals become obese even on a low-fat diet, and they suffer from premature death due to severe cardiac steatosis and dysfunction (Haemmerle et al. 2006, Schrammel et al. 2013). Furthermore, the normal fasting- or exercise-induced rise in plasma FFA is absent in ATGL-KO animals indicating a failure to increase lipolysis in WAT (Huijsman et al. 2009, Schoiswohl et al. 2010). Without sufficient fuel from lipid substrates, they rely primarily on carbohydrate metabolism for energy conversion resulting in rapid depletion of hepatic and SM glycogen stores (Huijsman et al. 2009, Schoiswohl et al. 2010). Consequently, when subjected to moderate exercise or short-term fasting, the mice become hypoglycemic, and if fasting is extended beyond a modest 8–12 h, they develop signs of severe energy starvation like hypothermia, lethargy, reduced oxygen consumption, and loss of lean mass (Haemmerle et al. 2006, Schoiswohl et al. 2010, Wu et al. 2012). Similarly, in spite of massively increased BAT mass, ATGL-KO mice are unable to increase thermogenesis in response to cold exposure, indicating that the mobilization of lipid fuel in BAT is defective (Haemmerle et al. 2006). In addition to the abnormal substrate metabolism, ATGL deficiency causes pancreatic steatosis leading to impaired insulin secretion and hypoinsulinemia (Peyot et al. 2009). Interestingly, however, despite the massive ectopic lipid accumulation and β-cell dysfunction, the ATGL-deficient mice have improved whole-body insulin sensitivity and glucose tolerance compared with WT animals (Haemmerle et al. 2006, Peyot et al. 2009). Specifically, muscle and WAT insulin signaling is improved, although in BAT and liver it is reduced (Kienesberger et al. 2009).

The key role of defective TG catabolism in the phenotype of ATGL-KO mice has recently been supported with the generation of transgenic mice with AT-specific overexpression of G0S2 (Heckmann et al. 2014). Like ATGL-deficient mice, WAT and BAT mass is increased in these animals due to impaired basal and stimulated lipolysis, but glucose and insulin tolerance is improved. Moreover, thermogenesis is attenuated leading to defective cold adaptation, and the fasting-induced switch from carbohydrate to fatty acid metabolism is severely impaired (Heckmann et al. 2014). Conversely, mice with global G0S2 KO are lean and resistant to high-fat diet-induced obesity and hepatic steatosis (Zhang et al. 2013). Furthermore, hepatic fatty acid metabolism is enhanced as G0S2 ablation accelerates ketogenesis and gluconeogenesis while glycogen breakdown is impaired (Zhang et al. 2013). Combined with the observations from Atg1-KO mice, these results support a defining role for ATGL-mediated lipolysis in whole-body substrate partitioning and metabolism, at least in mice.

ATGL in human obesity and metabolic disease

The available literature on the expression patterns of ATGL in human obesity is somewhat conflicting. In a study
among lean and obese women, no difference in ATGL protein levels were found in subcutaneous abdominal AT (Ryden et al. 2007). Conversely, in mixed populations of men and women, ATGL mRNA was increased in subcutaneous abdominal AT in obesity, but the protein levels were reduced (Steinberg et al. 2007, Yao-Borengasser et al. 2011) and a negative correlation was found between BMI and ATGL protein expression (Yao-Borengasser et al. 2011). Furthermore, in paired biopsies, ATGL mRNA expression was lower in visceral than in subcutaneous abdominal AT (Yao-Borengasser et al. 2011), and when comparing visceral AT from obese and lean subjects, ATGL mRNA was increased in obesity (Steinberg et al. 2007, Tinahones et al. 2010), while the protein levels were unaffected (Steinberg et al. 2007). Similarly, in a recent comparison of lean and obese men, only the mRNA of ATGL was increased in visceral fat in obesity, but in subcutaneous abdominal fat, ATGL protein was increased in the obese subjects (De Naeyer et al. 2011). Furthermore, among obese males and females with either normal or impaired insulin sensitivity, insulin resistance has been shown to be associated with reduced ATGL protein and mRNA in subcutaneous abdominal AT (Jocken et al. 2007) and reduced ATGL mRNA in visceral AT (Berndt et al. 2008). However, whereas the available data on ATGL expression in obesity is inconclusive, the expression of CGI-58 seems to be remarkably stable between depots (Yao-Borengasser et al. 2011) and in obesity (Steinberg et al. 2007).

Gender-specific differences may explain some of the discrepancies between the studies on ATGL, but in light of the substantial body of conflicting data, the role of human ATGL in the pathogenesis of metabolic disease in obesity is currently unclear.

By contrast, defective ATGL-mediated lipolysis has been unequivocally identified as the primary defect in the inherited monogenic disorder neutral lipid storage disease (NLSD; Lefevre et al. 2001, Lass et al. 2006, Fischer et al. 2007). Patients with loss-of-function mutations affecting ATGL are characterized by ectopic TG accumulation and visceral obesity, skeletal and cardiac myopathy, and variable degrees of hepatic and pancreatic steatosis (Schweiger et al. 2009, Laforet et al. 2013, Natali et al. 2013). However, the metabolic phenotype of these patients is heterogeneous. Thus, some are insulin resistant and develop type 2 diabetes (Laforet et al. 2013), while others have normal insulin sensitivity but impaired glucose tolerance (Natali et al. 2013). This probably reflects individual differences in the pattern of ectopic lipid deposition: patients with extensive pancreatic steatosis generally have an impaired insulin response to an oral glucose challenge (Natali et al. 2013), while insulin resistance is more common in patients with severe muscular involvement and hepatic steatosis (Laforet et al. 2013). The clinical manifestations of loss-of-function mutations in CGI-58 are similar to those observed in functional ATGL deficiency except that these patients do not develop myopathy (Igal et al. 1997). Instead, they suffer from severe ichthyosis (Chanarin et al. 1975, Lefevre et al. 2001) and, accordingly, the two types of NLSD are known as NLSD with myopathy (NLSDM) and NLSD with ichthyosis (NLSDI, also known as Chanarin–Dorfman syndrome) (Fischer et al. 2007). The epidermal defects observed in NLSDI are not present in NLSDM, suggesting an ATGL-independent function of CGI-58, possibly as a LPAAT in the synthesis pathway of glycerophospholipids and acylceramides required for the formation and maintenance of the skin permeability barrier (Igal & Coleman 1996, Radner et al. 2009). Consistently, KO of CGI-58 in mice results in a neonatal lethal phenotype caused by leaky skin, and the pups die from desiccation within hours after birth (Radner et al. 2009). An overview of studies on genetic deficiencies affecting ATGL function in humans and animal models is listed in Table 1.

Hormone-sensitive lipase

HSL was discovered in rat AT in the early 1960s as a lipolytic enzyme, which was inducible by fasting and stimulation with ACTH or adrenaline and inhibited by insulin (Hollenberg et al. 1961, Rizack 1964, Vaughan et al. 1964).

Like ATGL, HSL is expressed in most tissues examined, with the highest expression found in WAT and BAT (Kraemer et al. 1993). The mRNA is generated from a single gene controlled by a number of alternative promoters that produce several different tissue-specific isoforms of the HSL protein that range in size from ~85 kDa and up to 130 kDa (Langin et al. 1993, Mairal et al. 2002). The HSL isoform found in human AT is a 786 aa protein with an apparent molecular weight of ~88 kDa (Langin et al. 1993).

Efficient lipid hydrolysis by HSL requires the lipase to form a complex with cytosolic fatty acid-binding protein 4 (FABP4), which acts as a molecular chaperone by shuttling the FFA generated by HSL out of the cell (Fig. 5A; Furuhashi & Hotamisligil 2008). Upon stimulation of lipolysis, HSL and FABP4 associate in the cytosol and the complex translocate to LĐs (Jenkins-Kruchten et al. 2003, Smith et al. 2007). Consistently, in FABP4-KO mice, the lipolytic capacity is reduced, and the intracellular FFA
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TG and MG (Fredrikson affinity for DG is approximately tenfold higher than for the substrates and intermediates in TG lipolysis, the sites is different; phosphorylation of Ser 563 is thought to CE several lipid substrates, including TG, DG, MG, RE, and phosphorylated by HSL, which is a promiscuous lipase than ATGL and readily hydrolyze (Fig. 5 B). In terms of specificity, HSL is a much more (Daval et al. 1998, Watt et al. 1998, Wei et al. 1981, 1986). The functional role of these sites are Ser563, Ser659, and Ser660 (Stralfors et al. 1984, Garton et al. 1988, Anthonsen et al. 1998) corresponding to the human residues Ser552, Ser649, and Ser650 (Contreras et al. 1998, Watt et al. 2006). The functional role of these sites is different; phosphorylation of Ser563 is thought to promote the translocation of HSL from the cytosol to LDs (Daval et al. 2005), while phosphorylation of Ser659 and Ser660 is critical for activation of the intrinsic enzymatic activity (Anthonsen et al. 1998). Conversely, phosphorylation of rat HSL on Ser565 (human Ser554) by AMPK inhibits HSL activation, most likely by steric hindrance of phosphorylation of the adjacent Ser563, thus preventing the translocation of HSL to the LDs (Daval et al. 2005) (Fig. 5B). In terms of specificity, HSL is a much more promiscuous lipase than ATGL and readily hydrolyze several lipid substrates, including TG, DG, MG, RE, and CE in vitro (Fredriksen et al. 1981, Wei et al. 1997). Among the substrates and intermediates in TG lipolysis, the affinity for DG is approximately tenfold higher than for TG and MG (Fredriksen et al. 1981, 1986).

Animal models of HSL deficiency

The key role of HSL as a DG hydrolase in vivo was revealed with the generation of HSL-KO mice, which were found to accumulate intracellular DG in AT, SM, cardiac muscle, and testis (Haemmerle et al. 2002). However, in contrast to ATGL-KO mice, HSL-deficient mice do not suffer from severe systemic lipid accumulation, although they tend to have enlargement of internal organs like liver, heart, pancreas, and spleen (Harada et al. 2003). Surprisingly, the WAT mass is slightly reduced, and they are resistant to high-fat diet-induced obesity and peripheral insulin resistance (Osuga et al. 2000, Harada et al. 2003, Park et al. 2005). This unexpected observation was found to be caused by a compensatory reduction in fatty acid esterification and de novo lipogenesis to counteract the reduced release of FFA to the circulation (Zimmermann et al. 2003). However, similar to ATGL-KO mice, HSL-KO mice have increased BAT mass and enlargement of brown adipocytes (Harada et al. 2003), but this is not associated with impaired thermogenesis, as they retain a normal sensitivity to cold exposure (Osuga et al. 2000). An overview of animal studies on genetic deficiencies affecting HSL is listed in Table 2.

Table 1 Overview of genetic studies on ATGL and CGI-58 function

<table>
<thead>
<tr>
<th>Species</th>
<th>Diagnosis/genetic model</th>
<th>Affected protein</th>
<th>Highlights of the study</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>NLSDI</td>
<td>CGI-58</td>
<td>Case report: clinical manifestations and proposal of diagnostic criteria</td>
<td>Igal et al. (1997)</td>
</tr>
<tr>
<td>Human</td>
<td>NLSDI</td>
<td>CGI-58</td>
<td>Identification of defects in CGI-58 as cause of NLSDI</td>
<td>Lefevre et al. (2001)</td>
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<tr>
<td>Human</td>
<td>NLSDI</td>
<td>CGI-58</td>
<td>Activation of ATGL and rescue of NLSDI by CGI-58</td>
<td>Lass et al. (2006)</td>
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<tr>
<td>Human</td>
<td>NLSDM</td>
<td>ATGL</td>
<td>Case report: identification of truncations in human ATGL</td>
<td>Fischer et al. (2007)</td>
</tr>
<tr>
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<td>NLSDM</td>
<td>ATGL</td>
<td>Identification of biochemical defects in truncated ATGL</td>
<td>Kobayashi et al. (2008)</td>
</tr>
<tr>
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<td>NLSDM</td>
<td>ATGL</td>
<td>Case report: magnetic resonance imaging of muscles and metabolic characterization</td>
<td>Laforet et al. (2013)</td>
</tr>
<tr>
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<td>NLSDM</td>
<td>ATGL</td>
<td>Case report: body composition and lipid metabolism</td>
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<td>Mouse KO</td>
<td>ATGL and HSL</td>
<td>Measurement of lipolysis in AT explants from KO animals</td>
<td>Schweiger et al. (2006)</td>
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<td>ATGL</td>
<td>Phenotyping of KO animals</td>
<td>Haemmerle et al. (2006)</td>
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<td>Insulin sensitivity and glucose/lipid metabolism</td>
<td>Kienesberger et al. (2009)</td>
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<td>ATGL and HSL</td>
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<td>ATGL</td>
<td>Evaluation of effects on insulin secretion</td>
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<td>CGI-58</td>
<td>Phenotyping of KO animals</td>
<td>Radner et al. (2009)</td>
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<td>ATGL</td>
<td>Lipid/glucose metabolism during exercise</td>
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<tr>
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<td>ATGL</td>
<td>Phenotyping and fed/fasting metabolism</td>
<td>Wu et al. (2012)</td>
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<td>ATGL</td>
<td>Cardiac metabolism</td>
<td>Schrammel et al. (2013)</td>
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<tr>
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<td>G052</td>
<td>Phenotyping of transgenic animals</td>
<td>Zhang et al. (2013)</td>
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<tr>
<td>Mouse KO</td>
<td>G052</td>
<td>Phenotyping of transgenic animals</td>
<td>Heckmann et al. (2014)</td>
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</table>
HSL in human obesity and metabolic disease

While changes in ATGL expression patterns in obesity are of uncertain significance, the importance of HSL expression is somewhat more well established, although discrepancies certainly exist. Thus, the expression of HSL mRNA in subcutaneous abdominal AT in obesity has been reported to be increased (Ray et al. 2009), reduced (Large et al. 1999, Mairal et al. 2006), or not affected (Steinberg et al. 2007, De Naeyer et al. 2011). However, irrespective of gender, the majority of studies have found the corresponding HSL protein levels to be reduced in obesity (Large et al. 1999, Langin et al. 2005, Ryden et al. 2007, Ray et al. 2009). Similarly, in the obese state, insulin resistance is associated with a reduction in HSL mRNA and protein in subcutaneous abdominal AT (Jocken et al. 2007). In visceral AT, HSL mRNA levels have consistently been found to be upregulated in obesity (Mairal et al. 2006, Steinberg et al. 2007, Ray et al. 2009, De Naeyer et al. 2011), but the protein levels seem to be unaffected (De Naeyer et al. 2011) or possibly reduced (Ray et al. 2009).

So far, no human examples of loss-of-function mutations affecting HSL have been reported, and in light of the relatively mild phenotype of HSL-KO mice, it seems unlikely that HSL deficiency per se is associated with severe metabolic defects in humans.

Monoglyceride lipase

MGL was identified in rats as an MG-specific lipase with no affinity toward TG or DG (Tornqvist & Belfrage 1976). The first MGL-KO mouse model has recently been generated (Table 2), and these animals accumulate MG in WAT, brain, and liver (Taschler et al. 2011). Also, it was found that HSL partially compensated for the absence of MGL in AT, as the stimulated glycerol release was reduced by a modest 43% compared with WT mice. However, upon specific inhibition of HSL in cultured fat pads, the MG-hydrolase activity was almost completely abolished (Taschler et al. 2011). To date, no reports have been published indicating that MGL expression and enzymatic activity in AT is regulated by hormonal signals or nutritional status, suggesting that the enzyme is constitutively active in the lipolytic cascade.

LD-associated proteins: the CIDE family

The PLIN proteins and the CIDE proteins constitute the two major families of LD-associated proteins in adipocytes. The pro-apoptotic CIDE proteins (cell-death inducing DNA fragmentation factor-α-like effector) comprise three members: CIDEA, CIDEB, and CIDEC (also known as fat-specific protein of 27 kDa, Fsp27) (Yonezawa et al. 2011).
LD-associated proteins: the PLIN family

The other family of LD-associated proteins has been studied in much more detail. The PLIN proteins were originally called the PAT family after perilipin A, adipophilin, and tail-interacting protein of 47 kDa (TIP-47), and the family also includes the proteins S3-12 and myocyte LD protein (MLDP, also known as OXPAT) (Bickel et al. 2009). However, due to their evolutionary, structural, and functional relationship, they are now annotated as PLIN1 (perilipin A), PLIN2 (adipophilin), PLIN3 (TIP-47), PLIN4 (S3-12), and PLIN5 (MLDP/OXPAT) (Kimmel et al. 2010). Of the PLIN proteins, PLIN1 is particularly important in the regulation of AT lipolysis, while the other family members are involved in adipogenesis and LD formation (PLIN2), LD biosynthesis and stabilization (PLIN3), LD maturation (PLIN4) and regulation of lipolysis in oxidative tissues not expressing PLIN1 (PLIN5) (Bickel et al. 2009).

Multiple regulatory roles of murine PLIN1 have been described in the lipolytic cascade, and it has been suggested that PLIN1 is the ‘master regulator’ of PKA-stimulated lipolysis in mice (Miyoshi et al. 2007). As such, PLIN1 either directly or indirectly regulates the activity of ATGL and HSL as well as their access to lipid substrates in the LDs. The LD targeting of HSL is partly governed by PLIN1; in the basal state, as much as half of the total cellular HSL content is located in the cytoplasm, but PKA-mediated PLIN1 and HSL phosphorylation has been shown to significantly enhance the co-localization and association of the two proteins on LDs (Miyoshi et al. 2006). However, studies on mutant PLIN1 lacking all phosphosites have revealed that in the absence of phosphorylation of PLIN1, the PKA-mediated increase in HSL activity is blunted, although the lipase is phosphorylated and translocated (Miyoshi et al. 2006). In other words, the lipolytic action of LD-associated and phosphorylated HSL are critically dependent on PLIN1 phosphorylation in mouse adipocytes.

Similarly, the activation of murine ATGL depends on phosphorylation of PLIN1, although by a different mechanism. In the basal state, unphosphorylated PLIN1 has been shown to negatively regulate ATGL by efficiently sequestering CGI-58, thereby preventing activation of ATGL (Granneman et al. 2007, 2009). Upon stimulation of lipolysis, phosphorylation of PLIN1 causes the dissociation of CGI-58, which can then bind and activate ATGL (Granneman et al. 2009; Fig. 4). Interestingly, a single amino acid in PLIN1 has been identified as the crucial residue for regulation of HSL- and ATGL-mediated lipolysis, as mutation of Ser\(^{517}\) in the mouse sequence almost completely abolishes PKA-stimulated FFA and glycerol release (Miyoshi et al. 2007).

Animal models of deficiencies in LD-associated proteins

In mice, the CIDE proteins exhibit a distinct expression pattern. Thus, while CIDEA is predominantly expressed in BAT (Zhou et al. 2003), CIDEB is almost exclusively

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**Table 2** Overview of genetic studies on HSL, MGL, and FABP4

<table>
<thead>
<tr>
<th>Species</th>
<th>Genetic model</th>
<th>Affected protein</th>
<th>Highlights of the study</th>
<th>Reference</th>
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<tr>
<td>Mouse KO</td>
<td>FABP4</td>
<td>Phenotyping of KO animals</td>
<td>Coe et al. (1999)</td>
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<td>Mouse KO</td>
<td>HSL</td>
<td>Phenotyping of KO animals</td>
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<td>Mouse KO</td>
<td>HSL</td>
<td>Involvement of HSL in whole-body DG catabolism</td>
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<tr>
<td>Mouse KO</td>
<td>HSL</td>
<td>AT adaptations to HSL deficiency</td>
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<tr>
<td>Mouse KO</td>
<td>MGL</td>
<td>Phenotyping of KO animals</td>
<td>Taschler et al. (2011)</td>
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</table>

CIDEA and CIDEC from mice and humans have recently been shown to be negative regulators of lipolysis (Nordstrom et al. 2005, Puri et al. 2008, Christianson et al. 2010), although their specific role is not well characterized yet. However, it appears that they promote lipid storage through their involvement in LD formation, fusion, and stabilization (Puri et al. 2008, Christianson et al. 2010, Ito et al. 2010). Accordingly, human CIDEC has been found to interact with PLIN1 (see below) and the interaction between these two proteins is critical for the formation of large LD’s and unilocular adipocytes (i.e. cells containing a single big LD) (Grahn et al. 2013, Sun et al. 2013). The mechanism by which the CIDE proteins inhibit lipolysis is incompletely understood but it seems to involve shielding of the LDs from the action of lipases by providing a physical barrier around the lipid core (Christianson et al. 2010, Yang et al. 2013).
expressed in the liver (Li et al. 2007), and CIDEC is primarily found in WAT (Nishino et al. 2008), suggesting a tissue-specific role of these proteins. Consistently, CIDEA-deficient mice are characterized by elevations in body temperature and overall metabolic rate due to accelerated BAT lipolysis and thermogenesis (Zhou et al. 2003). Consequently, these mice are lean and resistant to diet-induce obesity and glucose intolerance. A liver-specific function of CIDEA has also been demonstrated, as hepatic CIDEA knockdown in genetically obese ob/ob mice reduces hepatic TG accumulation and LD size and accelerates overall energy expenditure (Zhou et al. 2012). Similarly, KO of CIDEB results in lean mice with improved glucose tolerance, insulin sensitivity, and resistance to hepatic steatosis, but BAT metabolism is normal in these animals (Li et al. 2007). Instead, ketogenesis and overall metabolic rate is accelerated, suggesting an overall shift in substrate utilization toward lipid metabolism (Li et al. 2007). In terms of metabolic rate, susceptibility to obesity, hepatic steatosis, and disturbances in glucose homeostasis and metabolism, the overall phenotype of CIDEA-KO mice is very similar to the phenotype of CIDEA- and CIDEB-deficient animals (Nishino et al. 2008, Toh et al. 2008). Notably, however, while the metabolic rate in BAT is reduced, mitochondrial biogenesis, oxygen consumption, and basal lipolytic rates are increased in WAT (Nishino et al. 2008). Consistently, WAT expression of specific factors inhibiting BAT differentiation is reduced and a concomitant increase in the expression of BAT-specific genes (e.g. UCP1) causes a shift toward a more brown-like phenotype (Toh et al. 2008). Thus, in spite of tissue-specific differences in the expression and regulation of the CIDE proteins, they seem to share a crucial role in the regulation of overall substrate partitioning and metabolism.

In agreement with the role of PLIN1 as a negative regulator of ATGL activity, PLIN1-deficient mice have been found to have dramatically decreased fat mass and increased basal lipolysis (Martinez-Botas et al. 2000, Tansey et al. 2001). This observation has been supported by in vitro studies; stimulation of 3T3-L1 cells with TNF-α was found to increase basal lipolysis due to a reduction in PLIN1 protein levels, and this effect could be reversed by simultaneous overexpression of PLIN1 (Souza et al. 1998). Furthermore, PLIN1-KO animals are lean and resistant to diet-induced obesity, but nevertheless they are prone to develop glucose intolerance and peripheral insulin resistance (Martinez-Botas et al. 2000, Tansey et al. 2001). Interestingly, in the absence of PLIN1, the expression of PLIN2 is increased and, consequently, PLIN2 is the major LD-associated protein in these animals (Tansey et al. 2001). In cultured cells, overexpression of PLIN2 has been shown to promote lipid storage by negatively regulating the access of ATGL to LDs (Listenberger et al. 2007), but the underlying mechanism is currently unknown, and it remains to be determined whether PLIN1 deficiency is associated with increased PLIN2 expression in humans in vivo.

LD-associated proteins in human obesity and metabolic disease

As for ATGL and HSL, the available data on the association between obesity and the expression of LD-associated proteins are rather inconclusive. In the obese state, PLIN1 mRNA expression has been reported to be increased in visceral AT (Ray et al. 2009, Tinahones et al. 2010) but unaffected in subcutaneous abdominal AT (Ray et al. 2009). However, others have found a negative correlation between BMI and visceral PLIN1 mRNA levels, suggesting that the expression is reduced in obesity (Moreno-Navarrete et al. 2013). In obesity, PLIN1 protein expression is reduced in both visceral and subcutaneous abdominal AT (Ray et al. 2009), and among obese subjects, the protein levels in subcutaneous abdominal AT are lower in insulin-resistant subjects than in insulin-sensitive subjects (Moreno-Navarrete et al. 2013).

Recently, two different loss-of-function mutations in PLIN1 have been identified in three patients diagnosed with a rare autosomal dominant partial lipodystrophy (Gandotra et al. 2011a). Both mutations introduced a frameshift causing the loss of three regulatory PKA sites, including the crucial Ser517. Consequently, these mutants fail to sequester CGI-58 leading to permanently elevated basal lipolysis due to constitutive activation of ATGL (Gandotra et al. 2011b). The clinical manifestations of these mutations included dyslipidemia, hypertension, lipatrophy, hepatic steatosis, severe insulin resistance, and type 2 diabetes. Remarkably, a very similar phenotype has been found in a patient carrying a loss-of-function mutation in the LD-targeting domain of CIDE, suggesting that the integrity of the interaction between CIDE and PLIN1 is crucial for proper LD dynamics and lipolytic control in humans in vivo (Rubio-Cabezas et al. 2009). Additionally, the CIDE-deficient AT had an unusually high occurrence of multilocular adipocytes supporting a role for CIDE in the formation and stabilization of large LD’s (Rubio-Cabezas et al. 2009). An overview of studies on genetic deficiencies affecting LD-associated proteins in humans and animal models is listed in Table 3.
Intracellular lipolysis in non-ATs

In this review, we have focused on lipolysis in AT. However, the ability to store and re-hydrolyze TG is not unique for adipocytes, and most cell types are able to form LDs by taking up and esterifying FFA into TG (Greenberg et al. 2011). In fact, the importance of tight lipolytic control in other tissues has become apparent with the characterization of the phenotypes associated with deficient lipase activity, particularly with respect to ATGL. Like the myocardial defects observed in Atgl KO mice, muscle-specific CGI-58 KO mice also suffer from cardiomyopathy and cardiac steatosis caused by a severe impairment of TG catabolism (Zierler et al. 2013). Conversely, myocardial specific ATGL overexpression in type 1 diabetic mice confers protection against diabetes-induced cardiomyopathy (Pulnilkunnil et al. 2013), supporting a key role of ATGL in the regulation of metabolism and lipid homeostasis in the heart. Similar results have been obtained in the liver, where ATGL deficiency causes hepatic steatosis and reduced β-oxidation (Ong et al. 2011, Wu et al. 2011) while liver-specific overexpression of ATGL or HSL reduces obesity-induced steatosis and increases β-oxidation (Reid et al. 2008, Ong et al. 2011). Surprisingly although, in spite of these effects of altered lipase expression on hepatic TG content and substrate utilization, liver-specific KO or overexpression of ATGL only has minor effects on hepatic insulin sensitivity and whole-body metabolic parameters (Turpin et al. 2011, Wu et al. 2011). In human SM, ATGL protein is increased significantly by endurance training, indicating that mobilization of intramyocellular TG stores involves ATGL (Alsted et al. 2009). Obesity is also associated with increased SM content of ATGL, whereas HSL is decreased, resulting in a substantial decrease in the ratio of DG to TG hydrolase activity (Jocken et al. 2010). Finally, in obese type 2 diabetic patients, basal SM lipolysis is elevated and the anti-lipolytic action of insulin is blunted (Jocken et al. 2013). Whether this impairment is a result of peripheral insulin resistance or a cause of it is unclear although, and at present, the mechanistic connection between intramyocellular lipolysis and insulin resistance is a matter of intense debate and research.

In summary, since the discovery of ATGL in 2004 tremendous progress has been made in the characterization of AT lipolysis. However, with the increasing number of newly identified enzymes and regulatory proteins, the remarkable complexity of the hormonal and intracellular signaling network regulating the lipolytic pathway has also become clear. Considering the severe phenotypes associated with defective lipolysis in AT, pancreas, liver, heart, and SM, it is evident that the balance between lipid mobilization, utilization, and storage is crucial in most tissues. Consequently, by delineating the processes regulating lipid metabolism in adipose and non-ATs alike, we can also advance our understanding of glucose metabolism and identify new pathways to target in the treatment of metabolic disease like type 2 diabetes.

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**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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**Table 3** Overview of genetic studies on LD-associated proteins

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Review


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