thematic review

Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores

Rudolf Zechner,[1] Petra C. Kienesberger, Guenter Haemmerle, Robert Zimmermann, and Achim Lass

Institute of Molecular Biosciences, University of Graz, Austria

Abstract **Fatty acids (FAs) are essential components of all lipid classes and pivotal substrates for energy production in all vertebrates. Additionally, they act directly or indirectly as signaling molecules and, when bonded to amino acid side chains of peptides, anchor proteins in biological membranes. In vertebrates, FAs are predominantly stored in the form of triacylglycerol (TG) within lipid droplets of white adipose tissue. Lipid droplet-associated TGs are also found in most nonadipose tissues, including liver, cardiac muscle, and skeletal muscle. The mobilization of FAs from all fat depots depends on the activity of TG hydrolases. Currently, three enzymes are known to hydrolyze TG, the well-studied hormone-sensitive lipase (HSL) and monoglyceride lipase (MGL), discovered more than 40 years ago, as well as the relatively recently identified adipose triglyceride lipase (ATGL). The phenotype of HSL- and ATGL-deficient mice, as well as the disease pattern of patients with defective ATGL activity (due to mutation in ATGL or in the enzyme's activator, CGI-58), suggest that the consecutive action of ATGL, HSL, and MGL is responsible for the complete hydrolysis of a TG molecule. The complex regulation of these enzymes by numerous, partially uncharacterized effectors creates the "lipolysome," a complex metabolic network that contributes to the control of lipid and energy homeostasis.** **This review focuses on the structure, function, and regulation of lipolytic enzymes with a special emphasis on ATGL.**—Zechner, R., P. C. Kienesberger, G. Haemmerle, R. Zimmermann, and A. Lass. **Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores.** ***J. Lipid Res.*** **2009.** 50: **3–21.**

Supplementary key words lipolysis • hydrolase • neutral lipid storage disease

Lipid homeostasis reflects a balance of processes, designed to generate fatty acids (FAs) and lipids, deliver them from their site of origin to target tissues, and catabolize them for metabolic purposes. Innumerable genes and signal components are responsible for an integrated communication network between many tissues and organs, including adipose tissue, liver, muscles, the digestive tract, pancreas, and the nervous system. This network ultimately accounts for the accurate regulation of lipid and energy homeostasis. Despite the central physiological importance of these processes for human health, many basic mechanisms regulating the synthesis, uptake, storage, and utilization of lipids remain insufficiently characterized.

FAs are vital components of essentially all known organisms. They are important substrates for oxidation and the production of cellular energy. FAs are essential precursors for all lipid classes, including those forming biological membranes. Finally, they are important for protein function in acylated proteins and as ligands for nuclear receptor transcription factors. In contrast to these "beneficial" characteristics, unesterified FAs can become deleterious for cells when present even at relatively low concentrations. The chronic exposure of nonadipose cells and tissues to elevated concentrations of FAs triggers adverse effects subsumed under the term of "lipotoxicity" (1, 2). Accordingly, when supplied with excessive nutrients, essentially all eukaryotes reesterify and deposit FAs as triacylglycerol (TG) droplets to provide an energy reserve for times of nutrient deprivation and to detoxify otherwise harmful compounds.

Until recently, lipid droplets were viewed as an inert storage pool of TG. It is now known that essentially all cells in the body generate lipid droplets composed of neutral lipids (TG and cholesteryl esters), phospholipids, and unesterified cholesterol at varying, tissue-specific concentrations. Additionally, numerous proteins are associated with lipid droplets (3–5). These include structural proteins, lipid-modifying enzymes, and proteins that regulate enzyme activities. To date, the physiological role of many of these factors remains elusive. However, from the limited knowledge that is available, it is apparent that lipid droplets represent remarkably flexible, dynamic organelles that are used for the production of membrane components,

This research was supported by a grant from Genomics of Lipid-Associated Disorders (GOLD), part of the Austrian Genome Project, Genome Research in Austria (GEN-AU), funded by the Austrian Ministry of Science and Research and by the Austrian Science Foundation (FWF), Grants W901-B05DK (Doktoratskolleg Molecular Enzymolgy) and F30-B05 (SFB Lipotox).

Manuscript received 6 October 2008 and in revised form 23 October 2008.

Published, JLR Papers in Press, October 23, 2008.
DOI 10.1194/jlr.R800031-JLR200

[1] To whom correspondence should be addressed.
e-mail: rudolf.zechner@uni-graz.at

energy substrates, and signaling molecules, including lipotoxic compounds (6, 7). Although lipid droplets are observed in many cell types, the majority of fat in mammals is found in adipocytes of white adipose tissue (WAT). The central contribution of WAT to the regulation of energy homeostasis is due to both the enormous lipid storage capacity as well as its function as an endocrine organ secreting numerous hormones and adipo-cytokines (8). Prevalent metabolic diseases such as obesity and type 2 diabetes emerge when TG synthesis and catabolism lose synchrony.

The key process in fat catabolism and the provision of energy substrate during times of nutrient deprivation (fasting) or enhanced energy demand (e.g., exercise) is the hydrolytic cleavage of stored TG, the generation of FAs and glycerol, and their release from adipocytes. A complex, hormonally controlled regulatory network controls the initiation of this process, called lipolysis, and ultimately activates key intracellular lipases to hydrolyze TG. Currently, three enzymes are known to have an established function in the lipolytic breakdown of fat in adipose and nonadipose tissues: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL).

REGULATION OF LIPOLYSIS

Numerous lipolytic and antilipolytic effectors control the catabolism of stored fat in various tissues (9, 10). These include hormones, cytokines, and adipokines. In adipose tissue, the most potent stimulatory signals are catecholamines acting on β-adrenergic receptors (11). Mouse adipocytes express three subtypes of β-adrenergic receptors (β-ARs): β_1-AR, β_2-AR, and β_3-AR. In human adipose tissue, only β_1 and β_2 receptors induce lipolysis. When catecholamines bind to these receptors, stimulatory G_s proteins activate adenylate cyclase, causing a rise in cAMP levels and elevated activity of cAMP-dependent protein kinase-A (PKA) (10, 12, 13). PKA-mediated phosphorlylation of target proteins, including lipolytic enzymes and lipid droplet-associated proteins, induces an increased release of FAs and glycerol from adipose tissue up to 100-fold. Other hormones that stimulate PKA via G_s protein-coupled receptors include glucagon, parathyroid hormone, thyrotropin, α-melanocyte-stimulating hormone, and adrenocorticotropin. Several antilipolytic factors have been shown to act through inhibitory G_i protein-coupled receptors (10). These factors include catecholamines acting through α_2-adrenergic receptors (11), adenosine (A1-adenosine receptor) (14), prostaglandin (E2 receptor) (15), NPY (NPY-1 receptor) (16), and nicotinic acid (GPR109A receptor) (17). The relative distribution of α- and β-adrenergic receptors therefore determines the lipolytic activity in a tissue- and cell type-specific manner.

Insulin and insulin-like growth factor represent the most potent inhibitory hormones in lipolysis (9, 18). Their effects are primarily communicated through the insulin receptor (IR), polyphosphorylation of insulin receptor substrates 1–4 (IRS1–4), activation of phosphatidylinositol-3 kinase (PI3K), and the induction of the protein kinase B/AKT (PKB/AKT). Complexity in this essentially linear pathway is added by the divergence at so-called critical nodes that interact with other signaling cascades (19). Critical nodes in the IR pathway include the IR and IRS interacting with cytokine and extracellular signal-regulated kinase (ERK) signaling and PI3K activating both 3-phosphoinositide-dependent protein kinases (PDK1 and 2) as well as atypical protein kinases C (PKCλ and ζ). At this point, a signaling network is established that regulates innumerable biological processes (possibly more than 1,000). Lipolysis is affected in multiple steps, including the phosphorylation of phosphodiesterase 3B, causing the degradation of cAMP and loss of PKA activation (18).

The mechanisms through which other effectors regulate lipolysis are less well characterized. These include tumor necrosis factor-α (TNFα), growth hormone, the Cide domain-containing proteins (CideN) family of proteins (CIDEA, -B, and -C), and the CopI-ARF vesicle transport machinery described below.

HSL, THE "CLASSIC" ENZYME IN LIPOLYSIS

The first enzyme discovered to facilitate the hormone-induced catabolism of fat was HSL. Although the initial observations of fasting-induced lipolytic activity in WAT of dogs (20) and man (21) were reported as early as 1932 and 1950, respectively, it was not until the early 1960s that a WAT-associated lipase was shown to be regulated by hormones and found to be different from lipoprotein lipase (22–25). In a landmark study, Vaughan, Berger, and Steinberg (26) discovered two independent lipolytic activities in WAT of various mammals and designated these enzymes HSL and MGL. The purification of HSL, cloning of the corresponding cDNA and gene, and high-level heterologous expression of the protein permitted an extensive study of the biochemical properties of the enzyme, its tissue-specific function, and its regulation by various agonists and antagonists. Several comprehensive reviews have been published recently to summarize these results (13, 27–30).

HSL enzymology

HSL exhibits broad substrate specificity capable of hydolyzing TG, diacylglycerol (DG), monoacylglycerol (MG), cholesteryl esters (CEs), retinyl esters (REs), and other ester substrates such as *p*-nitrophenyl butyrate (31). The relative maximal hydrolysis rates are in the range of 1: 10: 1: 4: 2 for TG: DG: MG:CE: RE. Thus, TGs are actually the worst substrate for HSL among all these natural lipid esters, whereas DGs are the best. HSL slightly favors unsaturated medium-chain FAs over saturated long-chain FAs in TG substrates (32). However, the substrate specificity toward the length or saturation grade of acyl chains within lipid esters is not very pronounced. Within the TG molecule, HSL preferentially hydrolyzes primary ester bonds in the *sn*-1 and *sn*-3 positions (33). Phosphorylation of HSL in vitro modestly increases enzyme activity for TG and CE hydrolysis by

1.5- to 2-fold (34, 35). The activity for DG or MG hydrolysis is not affected.

HSL gene, mRNA, and protein structure

The gene for human HSL (*LIPE*) spans a genomic region of 26 kb and is located on chromosome 19q13.2 (36). In addition to 10 exons that are transcribed into HSL mRNA in all human and mouse tissues, alternative exon usage results in a significant variation in the 5′-region of HSL transcripts (37–40). In adipose tissue, adrenal gland, and ovary, HSL transcription starts from multiple exons (exons A, B, C, D, or exon 1) within a 13 kb region. Because exons B, C, and D are noncoding, the alternative exon usage does not change the amino acid composition of the enzyme. In contrast, exon A contains coding information for 43 additional amino acids, leading to an alternative enzyme isoform. In testis, two tissue-specific exons (T1 and T2) are used as transcriptional start sites. Exon T1 codes for an additional 300 amino acids, whereas T2 contains no coding sequences. The high variability in exon usage results in various HSL mRNA and protein sizes in adipose tissue, pancreatic β-cells, ovaries, and testis. Multiple potential transcription factor binding elements upstream of each transcriptional start site suggest the possibility of differential transcriptional regulation of HSL in different tissues and under various physiological conditions.

According to the HSL domain structure model [the three-dimensional (3D) structure of the enzyme remains to be elucidated], the enzyme can be subdivided into three functional regions (41–44). The N-terminal domain (amino acids 1–300) is believed to mediate enzyme dimerization (45) and interaction with FABP4, a fatty acid binding protein known to enhance HSL enzyme activity (46–48). The C-terminal domain contains the catalytic triad composed of serine 423, aspartate 703, and histidine 733 (numbering relates to rat HSL, isoform 2) within an α/β hydrolase fold typically found in many lipases and esterases. The third domain represents the regulatory module of the enzyme. This loop region (amino acids 521–669) contains all known phosphorylation sites of HSL.

HSL regulation of enzyme activity

Two major mechanisms determine HSL activity: enzyme phosphorylation by protein kinases and interaction with auxiliary proteins. The pathway of β-adrenergic stimulation involves the PKA-mediated phosphorylation of HSL. Originally it was believed that phosphorylation at two serine residues (563 and 565) (numbering relates to rat HSL, isoform 2) was sufficient to mediate the cAMP-dependent activation of HSL (49, 50). Serine 565 was considered the basal phosphorylation site and serine 563 the regulatory site (51–53). However, PKA-mediated enzyme activation in an HSL variant in which Ser 563 was replaced by alanine led to the discovery of additional PKA phosphorylation sites (54). The identification of these additional serines that are targets for phosphorylation by PKA (Ser 659 and Ser 660) (54), ERK (Ser 600) (55), glycogen synthase kinase-4 (Ser 563) (56), Ca^{2+}/calmodulin-dependent kinase II (Ser 565) (57), and AMP-activated kinase (Ser 565) (57) has markedly increased the complexity of posttranslational HSL modification and regulation. Enzymes involved in the dephosphorylation of HSL include protein phosphatases 1, 2A, and 2C (58).

HSL phosphorylation by PKA in response to β-adrenergic stimulation induces the intrinsic HSL enzyme activity only moderately (approximately 2-fold). This is in sharp contrast to findings in intact cells where β-adrenergic stimulation and activation of PKA cause up to a 100-fold induction of FA and glycerol release. Thus, in addition to HSL modification, other mechanisms must contribute to hormone-induced lipolysis. This finding led to the discovery of perilipin (3, 59–61). Perilipin is expressed mostly in WAT and steroidogenic tissues, where it localizes to the surface of lipid droplets (62). β-adrenergic stimulation of adipocytes causes the PKA-mediated polyphosphorylation of six defined serine residues within the protein (Ser 81, -222, -276, -433, -492, and -517), which results in the translocation of HSL to the lipid droplet and initiation of hydrolysis (63–66). Although originally HSL binding to the lipid droplet was seen in association with perilipin dissociation, the characterization of perilipin-deficient mice and functional studies with perilipin mutants redefined and extended this "replacement" hypothesis (67–69). During hormone stimulation, perilipin is essential for the recruitment of HSL to lipid droplets and full enzyme activation (65). Notably, perilipin phosphorylation is not required for the translocation of HSL, because unphosphorylated perilipin mutants still recruit HSL to the surface of lipid droplets (64). In contrast, perilipin phosphorylation is absolutely crucial for the hydrolytic activity of HSL. Perilipin is mostly associated with small lipid droplets within fat cells and, in fact, contributes to the fragmentation of large lipid droplets during the lipolytic process. The latter activity of perilipin involves its phosphorylation at serine residue 492 (70, 71).

Perilipin belongs to the PAT family of proteins (as reviewed in Refs. 3, 72). These factors include perilipin, adipophilin (ADRP), Tip47, S3-12, and myocyte lipid droplet protein (MLDP, also termed OXPAT). Because perilipin expression is mostly restricted to adipose and steroidogenic cells, it is of great interest to determine whether other PAT family members can accomplish a similar regulatory role for HSL in nonadipose tissues. In one report, HSL interaction with lipotransin was shown to activate HSL-mediated lipolysis (73). However, this mechanism has not been confirmed since its original observation.

HSL deficiency in mice: HSL is not alone

For more than three decades, HSL was considered to be the only and therefore rate-limiting enzyme for the lipolytic catabolism of stored fat in adipose and nonadipose tissues. Because HSL was shown to hydrolyze both TG and DG substrates, it was believed that the enzyme represented the only lipase activated by hormonal stimulation. This view, however, changed when several independent laboratories reported on the phenotype of HSL-deficient mice (74–76). Although HSL deficiency causes infertility

in male mice, owing to a defect in sperm maturation, the animals are normal with regard to their lipid and energy metabolism. Unexpectedly, HSL knockout (HSL-ko) mice were not overweight or obese. To the contrary, with increased age, they had reduced WAT weight (77) and were resistant to genetically or diet-induced obesity (78). HSL-deficient adipocytes responded to β-adrenergic stimulation and, compared with control mice, exhibited only a moderate decrease in their capacity to release FA (~40%) (32, 79). Importantly, HSL deficiency resulted in DG accumulation in several tissues, indicating that HSL is rate-limiting for DG hydrolysis (32). These findings strongly suggested that at least one additional enzyme acted as TG hydrolase when HSL was absent and that this activity was either directly or indirectly "hormone sensitive." The findings also indicated that HSL was more important as DG hydrolase than as TG hydrolase.

ATGL: A NEW PLAYER IN THE LIPOLYSIS TEAM

In 2004, three groups independently published the discovery of an enzyme able to hydrolyze TG and named it ATGL (77), desnutrin (80), or calcium-independent phospholipase A2ζ (iPLA2ζ) (81). Very soon after these initial reports, ATGL orthologous genes and proteins were identified and characterized in other vertebrates, flies, fungi, and plants (82–86). Work with the *Drosophila melanogaster* enzyme "brummer," triacylglycerol lipase-4 in *Saccharomyces cerevisiae*, and sugar-dependent1 in *Arabidopsis thaliana* demonstrated that each of these proteins exhibits robust TG hydrolase activity and has a fundamental role in the regulation of TG homeostasis in the respective organism.

ATGL enzymology

ATGL exhibits 10-fold higher substrate specificity for TG than for DG and selectively performs the first step in TG hydrolysis, resulting in the formation of DG and FA (77). The stereospecificity of ATGL for the chemically distinct ester bonds within the TG molecule is currently not known. Therefore, it is also unclear whether the DG generated by ATGL can participate in signaling processes involving *sn*-1,2-DG, such as the activation of various PKC isoenzymes. ATGL was also reported to have transacylase (81, 87) and phospholipase activity (77, 81, 88) that was shown to be lower than its TG hydrolase activity (77, 81). In contrast to HSL, ATGL does not hydrolyze MG, CE, or RE. Smirnova et al. (89) demonstrated that the hydrolytic function of ATGL is not restricted to the catabolism of lipid droplets ("adiposomes") in adipose tissue and suggested the enzyme be renamed *adiposome triglyceride lipase* rather than *adipose triglyceride lipase* to more adequately reflect its function.

ATGL gene, mRNA, and protein structure

The mouse Atgl gene (*Pnpla2*) contains nine exons and spans a region of approximately 6 kb on chromosome 7F5. Transcription of the gene results in a 1.96 kb mRNA coding for a 486 amino acid protein with a molecular mass of 54 kDa. The ten exons of the human ATGL gene (*PNPLA2*) span 6.32 kb of genomic DNA, which are located on chromosome 11p15.5. Mammalian ATGL belongs to a gene family characterized by the presence of a patatin domain (Pfam01734). This structural motif was designated for patatin, the most abundant protein in the potato tuber, with established DG, MG, and phospholipase activity, but no TG hydrolase activity (90–92). ATGL is most closely related to a group of five genes and proteins named *patatin-like phospholipase domain-containing 1 to 5* (PNPLA1-5) (93, 94). Members of this protein family in addition to ATGL (PNPLA2) are PNPLA1, adiponutrin (PNPLA3), GS2 (PNPLA4), and GS2-like (PNPLA5). To date, no orthologous gene for GS2 has been identified in the mouse genome. More distantly related members of ATGL include neuropathy target esterase (NTE, PNPLA6), NTE-related esterase (NRE, PNPLA7), calcium-independent phospholipase A2γ (iPLA2γ, PNPLA8), and phospholipase A2 group VI (PLA2G6, PNPLA9). Like ATGL, adiponutrin, GS2, and GS2-like also exhibit hydrolase and transacylase activity in in vitro assays (81, 87). Low specific phospholipase activity was reported for ATGL, adiponutrin, and GS2-like (81, 88). Considering the structural and functional diversity within patatin domain-containing proteins, the name *patatin-like phospholipase domain-containing 1-9* for these proteins is somewhat misleading and should be changed to a more general name such as *patatin domain-containing lipid hydrolase 1-9* (PDLH1-9).

The primary structures of the human and murine ATGL enzyme share 84% sequence identity. Sequence identity is particularly high within the patatin domain (>95%) harboring the active site of the enzyme. A schematic representation of the domain structure of ATGL is shown in **Fig. 1**. Interestingly, unlike other typical TG hydrolases, the active site of patatin domain-containing enzymes is not composed of a catalytic triad. Instead, 3D structure determination of related members of the family (potato patatin) revealed that the enzyme mechanism depends on a catalytic dyad (95). In ATGL, mutational analyses identified serine 47 as the active site nucleophile located within a canonical GXSXG sequence (87, 89, 96). From homology considerations, it is assumed that aspartate 166 is the second amino acid critical for the catalytic dyad in ATGL. Sequence and 3D-structural similarities also indicate that the dyad is embedded within a three-layer α/β/α architecture commonly found in hydrolases/esterases (97). Similarly, as has been shown for other patatin domain enzymes, the transition state in ATGL might be stabilized by a glycine-rich oxyanion hole. The C-terminal region of ATGL exhibits only poor homology to the other members of the PNPLA family. A hydrophobic stretch from amino acids 315 to 360 was proposed to mediate lipid droplet binding (77). Additionally, two phosphorylation sites were identified in the C-terminal region of the enzyme (serine 404 and serine 428 in human ATGL) (77, 98). The functional roles of enzyme phosphorylation and involved protein kinases remain unknown. Notably, the human protein is 19 amino acids longer than the mouse ortholog and contains a proline-rich sequence on its very C terminus. Whether

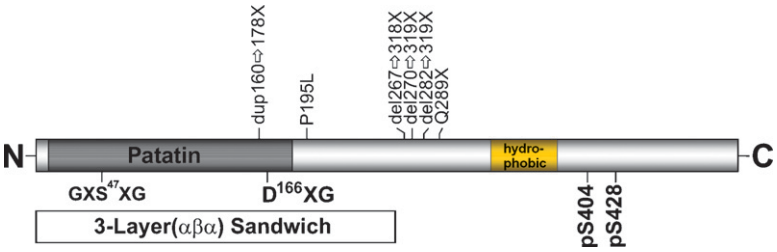

Fig. 1. Representation of the structural domains of human adipose triglyceride lipase (ATGL) protein. The crucial structural components for enzyme function are indicated, including the patatin domain, α/β hydrolase region, active site serine (S47), putative aspartic acid within the catalytic dyad (D166), potential lipid binding domain (hydrophobic), and two established phosphorylation sites (serine 404 and serine 428). Mutations associated with neutral lipid storage disease with myopathy are also indicated.

this peptide stretch contributes to species-specific differences in ATGL regulation and function requires clarification.

ATGL physiological function

The important role of ATGL in lipolysis became evident from observations in ATGL-deficient (ATGL-ko) mice (99). In contrast to HSL-deficient mice, ATGL-ko animals had a severe "lipid" phenotype (for phenotype comparison of HSL-ko and ATGL-ko mice, see **Table 1**). Absence of ATGL causes a reduction of FA release from WAT by more than 75%. ATGL-ko mice accumulated TG in essentially all organs and cell types analyzed, consistent with an important function of ATGL in TG catabolism in multiple tissues. Defective TG mobilization and massive TG accumulation cause severe myopathy in cardiac muscle, defective thermogenesis in brown adipose tissue (BAT), and an overall defect in energy homeostasis (see section on the tissue-specific function of ATGL below). The excessive TG accumulation in the heart causes cardiac dysfunction and premature death in ATGL-deficient mice. In WAT, TG hydrolase activity and hormone-stimulated lipolysis were also drastically reduced, which is consistent with the view that ATGL is "hormone sensitive" via either a direct or an indirect mechanism.

In contrast to HSL-ko animals, ATGL-deficient male mice are fertile, indicating that massive TG accumulation in testis per se is not causative for male infertility. Instead, it appears more likely that the impaired hydrolysis of DG, CE, and RE may cause a defect in germ cell maturation in HSL-deficient mice. The concentration of plasma FA, TG, and ketone bodies are decreased in both fasted HSL-ko and ATGL-ko mice, yet the absolute levels are lower in ATGL deficiency and are also decreased in fed ATGL-ko animals as compared with wild-type littermates. Interestingly, total cholesterol and HDL cholesterol concentrations are elevated in HSL-deficient mice and reduced in ATGL-deficient mice. The reason for this unexpected difference is unclear and requires elucidation.

TABLE 1. Phenotypes observed in ATGL-deficient and HSL-deficient mice

Parameter	ATGL-ko	HSL-ko
Life span	Reduced	Normal
Fertility	Normal	Infertile
Body weight	Increased	Normal
Fat mass	Increased	Reduced
Fat accumulation in nonadipose tissues	Severe	Reduced
Tissue DG accumulation	Normal	Severe
Thermogenesis	Defective	Normal
Plasma FA	Reduced	Reduced
Plasma TG	Reduced	Reduced
Plasma ketone bodies	Reduced	Reduced
Plasma cholesterol	Reduced	Increased
Plasma HDL cholesterol	Reduced	Increased
Glucose/insulin tolerance	Increased	Increased

ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; ko, knockout; DG, diacylglycerol; TG, triacylglycerol. Data assembled from (32, 74–76, 99).

Taken together, the analysis of ATGL-ko mice suggested that ATGL is rate-limiting for the first step in TG hydrolysis, generating DG and FA with an approximately 10-fold higher specificity toward TG than HSL (calculated). HSL efficiently degrades DG, generating MG and FA. The final step, resulting in the formation of glycerol and FA, is performed by MGL. Whether other TG hydrolases in addition to ATGL and HSL also contribute to the hydrolysis of TG in WAT was recently addressed by Schweiger et al. (100). Complete inhibition of HSL with a specific inhibitor (provided by Novo Nordisk) resulted in an almost complete absence of FA release in ATGL-deficient adipose tissue, suggesting that besides ATGL and HSL, additional lipases contribute little to the lipolytic capacity of white fat cells in mice. The role of alternative lipases, such as Ces3 (101) or TGH-2 (102), in WAT under specific physiological conditions or their contribution to lipolysis in nonadipose tissues remains to be determined.

Regulation of ATGL: hormones and cytokines

Although ATGL is expressed in most tissues of the body, the highest levels of mRNA and enzyme activity are found in WAT and BAT (77, 80, 81, 87, 103–108). During adipocyte differentiation of 3T3-L1 cells, ATGL expression is strongly induced, reaching maximal levels when the cells accumulate visible lipid droplets (77, 80, 81, 88, 93, 103, 109). Compared with WAT and BAT, ATGL mRNA levels are much lower in other tissues. Quantitative PCR analysis revealed that adrenals, testis, cardiac muscle, and skeletal muscle have approximately 25% of the ATGL mRNA levels (normalized to tissue protein content) found in WAT, other tissues around 10% (87, 109).

In contrast to a wealth of available information on the regulation of HSL in WAT, comparatively little is known about the molecular pathways leading to the activation of ATGL activity. The ability of HSL-ko WAT to respond to hormonal stimulation (32) and the finding that HSL inhibition in WAT leaves a "hormone-inducible" hydrolytic activity (100) suggest that ATGL activity is either directly or indirectly activated by hormonal signals. Several observations indicate that the molecular mechanism leading to ATGL activation is different from that described for HSL. First,

unlike HSL, ATGL is present on lipid droplets of adipocytes in similar amounts in the basal state and in the activated state (77). Second, although ATGL can be phosphorylated, it is not a target for PKA (77, 98). Third, ATGL activity is greatly enhanced by a protein annotated as α/β hydrolase domain-containing protein 5 (ABHD5) or comparative gene identification-58 (CGI-58) (96). CGI-58 does not affect HSL enzyme activity (96).

To date, most studies addressing the regulation of ATGL by hormonal or nutritional effectors have restricted their analyses to the measurement of ATGL mRNA levels and have not reported ATGL enzyme activities. Considering the likely posttranscriptional regulation of ATGL and HSL by phosphorylation and numerous modulating protein factors (see below), this is unfortunate, and conclusions drawn from these data must be viewed with caution when lipolytic activities are assumed from lipase mRNA concentrations. As expected for a TG hydrolase active in WAT, ATGL mRNA concentrations are markedly affected by nutritional status, and increase during fasting and decrease during refeeding (80, 87, 103, 109). ATGL mRNA levels during fasting are not paralleled by HSL mRNA levels that are downregulated during acute fasting and increase only after prolonged food deprivation (3–5 days) (110). From in vitro experiments in murine 3T3-L1 adipocytes, Villena et al. (80) concluded that glucocorticoids could be responsible for the increase of ATGL mRNA levels in the fasted state. The observation that ATGL mRNA is significantly downregulated in genetic models of obesity (*ob/ob* and *db/db* mice) suggested a possible contribution of ATGL in the pathogenesis of obesity (80, 103); however, this effect was not observed in all studies (87).

The enormous induction of FA and glycerol release from fat cells in response to β-adrenergic stimulation is not associated with increased levels of either ATGL or HSL mRNA. In fact, in some studies, isoproterenol treatment of 3T3-L1 cells or isolated adipocytes causes decreased mRNA concentrations of both lipases (111–113). This suggests that the β-adrenergic stimulation of lipolysis is exclusively regulated posttranscriptionally.

Insulin treatment reduces ATGL mRNA levels in murine 3T3-L1 adipocytes (103, 109, 111). Importantly, this inhibitory effect of insulin on ATGL expression was also demonstrated in vivo using mouse models of systemic insulin deficiency (streptozotocin-treated animals) and of adipose-specific insulin receptor deficiency (109). Both mouse models exhibited increased lipolysis and increased ATGL mRNA levels, indicating that the induction of ATGL gene expression might contribute to elevated FA mobilization under conditions of defective insulin signaling.

Cytokines, and specifically TNFα, have multiple effects on adipose tissue, and TNFα has been implicated in the pathogenesis of obesity and insulin resistance (114, 115). TNFα strongly stimulates lipolysis; as a consequence, massive lipid catabolism might contribute to the wasting seen in cancer cachexia. The finding that TNFα is able to stimulate lipolysis in HSL-ko adipocytes (79) suggested that the process was HSL-independent and that ATGL could be the actual TNFα target lipase. However, although lipolysis is increased, two studies have reported that both ATGL and HSL mRNA levels decrease in 3T3-L1 adipocytes in response to TNFα treatment (103, 111). This again suggests a dissociation of enzyme mRNA levels and enzyme activity. A possible explanation for low ATGL mRNA levels upon TNFα treatment relates to the fact that TNFα suppresses the expression of a large number of adipose-specific genes, leading to an "adipocyte dedifferentiation" process (116). One of these genes, peroxisome proliferator-activated receptor-γ (PPARγ), is a key nuclear receptor controlling adipocyte differentiation and metabolism (117). Kim et al. (103) demonstrated that ATGL is a direct transcriptional target gene for PPARγ, and PPARγ agonists such as rosiglitazone increase ATGL mRNA levels and induce lipolysis in various adipose models (103, 118–121). Therefore, it is conceivable that the TNFα-mediated inhibition of PPARγ reduces ATGL mRNA expression. How TNFα affects ATGL enzyme activity is currently unknown. In macrophages, ATGL has also been shown to be a target of PPARδ (122).

Contradicting views currently exist regarding the relative importance of ATGL in relation to HSL in human WAT. Langin (9) concluded from their studies in primary human adipocytes that HSL is the major lipase for catecholamine- and natriuretic peptide-stimulated lipolysis, whereas ATGL mediates TG hydrolysis mainly during basal lipolysis. Another study suggested that human HSL has a higher capacity to hydrolyze TG compared with ATGL (106). This study and a report by Ryden et al. (107) also found that, in contrast to HSL, ATGL mRNA and protein levels in adipose tissue are unaffected by obesity and weight reduction, arguing for a regulation of HSL, but not ATGL gene expression in response to obesity status. In contrast, other reports assign a crucial role to ATGL for TG hydrolysis in human WAT and show decreased ATGL mRNA and protein levels in obese individuals with insulin resistance (104, 108, 123). The availability of specimens from patients with ATGL deficiency might help to elucidate the functional role of ATGL in human WAT.

Regulation of ATGL: lipid droplet proteins

CGI-58. Mammalian TG hydrolases that act on water/lipid interphases frequently require cofactors for full enzyme function. For example, pancreatic lipase forms a complex with a colipase, and lipoprotein lipase (LPL) acts in concert with apolipoprotein C-II (apoC-II). ApoC-II is present on the surface of the major substrates for LPL, the TG-rich lipoproteins VLDL and chylomicrons. By analogy, it was not totally surprising when a lipid droplet protein, CGI-58 or ABHD5, was found to activate ATGL (96). In the presence of CGI-58, the TG hydrolase activity of mouse ATGL is induced approximately 20-fold. Human ATGL is also activated by CGI-58, although to a lesser degree (approximately 5-fold ATGL induction). Importantly, these findings provided a biochemical explanation for a human disorder. In 2001, Lefevre et al. (125) discovered that mutations in the gene for CGI-58 are causative for a lipid storage disorder designated "neutral lipid storage disease" or Chanarin Dorfman Syndrome (see below for discussion of human mutations). CGI-58 was originally

identified as a homologous gene in an alignment of the human and the *Caenorhabditis elegans* genomes. Mouse CGI-58 is ubiquitously expressed, with the highest expression levels found in testis and adipose tissue (96, 126).

CGI-58 is a 349 amino acid-long protein with a molecular mass of 40 kDa. As shown in **Fig. 2**, the protein belongs to the esterase/thioesterase/lipase subfamily of proteins structurally characterized by the presence of α/β hydrolase folds. In contrast to most other members of this family, the putative nucleophilic serine within the canonical esterase/lipase motif GXSXG is replaced by an asparagine in CGI-58 (125), effectively eliminating the possibility that CGI-58 functions as a lipase. The protein was shown to bind to lipid droplets by interaction with perilipin A in a hormone-dependent way (126–128). In nonstimulated adipocytes, CGI-58 is tightly associated with the lipid droplet, whereas upon β-adrenergic stimulation and concomitant phosphorylation of perilipin, CGI-58 dissociates and becomes cytosolic (126, 128). Reducing the cAMP levels of the cell reverses this dissociation process (126). Fluorescence resonance energy transfer and bimolecular fluorescence complementation experiments showed that CGI-58, once dissociated from perilipin, colocalizes in close proximity to ATGL (129), suggesting the involvement of CGI-58/ATGL interaction in stimulated lipolysis. CGI-58 is not involved in the vesicularization of lipid droplets during lipolysis (130). In summary, these findings support the following scenario: In the basal state, when adipocytes are not hormonally stimulated, CGI-58 binds to perilipin A and is unable to activate ATGL. Following hormonal stimulation, perilipin is phosphorylated at several serine residues, including serine 517, whereupon CGI-58 dissociates from perilipin, interacts with ATGL, and activates TG hydrolysis. Whether phosphorylation of serine 517 in perilipin or phosphorylation of ATGL affects the respective CGI-58 binding directly is currently not known. Concomitant with ATGL activation, HSL translocates from the cytosol to the lipid droplet and efficiently hydrolyzes DG, the lipolytic product of ATGL.

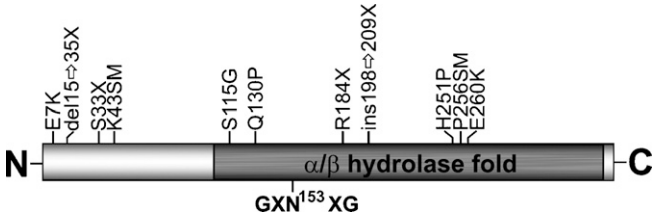

Fig. 2. Representation of the structural domains of human comparative gene identification-58 (CGI-58) α/β hydrolase domain-containing protein 5 (ABHD5) (CGI-58/ABHD5). The α/β hydrolase region and the asparagine residue replacing a serine within the consensus GXSXG of lipases and esterases are indicated. Additionally, mutations in CGI-58 associated with neutral lipid storage disease with ichthyosis are shown. Two mutations within splice acceptor consensus sequences cause protein truncations after exon 2 and exon 5. The final amino acids of the wild-type sequences are indicated (K43SM and P256SM).

Activation of ATGL might not be the only physiological activity of CGI-58. Importantly, a very recent publication showed that in addition to its function as ATGL activator, CGI-58 can also act as acylglycerolphosphate acyltransferase (AGPAT) (131). The role of this reaction in vivo remains to be determined (see below for discussion of human mutations).

PAT proteins. The crucial role of perilipin in the ATGL/CGI-58-mediated hydrolysis of TG became evident in an elegant study by Miyoshi et al. (63) showing that hormone-stimulated lipolysis depended on perilipin and ATGL. The authors demonstrated that perilipin phosphorylation of residue serine-517 is essential for ATGL-mediated lipolysis and represents a prerequisite for the function of subsequent lipase activity of HSL.

Perilipin expression is confined to adipose tissue and steroidogenic tissues. Lipolysis of lipid droplet-associated TG is, however, required in many other tissues, including those that do not express perilipin, such as skeletal and cardiac muscle, or the liver. Accordingly, alternative mechanisms must exist to control TG hydrolysis by ATGL and HSL (and possibly other lipases) in nonadipose tissues. These mechanisms are not well understood. Recently, two studies addressed the questions of whether and how nonperilipin PAT proteins affect lipolysis and ATGL. Listenberger et al. (132) demonstrated that ADRP controls ATGL access and TG lipolysis in HEK293 cells and other human cell lines. Bell et al. (133) studied the role of various PAT proteins in TG catabolism in hepatocyte-like AML12 cells and found that reduced expression of ADRP and TIP47 caused increased ATGL localization to lipid droplets and increased lipolytic rates. These findings are consistent with a crucial regulatory role for lipid droplet scaffold protein regulating the substrate access of functional ATGL.

Pigment epithelium-derived factor. In addition to PAT proteins, other proteins found on lipid droplets are also involved in the regulation of lipolysis. Surprisingly, searching for receptors and binding proteins for pigment epithelium-derived factor (PEDF), Notari et al. (88) identified ATGL as a PEDF binding protein and proposed to name the enzyme *PEDF-receptor*. Apparently, ATGL is highly expressed in the pigment epithelium and can be found on the plasma membrane, where it binds to PEDF and exhibits phospholipase activity. PEDF binding might also be important in cells and organs where ATGL is localized only within cells. For example, hepatocytes that lack PEDF were shown to accrue neutral lipid droplets, and lipid accumulation was reversed by the reexpression of PEDF (134). These results suggest that PEDF binds to ATGL on lipid droplets, inducing TG hydrolysis. The mechanism of this activation and the question of whether PEDF mediates the activation of ATGL also in other tissues remain to be determined.

CideN. Another group of lipid droplet binding proteins that regulate lipolysis belongs to the CideN family. CideN

proteins were originally discovered because of their structural similarity to DNA fragmentation factors and were believed to regulate cell death activation (135). Recently, members of the CideN family were shown to affect lipid droplet morphology and turnover. CideA and CideC/Fsp27 bind to lipid droplets and colocalize with perilipin (136, 137). Overexpression of these factors inhibits fat catabolism and induces cellular lipid accumulation (136). Consistent with these findings, mice that lack CideC/FSP27 have smaller, multilocular lipid droplets, decreased fat mass, lower levels of plasma FAs, and increased insulin sensitivity (138, 139). Similarly, CideA and CideB deficiency in mice is associated with a lean phenotype (140, 141). CideC/Fsp27 is also important for the regulation of TG catabolism in hepatocytes, because increased protein expression in *ob/ob* mice or in animals infected with CideC-expressing adenovirus causes hepatic lipid accumulation and steatosis (142). The mechanism by which members of the CideN family regulate the activity of lipases is currently unknown.

Arf1-CopI. In a genome-wide RNA interference screen in *Drosophila* S2 cells, Guo et al. (143) identified a large number of genes that affect lipid droplet biogenesis and morphology. Interestingly, the study identified a subset of the Arf1-CopI family of vesicular transport proteins that strongly affect lipid mobilization. Silencing of Arf79F or CopI resulted in smaller, more disperse lipid droplets and increased lipolysis, suggesting yet another currently unknown mechanism that regulates the activity of lipolytic enzymes.

Taken together, these results suggest that lipases are embedded in a complex "lipolysome" consisting of the actual lipolytic enzymes and numerous modulators of enzyme activity.

MUTATIONS IN ATGL OR CGI-58 CAUSE NEUTRAL LIPID STORAGE DISEASE IN HUMANS

Recently, mutations in the genes for ATGL and CGI-58 were identified and provided the molecular basis underlying neutral lipid storage disease (NLSD) in humans. NLSD is a rare, autosomal genetic disorder characterized by systemic accumulation of TG in all tissues of the body. It is diagnosed by increased TG storage in blood granulocytes (referred to as Jordans' anomaly) (144). Excessive lipid storage leads to variable forms of skeletal and cardiac myopathy and hepatic steatosis. Additionally, some patients suffer from ataxia, hearing loss, or mental retardation (145, 146). According to a recently proposed classification (147), NLSD can be subdivided into two distinct groups. Depending on whether or not the patients suffer from a skin defect (severe ichthyosis), they are diagnosed with either neutral lipid storage disease with ichthyosis (NLSDI, also known as Chanarin Dorfman Syndrome) or neutral lipid storage disease with myopathy (NLSDM), respectively. Importantly, this classification finds its molecular basis in the affected genes. Mutations in the gene for ATGL (*PNPLA2*) cause NLSDM, and mutations in the gene for CGI-58 cause NLSDI.

ATGL

In 2007, Fischer et al. (147) reported that mutations in the gene for ATGL cause NLSDM. Since then, several new mutations in the ATGL gene locus (*PNPLA2*) were discovered (148, 149). Currently, six mutations are known to cause aberrant ATGL proteins (indicated in Fig. 1). These include a point mutation (Pro195Leu), four frameshift mutations (at amino acids 160, 267, 270, or 283), and one nonsense mutation (Asn289×). Both frameshift and nonsense mutations result in the deletion of the C-terminal region of ATGL. Interestingly, the patatin domain with the active site serine 47/aspartate 166 dyad is present in most of the truncated ATGL variants. These ATGL mutants are enzymatically highly active and can be stimulated by CGI-58 when artificial lipid emulsions are used as substrates (147, 149, 150). However, ATGL lacking parts of the C-terminal half of the enzyme exhibited reduced binding to cellular lipid droplets, and it is assumed that defective substrate binding in a cellular context is responsible for the lipolytic defect. In one patient with severe myopathy, the mutation occurred within the patatin domain (frameshift mutation at amino acid 160); this mutation results in a truncated protein that lacks the active site aspartate 166 (148). Whether this mutation results in complete enzyme inactivation is not known. True null mutants that totally lack ATGL have not been found so far. More extensive biochemical characterization of ATGL deletion mutants retaining the patatin and α/β hydrolase domain but lacking the C-terminal region revealed a double function of the C terminus (150). First, it appears to mediate lipid droplet binding. Second, it has an inhibitory role in the hydrolytic reaction, because absence of the C-terminal region generates an enzyme with higher specific activity against artificial substrates. Whether the established phosphorylation sites present in this region (98) affect lipid binding or enzyme activity is presently unknown.

The clinical observations in patients with NLSDM resemble in many aspects the phenotypic characteristics of ATGL-ko mice. Both genetic deficiencies result in systemic lipid accumulation, Jordans' anomaly, and myopathy. Skin defects were not observed in either species. Two patients with NLSDM were reported to have died from cardiac failure (147, 149), but unfortunately it is not known whether their cardiac dysfunction resulted from excessive lipid accumulation as observed in mice. The identification and characterization of additional patients with *PNPLA2* gene mutations will help to elucidate the role of ATGL in human cardiac physiology.

CGI-58

Six years before mutations in ATGL were found to cause NLSDM, Lefèvre et al. (125) described eight mutations in the human gene for CGI-58 in families with a confirmed diagnosis of NLSDI. Subsequently, other groups reported additional mutations in the gene for CGI-58 causative for NLSDI (96, 151, 152). The locations of the known mutations in the CGI-58 sequence that cause NLSDI are included in Fig. 2. Patients share many clinical features with those affected with ATGL deficiency, but some strik-

ing differences are apparent. All patients with CGI-58 deficiency suffer from severe ichthyosis, and some of them have developmental defects, including deformation of the ear and mental retardation. These differences suggest that CGI-58 has additional functions that are independent of ATGL. Mutated CGI-58 with single amino acid substitutions (Q130P, E260K) or deletions totally fail to activate ATGL (96), suggesting that the defective ATGL stimulation by CGI-58 is causative for the multi-tissue TG accumulation observed in NLSDI. Interestingly, the same mutations in CGI-58 are unable to bind to perilipin and are not associated with lipid droplets (128). Alternative explanations for the molecular defect in NLSDI have also been proposed. Even before CGI-58 was discovered, it was shown that the molecular defect present in NLSDI prevents lipid remodeling from neutral lipids to glycerophospholipids (153, 154). Considering the recent finding that CGI-58 exhibits AGPAT activity (131) and might affect phospholipid synthesis, it seems conceivable that both defective TG hydrolysis and phospholipid synthesis contribute to the pathogenesis of NLSDI. However, several issues need clarification. First, the finding that mutations in CGI-58 causing NLSDI had no effect on its activity as acyltransferase raises the question of whether this activity is lacking in patients with NLSDI. Second, how does a defect in AGPAT activity of CGI-58 cause massive TG accumulation in light of the fact that the product of the AGPAT reaction, phosphatidic acid, is a common precursor for both TG and glycerophospholipids? Normally, AGPAT deficiency results in lipodystrophy and not excessive lipid accumulation (155).

Taken together, the clinical phenotype of patients affected with both forms of NLSD and the comparison to the phenotype of ATGL-ko mice suggest that excessive lipid accumulation results from decreased lipolysis due to defects in the enzyme (ATGL) or its activator (CGI-58). In the skin, however, CGI-58 has an additional, ATGL-independent function that is defective in NLSDI and responsible for the development of ichthyosis. Whether this function of CGI-58 involves the activation of another lipase, alterations in the metabolism of phospholipids, or a completely unrelated activity remains to be determined.

THE TISSUE-SPECIFIC ROLE OF ATGL/CGI-58

With the availability of genetically modified mice that lack ATGL (CGI-ko mice have not been reported to date) and the characterization of patients with NLSDM and NLSDI, a picture emerges of how ATGL affects lipid metabolism and energy homeostasis. As a general conclusion, it is evident that the physiological function of ATGL/CGI-58 is not restricted to adipose tissue but is also crucially important in many nonadipose tissues.

WAT

ATGL deficiency in mice is associated with a major defect in WAT lipolysis (99). Both TG hydrolase activity in WAT lysates and the release of FAs and glycerol from intact tissue samples are markedly increased. As a consequence, 8-week-old ATGL-deficient mice are obese, exhibiting double the fat mass of normal mice when kept on a normal chow diet. In contrast to mice, human patients with ATGL or CGI-58 deficiency are not overweight or obese. This has been used as argument that ATGL-mediated lipolysis in human WAT is less important than in mouse WAT. However, other explanations are also conceivable. First, defective lipolysis in WAT can result in a concomitant downregulation of lipogenesis. For example, the loss of WAT mass in HSL-deficient mice is caused by a drastic reduction of lipogenesis due to decreased PPARγ activity (156). Second, patients might change their eating habits as a consequence of their disease. Third, the absence of ATGL in human WAT might induce alternative lipase activities. The analysis of tissue samples from patients suffering from NLSDM or NLSDI will hopefully help to assess the role of ATGL in human WAT.

BAT

BAT serves as a TG storage organ with the unique ability to generate heat by "non-shivering" thermogenesis. In humans, brown adipocytes are abundant in neonates and diminish with age. In rodents and hibernating animals, BAT persists throughout life and is a major site for heat production in response to low environmental temperature. Similarly to the situation in WAT, β-adrenergic stimulation in BAT promotes the hydrolysis of stored TG by endogenous lipases, leading to the mobilization of FAs as fuel for thermogenesis. Defective norepinephrine and epinephrine synthesis (157) or deficiency of all three known β-adrenergic receptors (158) results in reduced lipolysis, increased BAT mass, and severe cold sensitivity due to defective thermogenesis. Remarkably, mice lacking HSL exhibited normal thermogenesis (75) and were not cold sensitive despite a lipolytic defect that resulted in brown adipocyte hypertrophy due to TG and DG accumulation. Apparently, in the absence of HSL, sufficient amounts of FAs are mobilized for mitochondrial heat production. In contrast, ATGL-ko mice are extremely cold sensitive and die after cold exposure of more than 6 h, indicating that the enzyme is essential for the provision of FA as substrate for thermogenesis (99).

Cardiac muscle

In the heart, continuous energy production is indispensable for the supply of ATP required for the permanent contractile function of the beating heart. It is estimated that 50–70% of the energy for myocardial contraction derives from the oxidation of FAs (159). Because cardiomyocytes do not synthesize FAs, they depend on their supply from two exogenous sources: first, WAT-derived, circulating unesterified FAs that are bound to plasma albumin; and second, TG-associated FAs released by LPL from TG-rich plasma lipoproteins. LPL is the only enzyme known to be responsible for the hydrolysis of plasma TG-rich lipoproteins in peripheral cells (160, 161). Experiments in transgenic and ko mouse models showed that the expression level of LPL in the heart largely determines the uptake rate of FA. Increased LPL activity in the heart result

in elevated FA utilization, increased lipid storage, decreased glucose utilization, and modest signs of cardiomyopathy (162–167). These studies and previous investigations showed that FAs, once absorbed by the heart, are not utilized directly for β-oxidation but, at least in part, are converted into TG (168). Subsequently, intracellular lipase(s) hydrolyze(s) these TG depots and provide(s) FA for oxidation. Accordingly, endogenous myocardial TGs provide a substantial amount of FAs for oxidation in perfused rat hearts, especially under conditions of overt diabetes (169). When hydrolyzed FAs are not utilized for oxidation, they are again esterified to TG. This creates a futile cycle of lipolysis and reesterification that reacts promptly to meet increased substrate demand when energy is needed.

Cardiomyocytes express HSL, and the hormone responsiveness of myocardial lipolysis suggested that HSL might be sufficient for TG hydrolysis in the heart (170, 171). However, HSL-deficient mice do not accumulate TG in the heart (32). In fact, overnight-fasted HSL-ko mice show markedly reduced myocardial TG levels and increased LPL activity (74), suggesting that both uptake of lipoprotein-associated FAs and intramyocardial TG mobilization are still functional when HSL is absent. ATGL-ko mice, in contrast, exhibit a prominent heart phenotype (99). As early as 6 weeks after birth, ATGL-deficient hearts accumulate lipids, as evident by increased number and size of lipid droplets; a process resulting in massive TG accumulation and yellowish discoloration of the heart. Lipid accumulation leads to an increased heart mass, decreased contractility, severe cardiac insufficiency, and premature death starting at about 12 weeks after birth. Remarkably, cardiac LPL activity is upregulated, indicating that despite massive TG accumulation, the FA uptake machinery is maximally induced. However, due to low VLDL levels in plasma during fasting, elevated LPL activities are probably not associated with increased FA uptake in cardiomyocytes. Decreased FA absorption from plasma is counterbalanced by increased uptake of glucose in ATGL-ko hearts. Thus, in the absence of ATGL, the release of FA from TG is blocked, leading to massive TG accumulation, decreased FA oxidation, and increased glucose utilization for energy production.

A recently discovered pathway to reduce excessive cardiac lipids involves the synthesis and secretion of apoB-containing lipoproteins (172). This mobilization of myocardial TG for lipoprotein secretion is thought to provide the heart with a "safety valve" for the disposal of excess lipids. It has been proposed that this process requires the hydrolysis of cytoplasmic TG stores and resynthesis of TG in the endoplamic reticulum. Whether this lipolytic step requires ATGL is presently unknown. Indirect evidence for such an involvement is provided by the fact that cardiac lipoprotein synthesis apparently cannot prevent the lethal lipid accumulation in hearts of ATGL-ko mice.

Whether defective ATGL function in humans with NLSDM or NLSDI also causes cardiac dysfunction is not clear from the few cases known so far. Cardiomyopathy was reported in patients with both conditions, NLSDM (147, 148) and NLSDI (173), although it appears to be much less severe or less frequent in the latter. Reportedly, two patients with ATGL deficiency died from cardiac failure, but it is not known whether excessive lipid accumulation caused the premature death.

Skeletal muscle

The release of FA from TG within skeletal myofibrillar lipid droplets requires lipases (174). The presence of HSL mRNA, protein, and enzyme activity has been documented in rodent (170, 175, 176) and in human skeletal muscle (177) by several laboratories. Compatible with its physiological function, HSL expression in skeletal muscle varies between fiber types, being higher in oxidative than in glycolytic fibers (175, 176). HSL in skeletal muscle is activated by PKA-mediated phosphorylation, a contraction-induced mechanism involving PKC, and the ERK pathway (175, 178, 179). An inhibitory effect of AMPK on HSL activity in resting and contracting muscle was reported by some (180–182) but not all studies (177). Despite the established role of HSL for the hydrolysis of stored TG in skeletal muscle, the absence of the enzyme in skeletal muscle of HSL-ko mice did not result in elevated muscular TG content or defects in muscle function (32, 75, 76). Instead, similarly to that observed in other tissues, HSL deficiency led to increased DG levels in skeletal muscle. Expression studies of genes involved in energy metabolism revealed that enzymes involved in carbohydrate metabolism are upregulated in HSL-ko skeletal muscles, whereas enzymes involved in FA biosynthesis are downregulated (183, 184).

In contrast to HSL-ko mice, ATGL-ko mice accumulated TG in skeletal muscle (99), supporting the concept that in addition to HSL, ATGL is also involved in the lipolytic cascade in myocytes. The absence of ATGL in skeletal muscle causes reduced lipolytic activity, neutral lipid droplet accumulation in oxidative muscle fibers, and elevated glucose uptake. Increased respiratory quotient (RQ) values during fasting in ATGL-ko compared with wild-type animals indicated increased glucose utilization in the absence of ATGL. Additional evidence for a functional role of ATGL was provided by Watt et al. (185), showing that overexpression of ATGL in skleletal muscle increases the oxidation of FA from TG stores and increases DG and ceramide production. Apparently, in this experimental setup, the endogenous HSL activity was not sufficient to hydrolyze excess DG. Accordingly, a dysequilibrium between the activities for ATGL and HSL might contribute to the production of lipotoxic intermediates and promote insulin resistance.

The crucial role of ATGL in skeletal muscle energy metabolism in humans is strikingly supported by recent findings in patients with NLSD. Individuals that lack ATGL (147–149, 151) or its activator, CGI-58 (144), accumulate TG in myocytes and develop muscle weakness and skeletal myopathy. Myopathy appears to be consistently more severe in patients with defective ATGL (NLSDM). The clinical phenotype of complete ATGL deficiency is not known because patients that completely lack ATGL have not been found so far. However, the severe myopathy in a patient with a frameshift mutation at amino acid 160 suggests that patients lacking the patatin domain are affected with a more severe form of the disease than those with an intact

patatin domain (147, 148). The observation of milder myopathy in CGI-58 deficient individuals (NLSDI) may be explained by the remnant ATGL activity in muscle that is still present when the ATGL activator is absent. The available information suggests that, similarly to tissues, HSL and ATGL work coordinately within the lipolytic pathway to provide FAs for oxidation and ATP production.

Other tissues

Despite the fact that ATGL expression in hepatocytes is relatively low compared with other tissues, both ATGL-ko mice and patients affected with NLSDM or NLSDI develop hepatosteatosis, suggesting that the lipase is functional in the liver. Increased ATGL expression in HepG2 cells causes increased lipid mobilization and a depletion of cellular TG stores (186). Similarly, overexpression of CGI-58 is associated with an increased lipolytic degradation of lipid droplets and increased secretion of TG-rich lipoproteins from cultivated hepatoma cells (187). Whether CGI-58 activates solely ATGL in hepatocytes or whether this process also affects lipoprotein biogenesis and secretion in vivo remains to be investigated. As mentioned above, hepatic ATGL is also regulated by PEDF (134). This interaction affects TG hydrolysis and might establish a new mechanism of ATGL regulation independent of CGI-58.

Other cell types and tissues with relatively high levels of ATGL/CGI-58 and HSL expression include macrophages, pancreatic β-cells, intestinal mucosa cells, pigment epithelial cells, and testis. Future studies are needed to elucidate the role of ATGL-mediated lipolysis and its integration with HSL and MGL activity in these tissues.

HSL AND ATGL IN CARBOHYDATE METABOLISM AND INSULIN ACTION

Elevated concentrations of plasma FAs and excess lipid deposition in insulin target tissues are major predisposing factors toward the development of insulin resistance and overt type 2 diabetes. This raises the possibility that enzymes participating in lipolysis influence carbohydrate metabolism and insulin sensitivity. Evidence for this concept was observed in mice lacking either HSL or ATGL.

Studies with HSL-ko mice revealed that HSL influences not only insulin sensitivity in peripheral tissues but also insulin secretion from pancreatic islet cells. However, reports have been controversial, probably due to the different genetic backgrounds of mice and nonuniformity in the experimental conditions. Using hyperinsulinemic euglycemic clamp studies, HSL-ko mice exhibited enhanced whole-body glucose uptake and protection from diet-induced insulin resistance in muscle, heart, and WAT on a high-fat diet (188). On a chow diet, HSL-ko mice showed increased hepatic insulin sensitivity (188, 189). These changes were associated with decreased tissue TG concentrations and fasting plasma FA levels (188, 189). Conversely, Mulder et al. (190) demonstrated decreased hepatic insulin action in HSL-ko mice. Insulin tolerance tests (ITTs) suggested whole-body insulin resistance in HSL-ko mice (190, 191). Consistently, ex vivo insulin-stimulated glucose transport into soleus muscle and lipogenesis in isolated adipocytes of HSL-ko mice were reduced (190). However, further examination indicated that the insulin sensitivity phenotype of HSL-ko mice depends on nutritional status and mouse gender (192). Importantly, pharmacological inhibition of HSL reduces hyperglycemia in streptozotozin-induced diabetic rats, suggesting improved insulin sensitivity (193). Moderately decreased glucose tolerance and the blunted response of plasma insulin levels upon glucose administration suggested impaired glucose-stimulated insulin secretion (GSIS) from pancreatic islets (191).

Extensive investigations with isolated islets of HSL-ko mice demonstrated that HSL plays a critical role in β-cell lipid metabolism, particularly neutral cholesteryl ester hydrolysis, and lipid signaling facilitating GSIS (191, 192). However, the effect of HSL deficiency on GSIS varied with gender, age, nutritional state (192), and genetic background of mice (194). Recently, a mechanism for HSL-facilitated GSIS from β-cells was suggested, wherein HSL serves an important role by providing free cholesterol for exocytosis of insulin granules (195). Besides its role in whole-body lipid metabolism and GSIS from pancreatic islets, HSL was also implicated in regulating adipokine mRNA and protein levels (78, 189). Moreover, HSL deficiency was associated with inflammation in and macrophage infiltration into WAT (196, 197), which is generally accepted as a causative factor in the development of insulin resistance in conjunction with obesity. This mechanism may provide another route of HSL affecting insulin sensitivity.

Because of the short time period since its discovery in 2004, much less information is currently available on the role of ATGL in carbohydrate metabolism. We previously demonstrated that ATGL-ko mice exhibit enhanced whole-body insulin sensitivity and glucose tolerance during ITTs and glucose tolerance tests (GTTs), respectively. This is remarkable, considering the massive lipid accumulation in multiple insulin target tissues, and implies an important role of ATGL in tissue insulin signaling and glucose metabolism (99). Whether changes in the concentration of other lipid species in ATGL-ko mice, such as DG, are involved is currently unknown. Furthermore, 2-deoxyglucose uptake under conditions similar to the GTT was significantly increased in skeletal muscle, heart, and liver. This observation is in accordance with enhanced glucose utilization in these tissues. The RQ was elevated in ATGL-ko mice during fasting, and ATGL-ko mice exhibited hypoglycemia upon short-term (4–6 h) fasting. Interestingly, plasma insulin concentrations were consistently decreased in fed ATGL-deficient mice. However, in vitro and in vivo studies employing overexpression and silencing of ATGL in rat skeletal muscle failed to demonstrate a connection between ATGL and muscle insulin sensitivity (185).

Data on parameters of carbohydrate metabolism are scarce in humans with ATGL deficiency. Interestingly, however, a Japanese patient exhibited decreased insulin levels despite normal insulin sensitivity (149). This data supports the concept that lipid accumulation in nonadipose tissue is not causative for insulin resistance and is consistent with a

study in humans showing an association between a nucleotide polymorphisms in the ATGL gene and increased concentrations of plasma FAs, TG, and glucose, as well as an increased risk for the development of type 2 diabetes (124). Accordingly, low ATGL activity may be associated with improved insulin sensitivity in mice and humans.

MGL, THE FINAL STEP IN TG HYDROLYSIS

Current data suggest that MGL is the rate-limiting enzyme in MG degradation (198). MGL was first purified in 1976 from rat adipose tissue (199). The enzyme specifically hydrolyzes MG and has no activity against DG or TG. Cloning of the mouse cDNA encoding MGL revealed that the enzyme is composed of 302 amino acids with a molecular mass of 33 kDa (200–202). Although not directly related to other lipases in the human genome, MGL belongs to the large family of α/β hydrolase fold proteins with a GXSXG motif. The amino acids building the active site consist of serine 122, aspartate 239, and histidine 269 (202). Mouse and human MGLs share 84% identity. The enzyme is ubiquitously expressed at relatively high levels and is found in the cytoplasm, the plasma membrane, and on lipid droplets. The high specific activity of the enzyme toward medium- and long-chain MG and its abundance in many tissues (highest in WAT) suggest that the final step in TG hydrolysis is not extensively regulated. However, definitive data on the physiological role of MGL are missing because mouse models that lack or overexpress MGL have not been reported to date. In addition to MGL, HSL (203), α/β hydrolase domain-containing protein 6 (ABHD6), ABHD12 (204), and fatty acid amide hydrolase (FAAH) (205, 206) were also identified as MG hydrolases in in vitro assays. The contribution of these enzymes to MG hydrolysis in vivo is not known.

Certain species of MG serve important signaling functions. The MG 2-arachidonoyl glycerol (2-AG) belongs to a family of compounds designated as endocannabinoids (ECs), which are endogenous agonists of cannabinoid (CB) receptors (207, 208). ECs are involved in the control of many biologic processes, including behavior, appetite regulation, pain, blood pressure, energy metabolism, inflammation, and cell growth. Their biological effect is mimicked by Δ^9-tetrahydrocannabinol, the major psychoactive component of marijuana. ECs are amides or esters of long-chain PUFAs and, besides 2-AG, anandamide [*N*-arachidonoylethanolamine (AEA)] has been identified as the main endogenous agonist of CB receptors (209). FAAH is believed to be responsible for the degradation of AEA, whereas MGL degrades 2-AG. Generally, MG, and specifically 2-AG, can be produced from three distinct lipolytic pathways. First, in the plasma membrane, glycerophospho-

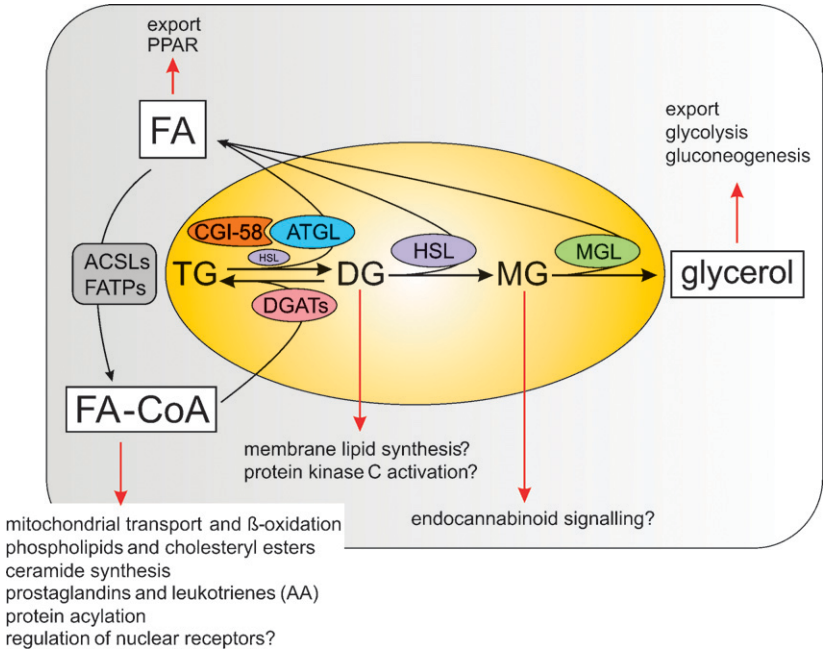

Fig. 3. Simplified summary of the lipolytic process and the involved metabolic intermediates. Several potential utilization pathways of these intermediates are indicated. In most tissues, lipid droplet-associated ATGL, together with its activator, CGI-58, hydrolyzes triacylglycerol (TG) to generate diacylglycerol (DG). The contribution of hormone-sensitive lipase (HSL) to TG hydrolysis may vary among tissues and species. Whether DGs can directly enter glycerophospholipid synthesis or operate as signaling molecules to activate various isoforms of protein kinase C requires clarification. HSL converts DG to monoacylglycerols (MGs). It is not known whether these MGs can contribute to signaling processes via the endocannabinoid system. The absence of acyl-CoA monoacylglycerol acyltransferase and the high activity of monoglyceride lipase (MGL) prevent reesterification of MG in most cell types and tissues (except intestinal mucosa cells). MGL hydrolyzes MG to glycerol and the last FA. Subsequent glycerol utilization in glycolytic or gluconeogenic pathways mostly occurs in the liver.

lipid degradation via phospholipase C results in DG formation, which is subsequently hydrolyzed by the *sn*-1-specific diacylglycerol lipase to yield 2-AG (210). Second, TG hydrolysis catalyzed by ATGL and the subsequent DG hydrolysis by HSL result in the production of MG within lipid droplets. Third, hydrolysis of plasma lipoprotein-associated TG by LPL results in the formation of FAs and MG, which are efficiently internalized by parenchymal cells (211). Whether all these sources contribute to the generation of bioactive MGs is currently not known, but it is generally assumed that MGL is the major enzyme for MG and 2-AG degradation, thereby inactivating CB receptor ligands (205, 206). MGL often colocalizes with CB1 receptors in the brain (212).

Importantly, the EC system plays a major role in the control of energy homeostasis and in the pathogenesis of metabolic diseases including obesity, metabolic syndrome, and type 2 diabetes. ECs affect energy balance via central orexigenic effects and by modulation of peripheral lipogenesis (213). CB receptor agonists are used as orexigenic agents in patients suffering from anorexia, whereas CB antagonists are used for the treatment of obesity-related disorders (214). The European Commission recently approved the CB1 receptor antagonist *Rimonabant* for diabetes treatment and as an anti-obesity drug (Trade name: Acomplia®). Inhibition of 2-AG-hydrolyzing enzymes, like MGL, is also considered a promising pharmacological approach to modulating MG levels (215, 216).

CONCLUSION AND PERSPECTIVES

Recent discoveries concerning new enzymes and coactivators have led to a revision of the lipolytic pathway, which catabolizes stored TG (summarized in **Fig. 3**). In conclusion, ATGL and HSL govern the capacity of adipose tissue and nonadipose tissues to mobilize FAs. ATGL is the major enzyme responsible for the first step in TG mobilization, generating DG and FA. HSL is rate-limiting for the subsequent degradation of DG. In the final hydrolytic step, MGL releases the third FA from the glycerol backbone. Each step provides a number of metabolic intermediates that act as precursors for a variety of metabolic pathways as well as bioactive molecules in cell signaling and gene regulation. In accord with ATGL's specific role in initiating the lipolytic cascade, the phenotype of ATGL-ko mice is more severe compared with HSL-ko mice.

However, important questions remain to be answered in order to understand the "lipolysome": Which other lipases besides the ones described so far contribute to the catabolism of stored fat in various tissues? Although ATGL, HSL, and MGL contribute more than 95% of the lipolytic capacity in WAT, it is conceivable that other lipases such as Ces 3 or TGH2 (101, 102) contribute to TG hydrolysis under certain physiological conditions in WAT and other tissues. How are the involved lipases, particularly ATGL, regulated in different tissues? The mechanisms of how established and newly identified lipid droplet proteins such as CGI-58, PAT proteins, CideN proteins, and the Arf-COPI vesicle transport system regulate the enzymatic activity of lipases require elucidation. How does lipolysis affect metabolic processes independently of FA production as energy substrates? Besides their role as energy substrates, the lipolytic products FA, DG, and MG fulfill other important physiological functions as precursors of various lipid classes, such as membrane lipids, prostaglandins and leukotrienes, and ceramides. Many of these lipids, as well as FA and DG per se, act as signaling molecules in signal transduction pathways and in the regulation of gene transcription (e.g., PPARs). An increased understanding of the role of lipases and lipolysis controlling these processes is of utmost importance. What is the role of ATGL and CGI-58 in the pathogenesis of metabolic disorders including inherited diseases like NLSD and acquired conditions like obesity, metabolic syndrome, and type 2 diabetes? The molecular basis of the phenotypical differences observed in ATGL deficiency versus CGI-58 deficiency is currently unknown and needs clarification. The characterization of CGI-58-deficient mice in comparison to ATGL-ko mice will help to solve this problem. Answers to these questions will facilitate the development of potential treatments for patients with NLSD. Additionally, better understanding of the "lipolysome" might lead to pharmacological treatment controlling the release of FA and other lipolytic products involved in the development of insulin resistance and type 2 diabetes.

The authors would like to thank Dr. Ellen Zechner and Mag. Caroline Schober for critically reviewing the manuscript and Mag. Renate Schreiber for her help in preparing the figures.

REFERENCES

1. Schaffer, J. E. 2003. Lipotoxicity: when tissues overeat. *Curr. Opin. Lipidol.* **14:** 281–287.
2. Unger, R. H. 2002. Lipotoxic diseases. *Annu. Rev. Med.* **53:** 319–336.
3. Brasaemle, D. L. 2007. Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. *J. Lipid Res.* **48:** 2547–2559.
4. Brasaemle, D. L., G. Dolios, L. Shapiro, and R. Wang. 2004. Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3–L1 adipocytes. *J. Biol. Chem.* **279:** 46835–46842.
5. Londos, C., D. L. Brasaemle, C. J. Schultz, J. P. Segrest, and A. R. Kimmel. 1999. Perilipins, ADRP, and other proteins that associate with intracellular neutral lipid droplets in animal cells. *Semin. Cell Dev. Biol.* **10:** 51–58.
6. Beckman, M. 2006. Cell biology. Great balls of fat. *Science.* **311:** 1232–1234.
7. Martin, S., and R. G. Parton. 2006. Lipid droplets: a unified view of a dynamic organelle. *Nat. Rev. Mol. Cell Biol.* **7:** 373–378.
8. Ahima, R. S., and M. A. Lazar. 2008. Adipokines and the peripheral and neural control of energy balance. *Mol. Endocrinol.* **22:** 1023–1031.
9. Langin, D. 2006. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacol. Res.* **53:** 482–491.
10. Holm, C., T. Osterlund, H. Laurell, and J. A. Contreras. 2000. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Annu. Rev. Nutr.* **20:** 365–393.
11. Lafontan, M., and M. Berlan. 1993. Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* **34:** 1057–1091.

12. Collins, S., W. Cao, and J. Robidoux. 2004. Learning new tricks from old dogs: beta-adrenergic receptors teach new lessons on firing up adipose tissue metabolism. *Mol. Endocrinol.* **18:** 2123–2131.
13. Holm, C. 2003. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Biochem. Soc. Trans.* **31:** 1120–1124.
14. Larrouy, D., J. Galitzky, and M. Lafontan. 1991. A1 adenosine receptors in the human fat cell: tissue distribution and regulation of radioligand binding. *Eur. J. Pharmacol.* **206:** 139–147.
15. Richelsen, B. 1992. Release and effects of prostaglandins in adipose tissue. *Prostaglandins Leukot. Essent. Fatty Acids.* **47:** 171–182.
16. Bradley, R. L., J. P. Mansfield, and E. Maratos-Flier. 2005. Neuropeptides, including neuropeptide Y and melanocortins, mediate lipolysis in murine adipocytes. *Obes. Res.* **13:** 653–661.
17. Offermanns, S. 2006. The nicotinic acid receptor GPR109A (HM74A or PUMA-G) as a new therapeutic target. *Trends Pharmacol. Sci.* **27:** 384–390.
18. Degerman, E., T. R. Landstrom, J. Wijkander, L. S. Holst, F. Ahmad, P. Belfrage, and V. Manganiello. 1998. Phosphorylation and activation of hormone-sensitive adipocyte phosphodiesterase type 3B. *Methods.* **14:** 43–53.
19. Taniguchi, C. M., B. Emanuelli, and C. R. Kahn. 2006. Critical nodes in signalling pathways: insights into insulin action. *Nat. Rev. Mol. Cell Biol.* **7:** 85–96.
20. Quagliarello, G., and G. Scoz. 1932. The existence of a lipase in adipose tissue. *Arch. Sci. Biol.* **17:** 513–529.
21. Renold, A. E., and A. Marble. 1950. Lipolytic activity of adipose tissue in man and rat. *J. Biol. Chem.* **185:** 367–375.
22. Bjorntorp, P., and R. H. Furman. 1962. Lipolytic activity in rat heart. *Am. J. Physiol.* **203:** 323–326.
23. Bjorntorp, P., and R. H. Furman. 1962. Lipolytic activity in rat epididymal fat pads. *Am. J. Physiol.* **203:** 316–322.
24. Hollenberg, C. H., M. S. Raben, and E. B. Astwood. 1961. The lipolytic response to corticotropin. *Endocrinology.* **68:** 589–598.
25. Rizack, M. A. 1964. Activation of an epinephrine-sensitive lipolytic activity from adipose tissue by adenosine 3′,5′-phosphate. *J. Biol. Chem.* **239:** 392–395.
26. Vaughan, M., J. E. Berger, and D. Steinberg. 1964. Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. *J. Biol. Chem.* **239:** 401–409.
27. Donsmark, M., J. Langfort, C. Holm, T. Ploug, and H. Galbo. 2005. Hormone-sensitive lipase as mediator of lipolysis in contracting skeletal muscle. *Exerc. Sport Sci. Rev.* **33:** 127–133.
28. Haemmerle, G., R. Zimmermann, and R. Zechner. 2003. Letting lipids go: hormone-sensitive lipase. *Curr. Opin. Lipidol.* **14:** 289–297.
29. Kraemer, F. B., and W. J. Shen. 2002. Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis. *J. Lipid Res.* **43:** 1585–1594.
30. Yeaman, S. J. 2004. Hormone-sensitive lipase—new roles for an old enzyme. *Biochem. J.* **379:** 11–22.
31. Yeaman, S. J. 1990. Hormone-sensitive lipase—a multipurpose enzyme in lipid metabolism. *Biochim. Biophys. Acta.* **1052:** 128–132.
32. Haemmerle, G., R. Zimmermann, M. Hayn, C. Theussl, G. Waeg, E. Wagner, W. Sattler, T. M. Magin, E. F. Wagner, and R. Zechner. 2002. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. *J. Biol. Chem.* **277:** 4806–4815.
33. Fredrikson, G., and P. Belfrage. 1983. Positional specificity of hormone-sensitive lipase from rat adipose tissue. *J. Biol. Chem.* **258:** 14253–14256.
34. Cook, K. G., S. J. Yeaman, P. Stralfors, G. Fredrikson, and P. Belfrage. 1982. Direct evidence that cholesterol ester hydrolase from adrenal cortex is the same enzyme as hormone-sensitive lipase from adipose tissue. *Eur. J. Biochem.* **125:** 245–249.
35. Fredrikson, G., P. Stralfors, N. O. Nilsson, and P. Belfrage. 1981. Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J. Biol. Chem.* **256:** 6311–6320.
36. Holm, C., T. G. Kirchgessner, K. L. Svenson, G. Fredrikson, S. Nilsson, C. G. Miller, J. E. Shively, C. Heinzmann, R. S. Sparkes, T. Mohandas, et al. 1988. Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-q13.3. *Science.* **241:** 1503–1506.
37. Blaise, R., J. Grober, P. Rouet, G. Tavernier, D. Daegelen, and D. Langin. 1999. Testis expression of hormone-sensitive lipase is conferred by a specific promoter that contains four regions binding testicular nuclear proteins. *J. Biol. Chem.* **274:** 9327–9334.
38. Blaise, R., T. Guillaudeux, G. Tavernier, D. Daegelen, B. Evrard, A. Mairal, C. Holm, B. Jegou, and D. Langin. 2001. Testis hormone-sensitive lipase expression in spermatids is governed by a short promoter in transgenic mice. *J. Biol. Chem.* **276:** 5109–5115.
39. Grober, J., H. Laurell, R. Blaise, B. Fabry, S. Schaak, C. Holm, and D. Langin. 1997. Characterization of the promoter of human adipocyte hormone-sensitive lipase. *Biochem. J.* **328:** 453–461.
40. Langin, D., H. Laurell, L. S. Holst, P. Belfrage, and C. Holm. 1993. Gene organization and primary structure of human hormone-sensitive lipase: possible significance of a sequence homology with a lipase of Moraxella TA144, an antarctic bacterium. *Proc. Natl. Acad. Sci. USA.* **90:** 4897–4901.
41. Holm, C., R. C. Davis, T. Osterlund, M. C. Schotz, and G. Fredrikson. 1994. Identification of the active site serine of hormone-sensitive lipase by site-directed mutagenesis. *FEBS Lett.* **344:** 234–238.
42. Osterlund, T., D. J. Beussman, K. Julenius, P. H. Poon, S. Linse, J. Shabanowitz, D. F. Hunt, M. C. Schotz, Z. S. Derewenda, and C. Holm. 1999. Domain identification of hormone-sensitive lipase by circular dichroism and fluorescence spectroscopy, limited proteolysis, and mass spectrometry. *J. Biol. Chem.* **274:** 15382–15388.
43. Osterlund, T., J. A. Contreras, and C. Holm. 1997. Identification of essential aspartic acid and histidine residues of hormone-sensitive lipase: apparent residues of the catalytic triad. *FEBS Lett.* **403:** 259–262.
44. Osterlund, T., B. Danielsson, E. Degerman, J. A. Contreras, G. Edgren, R. C. Davis, M. C. Schotz, and C. Holm. 1996. Domain-structure analysis of recombinant rat hormone-sensitive lipase. *Biochem. J.* **319:** 411–420.
45. Shen, W. J., S. Patel, R. Hong, and F. B. Kraemer. 2000. Hormone-sensitive lipase functions as an oligomer. *Biochemistry.* **39:** 2392–2398.
46. Shen, W. J., Y. Liang, R. Hong, S. Patel, V. Natu, K. Sridhar, A. Jenkins, D. A. Bernlohr, and F. B. Kraemer. 2001. Characterization of the functional interaction of adipocyte lipid-binding protein with hormone-sensitive lipase. *J. Biol. Chem.* **276:** 49443–49448.
47. Shen, W. J., K. Sridhar, D. A. Bernlohr, and F. B. Kraemer. 1999. Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein. *Proc. Natl. Acad. Sci. USA.* **96:** 5528–5532.
48. Smith, A. J., B. R. Thompson, M. A. Sanders, and D. A. Bernlohr. 2007. Interaction of the adipocyte fatty acid-binding protein with the hormone-sensitive lipase: regulation by fatty acids and phosphorylation. *J. Biol. Chem.* **282:** 32424–32432.
49. Stralfors, P., and P. Belfrage. 1983. Phosphorylation of hormone-sensitive lipase by cyclic AMP-dependent protein kinase. *J. Biol. Chem.* **258:** 15146–15152.
50. Stralfors, P., P. Bjorgell, and P. Belfrage. 1984. Hormonal regulation of hormone-sensitive lipase in intact adipocytes: identification of phosphorylated sites and effects on the phosphorylation by lipolytic hormones and insulin. *Proc. Natl. Acad. Sci. USA.* **81:** 3317–3321.
51. Garton, A. J., D. G. Campbell, P. Cohen, and S. J. Yeaman. 1988. Primary structure of the site on bovine hormone-sensitive lipase phosphorylated by cyclic AMP-dependent protein kinase. *FEBS Lett.* **229:** 68–72.
52. Garton, A. J., and S. J. Yeaman. 1990. Identification and role of the basal phosphorylation site on hormone-sensitive lipase. *Eur. J. Biochem.* **191:** 245–250.
53. Olsson, H., and P. Belfrage. 1987. The regulatory and basal phosphorylation sites of hormone-sensitive lipase are dephosphorylated by protein phosphatase-1, 2A and 2C but not by protein phosphatase-2B. *Eur. J. Biochem.* **168:** 399–405.
54. Anthonsen, M. W., L. Ronnstrand, C. Wernstedt, E. Degerman, and C. Holm. 1998. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. *J. Biol. Chem.* **273:** 215–221.
55. Greenberg, A. S., W. J. Shen, K. Muliro, S. Patel, S. C. Souza, R. A. Roth, and F. B. Kraemer. 2001. Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J. Biol. Chem.* **276:** 45456–45461.
56. Olsson, H., P. Stralfors, and P. Belfrage. 1986. Phosphorylation of the basal site of hormone-sensitive lipase by glycogen synthase kinase-4. *FEBS Lett.* **209:** 175–180.
57. Garton, A. J., D. G. Campbell, D. Carling, D. G. Hardie, R. J. Colbran, and S. J. Yeaman. 1989. Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. *Eur. J. Biochem.* **179:** 249–254.
58. Olsson, H., and P. Belfrage. 1988. Phosphorylation and dephosphorylation of hormone-sensitive lipase. Interactions between

the regulatory and basal phosphorylation sites. *FEBS Lett.* **232:** 78–82.
59. Granneman, J. G., and H. P. Moore. 2008. Location, location: protein trafficking and lipolysis in adipocytes. *Trends Endocrinol. Metab.* **19:** 3–9.
60. Londos, C., C. Sztalryd, J. T. Tansey, and A. R. Kimmel. 2005. Role of PAT proteins in lipid metabolism. *Biochimie.* **87:** 45–49.
61. Tansey, J. T., C. Sztalryd, E. M. Hlavin, A. R. Kimmel, and C. Londos. 2004. The central role of perilipin a in lipid metabolism and adipocyte lipolysis. *IUBMB Life.* **56:** 379–385.
62. Greenberg, A. S., J. J. Egan, S. A. Wek, N. B. Garty, E. J. Blanchette-Mackie, and C. Londos. 1991. Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *J. Biol. Chem.* **266:** 11341–11346.
63. Miyoshi, H., J. W. Perfield II, S. C. Souza, W. J. Shen, H. H. Zhang, Z. S. Stancheva, F. B. Kraemer, M. S. Obin, and A. S. Greenberg. 2007. Control of adipose triglyceride lipase action by serine 517 of perilipin A globally regulates protein kinase A-stimulated lipolysis in adipocytes. *J. Biol. Chem.* **282:** 996–1002.
64. Miyoshi, H., S. C. Souza, H. H. Zhang, K. J. Strissel, M. A. Christoffolete, J. Kovsan, A. Rudich, F. B. Kraemer, A. C. Bianco, M. S. Obin, et al. 2006. Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and -independent mechanisms. *J. Biol. Chem.* **281:** 15837–15844.
65. Sztalryd, C., G. Xu, H. Dorward, J. T. Tansey, J. A. Contreras, A. R. Kimmel, and C. Londos. 2003. Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. *J. Cell Biol.* **161:** 1093–1103.
66. Tansey, J. T., A. M. Huml, R. Vogt, K. E. Davis, J. M. Jones, K. A. Fraser, D. L. Brasaemle, A. R. Kimmel, and C. Londos. 2003. Functional studies on native and mutated forms of perilipins. A role in protein kinase A-mediated lipolysis of triacylglycerols. *J. Biol. Chem.* **278:** 8401–8406.
67. Clifford, G. M., C. Londos, F. B. Kraemer, R. G. Vernon, and S. J. Yeaman. 2000. Translocation of hormone-sensitive lipase and perilipin upon lipolytic stimulation of rat adipocytes. *J. Biol. Chem.* **275:** 5011–5015.
68. Martinez-Botas, J., J. B. Anderson, D. Tessier, A. Lapillonne, B. H. Chang, M. J. Quast, D. Gorenstein, K. H. Chen, and L. Chan. 2000. Absence of perilipin results in leanness and reverses obesity in Lepr(db/db) mice. *Nat. Genet.* **26:** 474–479.
69. Tansey, J. T., C. Sztalryd, J. Gruia-Gray, D. L. Roush, J. V. Zee, O. Gavrilova, M. L. Reitman, C. X. Deng, C. Li, A. R. Kimmel, et al. 2001. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc. Natl. Acad. Sci. USA.* **98:** 6494–6499.
70. Marcinkiewicz, A., D. Gauthier, A. Garcia, and D. L. Brasaemle. 2006. The phosphorylation of serine 492 of perilipin a directs lipid droplet fragmentation and dispersion. *J. Biol. Chem.* **281:** 11901–11909.
71. Moore, H. P., R. B. Silver, E. P. Mottillo, D. A. Bernlohr, and J. G. Granneman. 2005. Perilipin targets a novel pool of lipid droplets for lipolytic attack by hormone-sensitive lipase. *J. Biol. Chem.* **280:** 43109–43120.
72. Ducharme, N. A., and P. E. Bickel. 2008. Lipid droplets in lipogenesis and lipolysis. *Endocrinology.* **149:** 942–949.
73. Syu, L. J., and A. R. Saltiel. 1999. Lipotransin: a novel docking protein for hormone-sensitive lipase. *Mol. Cell.* **4:** 109–115.
74. Haemmerle, G., R. Zimmermann, J. G. Strauss, D. Kratky, M. Riederer, G. Knipping, and R. Zechner. 2002. Hormone-sensitive lipase deficiency in mice changes the plasma lipid profile by affecting the tissue-specific expression pattern of lipoprotein lipase in adipose tissue and muscle. *J. Biol. Chem.* **277:** 12946–12952.
75. Osuga, J., S. Ishibashi, T. Oka, H. Yagyu, R. Tozawa, A. Fujimoto, F. Shionoiri, N. Yahagi, F. B. Kraemer, O. Tsutsumi, et al. 2000. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. *Proc. Natl. Acad. Sci. USA.* **97:** 787–792.
76. Wang, S. P., N. Laurin, J. Himms-Hagen, M. A. Rudnicki, E. Levy, M. F. Robert, L. Pan, L. Oligny, and G. A. Mitchell. 2001. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. *Obes. Res.* **9:** 119–128.
77. Zimmermann, R., J. G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger, M. Riederer, A. Lass, G. Neuberger, F. Eisenhaber, A. Hermetter, et al. 2004. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science.* **306:** 1383–1386.
78. Harada, K., W. J. Shen, S. Patel, V. Natu, J. Wang, J. Osuga, S. Ishibashi, and F. B. Kraemer. 2003. Resistance to high-fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice. *Am. J. Physiol. Endocrinol. Metab.* **285:** E1182–E1195.
79. Okazaki, H., J. Osuga, Y. Tamura, N. Yahagi, S. Tomita, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, S. Kimura, et al. 2002. Lipolysis in the absence of hormone-sensitive lipase: evidence for a common mechanism regulating distinct lipases. *Diabetes.* **51:** 3368–3375.
80. Villena, J. A., S. Roy, E. Sarkadi-Nagy, K. H. Kim, and H. S. Sul. 2004. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. *J. Biol. Chem.* **279:** 47066–47075.
81. Jenkins, C. M., D. J. Mancuso, W. Yan, H. F. Sims, B. Gibson, and R. W. Gross. 2004. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J. Biol. Chem.* **279:** 48968–48975.
82. Eastmond, P. J. 2006. SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. *Plant Cell.* **18:** 665–675.
83. Gronke, S., A. Mildner, S. Fellert, N. Tennagels, S. Petry, G. Muller, H. Jackle, and R. P. Kuhnlein. 2005. Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. *Cell Metab.* **1:** 323–330.
84. Kurat, C. F., K. Natter, J. Petschnigg, H. Wolinski, K. Scheuringer, H. Scholz, R. Zimmermann, R. Leber, R. Zechner, and S. D. Kohlwein. 2006. Obese yeast: triglyceride lipolysis is functionally conserved from mammals to yeast. *J. Biol. Chem.* **281:** 491–500.
85. Saarela, J., G. Jung, M. Hermann, J. Nimpf, and W. J. Schneider. 2008. The patatin-like lipase family in Gallus gallus. *BMC Genomics.* **9:** 281.
86. Shan, T., Y. Wang, T. Wu, J. Guo, J. Liu, J. Feng, and Z. Xu. 2008. Porcine adipose triglyceride lipase complementary deoxyribonucleic acid clone, expression pattern, and regulation by resveratrol. *J. Anim. Sci.* **86:** 1781–1788.
87. Lake, A. C., Y. Sun, J. L. Li, J. E. Kim, J. W. Johnson, D. Li, T. Revett, H. H. Shih, W. Liu, J. E. Paulsen, et al. 2005. Expression, regulation, and triglyceride hydrolase activity of Adiponutrin family members. *J. Lipid Res.* **46:** 2477–2487.
88. Notari, L., V. Baladron, J. D. Aroca-Aguilar, N. Balko, R. Heredia, C. Meyer, P. M. Notario, S. Saravanamuthu, M. L. Nueda, F. Sanchez-Sanchez, et al. 2006. Identification of a lipase-linked cell membrane receptor for pigment epithelium-derived factor. *J. Biol. Chem.* **281:** 38022–38037.
89. Smirnova, E., E. B. Goldberg, K. S. Makarova, L. Lin, W. J. Brown, and C. L. Jackson. 2006. ATGL has a key role in lipid droplet/adiposome degradation in mammalian cells. *EMBO Rep.* **7:** 106–113.
90. Andrews, D. L., B. Beames, M. D. Summers, and W. D. Park. 1988. Characterization of the lipid acyl hydrolase activity of the major potato (Solanum tuberosum) tuber protein, patatin, by cloning and abundant expression in a baculovirus vector. *Biochem. J.* **252:** 199–206.
91. Jimenez-Atienzar, M., J. Cabanes, F. Gandia-Herrero, J. Escribano, F. Garcia-Carmona, and M. Perez-Gilabert. 2003. Determination of the phospholipase activity of patatin by a continuous spectrophotometric assay. *Lipids.* **38:** 677–682.
92. Senda, K., H. Yoshioka, N. Doke, and K. Kawakita. 1996. A cytosolic phospholipase A2 from potato tissues appears to be patatin. *Plant Cell Physiol.* **37:** 347–353.
93. Wilson, P. A., S. D. Gardner, N. M. Lambie, S. A. Commans, and D. J. Crowther. 2006. Characterization of the human patatin-like phospholipase family. *J. Lipid Res.* **47:** 1940–1949.
94. Zechner, R., J. G. Strauss, G. Haemmerle, A. Lass, and R. Zimmermann. 2005. Lipolysis: pathway under construction. *Curr. Opin. Lipidol.* **16:** 333–340.
95. Rydel, T. J., J. M. Williams, E. Krieger, F. Moshiri, W. C. Stallings, S. M. Brown, J. C. Pershing, J. P. Purcell, and M. F. Alibhai. 2003. The crystal structure, mutagenesis, and activity studies reveal that patatin is a lipid acyl hydrolase with a Ser-Asp catalytic dyad. *Biochemistry.* **42:** 6696–6708.
96. Lass, A., R. Zimmermann, G. Haemmerle, M. Riederer, G. Schoiswohl, M. Schweiger, P. Kienesberger, J. G. Strauss, G. Gorkiewicz, and R. Zechner. 2006. Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. *Cell Metab.* **3:** 309–319.

97. Schneider, G., G. Neuberger, M. Wildpaner, S. Tian, I. Berezovsky, and F. Eisenhaber. 2006. Application of a sensitive collection heuristic for very large protein families: evolutionary relationship between adipose triglyceride lipase (ATGL) and classic mammalian lipases. *BMC Bioinformatics.* **7:** 164.
98. Bartz, R., J. K. Zehmer, M. Zhu, Y. Chen, G. Serrero, Y. Zhao, and P. Liu. 2007. Dynamic activity of lipid droplets: protein phosphorylation and GTP-mediated protein translocation. *J. Proteome Res.* **6:** 3256–3265.
99. Haemmerle, G., A. Lass, R. Zimmermann, G. Gorkiewicz, C. Meyer, J. Rozman, G. Heldmaier, R. Maier, C. Theussl, S. Eder, et al. 2006. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science.* **312:** 734–737.
100. Schweiger, M., R. Schreiber, G. Haemmerle, A. Lass, C. Fledelius, P. Jacobsen, H. Tornqvist, R. Zechner, and R. Zimmermann. 2006. Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. *J. Biol. Chem.* **281:** 40236–40241.
101. Soni, K. G., R. Lehner, P. Metalnikov, P. O'Donnell, M. Semache, W. Gao, K. Ashman, A. V. Pshezhetsky, and G. A. Mitchell. 2004. Carboxylesterase 3 (EC 3.1.1.1) is a major adipocyte lipase. *J. Biol. Chem.* **279:** 40683–40689.
102. Okazaki, H., M. Igarashi, M. Nishi, M. Tajima, M. Sekiya, S. Okazaki, N. Yahagi, K. Ohashi, K. Tsukamoto, M. Amemiya-Kudo, et al. 2006. Identification of a novel member of the carboxylesterase family that hydrolyzes triacylglycerol: a potential role in adipocyte lipolysis. *Diabetes.* **55:** 2091–2097.
103. Kim, J. Y., K. Tillison, J. H. Lee, D. A. Rearick, and C. M. Smas. 2006. The adipose tissue triglyceride lipase ATGL/PNPLA2 is downregulated by insulin and TNF-alpha in 3T3-L1 adipocytes and is a target for transactivation by PPARgamma. *Am. J. Physiol. Endocrinol. Metab.* **291:** E115–E127.
104. Jocken, J. W., D. Langin, E. Smit, W. H. Saris, C. Valle, G. B. Hul, C. Holm, P. Arner, and E. E. Blaak. 2007. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. *J. Clin. Endocrinol. Metab.* **92:** 2292–2299.
105. Langin, D., A. Dicker, G. Tavernier, J. Hoffstedt, A. Mairal, M. Ryden, E. Arner, A. Sicard, C. M. Jenkins, N. Viguerie, et al. 2005. Adipocyte lipases and defect of lipolysis in human obesity. *Diabetes.* **54:** 3190–3197.
106. Mairal, A., D. Langin, P. Arner, and J. Hoffstedt. 2006. Human adipose triglyceride lipase (PNPLA2) is not regulated by obesity and exhibits low in vitro triglyceride hydrolase activity. *Diabetologia.* **49:** 1629–1636.
107. Ryden, M., J. Jocken, V. van Harmelen, A. Dicker, J. Hoffstedt, M. Wiren, L. Blomqvist, A. Mairal, D. Langin, E. Blaak, et al. 2007. Comparative studies of the role of hormone-sensitive lipase and adipose triglyceride lipase in human fat cell lipolysis. *Am. J. Physiol. Endocrinol. Metab.* **292:** E1847–E1855.
108. Steinberg, G. R., B. E. Kemp, and M. J. Watt. 2007. Adipocyte triglyceride lipase expression in human obesity. *Am. J. Physiol. Endocrinol. Metab.* **293:** E958–E964.
109. Kershaw, E. E., J. K. Hamm, L. A. Verhagen, O. Peroni, M. Katic, and J. S. Flier. 2006. Adipose triglyceride lipase: function, regulation by insulin, and comparison with adiponutrin. *Diabetes.* **55:** 148–157.
110. Sztalryd, C., and F. B. Kraemer. 1994. Regulation of hormone-sensitive lipase during fasting. *Am. J. Physiol.* **266:** E179–E185.
111. Kralisch, S., J. Klein, U. Lossner, M. Bluher, R. Paschke, M. Stumvoll, and M. Fasshauer. 2005. Isoproterenol, TNFalpha, and insulin downregulate adipose triglyceride lipase in 3T3-L1 adipocytes. *Mol. Cell. Endocrinol.* **240:** 43–49.
112. Slavin, B. G., J. M. Ong, and P. A. Kern. 1994. Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. *J. Lipid Res.* **35:** 1535–1541.
113. Plee-Gautier, E., J. Grober, E. Duplus, D. Langin, and C. Forest. 1996. Inhibition of hormone-sensitive lipase gene expression by cAMP and phorbol esters in 3T3-F442A and BFC-1 adipocytes. *Biochem. J.* **318:** 1057–1063.
114. Hotamisligil, G. S. 2006. Inflammation and metabolic disorders. *Nature.* **444:** 860–867.
115. Wellen, K. E., and G. S. Hotamisligil. 2005. Inflammation, stress, and diabetes. *J. Clin. Invest.* **115:** 1111–1119.
116. Ruan, H., and H. F. Lodish. 2003. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. *Cytokine Growth Factor Rev.* **14:** 447–455.
117. Zhang, B., J. Berger, E. Hu, D. Szalkowski, S. White-Carrington, B. M. Spiegelman, and D. E. Moller. 1996. Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha. *Mol. Endocrinol.* **10:** 1457–1466.
118. Festuccia, W. T., M. Laplante, M. Berthiaume, Y. Gelinas, and Y. Deshaies. 2006. PPARgamma agonism increases rat adipose tissue lipolysis, expression of glyceride lipases, and the response of lipolysis to hormonal control. *Diabetologia.* **49:** 2427–2436.
119. Kershaw, E. E., M. Schupp, H. P. Guan, N. P. Gardner, M. A. Lazar, and J. S. Flier. 2007. PPARgamma regulates adipose triglyceride lipase in adipocytes in vitro and in vivo. *Am. J. Physiol. Endocrinol. Metab.* **293:** E1736–E1745.
120. Kim, J. Y., Y. Wu, and C. M. Smas. 2007. Characterization of ScAP-23, a new cell line from murine subcutaneous adipose tissue, identifies genes for the molecular definition of preadipocytes. *Physiol. Genomics.* **31:** 328–342.
121. Shen, W. J., S. Patel, Z. Yu, D. Jue, and F. B. Kraemer. 2007. Effects of rosiglitazone and high fat diet on lipase/esterase expression in adipose tissue. *Biochim. Biophys. Acta.* **1771:** 177–184.
122. Lee, C. H., K. Kang, I. R. Mehl, R. Nofsinger, W. A. Alaynick, L. W. Chong, J. M. Rosenfeld, and R. M. Evans. 2006. Peroxisome proliferator-activated receptor delta promotes very low-density lipoprotein-derived fatty acid catabolism in the macrophage. *Proc. Natl. Acad. Sci. USA.* **103:** 2434–2439.
123. Berndt, J., S. Kralisch, N. Kloting, K. Ruschke, M. Kern, M. Fasshauer, M. R. Schon, M. Stumvoll, and M. Bluher. 2008. Adipose triglyceride lipase gene expression in human visceral obesity. *Exp. Clin. Endocrinol. Diabetes.* **116:** 203–210.
124. Schoenborn, V., I. M. Heid, C. Vollmert, A. Lingenhel, T. D. Adams, P. N. Hopkins, T. Illig, R. Zimmermann, R. Zechner, S. C. Hunt, et al. 2006. The ATGL gene is associated with free fatty acids, triglycerides, and type 2 diabetes. *Diabetes.* **55:** 1270–1275.
125. Lefevre, C., F. Jobard, F. Caux, B. Bouadjar, A. Karaduman, R. Heilig, H. Lakhdar, A. Wollenberg, J. L. Verret, J. Weissenbach, et al. 2001. Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin-Dorfman syndrome. *Am. J. Hum. Genet.* **69:** 1002–1012.
126. Subramanian, V., A. Rothenberg, C. Gomez, A. W. Cohen, A. Garcia, S. Bhattacharyya, L. Shapiro, G. Dolios, R. Wang, M. P. Lisanti, et al. 2004. Perilipin A mediates the reversible binding of CGI-58 to lipid droplets in 3T3-L1 adipocytes. *J. Biol. Chem.* **279:** 42062–42071.
127. Vallet-Erdtmann, V., G. Tavernier, J. A. Contreras, A. Mairal, C. Rieu, A. M. Touzalin, C. Holm, B. Jegou, and D. Langin. 2004. The testicular form of hormone-sensitive lipase HSLtes confers rescue of male infertility in HSL-deficient mice. *J. Biol. Chem.* **279:** 42875–42880.
128. Yamaguchi, T., N. Omatsu, S. Matsushita, and T. Osumi. 2004. CGI-58 interacts with perilipin and is localized to lipid droplets. Possible involvement of CGI-58 mislocalization in Chanarin-Dorfman syndrome. *J. Biol. Chem.* **279:** 30490–30497.
129. Granneman, J. G., H. P. Moore, R. L. Granneman, A. S. Greenberg, M. S. Obin, and Z. Zhu. 2007. Analysis of lipolytic protein trafficking and interactions in adipocytes. *J. Biol. Chem.* **282:** 5726–5735.
130. Yamaguchi, T., N. Omatsu, E. Morimoto, H. Nakashima, K. Ueno, T. Tanaka, K. Satouchi, F. Hirose, and T. Osumi. 2007. CGI-58 facilitates lipolysis on lipid droplets but is not involved in the vesiculation of lipid droplets caused by hormonal stimulation. *J. Lipid Res.* **48:** 1078–1089.
131. Ghosh, A. K., G. Ramakrishnan, C. Chandramohan, and R. Rajasekharan. 2008. CGI-58, the causative gene for Chanarin-Dorfman Syndrome, mediates acylation of lysophosphatidic acid. *J. Biol. Chem.* **283:** 24525–24533.
132. Listenberger, L. L., A. G. Ostermeyer-Fay, E. B. Goldberg, W. J. Brown, and D. A. Brown. 2007. Adipocyte differentiation-related protein reduces the lipid droplet association of adipose triglyceride lipase and slows triacylglycerol turnover. *J. Lipid Res.* **48:** 2751–2761.
133. Bell, M., H. Wang, H. Chen, J. C. McLenithan, D. W. Gong, R. Z. Yang, D. Yu, S. K. Fried, M. J. Quon, C. Londos, et al. 2008. Consequences of lipid droplet coat protein downregulation in liver cells: abnormal lipid droplet metabolism and induction of insulin resistance. *Diabetes.* **57:** 2037–2045.
134. Chung, C., J. A. Doll, A. K. Gattu, C. Shugrue, M. Cornwell, P. Fitchev, and S. E. Crawford. 2008. Anti-angiogenic pigment epithelium-derived factor regulates hepatocyte triglyceride content through adipose triglyceride lipase (ATGL). *J. Hepatol.* **48:** 471–478.

135. Inohara, N., T. Koseki, S. Chen, X. Wu, and G. Nunez. 1998. CIDE, a novel family of cell death activators with homology to the 45 kDa subunit of the DNA fragmentation factor. *EMBO J.* **17:** 2526–2533.
136. Puri, V., S. Konda, S. Ranjit, M. Aouadi, A. Chawla, M. Chouinard, A. Chakladar, and M. P. Czech. 2007. Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage. *J. Biol. Chem.* **282:** 34213–34218.
137. Puri, V., S. Ranjit, S. Konda, S. M. Nicoloro, J. Straubhaar, A. Chawla, M. Chouinard, C. Lin, A. Burkart, S. Corvera, et al. 2008. Cidea is associated with lipid droplets and insulin sensitivity in humans. *Proc. Natl. Acad. Sci. USA.* **105:** 7833–7838.
138. Nishino, N., Y. Tamori, S. Tateya, T. Kawaguchi, T. Shibakusa, W. Mizunoya, K. Inoue, R. Kitazawa, S. Kitazawa, Y. Matsuki, et al. 2008. FSP27 contributes to efficient energy storage in murine white adipocytes by promoting the formation of unilocular lipid droplets. *J. Clin. Invest.* **118:** 2808–2821.
139. Toh, S. Y., J. Gong, G. Du, J. Z. Li, S. Yang, J. Ye, H. Yao, Y. Zhang, B. Xue, Q. Li, et al. 2008. Up-regulation of mitochondrial activity and acquirement of brown adipose tissue-like property in the white adipose tissue of fsp27 deficient mice. *PLoS ONE.* **3:** e2890.
140. Li, J. Z., J. Ye, B. Xue, J. Qi, J. Zhang, Z. Zhou, Q. Li, Z. Wen, and P. Li. 2007. Cideb regulates diet-induced obesity, liver steatosis, and insulin sensitivity by controlling lipogenesis and fatty acid oxidation. *Diabetes.* **56:** 2523–2532.
141. Zhou, Z., S. Yon Toh, Z. Chen, K. Guo, C. P. Ng, S. Ponniah, S. C. Lin, W. Hong, and P. Li. 2003. Cidea-deficient mice have lean phenotype and are resistant to obesity. *Nat. Genet.* **35:** 49–56.
142. Matsusue, K., T. Kusakabe, T. Noguchi, S. Takiguchi, T. Suzuki, S. Yamano, and F. J. Gonzalez. 2008. Hepatic steatosis in leptin-deficient mice is promoted by the PPARgamma target gene Fsp27. *Cell Metab.* **7:** 302–311.
143. Guo, Y., T. C. Walther, M. Rao, N. Stuurman, G. Goshima, K. Terayama, J. S. Wong, R. D. Vale, P. Walter, and R. V. Farese. 2008. Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature.* **453:** 657–661.
144. Pena-Penabad, C., M. Almagro, W. Martinez, J. Garcia-Silva, J. Del Pozo, M. T. Yebra, C. Sanchez-Manzano, and E. Fonseca. 2001. Dorfman–Chanarin syndrome (neutral lipid storage disease): new clinical features. *Br. J. Dermatol.* **144:** 430–432.
145. Chanarin, I., A. Patel, G. Slavin, E. J. Wills, T. M. Andrews, and G. Stewart. 1975. Neutral-lipid storage disease: a new disorder of lipid metabolism. *BMJ.* **1:** 553–555.
146. Dorfman, M. L., C. Hershko, S. Eisenberg, and F. Sagher. 1974. Ichthyosiform dermatosis with systemic lipidosis. *Arch. Dermatol.* **110:** 261–266.
147. Fischer, J., C. Lefevre, E. Morava, J. M. Mussini, P. Laforet, A. Negre-Salvayre, M. Lathrop, and R. Salvayre. 2007. The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy. *Nat. Genet.* **39:** 28–30.
148. Akiyama, M., K. Sakai, M. Ogawa, J. R. McMillan, D. Sawamura, and H. Shimizu. 2007. Novel duplication mutation in the patatin domain of adipose triglyceride lipase (PNPLA2) in neutral lipid storage disease with severe myopathy. *Muscle Nerve.* **36:** 856–859.
149. Kobayashi, K., T. Inoguchi, Y. Maeda, N. Nakashima, A. Kuwano, E. Eto, N. Ueno, S. Sasaki, F. Sawada, M. Fujii, et al. 2008. The lack of the C-terminal domain of adipose triglyceride lipase causes neutral lipid storage disease through impaired interactions with lipid droplets. *J. Clin. Endocrinol. Metab.* **93:** 2877–2884.
150. Schweiger, M., G. Schoiswohl, A. Lass, F. P. Radner, G. Haemmerle, R. Malli, W. Graier, I. Cornaciu, M. Oberer, R. Salvayre, et al. 2008. The C-terminal region of human adipose triglyceride lipase affects enzyme activity and lipid droplet binding. *J. Biol. Chem.* **283:** 17211–17220.
151. Akiyama, M., D. Sawamura, Y. Nomura, M. Sugawara, and H. Shimizu. 2003. Truncation of CGI-58 protein causes malformation of lamellar granules resulting in ichthyosis in Dorfman-Chanarin syndrome. *J. Invest. Dermatol.* **121:** 1029–1034.
152. Ben Selma, Z., S. Yilmaz, P. O. Schischmanoff, A. Blom, C. Ozogul, L. Laroche, and F. Caux. 2007. A novel S115G mutation of CGI-58 in a Turkish patient with Dorfman-Chanarin syndrome. *J. Invest. Dermatol.* **127:** 2273–2276.
153. Igal, R. A., and R. A. Coleman. 1996. Acylglycerol recycling from triacylglycerol to phospholipid, not lipase activity, is defective in neutral lipid storage disease fibroblasts. *J. Biol. Chem.* **271:** 16644–16651.
154. Williams, M. L., R. A. Coleman, D. Placezk, and C. Grunfeld. 1991. Neutral lipid storage disease: a possible functional defect in phospholipid-linked triacylglycerol metabolism. *Biochim. Biophys. Acta.* **1096:** 162–169.
155. Agarwal, A. K., and A. Garg. 2003. Congenital generalized lipodystrophy: significance of triglyceride biosynthetic pathways. *Trends Endocrinol. Metab.* **14:** 214–221.
156. Zimmermann, R., G. Haemmerle, E. M. Wagner, J. G. Strauss, D. Kratky, and R. Zechner. 2003. Decreased fatty acid esterification compensates for the reduced lipolytic activity in hormone-sensitive lipase-deficient white adipose tissue. *J. Lipid Res.* **44:** 2089–2099.
157. Thomas, S. A., and R. D. Palmiter. 1997. Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline. *Nature.* **387:** 94–97.
158. Bachman, E. S., H. Dhillon, C. Y. Zhang, S. Cinti, A. C. Bianco, B. K. Kobilka, and B. B. Lowell. 2002. betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science.* **297:** 843–845.
159. van der Vusse, G. J., M. van Bilsen, and J. F. Glatz. 2000. Cardiac fatty acid uptake and transport in health and disease. *Cardiovasc. Res.* **45:** 279–293.
160. Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* **37:** 693–707.
161. Preiss-Landl, K., R. Zimmermann, G. Hammerle, and R. Zechner. 2002. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. *Curr. Opin. Lipidol.* **13:** 471–481.
162. Augustus, A., H. Yagyu, G. Haemmerle, A. Bensadoun, R. K. Vikramadithyan, S. Y. Park, J. K. Kim, R. Zechner, and I. J. Goldberg. 2004. Cardiac-specific knock-out of lipoprotein lipase alters plasma lipoprotein triglyceride metabolism and cardiac gene expression. *J. Biol. Chem.* **279:** 25050–25057.
163. Levak-Frank, S., W. Hofmann, P. H. Weinstock, H. Radner, W. Sattler, J. L. Breslow, and R. Zechner. 1999. Induced mutant mouse lines that express lipoprotein lipase in cardiac muscle, but not in skeletal muscle and adipose tissue, have normal plasma triglyceride and high-density lipoprotein-cholesterol levels. *Proc. Natl. Acad. Sci. USA.* **96:** 3165–3170.
164. Noh, H. L., K. Okajima, J. D. Molkentin, S. Homma, and I. J. Goldberg. 2006. Acute lipoprotein lipase deletion in adult mice leads to dyslipidemia and cardiac dysfunction. *Am. J. Physiol. Endocrinol. Metab.* **291:** E755–E760.
165. Augustus, A. S., J. Buchanan, T. S. Park, K. Hirata, H. L. Noh, J. Sun, S. Homma, J. D'Armiento, E. D. Abel, and I. J. Goldberg. 2006. Loss of lipoprotein lipase-derived fatty acids leads to increased cardiac glucose metabolism and heart dysfunction. *J. Biol. Chem.* **281:** 8716–8723.
166. Pillutla, P., Y. C. Hwang, A. Augustus, M. Yokoyama, H. Yagyu, T. P. Johnston, M. Kaneko, R. Ramasamy, and I. J. Goldberg. 2005. Perfusion of hearts with triglyceride-rich particles reproduces the metabolic abnormalities in lipotoxic cardiomyopathy. *Am. J. Physiol. Endocrinol. Metab.* **288:** E1229–E1235.
167. Yagyu, H., G. Chen, M. Yokoyama, K. Hirata, A. Augustus, Y. Kako, T. Seo, Y. Hu, E. P. Lutz, M. Merkel, et al. 2003. Lipoprotein lipase (LpL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. *J. Clin. Invest.* **111:** 419–426.
168. Swanton, E. M., and E. D. Saggerson. 1997. Effects of adrenaline on triacylglycerol synthesis and turnover in ventricular myocytes from adult rats. *Biochem. J.* **328:** 913–922.
169. Saddik, M., and G. D. Lopaschuk. 1994. Triacylglycerol turnover in isolated working hearts of acutely diabetic rats. *Can. J. Physiol. Pharmacol.* **72:** 1110–1119.
170. Holm, C., P. Belfrage, and G. Fredrikson. 1987. Immunological evidence for the presence of hormone-sensitive lipase in rat tissues other than adipose tissue. *Biochem. Biophys. Res. Commun.* **148:** 99–105.
171. Small, C. A., A. J. Garton, and S. J. Yeaman. 1989. The presence and role of hormone-sensitive lipase in heart muscle. *Biochem. J.* **258:** 67–72.
172. Nielsen, L. B., M. Veniant, J. Boren, M. Raabe, J. S. Wong, C. Tam, L. Flynn, T. Vanni-Reyes, M. D. Gunn, I. J. Goldberg, et al. 1998. Genes for apolipoprotein B and microsomal triglyceride transfer protein are expressed in the heart: evidence that the heart has the capacity to synthesize and secrete lipoproteins. *Circulation.* **98:** 13–16.
173. Igal, R. A., J. M. Rhoads, and R. A. Coleman. 1997. Neutral lipid storage disease with fatty liver and cholestasis. *J. Pediatr. Gastroenterol. Nutr.* **25:** 541–547.

174. Blaak, E. E. 2005. Metabolic fluxes in skeletal muscle in relation to obesity and insulin resistance. *Best Pract. Res. Clin. Endocrinol. Metab.* **19:** 391–403.
175. Langfort, J., T. Ploug, J. Ihlemann, M. Saldo, C. Holm, and H. Galbo. 1999. Expression of hormone-sensitive lipase and its regulation by adrenaline in skeletal muscle. *Biochem. J.* **340:** 459–465.
176. Peters, S. J., D. J. Dyck, A. Bonen, and L. L. Spriet. 1998. Effects of epinephrine on lipid metabolism in resting skeletal muscle. *Am. J. Physiol.* **275:** E300–E309.
177. Roepstorff, C., B. Vistisen, M. Donsmark, J. N. Nielsen, H. Galbo, K. A. Green, D. G. Hardie, J. F. Wojtaszewski, E. A. Richter, and B. Kiens. 2004. Regulation of hormone-sensitive lipase activity and Ser563 and Ser565 phosphorylation in human skeletal muscle during exercise. *J. Physiol.* **560:** 551–562.
178. Donsmark, M., J. Langfort, C. Holm, T. Ploug, and H. Galbo. 2003. Contractions activate hormone-sensitive lipase in rat muscle by protein kinase C and mitogen-activated protein kinase. *J. Physiol.* **550:** 845–854.
179. Langfort, J., T. Ploug, J. Ihlemann, C. Holm, and H. Galbo. 2000. Stimulation of hormone-sensitive lipase activity by contractions in rat skeletal muscle. *Biochem. J.* **351:** 207–214.
180. Muoio, D. M., K. Seefeld, L. A. Witters, and R. A. Coleman. 1999. AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochem. J.* **338:** 783–791.
181. Smith, A. C., C. R. Bruce, and D. J. Dyck. 2005. AMP kinase activation with AICAR further increases fatty acid oxidation and blunts triacylglycerol hydrolysis in contracting rat soleus muscle. *J. Physiol.* **565:** 547–553.
182. Watt, M. J., G. R. Steinberg, S. Chan, A. Garnham, B. E. Kemp, and M. A. Febbraio. 2004. Beta-adrenergic stimulation of skeletal muscle HSL can be overridden by AMPK signaling. *FASEB J.* **18:** 1445–1446.
183. Pinent, M., H. Hackl, T. R. Burkard, A. Prokesch, C. Papak, M. Scheideler, G. Hammerle, R. Zechner, Z. Trajanoski, and J. G. Strauss. 2008. Differential transcriptional modulation of biological processes in adipocyte triglyceride lipase and hormone-sensitive lipase-deficient mice. *Genomics.* **92:** 26–32.
184. Hansson, O., M. Donsmark, C. Ling, P. Nevsten, M. Danfelter, J. L. Andersen, H. Galbo, and C. Holm. 2005. Transcriptome and proteome analysis of soleus muscle of hormone-sensitive lipase-null mice. *J. Lipid Res.* **46:** 2614–2623.
185. Watt, M. J., B. J. van Denderen, L. A. Castelli, C. R. Bruce, A. J. Hoy, E. W. Kraegen, L. Macaulay, and B. E. Kemp. 2008. Adipose triglyceride lipase regulation of skeletal muscle lipid metabolism and insulin responsiveness. *Mol. Endocrinol.* **22:** 1200–1212.
186. Reid, B. N., G. P. Ables, O. A. Otlivanchik, G. Schoiswohl, R. Zechner, W. S. Blaner, I. J. Goldberg, R. F. Schwabe, S. C. Chua, Jr., and L. S. Huang. 2008. Hepatic overexpression of hormone-sensitive lipase and adipose triglyceride lipase promotes fatty acid oxidation, stimulates direct release of free fatty acids, and ameliorates steatosis. *J. Biol. Chem.* **283:** 13087–13099.
187. Brown, J. M., S. Chung, A. Das, G. S. Shelness, L. L. Rudel, and L. Yu. 2007. CGI-58 facilitates the mobilization of cytoplasmic triglyceride for lipoprotein secretion in hepatoma cells. *J. Lipid Res.* **48:** 2295–2305.
188. Park, S. Y., H. J. Kim, S. Wang, T. Higashimori, J. Dong, Y. J. Kim, G. Cline, H. Li, M. Prentki, G. I. Shulman, et al. 2005. Hormone-sensitive lipase knockout mice have increased hepatic insulin sensitivity and are protected from short-term diet-induced insulin resistance in skeletal muscle and heart. *Am. J. Physiol. Endocrinol. Metab.* **289:** E30–E39.
189. Voshol, P. J., G. Haemmerle, D. M. Ouwens, R. Zimmermann, R. Zechner, B. Teusink, J. A. Maassen, L. M. Havekes, and J. A. Romijn. 2003. Increased hepatic insulin sensitivity together with decreased hepatic triglyceride stores in hormone-sensitive lipase-deficient mice. *Endocrinology.* **144:** 3456–3462.
190. Mulder, H., M. Sorhede-Winzell, J. A. Contreras, M. Fex, K. Strom, T. Ploug, H. Galbo, P. Arner, C. Lundberg, F. Sundler, et al. 2003. Hormone-sensitive lipase null mice exhibit signs of impaired insulin sensitivity whereas insulin secretion is intact. *J. Biol. Chem.* **278:** 36380–36388.
191. Roduit, R., P. Masiello, S. P. Wang, H. Li, G. A. Mitchell, and M. Prentki. 2001. A role for hormone-sensitive lipase in glucose-stimulated insulin secretion: a study in hormone-sensitive lipase-deficient mice. *Diabetes.* **50:** 1970–1975.
192. Peyot, M. L., C. J. Nolan, K. Soni, E. Joly, R. Lussier, B. E. Corkey, S. P. Wang, G. A. Mitchell, and M. Prentki. 2004. Hormone-sensitive lipase has a role in lipid signaling for insulin secretion but is non-essential for the incretin action of glucagon-like peptide 1. *Diabetes.* **53:** 1733–1742.
193. Claus, T. H., D. B. Lowe, Y. Liang, A. I. Salhanick, C. K. Lubeski, L. Yang, L. Lemoine, J. Zhu, and K. B. Clairmont. 2005. Specific inhibition of hormone-sensitive lipase improves lipid profile while reducing plasma glucose. *J. Pharmacol. Exp. Ther.* **315:** 1396–1402.
194. Fex, M., C. S. Olofsson, U. Fransson, K. Bacos, H. Lindvall, M. Sorhede-Winzell, P. Rorsman, C. Holm, and H. Mulder. 2004. Hormone-sensitive lipase deficiency in mouse islets abolishes neutral cholesterol ester hydrolase activity but leaves lipolysis, acylglycerides, fat oxidation, and insulin secretion intact. *Endocrinology.* **145:** 3746–3753.
195. Larsson, S., N. Wierup, F. Sundler, L. Eliasson, and C. Holm. 2008. Lack of cholesterol mobilization in islets of hormone-sensitive lipase deficient mice impairs insulin secretion. *Biochem. Biophys. Res. Commun.* **376:** 558–562.
196. Cinti, S., G. Mitchell, G. Barbatelli, I. Murano, E. Ceresi, E. Faloia, S. Wang, M. Fortier, A. S. Greenberg, and M. S. Obin. 2005. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J. Lipid Res.* **46:** 2347–2355.
197. Hansson, O., K. Strom, N. Guner, N. Wierup, F. Sundler, P. Hoglund, and C. Holm. 2006. Inflammatory response in white adipose tissue in the non-obese hormone-sensitive lipase null mouse model. *J. Proteome Res.* **5:** 1701–1710.
198. Fredrikson, G., H. Tornqvist, and P. Belfrage. 1986. Hormone-sensitive lipase and monoacylglycerol lipase are both required for complete degradation of adipocyte triacylglycerol. *Biochim. Biophys. Acta.* **876:** 288–293.
199. Tornqvist, H., and P. Belfrage. 1976. Purification and some properties of a monoacylglycerol-hydrolyzing enzyme of rat adipose tissue. *J. Biol. Chem.* **251:** 813–819.
200. Karlsson, M., K. Reue, Y. R. Xia, A. J. Lusis, D. Langin, H. Tornqvist, and C. Holm. 2001. Exon-intron organization and chromosomal localization of the mouse monoglyceride lipase gene. *Gene.* **272:** 11–18.
201. Karlsson, M., H. Tornqvist, and C. Holm. 2000. Expression, purification, and characterization of histidine-tagged mouse monoglyceride lipase from baculovirus-infected insect cells. *Protein Expr. Purif.* **18:** 286–292.
202. Karlsson, M., J. A. Contreras, U. Hellman, H. Tornqvist, and C. Holm. 1997. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J. Biol. Chem.* **272:** 27218–27223.
203. Yeaman, S. J., G. M. Smith, C. A. Jepson, S. L. Wood, and N. Emmison. 1994. The multifunctional role of hormone-sensitive lipase in lipid metabolism. *Adv. Enzyme Regul.* **34:** 355–370.
204. Blankman, J. L., G. M. Simon, and B. F. Cravatt. 2007. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* **14:** 1347–1356.
205. Dinh, T. P., D. Carpenter, F. M. Leslie, T. F. Freund, I. Katona, S. L. Sensi, S. Kathuria, and D. Piomelli. 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA.* **99:** 10819–10824.
206. Saario, S. M., J. R. Savinainen, J. T. Laitinen, T. Jarvinen, and R. Niemi. 2004. Monoglyceride lipase-like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. *Biochem. Pharmacol.* **67:** 1381–1387.
207. Pertwee, R. G. 2006. The pharmacology of cannabinoid receptors and their ligands: an overview. *Int. J. Obes. (Lond.).* **30 (Suppl.):** 13–18.
208. Sugiura, T., S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, and K. Waku. 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215:** 89–97.
209. Cravatt, B. F., K. Demarest, M. P. Patricelli, M. H. Bracey, D. K. Giang, B. R. Martin, and A. H. Lichtman. 2001. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc. Natl. Acad. Sci. USA.* **98:** 9371–9376.
210. Bisogno, T., F. Howell, G. Williams, A. Minassi, M. G. Cascio, A. Ligresti, I. Matias, A. Schiano-Moriello, P. Paul, E. J. Williams,

et al. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163:** 463–468.
211. Mead, J. R., S. A. Irvine, and D. P. Ramji. 2002. Lipoprotein lipase: structure, function, regulation, and role in disease. *J. Mol. Med.* **80:** 753–769.
212. Gulyas, A. I., B. F. Cravatt, M. H. Bracey, T. P. Dinh, D. Piomelli, F. Boscia, and T. F. Freund. 2004. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur. J. Neurosci.* **20:** 441–458.
213. Ravinet Trillou, C., C. Delgorge, C. Menet, M. Arnone, and P. Soubrie. 2004. CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int. J. Obes. Relat. Metab. Disord.* **28:** 640–648.
214. Carai, M. A., G. Colombo, and G. L. Gessa. 2005. Rimonabant: the first therapeutically relevant cannabinoid antagonist. *Life Sci.* **77:** 2339–2350.
215. Bari, M., N. Battista, F. Fezza, V. Gasperi, and M. Maccarrone. 2006. New insights into endocannabinoid degradation and its therapeutic potential. *Mini Rev. Med. Chem.* **6:** 257–268.
216. Vandevoorde, S., and D. M. Lambert. 2005. Focus on the three key enzymes hydrolysing endocannabinoids as new drug targets. *Curr. Pharm. Des.* **11:** 2647–2668.