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MOLECULAR MECHANISMS REGULATING HORMONE-SENSITIVE LIPASE AND LIPOLYSIS

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■ **Abstract** Hormone-sensitive lipase, the rate-limiting enzyme of intracellular TG hydrolysis, is a major determinant of fatty acid mobilization in adipose tissue as well as other tissues. It plays a pivotal role in lipid metabolism, overall energy homeostasis, and, presumably, cellular events involving fatty acid signaling. Detailed knowledge about its structure and regulation may provide information regarding the pathogenesis of such human diseases as obesity and diabetes and may generate concepts for new treatments of these diseases. The current review summarizes the recent advances with regard to hormone-sensitive lipase structure and molecular mechanisms involved in regulating its activity and lipolysis in general. A summary of the current knowledge regarding regulation of expression, potential involvement in lipid disorders, and role in tissues other than adipose tissue is also provided.

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INTRODUCTION TO HORMONE-SENSITIVE LIPASE (HSL)

Molecular components involved in regulation of lipolysis play a pivotal role in lipid metabolism and overall energy homeostasis, as well as in various cellular events involving fatty acid signaling. Fatty acids stored in the form of triglycerides (TGs) are the main source of energy in the absence of dietary substrates. From adipose tissue, up to 150 g of fatty acids can be liberated daily, corresponding to approximately half of the daily caloric needs. In addition, stored TGs constitute a pool of fatty acid–derived signaling molecules. Hydrolysis of TGs occurs through three consecutive reactions and is catalyzed by two enzymes: hormone-sensitive lipase (HSL) and monoglyceride lipase (MGL). HSL alone catalyzes the hydrolysis of TGs and diglycerides (DGs), whereas the participation of MGL is required to obtain complete hydrolysis of monoglycerides (MGs) (43). This chapter is focused on the rate-limiting enzyme in intracellular TG hydrolysis, HSL, and the molecular mechanisms regulating this enzyme.

Identification of HSL in Adipose Tissue

The first description of a hormone-responsive lipolytic activity of adipose tissue dates back to the early 1960s, when a lipolytic activity with properties different from those of lipoprotein lipase (LPL) was described (10, 66, 138). This activity was given the name hormone-sensitive lipase in 1964 by Vaughan et al (173), who also made the distinction between HSL and MGL, based on differences in pH optimum and substrate specificity. HSL purified from adipose tissue is a hydrophobic and labile protein with an apparent M_r of 84,000 (6, 42, 112–114). Following the cDNA cloning of HSL (73), powerful expression systems were established based on baculovirus/insect cell technology, providing large amounts of homogenous protein for structural and functional studies (21, 120c).

Biochemical and Enzymological Properties of HSL

Compared with other lipases, HSL has a uniquely broad substrate specificity. It hydrolyzes all acylglycerols: TGs, DGs, and MGs (42, 173, 120c) and also cholesteryl esters (42, 120c), steroid fatty acid esters (99), retinyl esters (177), and *para*-nitrophenyl esters (172, 120c). Noteworthy, however, is the fact that HSL lacks phospholipase activity. The activity against DGs is higher than the activity against TGs and MGs, by 10- to 12-fold and 5- to 10-fold, respectively (41, 120c). This, in combination with the action of MGL, assures no accumulation of intermediate metabolites (41–43). The cholesteryl ester hydrolase (CEH) activity of HSL is twice as high as the activity toward TGs (42, 120c) and, thus, is relatively much higher than that of bile-salt–stimulated lipase, another TG lipase exhibiting CEH activity. The esterase activity of HSL, measured as its activity against *para*-nitrophenyl esters, is also remarkably high, more than 20-fold that of TGs (120c). The relative maximal rates of hydrolysis, i.e. 1 : 11 : 2.1 : 23 for trioleoin, diolein, cholesterol oleate, and *para*-nitrophenyl butyrate, respectively, are based on measurements performed in vitro using purified, detergent-solubilized and non-phosphorylated enzyme. Phosphorylation under standard in vitro assay conditions increases moderately the activity against TGs and cholesteryl esters, whereas the activity against DGs and MGs is unaffected (42, 120c). Whether DG and MG lipase activity is unaffected by phosphorylation also in vivo is not known (69).

HSL exhibits a preference, although not absolute, for the 1- or 3-ester bond of its acylglycerol susbtrate, with approximately three- to fourfold higher hydrolysis rates of the 1(3)-ester bonds than of the 2-ester bond (41). With regard to specificity for fatty acids, studies on crude preparations of rat HSL have shown a preferential release of polyunsaturated (n-3/n-6) fatty acids (PUFA) from TGs (51). Similar data have been obtained from studies on isolated human fat cells (127). Moreover, studies on the fatty acid composition of adipose tissue from golden hamsters also suggested a preferential release of PUFA during arousal from the hibernating state (17). Whether the preferential release of PUFA indeed reflects a property of HSL or whether it can be attributed to an increased accessibility of PUFA at the lipid/solvent interface, through differences in partition coefficient between the interfacial phase and the apolar phase inside the lipid droplet, is not known (127). Fatty acids, i.e. oleic acid and oleoyl coenzyme A (CoA), and MGs have been shown to inhibit HSL. This feedback inhibition was proposed to prevent the accumulation of fatty acids and free cholesterol in the cell and to assure that the capacity of MGL to hydrolyze MGs is not exceeded (83). Recently, adipocyte lipid-binding protein (ALBP) was shown to interact with HSL, raising the possibility that ALBP sequesters fatty acids resulting from HSL-catalyzed lipolysis and thus prevents the feedback inhibition by lipolytic products (149).

Structure of HSL

cDNA and Genomic Cloning The first cDNA cloning of HSL was accomplished in 1988 from rat adipocytes (73). This cDNA predicts 768 amino acids and a

molecular mass of 84,073 (70, 73, 102), which is in good agreement with the M_r of 84,000 (42). The human adipocyte HSL cDNA, obtained concomitantly with the cloning of the human HSL gene, predicts 775 amino acids and a molecular mass of 84,032 (21, 94). The identity between the amino acid sequence of rat and human HSL is 82%. Despite the similarity in molecular mass and sequence between rat and human adipocyte HSL, the human protein migrates as a slightly larger protein than the rat protein on sodium dodecyl sulfate–polyacrylamide gel electrophoresis: 88 vs 84 kDa (42, 68). On isolation and characterization of the human HSL adipocyte promoter, a closer analysis of the human HSL mRNA showed that it is 2.8 kb in size (56) rather than 3.3 kb as originally reported (68). Because rat and human adipocyte HSL mRNA comigrate on Northern blot analysis (68), this correction presumably also applies to the rat adipocyte HSL mRNA.

Northern blot analyses of HSL mRNA in rat and human tissues have shown that HSL is expressed in a variety of tissues, including white and brown adipose tissue, steroidogenic tissues, mammary gland, muscle tissues, macrophages, and endocrine pancreas (22, 73, 75, 85, 111, 131). Several distinct mRNA species exhibiting a tissue-specific distribution have been observed. The larger testicular HSL mRNA has been cloned from both rat and human testes and encodes an additional 300 and 301 N-terminal amino acids, respectively, compared with the adipocyte mRNA (75). This is consistent with the presence of HSL protein species in the range of 116–130 kDa in crude homogenates of rat and human testes. In contrast to the sequence identity of 82% between rat and human adipocyte HSL, the identity between the respective testis-specific parts is only 37%.

The exon-intron organization reported for human (94) and mouse HSL (102, 167) are very similar. Both genes comprise nine main coding exons, spanning approximately 11 kb (man) and 10 kb (mouse), respectively (Figure 1). The transcriptional start site was originally determined to 632 and 593 nucleotides (nt), respectively, upstream from the translation start site, which was in good agreement with the 600-nt-long 5′ leader sequence reported for the rat HSL cDNA (73). However, subsequent more detailed analyses of this region of the human gene have revealed a greater degree of complexity. Two 5′ leader sequences of 170 and 70 nt, respectively, have been identified (56). Except for the last 20 nt, which precede the ATG initiation codon in exon 1, these 5′ leader sequences originate from separate, single exons designated A and B, respectively. These are mutually exclusive and only exon B appears to be represented among transcripts in human adipocytes. Besides exon A and B, one additional upstream exon has been identified in the human HSL gene, exon T, located 16 kb upstream of exon 1 (75). Exon T, together with exons 1–9, encode the larger testicular isoform, HSL_{tes}. Recently, a testis-specific promoter that binds members of the Sox family of testicular nuclear proteins was identified upstream of exon T (11).

In summary, besides the commonly expressed coding exons 1–9, several upstream noncoding and coding exons appear to be used in a mutually exclusive and tissue-specific manner. All known upstream exons splice to exon 1 at the same acceptor splice site, 20 nt upstream of the translational start site and, in the case of

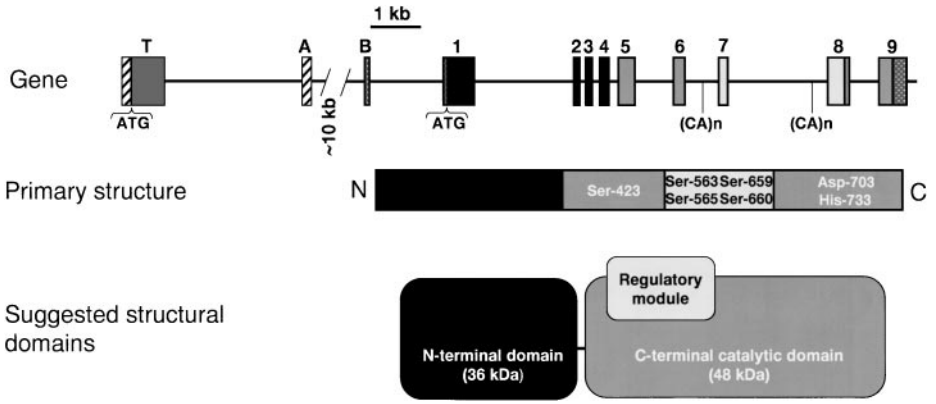

Figure 1 Organization of the human hormone-sensitive lipase (HSL) gene and domain structure of adipocyte HSL (HSL_{adi}). (*Top*) Exon/intron organization of the human HSL gene. The two known translation start sites for the HSL_{adi} and testicular HSL, respectively, are indicated, as well as the position of two reported CA repeats. (*Middle*) Linear representation of the HSL amino acid sequence (numbering from the rat sequence), divided into three functional regions as described in the text. Exons encoding each of these regions are *shaded* accordingly. (*Bottom*) Proposed domain structure of HSL_{adi} with two major structural domains: an N-terminal domain and a C-terminal catalytic domain. The latter harbors the catalytic triad (Ser-423, Asp-703, and His-733) and a regulatory module with four phosphorylation sites (Ser-563, -565, -659, and -660).

exon T, in frame with this. Although only one tissue-specific promoter has been described, the identification of more can be anticipated.

Domain Structure Early sequence comparisons revealed that HSL is closely related to several bacterial lipases and esterases (62, 93, 94). In recent years, the number of proteins found to be related to HSL proteins has increased to form a small family of putative lipases and esterases. The only other known mammalian member of this family is an arylacetamide deacetylase from human liver (125). From a structural point of view, HSL is the most complex of the proteins in the family. Alignment of HSL from different species (man, rat, and mouse) and the other proteins in the HSL family allows the conceptual division of the HSL sequence in three distinct regions (120c) (see Figure 1). The N-terminal region (residues 1–315) is only present in HSL and shows a very high degree of conservation between man and rodents (91% identity). This is followed by a short stretch of 18 amino acids, which is poorly conserved among HSL from different species (44% identity) and is believed to represent a linker between domains. The second region corresponds to the part of the sequence showing homology with the other members of the family and is expected to constitute the catalytic core of the enzyme (see below). This region is split in two by the insertion of approximately 150 residues that are not present in the bacterial relatives of HSL and that constitute the third distinct region of the HSL sequence. It is interesting to note

that this insertion contains the three serine residues known to be phosphorylated by the protein kinase A (PKA) (2) and is expected to form a regulatory module in HSL. Thus, from the sequence analysis it is expected that the HSL protein is composed of at least two domains: One constitutes the catalytic core and the regulatory module, and the other may play its role in binding to the lipid droplets and to other putative interacting proteins. In this respect, it has recently been proposed that the N-terminal domain of HSL interacts with ALBP, which acts as an intracellular fatty acid carrier (149). A second protein proposed to interact with HSL is the recently cloned lipotransin (164), but the region of HSL that interacts with this protein has not been identified.

Several pieces of experimental evidence support the conceptual division of HSL in more than one domain. Limited proteolysis data demonstrate the existence of stable fragments that are highly resistant to proteolysis (154, 155, 120a, 120c). A major stable peptide, comprising residues 1–323, is obtained after prolonged cleavage with endoproteinase Lys-C, providing experimental evidence that this constitutes a separate structural domain (120a). Circular dichroism and fluorescence spectroscopy analyses during denaturation with guanidine hydrochloride and heat have revealed two major steps of unfolding (120a), supporting the notion of two separate structural domains.

Three-Dimensional Model of the Catalytic Core of HSL Intense research on lipases and esterases during the past decade has revealed that these proteins share a common three-dimensional fold, denominated the α/β-hydrolase fold, that harbors a catalytic triad formed by serine, a carboxylic acid (aspartic or glutamic), and histidine (116). The common feature of the α/β-hydrolase fold is a central β-sheet surrounded by a variable number of α-helices. Some of these central structures are strikingly well conserved, even among proteins that share very low sequence homology (for further details on the α/β-hydrolase fold, see 30, 116). In the case of HSL, the secondary structure elements that constitute this scaffold are expected to be within the regions showing homology to the other members of the HSL family, namely residues 332–535 and 688–766 (in rat HSL). Secondary structure prediction in this region and a threading approach allowed the identification of the central elements of the α/β-hydrolase in the HSL sequence, and the building of a three-dimensional model for this domain of the protein (23). The validity of this model has recently been corroborated by the elucidation of the crystal structure of Brefeldin A esterase from *Bacillus subtilis* (178), a protein that shows significant homology to HSL. The catalytic triad of rat HSL, predicted from the model and confirmed by site-directed mutagenesis, is constituted by residues serine 423, aspartate 703, and histidine 733 (70, 120b).

Subunit Structure Early experiments with gel chromatography and sucrose gradient centrifugation, performed on rat adipose tissue HSL, indicated that the enzyme is a dimer in solution (7; G Edgren & C Holm, unpublished results). With the availability of large amounts of homogenous recombinant HSL, this has

recently been confirmed using sedimentation equilibrium analysis (120a). The subunit structure in relation to the functional state of the enzyme has not been elucidated, however.

EXPRESSION AND ROLE OF HSL IN TISSUES OTHER THAN ADIPOSE TISSUE

Steroidogenic Tissues

Soon after the partial purification of HSL from rat adipose tissue and the discovery that HSL has CEH activity (42), purification of a CEH from bovine adrenal cortex and corpus luteum was reported (25, 26). Enzyme inhibition, phosphorylation, and labeling indicated that this neutral CEH was most likely identical to HSL (26). Later, Western and Northern blot analyses identified HSL protein and transcript in adrenal glands, ovaries, placenta, and testes (67, 73, 75, 89), which suggests a role for HSL in steroidogenesis by providing free cholesterol for steroid synthesis (25, 26, 187). Furthermore, several steroid hormones are stored in the cells in the form of fatty acid esters, and HSL is likely to be the neutral esterase hydrolyzing these steroid esters (99). Recently, most attention with regard to HSL in steroidogenic tissues has been paid to testes and the particular isoform found there (see above). However, HSL is present not in the steroid-producing Leydig cells of rat testes but rather in Sertoli cells and haploid germ cells (74, 75). Thus, HSL in testes is probably not involved in steroidogenesis but could instead play a role in spermatogenesis and possibly reproductive function. It is not unlikely that HSL functions as a TG lipase in testes and a CEH in adrenal glands, but further investigations are needed in order to establish this.

As discussed above, the testis-specific isoform of HSL, HSL_{tes}, has an extra N-terminal domain of unknown function. This domain has a putative motif for SH3 binding, which suggests direct interaction of HSL_{tes} with other proteins (75). The domain is particularly rich in prolines (P), glutamic acids (E), serines (S), and threonines (T). Proteins rich in these amino acids (PEST sequences) are potentially susceptible to rapid proteolytic degradation (141). It is possible that a short half-life, together with a tight transcriptional and translational control, account for the observed stage-dependent expression of HSL in germ cells with mRNA expression in stages X–XIV of spermatogenesis and delayed protein expression in stages XIII–VII (11, 74, 75).

Muscle Tissues

By 1969, the presence of an HSL-like activity in heart myocytes had been proposed (19). The nature of this enzyme activity remained controversial for many years. Based on a number of observed enzyme characteristics, it was proposed

that the enzyme was an HSL with properties of LPL, named HSL-L (123), or possibly an intracellular pool of LPL (122). Similar arguments were also brought forward for the intracellular lipase of skeletal muscle (120). With the aid of antibodies and cDNA probes as tools, several groups have subsequently established the expression of HSL in heart and skeletal muscle (52, 67, 73, 75, 152). In a more recent study, isolated skeletal muscle fibers were used to demonstrate that HSL is indeed expressed in myocytes and not only in adipocytes interlaced between muscle fibers (92). The expression is correlated to fiber type, being higher in oxidative than in glycolytic fibers. By using neutralizing antibodies it was furthermore confirmed that HSL accounts for a significant fraction of the TG lipase activity in skeletal muscle fibers. The TG stores found in the cytoplasm of myocytes are mobilized by catecholamines and by exercise, and based on existing data, it seems reasonable to propose that HSL accounts for this mobilization. The concomitant activation of both HSL and glycogen phosphorylase in response to epinephrine and contractions suggests simultaneous activation of glycogen and TG breakdown (92). By using isolated rat skeletal muscle fibers a dual activation of HSL by epinephrine and contractions was demonstrated (91). The mechanisms involved in the β-adrenergic–induced activation of HSL are presumably the same as in adipocytes and are discussed below. The mechanism for the contraction-induced activation, which appears to be independent of the sympathetic nervous system, is intriguing and completely unknown.

Brown Adipose Tissue

By 1965, it was suggested that HSL was the intracellular TG lipase in brown adipose tissue (58), and through immunoprecipitation experiments, direct evidence for its presence in this tissue has been provided (71). Fatty acids mobilized from intracellular stores through the action of HSL are used in nonshivering thermogenesis, presumably both as substrate and as physiological activators of uncoupling protein. In relation to this it is interesting to note that HSL has been shown to retain high enzyme activity at low temperatures (94; H Laurell et al, manuscript in preparation). This psychrotolerant property of HSL may be critical for nonshivering thermogenesis and overall energy metabolism in hibernating animals. At the transcriptional level, however, there is no cold-induced activation of HSL (71), in contrast to a number of other genes, such as LPL and uncoupling protein, which dramatically increase their expression during cold exposure. Phosphorylation and activation of a constitutively highly expressed, cold-adapted HSL, due to catecholamine release, may constitute the first line of defense on sudden cold stress. Prolonged duration of the cold stress, which severely depletes the endogenous TG reserves, activates the second line of defense, namely cAMP-mediated transcriptional activation of a number of critical proteins, including LPL (18). This leads to increased uptake of lipids from the circulation concomitant with hypertrophy and general recruitment of brown adipocytes (see 16, and references therein).

Macrophages

HSL protein and mRNA have been detected in macrophages from murine origin and in several macrophage-like cell lines (22, 85, 152, 153). Therefore, HSL is one of the neutral lipases in these cell types. However, the involvement of HSL in the course of human arteriosclerosis is uncertain. Although small amounts of HSL mRNA have been detected in human macrophages using a polymerase chain reaction approach (131), this finding contrasts with two independent reports showing that HSL mRNA is undetectable in human monocyte-derived macrophages (24, 101). Li & Hui (101) found that HSL mRNA was detectable by reverse transcription–polymerase chain reaction in human monocytes and in THP-1 monocytes, but it was undetectable in mature macrophages and in differentiated THP-1 macrophages, which suggests a down-regulation of the gene during differentiation (101). Furthermore, HSL expression in murine macrophages is down-regulated by the intracellular content of cholesterol (82). Thus, variance in the differentiation state and culture conditions may account for the discrepancies in the detection of HSL mRNA in human macrophages mentioned above.

Independent of the presence or absence of HSL in human macrophages, it has repeatedly been postulated that stimulation of the neutral CEH activity in macrophages may prevent the progression of atherosclerosis, by reducing the accumulation of cholesteryl esters in the foam cells. However, because cholesteryl ester formation in the macrophage is a mechanism likely to be defensive against the elevation of intracellular free cholesterol levels (183), the benefits of increasing a neutral CEH activity is questionable in a situation of massive uptake of lipoproteins, as is the case of the macrophages in the artery wall. Overexpression of HSL may result in an increased cellular stress caused by the considerable energetic effort required to maintain intracellular free cholesterol content below toxic levels, by means of futile reesterification into cholesteryl esters. Because atherogenesis is an inflammatory process (143), macrophage stress should aggravate rather than alleviate it. Consistent with this view, a recent study demonstrates that transgenic mice overexpressing HSL in macrophages suffered larger and more advanced atherosclerotic lesions than did their control littermates (37). This was despite the fact that overexpression of HSL in macrophages in culture actually resulted in a net reduction of the cholesteryl ester load in these cells (36). Thus, increasing neutral CEH activity in macrophages would require a parallel increase in the mechanisms of free cholesterol efflux from the macrophages in order to reduce rather than enhance atherosclerosis.

Pancreatic β-cells

Recently, HSL was shown to be expressed in clonal β-cells, as well as in isolated mouse and rat islets of Langerhans (111). The role of HSL in these cells is not known, but an attractive hypothesis is that HSL is responsible for generating the lipid-derived signal that appears to be necessary for stimulus-secretion coupling in these cells. The nature of this lipid-derived signal is not known, but among proposed

candidates are malonyl-CoA, long-chain acyl CoA, and DGs. The HSL mRNA, as well as the main HSL isoform in islets and clonal β-cells, is slightly larger than in adipocytes, raising the possibility of the presence of additional upstream exons in the HSL gene, besides the testis-specific exon and exons A and B (see above and Figure 1).

MOLECULAR MECHANISMS IN REGULATED LIPOLYSIS

Regulation of Adipocyte Lipolysis by Catecholamines and Insulin

Lipolysis as a regulated event has been known since the early 1960s, when it was established that fast-acting hormones such as ACTH and epinephrine increased lipolysis (66, 138), and that insulin counteracted this activation (44, 53, 140). It was soon recognized that cAMP was involved in the regulation of the catecholamine-sensitive lipolytic activity in adipose tissue (139). Today, it is generally accepted that lipolysis is controlled mainly by the activity of the sympathetic nervous system and by plasma insulin levels. Activation of lipolysis is mediated by an increment of intracellular cAMP concentrations and activation of PKA. The two main targets for PKA-mediated phosphorylation in the adipocyte are HSL and the perilipins, and phosphorylation of these proteins dramatically increases lipolysis. Catecholamines are able either to stimulate lipolysis via three subtypes of β-adrenergic receptors, which are positively coupled to adenylyl cyclase by Gs proteins, or to inhibit lipolysis via α2-adrenergic receptors, negatively coupled to the enzyme by Gi proteins. Concerning the functional significance of adrenoceptor-mediated increases in cAMP levels, two points should be emphasized. First, lipolytic agents generally increase the levels of cAMP far above the concentrations required for maximal activation of PKA (38, 76). Second, considerable species and tissue specificity exists with regard to the distribution of adrenoceptor subtypes. In humans, for instance, the interplay between α2- and β-adrenergic receptors plays an important role in modulating cAMP levels in adipocytes (for a review, see 90).

Insulin, the most important physiological inhibitor of catecholamine-induced lipolysis, induces phosphorylation and activation of the phosphodiesterase type 3B (PDE3B), leading to a decrease in cAMP levels and concomitant decrease of PKA activity (for a review, see 29). Specific inhibition of PDE3B completely blocks the antilipolytic effect of insulin (34, 35, 147), indicating that cAMP degradation is the main mechanism whereby insulin antagonizes catecholamine-induced lipolysis. The signaling pathway leading to activation of the PDE3B involves the insulin receptor, insulin receptor substrates, phosphatidyl inositol-3 kinase, and probably protein kinase B (also known as Akt) (115, 128, 129, 179, 180).

Ultimately, the rate of lipolysis in the adipose tissue is dependent on the phosphorylation state of at least two PKA target proteins: HSL and perilipin. The next

sections deal with current knowledge regarding molecular mechanisms underlying lipolysis activation.

Regulation of HSL by Reversible Protein Phosphorylation

Phosphorylation by Protein Kinase A Phosphorylation of partially purified HSL by PKA, leading to a moderate activation of the lipase activity in vitro, was described in the early 1970s (79, 80). Subsequent phosphopeptide mapping and phosphoamino acid analysis suggested that HSL was phosphorylated on a single serine residue, named the regulatory site (159). Partial amino acid sequencing of phosphopeptides generated from bovine HSL (49), together with determination of the primary structure of rat HSL (73), allowed identification of Ser-563 as the regulatory site. However, more recent data have dramatically challenged this view on the short-term regulation of HSL. The finding that mutation of the "regulatory" serine did not abolish PKA-induced activation of HSL led to the identification of two novel PKA sites, Ser-659 and Ser-660, that are responsible for in vitro activation of HSL (2). It is noteworthy that these sites are phosphorylated in intact primary adipocytes in response to isoproterenol stimulation (2). The role of phosphorylation of Ser-563 remains elusive.

Phosphorylation by Other Kinases Apart from the three PKA phosphorylation sites described above, HSL is phosphorylated in vivo in hormonally quiescent cells at a site named the basal site (161), corresponding to Ser-565 in rat HSL (49, 73), i.e. two residues C-terminal to Ser-563. Glycogen synthase kinase-4 (118), Ca^{2+}/calmodulin-dependent kinase II, and AMP-activated protein kinase (AMPK) (48) phosphorylate Ser-565 in vitro without any direct effect on enzyme activity. AMPK has been proposed to be the physiologically relevant kinase (48), based on its involvement in other aspects of lipid metabolism and on its proposed role as fuel gauge (for a review, see 59). Furthermore, because phosphorylation by AMPK prevented subsequent phosphorylation of Ser-563 and vice versa, it was proposed that phosphorylation of Ser-565 exerts an antilipolytic role (48). This proposal is supported by experiments showing that preincubation with AICAR (5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside)—an activator of AMPK—causes a moderate reduction of the lipolytic response to catecholamines in primary adipocytes (27, 162). However, the recent finding that Ser-563 is not essential for HSL activation (2) raises some questions regarding this antilipolytic role of Ser-565 phosphorylation. Also, the moderate antilipolytic effect of AICAR should be interpreted with caution because this substance has many cellular effects and has been questioned as a specific AMPK activator (84).

Protein Phosphatases Acting on HSL Two major studies have been devoted to clarification of the action of protein phosphatases (PPs) on HSL (117, 182). Even though the source of both HSL and PPs differed in the two studies, both demonstrated a severalfold higher activity of PP2A and PP2C than PP1 toward PKA sites

(117, 182). The same was true for the basal site, i.e. Ser-565 (117). Because of their higher abundance in adipose tissue, PP2A and PP1 are believed to be the main PPs acting on HSL in vivo. Wood et al (182) suggested a role for PP1 in selective dephosphorylation of the basal site. This, however, was not supported by another study, which showed a higher preference of PP1 for the PKA sites than for the basal site (117). The dephosphorylation of the novel PKA sites, Ser-659 and Ser-660, as well as the possible existence of specific HSL phosphatases, remains to be investigated.

Mechanisms Behind cAMP-Mediated Activation of Lipolysis

Although the molecular mechanisms that underlie activation of lipolysis are not known in detail, a number of recent findings has provided some insight into the process. Stimulation of adipocytes with catecholamines triggers the translocation of HSL from the cytoplasmatic compartment to the surface of the lipid droplets, a phenomenon that has been demonstrated both by subcellular fractionation experiments on primary adipocytes (33, 64) and by direct immunocytochemistry on 3T3-L1 adipocytes (15). The process seems to be more complex than a simple conformational change of HSL, leading to a higher affinity for the lipidic components of the droplet. In vitro, even nonphosphorylated HSL binds readily to phospholipid vesicles (72) and mixed phospholipid/diglyceride monolayers (69). However, in intact cells, the interaction with the lipid droplets only takes place on stimulation of the cell and phosphorylation of HSL. A mutant variant in which the newly described phosphorylation sites (Ser-659, Ser-660) are replaced by alanines is unable to translocate (C-L Su et al, manuscript in preparation). By analogy with other lipases, it can be speculated that HSL may exist in two conformational states, an active form—corresponding to the "open" forms exposing a large hydrophobic area observed in other lipases—and an inactive ("closed") form. In intact cells, phosphorylation of HSL would be required to trigger the transition from the closed to the open form, exposing a hydrophobic area that would interact with the lipids. In vitro, HSL may be permanently stabilized in the open conformation by the nonionic detergent required to keep the enzyme soluble and active in its purified form—or by the abundant tensioactive molecules present in cell homogenates—thus making phosphorylation unnecessary. This mechanism of stabilization of the open conformation by a micelle of tensioactive molecules has been elegantly demonstrated for pancreatic lipase (63).

A complementary mechanism precluding HSL binding to the lipid droplets in nonstimulated intact cells seems to rely on the perilipins. These are a family of closely related proteins located on the surface of the lipid droplets in adipocytes (12, 55). It has been proposed that perilipins may create a protective barrier for the interaction of HSL with the lipids (104). On stimulation of the adipocytes, perilipin phosphorylation would relieve the restraint and allow phosphorylated HSL free access to the lipid droplet substrate, leading to initiation of TG hydrolysis. Compelling evidence for this barrier role of perilipin has recently been provided (156).

Very recently, two proteins have been proposed to interact with HSL: ALBP (149) and lipotransin, a newly cloned droplet-associated protein (164). The interaction of ALBP with HSL may facilitate rapid evacuation of lipolytic products. Lipotransin has been proposed to dock HSL to the lipid droplet surface and to play a role in the arrest of lipolysis by insulin (164). The current knowledge and hypotheses regarding molecular mechanisms involved in activation of lipolysis are summarized in Figure 2 (see color insert).

Alternative Mechanisms for Control of Lipolysis and HSL

Several observations indicate the involvement of mechanisms other than those described above in the control of lipolysis and HSL. The contraction-induced activation of HSL observed in skeletal muscle (91), for instance, suggests the involvement of calcium in lipolysis control, possibly via Ca^{2+}/calmodulin-dependent kinase(s). Although early observations pointed to a role of calcium at different levels of the adipocyte lipolytic cascade (1, 32, 146), this has been contradicted by recent reports suggesting that an increase in intracellular calcium concentrations inhibits lipolysis (170, 184). However, it is certainly possible that tissue-specific mechanisms exist for lipolysis control. Studies of rodents have demonstrated that leptin induces lipolysis both in vitro (46, 175) and in vivo (45). The effect was absent in animals lacking a functional leptin receptor, i.e. db/db mice (45, 46) and in Zucker rats (175). It is intriguing to note that fatty acid release does not accompany glycerol release in leptin-induced lipolysis, which suggests simultaneous induction of fatty acid oxidation (175). In these studies, effects on HSL expression were not investigated. Thus it is not known whether the lipolytic effect of leptin is mediated via a phosphorylation-induced activation of HSL, an induction of HSL expression, a combination of both, or some other mechanism. In another study, however, long-term treatment of mice with leptin increased HSL mRNA expression by 30% in white adipose tissue, whereas no effect was seen in brown adipose tissue (145). The leptin signal is presumably transduced via the JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway. Growth hormone is another hormone signaling via the JAK/STAT pathway and with documented lipolytic effects, although with variable efficiency depending on species and experimental system (for a review, see 28). cGMP has been shown to mimic cAMP as an activator of lipolysis (139). This is supported by in vitro studies showing that cGMP-dependent protein kinase phosphorylates and activates HSL (86, 160). cGMP-dependent protein kinase is present at very low levels in adipose tissue (103), and the physiological role of this kinase and cGMP for activation of lipolysis, if any, is not known. Recently, nitric oxide was shown to exert modulatory actions on lipolysis in adipose tissue via cGMP-independent mechanisms (50). The effects were complex, with different redox forms acting as either stimulatory or inhibitory. Finally, a recent investigation demonstrated lipolytic action of glucose-dependent insulinotropic polypeptide on 3T3- L1 adipocytes. The effect appeared to be cAMP mediated and could be blocked by insulin in a wortmannin-sensitive manner (109). A species-specific in-frame skipping of exon 6 occurs during the splicing of human HSL

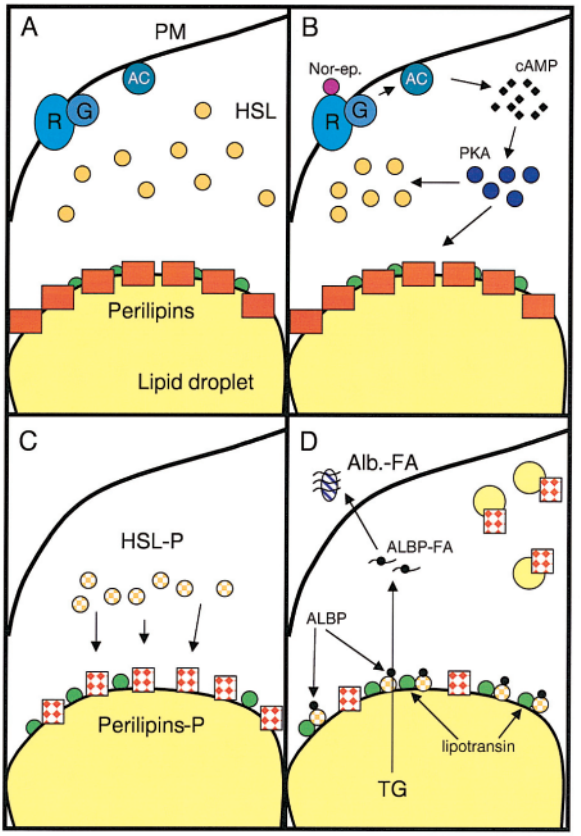

Figure 2 Current hypotheses of the mechanisms of lipolysis regulation. (A) In nonstimulated adipocytes, lipolysis is minimal because of the lack of phosphorylation of hormone-sensitive lipase (HSL) and because of a barrier created by perilipins on the surface of the lipid droplet. (B) Catecholamine stimulation triggers an elevation of cAMP concentration that activates protein kinase A (PKA), resulting in the phosphorylation of HSL and the perilipins. (C) Perilipin phosphorylation probably relieves the restraint and allows the translocation of phosphorylated (P) HSL to the lipid droplet. (D) Interaction of HSL with adipocyte lipid-binding protein (ALBP) favors a rapid evacuation of the fatty acids resulting from the hydrolysis of triglycerides (TG). An interaction between phosphorylated HSL and lipotransin occurs at the surface of the lipid droplet. Whether this interaction is required for the docking of HSL to the droplets or plays a role in the subsequent inhibition of lipolysis by insulin is not known.

mRNA. Even though the resulting protein lacks both lipase and esterase activity, it is possible that this HSL variant plays a role in lipolysis regulation (98).

LONG-TERM REGULATION OF HSL

The Regulatory Regions of the HSL Gene and Chromosomal Mapping

As mentioned above, a complex picture of the HSL gene, with several upstream exons and alternate promoters that are used in a tissue-specific manner, has begun to emerge. However, information regarding regulatory elements is still scarce. The minimal promoter controlling the HSL expression in human adipocytes has been characterized. However, the *cis*-regulatory elements conferring the tissue specificity do not appear to reside within the 2.1 kb of sequence immediately upstream of exon B (56). Regarding the expression in testes, Blaise et al (11) recently showed that tissue specificity is governed by the 0.5 kb immediately upstream of exon T (11). Several other potential elements for tissue-specific expression, as well as other regulatory regions, have been described both in the mouse (102) and the human gene (168). However, the functionality of these elements remains to be investigated.

The human HSL gene has been mapped to a locus at the long arm of chromosome 19, designated the LIPE locus (19q13.1–19q13.2) (73, 97, 100, 148). The mouse HSL gene has been mapped to the homologous region on chromosome 7 and is distinct from the two mouse obesity loci, Ad (adult obesity and diabetes) and Tub (tubby), found in this region (176).

Factors Controlling HSL Expression

Existing adipocyte-like cell lines, such as 3T3-L1, 3T3-F442A, and BFC-1, together with primary adipocyte cultures, have been used to investigate the effect of different nutritional and hormonal factors on HSL expression. cAMP and phorbol esters have been found to decrease HSL expression in mature 3T3-F442A and BFC-1 adipocytes via two apparently distinct mechanisms (124). It was also reported that insulin, growth hormone, and retinoic acid (124) and oleic acid (3, 126) had no effect on HSL expression in mature 3T3-F442A adipocytes. In a study on isolated rat adipocytes in culture, dexamethasone increased HSL mRNA fourfold, whereas adrenaline and growth hormone had no effect (151). The effect of dexamethasone contrasts with the study by Plée-Gautier et al (124), who reported no effect by dexamethasone alone, but a potentiation of the cAMP effect. The reason for the discrepancy is not known but may be attributed to the different experimental systems. Glucose deprivation has been shown to reduce HSL mRNA levels by a factor of 2.5–3 in 3T3-F442A cells (126), whereas another study, performed in primary cultures of rat adipocytes, showed no effect of glucose alone but an up-regulation in response to a combination of high glucose and insulin (13). Again, the discrepancy may be attributed to differences in experimental systems. The physiological significance of the glucose/insulin effects on HSL

expression remains to be determined, but it may reflect an adaptation to nutritional changes.

The role of the cytokine tumor necrosis factor alpha (TNFα), implicated in obesity and obesity-linked insulin resistance (77), on HSL expression has been addressed in several studies. An early study showed a down-regulation of HSL gene expression on TNFα treatment of 3T3-L1 cells, as measured by Northern blot analysis and enzyme activity measurements (163). A similar effect, although much more moderate, was seen at the protein level in a more recent study (156). In a study on primary rat adipocytes, however, no alteration of the levels of HSL protein occurred on treatment with TNFα (54). The down-regulation of HSL observed in 3T3-L1 cells may reflect the known dedifferentiating effect of TNFα in these cells (142), whereas the lipolytic effect of TNFα could be explained by the decrease in LPL activity and intracellular reesterification rates (78) and/or down-regulation of perilipin expression (156, 157).

Regulation of HSL in Different Physiological Situations

In several physiological situations, such as pregnancy, lactation, hibernation, and fasting, a reciprocal regulation between HSL and LPL in adipose tissue has been demonstrated. Studies performed in pregnant rats have shown that during the anabolic phase, HSL-to-LPL mRNA and activity ratios are low, whereas this ratio increases during the catabolic phase (106). The same study showed that during lactation, HSL expression returned to control levels, whereas the LPL mRNA levels remained reduced. Moreover, Vernon et al (174) have suggested that the decrease in lipolytic response to catecholamines that occurs on natural weaning or litter removal is due to a reduced efficiency in the translocation of HSL, rather than to alterations in the HSL activity itself.

During the hibernation period of the yellow-bellied marmot (*Marmota flaviventris*), the HSL-to-LPL mRNA ratio was found to be twice the ratio during the summer and fall (181). Thus, LPL mRNA levels were high during the mass-gain phase, whereas HSL expression was significantly up-regulated during the fasting hibernating period.

Fasting induces an increase in plasma nonesterified fatty acids (NEFA) because of the combination of accelerated rate of lipolysis and decreased rate of fatty acid reesterification (39). LPL activity in adipose tissue decreases during fasting, resulting in channeling of fatty acids to heart and skeletal muscle, where LPL activity is up-regulated (8, 31, 119). Sztalryd & Kraemer (165) showed that in rats, HSL activity remained relatively unchanged up to day 3 of fasting, whereas a twofold increase in HSL activity, as well as HSL mRNA and protein levels, was observed during prolonged starvation. Thus, increase in NEFA levels during short-term fasting appears to involve either posttranslational control of HSL or the regulation of other proteins, whereas during long-term fasting pretranslational mechanisms seem to predominate.

In humans, most of the recent studies in this field have focused on obese subjects subjected to dietary calorie restriction. An 8- to 12- week weight reduction program

for women with upper-body obesity was shown to increase lipolytic noradrenaline sensitivity and decrease basal lipolysis rate (136). These changes were attributed to an increase in β2-adrenergic receptor sensitivity and a decrease of HSL activity, respectively (136). Klein et al (88) also showed that subjects with severe and upper-body obesity have decreased basal whole-body lipolytic rates after a diet-induced weight loss and decreased expression of HSL in subcutaneous abdominal fat deposits. On the other hand, in obese female monozygotic twins subjected to a 4-week very-low-calorie diet, basal and stimulated lipolysis, as well as HSL protein and activity levels in subcutaneous abdominal fat deposits, were increased twofold (158).

Different Adipose Tissue Depots

The regional distribution of body fat seems to be important with regard to the involvement of obesity in metabolic disorders and cardiovascular diseases (for a review, see 14). In particular, accumulation of visceral fat is associated with a higher risk for cardiovascular disease (9) and precedes the development of insulin resistance (57). Lipolytic activity of fat cells has been shown to vary according to the anatomic location of fat deposit (for a review, see 90). In humans, isolated omental adipocytes exhibit a higher lipolytic response to catecholamines than do subcutaneous adipocytes (107, 120d). The underlying mechanisms are poorly understood, but differences in the expression of β-adrenergic receptors (60) and variations in the balance of α2- and β-adrenergic receptors (107) could be contributing factors. In contrast to the general notion of higher lipolytic responsiveness of omental compared with subcutaneous adipocytes, a study addressing the issue of HSL expression in different depots showed maximal lipolytic capacity, as well as HSL mRNA and activity levels, that were higher in subcutaneous than in omental fat cells (133). Another study, however, showed no difference in HSL mRNA levels between subcutaneous and omental adipose tissue (99a).

In rats, higher rates of lipolysis were observed in internal fat deposits (epididymal, retroperitoneal) than in subcutaneous fat deposits (dorsal-subcutaneous). These differences seemed to be due to variations in the expression of HSL because HSL mRNA, protein, and activity were twofold higher in internal adipocytes than in subcutaneous adipocytes (166). Other studies have also showed a lower lipolytic response in rat subcutaneous adipocytes, which was related to lower levels of HSL activity (110) and HSL mRNA (169).

HSL AND PATHOPHYSIOLOGY

Familial Combined Hyperlipidemia

Familial combined hyperlipidemia (FCHL), characterized by elevated apolipoprotein (apo)B levels, hypertriglyceridemia, and/or hypercholesterolemia in multiple individuals within one family, is a common metabolic disorder (prevalence 0.5%) of unknown etiology. A lipolytic defect, associated with reduced HSL

activity, has been observed in subcutaneous adipocytes from men with FCHL (135). LPL activity, however, was unaltered (132), which is inconsistent with other studies showing reduced LPL activity in FCHL patients (5, 47). Several studies have also reported defects in the LPL gene or in the promoter of the LPL gene in patients with FCHL (65, 185, 186). However, a recent study of Finnish families, with members suffering from FCHL-like dyslipidemia, showed that the genes encoding HSL, LPL, and hepatic lipase do not represent major loci influencing the expression of FCHL phenotype (121). In similar studies, no linkage between FCHL and the genes for apoB, apoA1, apoE/CI/CII, LPL and high-density lipoprotein–binding protein was observed (4, 144). Hence, the reduced HSL activities in FCHL is most likely secondary to an unknown primary defect.

The Metabolic Syndrome and Diabetes

It has been suggested that elevated circulating FFA, a major feature observed in subjects with type II diabetes and/or the metabolic syndrome, is due to a dysregulation of adipose tissue function (40, 130). Because of its role in fatty acid mobilization, HSL is among the candidates for such a dysregulation, and furthermore, HSL has been suggested as a target for the development of new antidiabetic drugs. In adipocytes from elderly male subjects with several manifestations of the metabolic syndrome, a marked resistance to the lipolytic effect of noradrenaline due to a decrease in the number of β2-adrenergic receptor was observed (134). In addition, the maximum lipolytic effects of isoproterenol, forskolin, and dibutyryl cyclic AMP were also markedly decreased, which suggests alterations at the level of the PKA/HSL complex (134). However, in a more recent study, HSL activity measured in vitro was found not to differ significantly between patients suffering from the metabolic syndrome and healthy, nonobese subjects (132).

With regard to genetic studies, a polymorphism in exon 4 of the HSL gene, changing an arginine to a cysteine, showed no difference in allele frequency between type II diabetic patients and healthy subjects (150). In another study, a polymorphic marker in intron 7 of the human HSL gene showed a significant difference in allele frequency distribution between type II diabetic patients, in particular abdominally obese type II diabetics with the metabolic syndrome, and control subjects (87). Although in agreement with another study, showing positive association between a different polymorphic marker in the HSL gene and type II diabetes (105), transmission disequilibrium tests suggested linkage disequilibrium between the HSL gene marker and an allele or gene that increases susceptibility to abdominal obesity and thereby possibly type II diabetes (87). In conclusion, although most available data seem to argue against a direct involvement of HSL aberrations in the development of the metabolic syndrome/type II diabetes, more studies are required to clarify this issue.

Obesity

In vivo and in vitro studies have shown that the lipolytic effect of catecholamines is blunted in obese subjects (20, 81, 137). This could be attributed to a decreased number of β2-adrenergic receptors, changes in the functional balance between α2- and β-adrenergic receptors, or postreceptor alterations (108, 137). In adipocytes from normal-weight subjects with obesity among first-degree relatives, Hellström et al (61) showed that the impaired lipolytic action of catecholamines was due mainly to reduced HSL activity. What is curious is that HSL mRNA levels were unaffected, which suggests the involvement of posttranscriptional mechanisms (61). Similarly, a very recent study showed that HSL mRNA, protein, and activity levels, as well as the maximum lipolytic capacity, were decreased in subcubtaneous adipocytes of obese subjects compared with healthy controls. The effects were less pronounced at the mRNA level than at the protein level (96). Several important conclusions can be drawn from these studies. First, HSL expression is a major determinant of the maximum lipolytic capacity of human adipocytes, which is further supported by a study performed on identically sized adipocytes with either low or high lipolytic capacity (95). Second, both transcriptional and posttranscriptional mechanisms appear to be involved in determining the amount of enzymatically active HSL in human adipocytes. Third, a dysfunction in lipolysis due to impaired HSL expression may play an important role in the development of obesity and may precede the actual development of obesity. In agreement with the latter proposal, a genetic association study showed a difference in allele frequency distribution for a polymorphic marker in intron 6 between obese subjects and healthy controls (105).

Cancer

In cancer patients, altered lipid metabolism associated with hyperlipidaemia, depletion of lipid from adipose tissue, and progressive weight loss is frequently observed. A study performed on adipose tissue of cancer patients showed a twofold increase in HSL mRNA that correlated with an increase in NEFA levels (171). However, the factors responsible for this increased expression of HSL are not known.

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