thematic review

Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis

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Abstract Hormone-sensitive lipase (HSL) is an intracellular neutral lipase that is capable of hydrolyzing triacylglycerols, diacylglycerols, monoacylglycerols, and cholesteryl esters, as well as other lipid and water soluble substrates. HSL activity is regulated post-translationally by phosphorylation and also by pretranslational mechanisms. The enzyme is highly expressed in adipose tissue and steroidogenic tissues, with lower amounts expressed in cardiac and skeletal muscle, macrophages, and islets. Studies of the structure of HSL have identified several amino acids and regions of the molecule that are critical for enzymatic activity and regulation of HSL. This has led to important insights into its function, including the interaction of HSL with other intracellular proteins, such as adipocyte lipid binding protein. Accumulating evidence has defined important functions for HSL in normal physiology, affecting adipocyte lipolysis, steroidogenesis, spermatogenesis, and perhaps insulin secretion and insulin action; however, direct links between abnormal expression or genetic variations of HSL and human disorders, such as obesity, insulin resistance, type 2 diabetes, and hyperlipidemia, await further clarification. The published reports examining the regulation, and function of HSL in normal physiology and disease are reviewed in this paper.—Kraemer, F. B., and W-J. Shen. **Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis.** ***J. Lipid Res.* 2002. 43: 1585–1594.**

Supplementary key words lipolysis • free fatty acids • adipose • adrenal • macrophage • testis • islet

Hormone-sensitive lipase (HSL) activity was first identified as an epinephrine-sensitive lipolytic activity in adipose tissue. Its name was coined to reflect the ability of hormones such as catecholamines, ACTH, and glucagon to stimulate the activity of this intracellular neutral lipase (1). Hormonal activation of HSL occurs via cyclic AMP dependent protein kinase (PKA), which phosphorylates HSL (2). As the enzyme responsible for the release of free fatty acids (FFA) from adipose tissue, HSL is felt to play a pivotal role in providing the major source of energy for most tissues. Although its expression is highest in adipose tissue, HSL is also expressed in adrenal, ovary, testis, and to a lesser extent in skeletal and cardiac muscle and macrophages (3, 4). Following the purification of the enzyme and the cloning of the cDNA encoding HSL, many research efforts have focused on understanding the activity, regulation, expression and function of this protein.

STRUCTURAL AND BIOCHEMICAL PROPERTIES

The HSL gene is located on chromosome 19q13.3 (3) and was initially described to contain 9 exons spanning approximately 11 and 10 kB in human (5) and mouse (6), respectively, that encode an mRNA of ~2.8 kB (7). Subsequently, two additional exons (termed A and B) that differentially encode 170 and 70 nt 5′ untranslated regions were identified approximately 12.5 and 1.5 kB upstream of exon 1, respectively (7). Only the smaller HSL mRNA product is expressed in human adipose tissue. In contrast, five different exons have been reported within 7 kB of the translation start site of exon 1 in mouse HSL, each of which can be alternatively utilized and expressed in mouse adipose tissue to varying degrees (8). In addition to these alternative exons encoding 5′ untranslated regions, several isoforms of HSL have been reported. A testis specific exon 15.5 kB upstream of exon 1 of human adipocyte HSL yields a 3.9 kB testicular HSL mRNA and encodes a larger protein (9). A second testis specific exon was identified ~12 kB upstream of exon 1 and encodes a protein identical to adipocyte HSL (10). β cells may have a specific exon (11) or an alternate translation start site may be 7 kB upstream of exon 1 (8).

The purified rat enzyme has a molecular weight of approximately 84,000 Da on SDS-PAGE, corresponding to

Abbreviations: ALBP, adipocyte lipid-binding protein; ERK, extracellular signal-regulated kinase; FFA, free fatty acids; HSL, hormone-sensitive lipase; PKA, cyclic AMP dependent protein kinase.

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the 768 amino acid protein with a molecular size of 82,820 Da predicted from the primary translation product of rat HSL cDNA (3). The human HSL cDNA encodes a 775 amino acid protein with a molecular size of 84,032 Da, which corresponds to an 88 kDa immunoreactive protein seen on SDS-PAGE (5). However, a truncated, catalytically inactive form of HSL due to alternative splicing which eliminates exon 6 has been described in human, but not rat, adipose tissue (12). At least three additional isoforms of HSL have been reported. The testis appears to express two isoforms, one encoded by the larger testicular mRNA produces a protein with an additional 300 (rat) or 301 (human) amino acids N-terminal to the normal adipose form (9). So in addition to an 84 kDa protein that is similar to adipose HSL, a second larger isoform of ~120–130 kDa is encoded by a unique testis mRNA (10). Islets and β cells have an HSL isoform that contains an additional 43 amino acids N-terminal to the normal adipose form (11). HSL exists as a functional dimer composed of homologous subunits; dimeric HSL has greater hydrolytic activity when compared with monomeric HSL but no difference in substrate affinity (13).

The primary sequence of HSL is unrelated to any of the other known mammalian lipases; however, it shares some sequence homology with lipase 2 of an antarctic bacterium, *Moraxella* TA144 (5). This homology aided in locating a G-X-S-X-G motif, which represents a consensus lipid binding sequence that contains the active site serine in other lipases, such as pancreatic lipase. This region was proposed (14), and later shown by site-directed mutagenesis (15), to contain the catalytically active serine at position 423 in rat HSL. Limited proteolysis and denaturation studies (16) suggested that HSL, like other lipases (17) contains two major domains (**Fig. 1**). The N-terminal 320 amino acid domain is encoded by exons 1–4 and has no primary or secondary structural similarity with known proteins; however, the N-terminal domain has been shown to interact with adipocyte lipid-binding protein (ALBP) and has been proposed to function as a docking domain for protein-protein interactions (18). The C-terminal portion of HSL is similar to acetylcholinesterase, bile salt-stimulated lipase and several fungal lipases, and is composed of α/β-hydrolase folds that accommodate the catalytic site. A significant advance in understanding the structure of HSL was made when it was observed that, even in the absence of primary sequence homology, the organization of the secondary structure predicted for the C-terminal ~450 amino acids of HSL was similar to the secondary structure of acetylcholinesterase and of two fungal lipases from *Geotrichum candidum* and *Candida rugosa* (19). Using molecular modeling, it was proposed, and later confirmed by site-directed mutagenesis, that Ser-423, Asp-703, and His-733 (numbered for rat HSL) constitute the catalytic triad for HSL and are found within this C-terminal portion (20). Also located within the C-terminal portion is a 150 amino acid stretch that is not predicted to be composed of α helices or β sheets, but contains known phosphorylation sites and has been termed the regulatory module.

HSL has broad substrate specificity; in addition to triacylglycerol, HSL can also catalyze the hydrolysis of diacylglycerol, 1(3) monoacylglycerol, cholesteryl esters, lipoidal esters of steroid hormones, and retinyl esters in adipose tissue, as well as water-soluble butyrate substrates (21–24); however, in contrast to many other lipases, HSL has no phospholipase activity. The activity against diacylglycerol is about 10-fold and 5-fold higher than the activity against triacylglycerol and monoacylglycerol, respectively, whereas the activity against cholesteryl esters is about twice the activity toward triacylglycerol. The esterase activity against water-soluble substrates is more than 20-fold that of triacylglycerols. HSL shows a preference for the *sn* 1- or 3-ester bond over the *sn* 2-ester bond as its substrate, with the relative activity against the *sn* 3-ester bond three to four times higher than the *sn* 2-ester bond. Although fatty acids appear to be more readily mobilized from adipose cells as their chain length shortens (between 12–24 carbons) and as their degree of unsaturation increases, examination of the ability of recombinant HSL to release individual fatty acids from triacylglycerol substrates in vitro does not support a large contribution of HSL to this selective mobilization (25). Nonetheless, the relative hydrolysis of 12–24 carbon atom saturated fatty acids by HSL does increase with decreasing chain length (26), and there is a tendency for a decrease in release as the number of unsaturated bonds increases, except for C20 fatty acids (25). HSL may preferentially hydrolyze oxidized cholesteryl esters (at least 13-HODE cholesteryl ester) compared with cholesteryl linoleate (27).

One of the unique features of HSL that differentiates it from most other lipases is that its activity against triacylglycerol and cholesteryl ester substrates appears to be regulated by reversible phosphorylation; however, hydrolytic activity against diacylglycerol, monoacylglycerol and water-soluble substrates is unaffected by phosphorylation (2). PKA increases the hydrolytic activity of HSL by phosphorylation of a single site that was initially identified as S563 in rat HSL (2) and is located within the regulatory module (Fig. 1). Although evidence to support the phosphorylation of S563 by PKA has been provided from mutagenesis experiments (28), other investigators have reported that S659 and S660 were phosphorylated by PKA in vitro and were required for the phosphorylation-induced increase in hydrolytic activity against triacylglycerol substrate (29). Additionally, lipolytic hormones not only can activate PKA, but also the mitogen activated protein kinase pathway and extracellular signal-regulated kinase (ERK). Activation of the ERK pathway appears to be able to regulate adipocyte lipolysis by phosphorylating HSL on S600 and increasing the activity of HSL (30). In contrast to activation of activity seen with PKA or ERK phosphorylation, other kinases such as glycogen synthase kinase-4, Ca^{++}/calmodulin-dependent protein kinase II, and AMP-activated protein kinase phosphorylate HSL at a secondary basal site S565 in rat HSL (2). Phosphorylation at S565 impairs the phosphorylation of S563 by PKA (2). HSL activity can be inactivated by protein phosphatases. The most active phosphatases against S563 are phosphatase 2A

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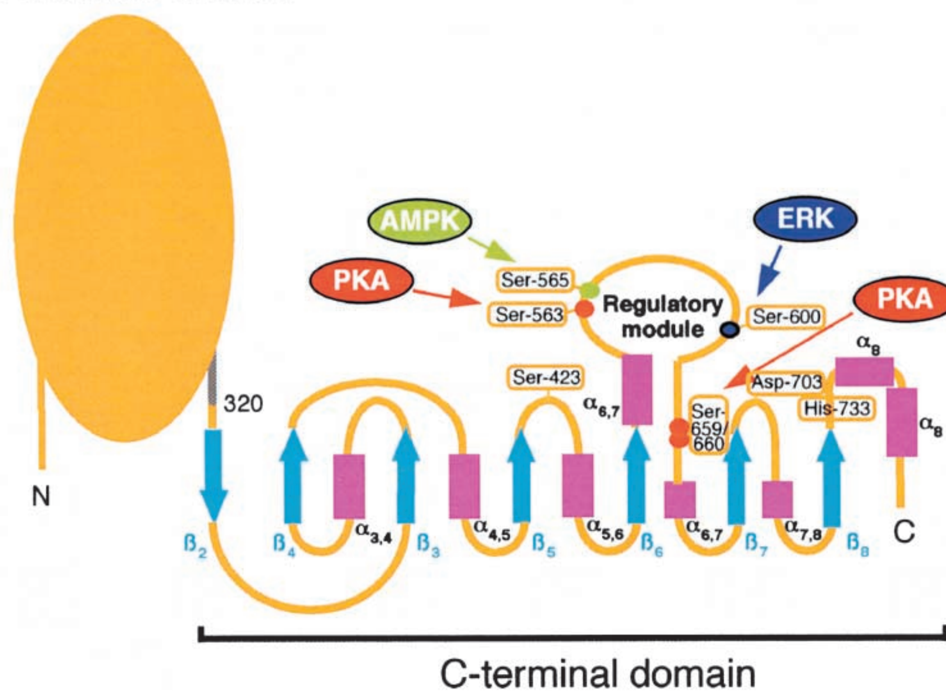

Fig. 1. Schematic structure of rat HSL. The N-terminal 320 amino acids are depicted as a globular structure since its precise structure cannot currently be modeled. The C-terminal portion forms an α/β hydrolase structure and contains the catalytic triad: Ser-423, Asp 703, His-733. The α helices are denoted in purple and the β sheets in cyan. The C-terminal portion is interrupted by the 150 amino acid "regulatory module" which contains important serine residues that can be phosphorylated by the different kinases shown.

and 2C, while S565 is predominately dephosphorylated by phosphatase 2A (31). Thus, several different kinases phosphorylate HSL at unique serines within the regulatory module and modulate HSL activity.

PHYSIOLOGICAL FUNCTIONS

Adipose tissue

The primary action attributed to HSL is hydrolysis of stored triacylglycerols in adipose tissue, i.e., lipolysis. The control of lipolysis is complex and involves multiple mechanisms (32, 33). These include lipolytic (β-adrenergic agonists, ACTH, etc.) and anti-lipolytic (insulin, adenosine, etc.) hormones, their cognate receptors and signaling pathways, lipid droplet-associated proteins such as perilipins, as well as HSL or other as yet unidentified lipases. In addition to the activation of HSL hydrolytic activity, other mechanisms involving HSL have been suggested to account for lipolysis. Evidence has been provided using subcellular fractionation to show that catecholamine-induced stimulation of lipolysis in vitro in 3T3-L1 adipocytes or in rat adipose cells is due to the translocation of phosphorylated HSL from an aqueous cytosolic compartment to the lipid droplet (34, 35). Using immunofluorescence microscopy, HSL was observed diffusely distributed throughout the cytosol of 3T3-L1 adipocytes and, upon catecholamine stimulation, HSL translocated from the cytosol to the surfaces of intracellular lipid droplets concomitant with the onset of lipolysis (36). It has been suggested that translocation of HSL to the lipid droplet is the critical event in regulating lipolysis induced by a variety of lipolytic agents, such as isoproterenol, forskolin, cyclic AMP, theophylline, and okadaic acid (37, 38); however, this is not true for all physiological conditions, since translocation of HSL was not observed with lipolytic stimulation in adipocytes from old (35) or lactating rats (39). Although the mechanisms mediating the translocation of HSL have not been well studied, disruption of microtubules or microfilaments appears to have minimal effects on isoproterenol-stimulated glycerol release and no visible effects on the translocation of HSL determined by immunofluorescence light microscopy (36). However, a protein (lipotransin) was identified in a yeast two-hybrid screen that interacts with HSL and was proposed to be a potential participant in the process of the translocation of HSL to the lipid droplet (40). Lipotransin is homologous to p60 katanin and is a member of the AAA protein superfamily, possessing ATPase and microtubule severing activities (41). The function of lipotransin in interacting with HSL and influencing lipolysis has yet to be elucidated. A proposed model for hormone-induced lipolysis is depicted in **Fig. 2**.

Fatty acids and monoacylglycerol exert product inhibition on HSL activity. This is interesting in light of the ob-

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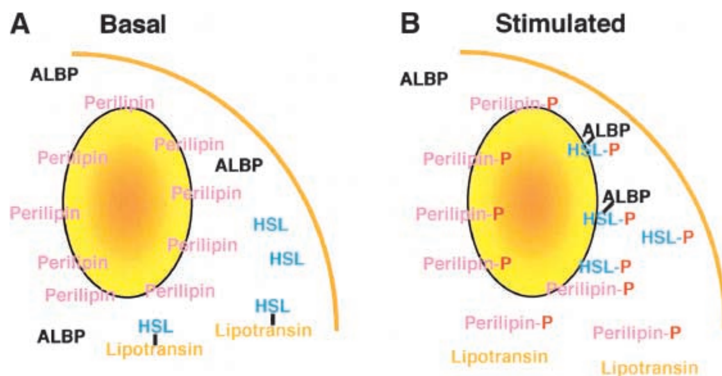

Fig. 2. Model of the mechanism of hormone-stimulated lipolysis. Under basal conditions HSL is not associated with the lipid droplet and is perhaps tethered to lipotransin, while perilipin decorates the lipid droplet and hinders access of the droplet to HSL. ALBP and other fatty acid binding proteins are found abundantly in the cytosol. Following hormonal stimulation, HSL and perilipin are phosphorylated and HSL translocates to the lipid droplet. ALBP binds to HSL, preventing fatty acid inhibition of the enzyme's hydrolytic activity, and sequesters and transports the released fatty acids.

servation that HSL specifically interacts with ALBP, a member of the family of intracellular lipid-binding proteins which bind fatty acids and other hydrophobic ligands (18). Mutational analysis has identified several amino acids within the N-terminal domain of HSL (H194 and E199) as critical for mediating the interaction of HSL with ALBP (42). Incubation or co-expression of ALBP with HSL increased substrate hydrolysis, which was lost when the binding of ALBP to HSL was disrupted by mutagenesis. In addition, the ability of fatty acids to inhibit HSL hydrolytic activity was attenuated by co-incubation with ALBP. These observations suggest that ALBP and HSL constitute a lipolytic complex that increases the hydrolytic activity of HSL through the physical interaction of HSL with ALBP, and because ALBP sequesters fatty acids and prevents product inhibition. This is consistent with experiments in ALBP null mice where basal and isoproterenol-stimulated lipolysis are decreased ~40% (43).

Indirect evidence has suggested that HSL is the rate limiting enzyme in intracellular lipolysis; overexpression of HSL in 3T3-F442A cells prevents differentiated adipocytes from accumulating triglyceride (44). Recently, the functional significance of HSL in adipose tissue metabolism has begun to be clarified in studies using HSL null mice (45–47). Inactivation of HSL by homologous recombination resulted in the complete absence of neutral cholesteryl ester hydrolase activity in adipose tissue (both white and brown); however, triacylglycerol lipase activity in white adipose tissue was reduced by only 40% and triacylglycerol lipase activity in brown adipose tissue was similar to wild-type mice (45). Basal lipolysis, i.e., glycerol release, was reduced in isolated adipose cells from HSL null mice in one study (45), but was unaffected and perhaps increased in another (46). Nonetheless, there was a marked defect or complete absence of catecholamine-stimulated glycerol release in adipose cells from HSL null mice (45, 46), whereas catecholamine-stimulated FFA release was still observed, but attenuated (45). This apparent discrepancy in the release of glycerol and FFA from adipose cells of HSL null mice has been clarified by the observation that diacylglycerol content increased markedly in adipose tissue of HSL null mice (47). In both white and brown adipose tissue from HSL null mice, catecholamine-stimulation caused the release of small amounts of FFA without any stimulated glycerol release, and a marked accumulation of diacylglycerol (47). Therefore, studies with HSL null mice appear to substantiate that HSL is the rate-limiting enzyme for diacylglycerol hydrolysis in adipose tissue and is essential for hormone stimulated lipolysis. The absence of HSL is not associated with the development of obesity; however, adipose cells from HSL null mice, while displaying size heterogeneity, tend to be hypertrophic (45, 46). Moreover, due to defective lipolysis during fasting, there is a reduction in circulating FFA and a decreased hepatic production of VLDL triglyceride secondary to the diminished release of FFA from adipose tissue (48). This is associated with an induction of LPL in white adipose tissue, as well as skeletal and cardiac muscle, but a decrease of LPL in brown adipose tissue (48).

Although HSL is primarily regulated by post-translational mechanisms, pretranslational mechanisms are important under some physiological settings. The expression of HSL protein and mRNA levels are lower in subcutaneous fat stores compared with internal fat depots in the rat (49), suggesting a possible basis for the differences in the rate of lipolysis among various fat depots. In contrast, subcutaneous fat in humans was reported to have higher HSL mRNA expression and HSL activity than omental fat. Human subcutaneous fat cells are larger and there is a positive correlation between fat cell size and HSL expression (50). When controlled for adipocyte cell size, the amount of HSL protein and HSL mRNA levels in subcutaneous adipocytes show a strong correlation with maximum lipolytic activity (51). A positive relationship between fat cell size and HSL expression was also seen with high fat feeding in rats, where fat feeding was associated with an increase in adipocyte cell size and an increase in both basal and stimulated HSL activity (52). In contrast, following several days of food deprivation in the rat, there is a ~2-fold increase in HSL activity, immunoreactive protein, and mRNA levels in adipose tissue that is not observed with short term fasting and is associated with a reduction in fat cell size (53). HSL mRNA levels have also been shown to be increased in adipose tissue in hibernating marmots during their time of fasting (54). Moreover, HSL activity, immunoreactive protein, and mRNA levels in adipose tissue were increased in response to streptozotocin-induced insulin deficiency in the rat, whereas short-

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term treatment with insulin returned HSL activity to normal without altering the increased amounts of HSL immunoreactive protein and mRNA (55). Thus, HSL activity appears to be regulated by pretranslational mechanisms under prolonged conditions, while short-term treatment with insulin controlled HSL by post-translational mechanisms. Using primary rat adipocytes, epinephrine, glucagon, growth hormone, and dexamethasone were all found to increase HSL activity, i.e., lipolysis, but only dexamethasone caused an increase in HSL mRNA levels (56), supporting a role for both post-translational and pretranslational mechanisms in controlling HSL expression and lipolysis. Glucose deprivation in adipocytes results in a decline in HSL expression, whereas incubation with glucose and insulin maintains HSL expression during adipocyte culture and results in an increase of basal and stimulated lipolysis (57). Recently, a glucose-responsive region was mapped within the proximal promoter of human HSL and the involvement of upstream stimulatory factor 1 and 2 binding to a consensus E-box within this region was shown to be responsible for transcriptional regulation in response to glucose metabolism between glucose-6-phosphate and triose phosphates (58). In contrast to induction of HSL, TNFα causes a marked reduction in HSL gene expression (59), while stimulating lipolysis in adipocytes; the mechanisms appear to involve other components of the lipolytic machinery, particularly perilipin (60).

OTHER TISSUES

Macrophages

HSL is definitely expressed in murine macrophages and macrophage cell lines (61, 62); however, its expression in human monocytes and macrophages has been controversial, with some studies unable to detect HSL (63, 64) and others documenting low levels of HSL expression (65, 66) or suggesting that the testicular HSL isoform is preferentially expressed (67). Further questioning a role for HSL in macrophages, neutral cholesteryl ester hydrolase activity has been reported to be unchanged in peritoneal macrophages isolated from HSL null mice (45, 68), and cholesteryl ester stores in HSL null macrophages are mobilized similar to wild-type macrophages (68), suggesting that HSL is not the major neutral cholesteryl ester hydrolase in macrophages. Nonetheless, it is possible that the findings with HSL null mice are due to a compensatory induction of other neutral cholesteryl ester hydrolases. HSL expression and neutral cholesteryl ester hydrolase activity are co-regulated in murine macrophages. Sterol loading of macrophages decreased HSL immunoreactive protein and neutral cholesteryl ester hydrolase activity (69), whereas insulin has been reported to rapidly decrease and leptin to rapidly increase HSL activity (70). Transgenic (71) or adenovirus-mediated (72) overexpression of HSL in macrophages results in an increase in the hydrolysis of cholesteryl ester stores and an increased cholesterol efflux, along with a decrease in the uptake of lipoproteins via scavenger receptors (72). Paradoxically, however, macrophage-specific transgenic expression of HSL resulted in more advanced atherosclerosis than in control mice fed a high fat, high cholesterol diet (73), perhaps due to indirect effects on inflammation.

Muscle

HSL of 84 kDa size has been reported to be expressed in cardiac (4) and skeletal muscle (74, 75). In skeletal muscle the expression of HSL is higher in oxidative than glycolytic muscle (74, 75), and HSL expression is reduced in 24-month-old rats (76), perhaps contributing to the increase in muscle triglyceride content observed with aging. HSL activity in muscle is stimulated by catecholamines (74, 75) acting via β-adrenergic receptors and cyclic AMP and by contraction acting independently of sympathetic tone or catecholamines (77). Exercise training does not affect the expression of HSL protein in muscle, but decreases the sensitivity of stimulation of muscle HSL activity by epinephrine (78). Heart-specific transgenic overexpression of HSL prevents the accumulation of cardiac triglyceride normally seen in fasted rodents (79). In addition, heart-specific overexpression of HSL alters the expression of cardiac genes for fatty acid oxidation, transcription factors, signaling molecules, cytoskeletal proteins, and histocompatibility antigens. Thus, HSL in cardiac and skeletal muscle plays a role in controlling the accumulation of triglyceride droplets and in energy utilization.

Adrenal

The neutral cholesterol ester hydrolase activity purified from the adrenal has been shown to be identical to HSL purified from adipose tissue (22). Moreover, immunoreactive HSL (4, 80) and HSL mRNA (3, 81) can be detected in adrenal, just as in adipose tissue. Less HSL is expressed in the glomerulosa than the inner cortex, and subfractionation of adrenals showed that immunoreactive HSL was prominently expressed in microsomes, with lesser amounts in the cytosol and little to no HSL in other fractions or the lipid droplet (82). HSL appears to be responsible for the vast majority, if not all, of the neutral cholesterol ester hydrolase activity in the adrenal since adrenals of HSL null mice have $<2\%$ of the neutral cholesterol ester hydrolase activity of wild-type mice (82). Although basal corticosterone levels are normal, the absence of HSL resulted in a reduction in corticosterone response to ACTH, suggesting that the actions of HSL are involved in the delivery of cholesterol for steroidogenesis.

Testis

The rat testis expresses a single 3.9 kB HSL mRNA due to a testis specific exon 15.5 kB upstream of exon 1 that encodes a ∼120–130 kDa immunoreactive protein, which contains an additional 300 amino acids N-terminal to the normal adipose form (9). The human testis contains both 3.9 kB and 3.3 kB HSL mRNAs, due to a testis specific exon 15.5 kB upstream of exon 1 that encodes a ∼120–130 kDa immunoreactive protein containing an additional 301 amino acids N-terminal to the normal adipose

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form and due to a second testis specific exon ~12 kB upstream of exon 1 that encodes a protein identical to adipocyte HSL (10). HSL expression in the testis shows marked developmental changes. HSL mRNA is undetectable in testis in the first few weeks of age and increases 25-fold to stable adult levels between 20 and 90 days (81). Whereas HSL activity appears to be regulated almost exclusively by post-translational mechanisms in most other tissues, treatment of sexually immature rats with chorionic gonadotropin increases neutral cholesteryl ester hydrolase activity by increasing HSL mRNA levels and the amount of HSL immunoreactive protein (83). In the rat, HSL is not expressed in Leydig cells, but HSL is localized to Sertoli cells and spermatids after spermiation, where in situ hybridization and immunohistochemistry have shown the lowest intensity of labeling in stages III–VII and the highest intensity of labeling in elongated spermatids in stages X–XIV (9, 84). In contrast, HSL has been observed by immunohistochemistry in Leydig cells, as well as Sertoli cells and sperm in guinea pig (85) and human testes (10) where two HSL mRNA species are expressed. It appears that only the larger 120 kDa isoform is expressed in spermatids (10). The testis-specific and spermatid-specific, expression of HSL has been attributed to a possible germ cell-specific zinc finger transcription factor that binds between −46 and −29 base pairs upstream of the testis-specific promoter (86, 87). In studies of HSL null mice, no immunoreactive HSL was observed in testis and no neutral cholesteryl ester hydrolase activity could be detected in testis of HSL null mice (45). Thus, it appears that HSL is responsible for all of the neutral cholesteryl ester hydrolase activity in testis even though previous reports have suggested that other lipases might be involved. Serum testosterone, LH, and FSH values were similar in HSL null and wild-type mice, which is consistent with HSL not being expressed in Leydig cells in rodents; however, HSL protein and enzymatic activity in interstitial tissue have been positively correlated with testosterone levels during development in the guinea pig (85). The absence of HSL in the testis resulted in a 2-fold increase in testicular cholesteryl ester (45) and diacylglycerol (47) content, as well as severe oligospermia in HSL null mice (45, 88). The sperm that do form display severe morphological abnormalities (88). Therefore, consonant with its expression in germ cells, these observations from HSL null mice suggest that HSL plays an important role in supporting normal spermiogenesis.

Islets

HSL is predominantly expressed in pancreatic islets as a 3.1 kB mRNA encoding an ~89 kDa isoform containing an additional 43 amino acids N-terminal to the adipocyte form of the protein, as well as the 2.8 kB mRNA encoding the 84 kDa adipocyte form (11). HSL immunoreactivity in islets is detected primarily in β cells, but some HSL is also observed in α cells (11). Long term incubation of β cells or islets with high concentrations of glucose increases HSL mRNA and protein expression, as well as HSL activity (89). This induction of HSL expression appears to be regulated transcriptionally and to depend on the metabolism of glucose. Treatment of ob/ob mice with leptin increased HSL expression in islets, along with a decrease in islet triglyceride content and an improvement in insulin secretion (90). HSL is definitely functional in islets, since islet triglyceride content is increased 2–2.5-fold in HSL null mice compared with wild-type mice (91). Although HSL null mice are normoglycemic and normoinsulinemic when evaluated under fed and overnight fasted conditions, they are glucose intolerant and display a lack of rise in insulin in response to a glucose load (91). In addition, isolated islets from HSL null mice secrete higher amounts of insulin when exposed to basal glucose concentrations, but do not release insulin in response to glucose; however, their response to KCl-induced insulin secretion is normal (91). These observations suggest that HSL might play an important role in glucose-induced insulin secretion and are consistent with the current view that fatty acid metabolites are critical for insulin secretion, but that excessive fatty acids can lead to lipotoxicity and dysfunction of β cell (92). The exact function of HSL in insulin secretion from β cells awaits further study.

Pathophysiology

Although HSL has not been identified as a major gene that is responsible for a unique metabolic disease, diminished activity of HSL or genetic polymorphisms of HSL have been described in various metabolic disorders. For instance, maximum enzymatic activity of HSL was reported to be decreased ~40% in 10 patients with familial combined hyperlipidemia in Sweden (93, 94); however, no differences in HSL activity were demonstrated in 48 subjects with familial combined hyperlipidemia in Finland when compared with normolipidemic spouses (95). Moreover, no differences in steady state HSL mRNA levels in adipose tissue (96) or in the frequency of a −60C/G polymorphism in the HSL promoter (97) were observed in Finnish subjects with familial combined hyperlipidemia. Nonetheless, a sib-pair linkage analysis using a CA dinucleotide repeat in intron 7 of HSL demonstrated a linkage between HSL and plasma triglyceride levels in 126 dizygotic pairs of women twins (98). Thus, it is unclear whether variations in HSL expression and activity are related to familial combined hyperlipidemia. Maximum stimulated lipolysis, and by inference HSL, was initially reported to be markedly reduced in adipocytes from subjects with insulin resistance (99); however, this difference was not observed later by the same investigators (94). Three polymorphisms in the coding region of HSL, Arg262Met, Glu620Asp, and Ser681Ile, do not contribute measurably to biological variation of insulin sensitivity (100). Nonetheless, the −60C/G polymorphism in the HSL promoter does appear to have effects on insulin sensitivity with the −60G variant displaying increased insulin stimulated glucose uptake (97) or lower insulin or FFA levels (101).

As with familial combined hyperlipidemia and insulin resistance, there are varying data as to the relationship of HSL with obesity. Thus, HSL protein and mRNA expres-

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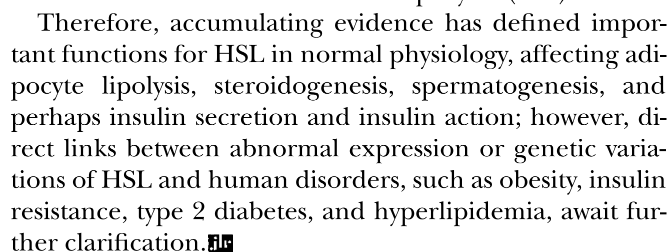
sion, as well as maximum lipolytic capacity of subcutaneous adipocytes, has been reported to be reduced in obese men and women (102). Furthermore, non-obese subjects with a family history of obesity have been reported to have a reduced maximum lipolytic capacity when compared with lean subjects without a family history of obesity (103); however, steady state mRNA levels of HSL were not different (104). Middle-aged men have an increase in percentage body fat compared with young men, and their fat cells have a lower maximal lipolytic response; yet HSL mRNA levels are higher in adipocytes from middle-aged men (105). A CA dinucleotide polymorphism within intron 7 of HSL has been reported to be associated with abdominal obesity in patients with type 2 diabetes (106); however, no linkage was found between an intragenic marker of HSL and morbid obesity (107). In addition, the −60C/G polymorphism in the HSL promoter, which results in a ~40% lower transcriptional activity of the −60G promoter (108), is associated with BMI, fat mass, and percentage body fat in women, but not in men. The −60G polymorphism has a positive relationship in black women and a negative relationship in white women that was lost after adjusting for fasting insulin concentrations (109). Interestingly, a polymorphism of a CA dinucleotide repeat in intron 6 has been reported to be associated with obesity and with type 2 diabetes (110). Importantly, this polymorphism is associated with a 50% decrease in the lipolytic rate of subcutaneous abdominal adipocytes (111).



Therefore, accumulating evidence has defined important functions for HSL in normal physiology, affecting adipocyte lipolysis, steroidogenesis, spermatogenesis, and perhaps insulin secretion and insulin action; however, direct links between abnormal expression or genetic variations of HSL and human disorders, such as obesity, insulin resistance, type 2 diabetes, and hyperlipidemia, await further clarification.

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