

REVIEW ARTICLE

Hormone-sensitive lipase – new roles for an old enzyme

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Although described initially as an intracellular adipocyte-specific triacylglycerol lipase, it is now clear that HSL (hormone-sensitive lipase) is expressed in multiple tissues and plays a number of roles in lipid metabolism, including that of a neutral cholesteryl ester hydrolase. The major isoform is a single polypeptide with a molecular mass of approx. 84 kDa and which comprises three major domains: a catalytic domain, a regulatory domain encoding several phosphorylation sites and an N-terminal domain involved in protein–protein and protein–lipid interactions. The activity of HSL is regulated acutely by several mechanisms, including reversible phosphorylation by a number of different protein kinases, translocation to different sites within the cell and interaction with

a number of proteins, some of which may serve to direct the inhibitory products of HSL away from the protein. It is also apparent from work with HSL null mice that more than one enzyme species may be classified as a hormone-sensitive lipase. The possible presence of HSL in macrophages remains controversial, and the role of the protein in pancreatic β -cells has yet to be fully elucidated. Altered expression of HSL in different cell types may be associated with a number of pathological states, including obesity, atherosclerosis and Type II diabetes.

Key words: β -cell, cholesterol metabolism, hormone-sensitive lipase, lipolysis, macrophage, perilipin.

HISTORICAL BACKGROUND

Up until around 1980, HSL (hormone-sensitive lipase) was regarded as an adipose tissue-specific enzyme, with the sole metabolic role of catalysing hormone-stimulated lipolysis in that tissue. Much of its early prominence came from the fact that HSL was one of the first enzymes for which evidence was presented for its regulation by reversible phosphorylation, it being the third reported substrate protein for cAMP-dependent protein kinase. However, progress was hindered for many years by the inability of workers to obtain purified preparations of the protein, which prevented identification of the HSL polypeptide(s) and any detailed understanding of the molecular mechanism of its regulation, etc. (reviewed in [1]).

Lipolysis is the hydrolysis of the ester bonds in triacylglycerol, which is of course composed of three fatty acids esterified to glycerol (Figure 1). Another storage form of fatty acids is cholesteryl ester, where the fatty acid moiety is esterified to the 3-position of cholesterol. One of the important roles of these cholesteryl esters is to serve as a source of cholesterol for steroidogenesis in tissues such as adrenal cortex. Work in the late 1970s demonstrated that nCEH (neutral cholesteryl ester hydrolase) in steroidogenic tissues shared several properties with HSL in adipose tissue, principally in its activation in response to hormones that elevate intracellular levels of cAMP (reviewed in [2]). This raised the possibility that the same enzyme might be responsible for the two activities in the different tissues. The first solid evidence in support of this came from the work of Per Belfrage's laboratory, who achieved the successful purification of HSL from rat epididymal fat pads, and demonstrated that the purified HSL protein had lipolytic activity against not only triacylglycerol, but also against diacylglycerol, monoacylglycerol and, crucially, chole-

steryl esters [3]. HSL also has activity against lipoidal and retinyl esters [4,5] but, perhaps surprisingly, the enzyme has no detectable phospholipase activity.

Shortly after the successful purification of HSL, evidence was presented that nCEH in the adrenal cortex showed a number of physicochemical similarities to HSL, in particular the same subunit molecular mass and susceptibility to inhibition by DFP (di-isopropyl fluorophosphate) [6,7]. Subsequently evidence was presented, based principally on selective labelling of an 84 kDa polypeptide with [³H]DFP and immunoblotting with anti-HSL antibodies, that HSL is present in other steroidogenic tissues, such as corpus luteum and testis, and in other cell types, including heart, mammary gland, skeletal muscle and macrophages [8–13]. More recent has been the demonstration of the enzyme in intestinal mucosa [14], with an exciting development being the clear demonstration of the presence of active HSL in the β -cells of the endocrine pancreas [15]. A striking exception to this relatively broad tissue distribution is liver, where there is no detectable HSL [13]. The presence of HSL in different tissues, coupled with its relatively broad lipid substrate specificity, allows it to play a number of roles in metabolism and its control (Table 1).

STRUCTURAL AND ENZYMIC STUDIES ON HSL

Use of cDNA cloning allowed prediction of the primary sequence of HSL [16,17]. This indicated that HSL is apparently unrelated to any other mammalian lipase, although subsequent analysis of the sequence of human HSL, containing 775 amino acids, indicated that it shares some identity with a lipase from the antarctic bacterium *Moraxella* TA144 [18,19]. Further sequence analysis indicated close similarity to several other bacterial proteins and

Abbreviations used: ACAT, acyl-CoA:cholesterol acyltransferase; ADRP, adipocyte differentiation-related protein; ANP, atrial natriuretic peptide; DFP, di-isopropyl fluorophosphate; ERK, extracellular-signal-regulated kinase; FABP, fatty acid-binding protein; HSL, hormone-sensitive lipase; nCEH, neutral cholesteryl ester hydrolase; PDE3B, phosphodiesterase 3B; PP2A, protein phosphatase 2A.

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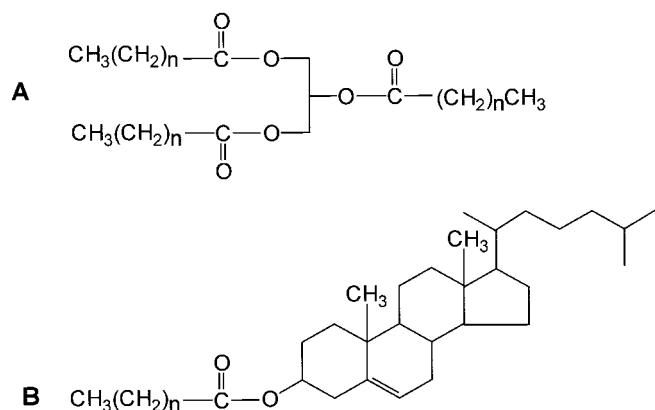


Figure 1 Chemical structures of the major physiological substrates of HSL: (A) triacylglycerol and (B) cholesteryl ester

Table 1 Fates of HSL products in different cell types

Tissue	Major product	Function
Adipose tissue	Fatty acids	Export for oxidation
Muscle, heart	Fatty acids	Oxidation
Pancreatic β -cells	Fatty acids	Signalling?
Adrenals, testes, ovaries	Cholesterol	Substrate for steroidogenesis
Macrophages	Cholesterol	Export (via high-density lipoprotein)
Placenta, ovaries	Steroids	Transcriptional control
Mammary gland	Cholesterol	Milk component, membranogenesis

more distant similarity to acetylcholinesterase and lipoprotein lipase, leading to the proposal of a lipase/esterase superfamily of proteins of which HSL is a member [20].

HSL is a serine hydrolase, inhibitable by DFP [3], with the active-site serine being identified by site-specific mutagenesis at position 423 in the rat and 424 in the human sequence, located

in a characteristic Gly-Xaa-Ser-Xaa-Gly motif found in lipases and esterases [21]. Molecular modelling led to the proposal that Asp-703 in rat HSL [22] (Asp-693 in human) and His-733 in rat HSL (His-723 in human) [20,22] are the other members of the catalytic triad. This was confirmed subsequently by site-specific mutagenesis studies [23].

Limited proteolytic studies, sequence analysis and molecular modelling have led to a proposed multi-domain structure for HSL [24–26] (Figure 2). The N-terminal portion of the adipocyte enzyme apparently comprises a stable domain of approx. 300 residues, to which a clear function has not been ascribed unequivocally, certainly *in vivo*. This region of the protein bears no detectable sequence identity with any other protein. However, there is increasing evidence that this domain mediates protein–protein interactions, and possibly binding to lipid. There is evidence that the functional form of HSL is a homodimer, although the monomeric form also has significant enzymic activity [27], and deletion studies suggest that the N-terminal region may play a role in dimerization. Interestingly, both HSL-(1–300) and HSL-(300–767) are able to interact with full-length HSL, suggesting a ‘nose to tail’ interaction between the two polypeptides in the dimer. It is also tempting to speculate that the N-terminal 300-residue region may be involved in the regulation of HSL, perhaps by interacting directly with other proteins involved in lipolysis or via interactions with the substrates or products of the HSL-catalysed reaction. Indeed, there is now some evidence in support of this proposal [28]. Demonstration that HSL interacts with FABP4 (fatty acid-binding protein 4; adipocyte lipid-binding protein) has been provided by a number of approaches, including the yeast two-hybrid system and co-precipitation of the two proteins from rat adipocyte extracts. The interaction between the two proteins is lost upon deletion of the first 300 residues of HSL. Further studies have identified residues 192–200 as being critical for the interaction with FABP4, with site-specific mutagenesis indicating a specific role for His-194 and Glu-199 [29]. Recent evidence suggests that fatty acids are required for the interaction between HSL and several FABPs [30]. It is obviously necessary to further dissect this large N-terminal portion of HSL in order to understand the roles of specific residues and regions in interacting with other molecular species.

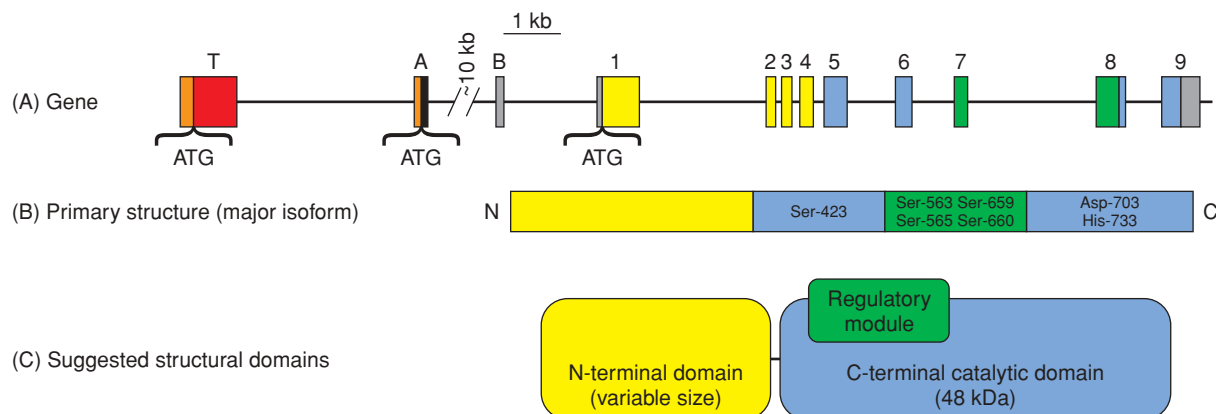


Figure 2 Outline structure of the human HSL gene and the corresponding protein

(A) Exon/intron organization of the human HSL gene. Exons T and A encode 300 and 43 additional amino acids respectively, located at the N-terminus. (B) Linear representation of the amino acid sequence of adipocyte HSL (numbering of the rat sequence), divided into three functional regions. Exons encoding each of these regions are coloured accordingly in (A). (C) Proposed domain structure of HSL, with two major structural domains: an N-terminal binding domain and a C-terminal catalytic domain, harbouring the catalytic triad and a regulatory module containing multiple phosphorylation sites. This Figure was kindly provided by Professor Cecilia Holm (University of Lund, Sweden).

The C-terminal section of the protein comprises two distinct domains, one containing the catalytic triad and a second constituting a regulatory 'loop'. The catalytic domain shows structural similarities to other lipases and is predicted to adopt a structural α/β hydrolase fold, as found in a number of other lipases [22,24]. The regulatory loop, running approximately from residue 521 to residue 669 in rat HSL [19] and mapping principally to exon 7 and most of exon 8 (Figure 2), contains all of the known phosphorylation sites (see below) and, consistent with the fact that phosphorylation of other lipases has not been demonstrated, such a loop is not found in any other lipase. An obvious, outstanding question is how phosphorylation of the different serine residues within the regulatory loop influences the intrinsic activity of the HSL catalytic domain, the interaction with the lipid substrates and products, and the movement of the HSL protein to and from the lipid droplet (see below).

As discussed above, HSL is active against a number of lipid substrates. When acting on triacylglycerol, it shows a marked preference for the *sn* 1-ester or 3-ester bonds [31], with a distinct 2-ester monoacylglycerol lipase acting in tandem in the fat cell to give complete breakdown of triacylglycerol [32]. Perhaps surprisingly, HSL shows little preference for the fatty acids in the triacylglycerol, although there is some increase in activity with decreasing chain length [33]. HSL is also active against short-chain esters *in vitro*, an activity that is retained after lipase activity has been destroyed by limited proteolysis [24,25,34]. This is consistent with a lipid-binding site being required in addition to the catalytic site, as is the case with other lipases.

PHOSPHORYLATION OF HSL

A key feature of HSL is its regulation by reversible phosphorylation, which mediates its activation by lipolytic hormones. In general, the extent of activation of HSL against triacylglycerol as substrate resulting from phosphorylation of HSL by cAMP-dependent protein kinase is of the order of 2-fold [3,7]. It is often quoted that phosphorylation of HSL does not activate it against other lipid substrates or against short-chain esters (although there is evidence that activation against cholesteryl esters occurs in response to phosphorylation [7,8,35]), but it may well be that the assay conditions for these substrates have not been optimized for the detection of the activation of HSL.

Early work from Per Belfrage's laboratory indicated that HSL was phosphorylated at two sites within the polypeptide, with one site being phosphorylated under basal conditions, and a second site being phosphorylated under lipolytic stimulation by cAMP-dependent protein kinase [36,37]. Peptide mapping and sequencing studies identified both sites, with the basal site being at Ser-565 in the rat polypeptide and the regulatory site being at Ser-563 [38–40]. The proximity of the two sites suggested that there may be some interaction between them, and it was reported that they are mutually exclusive, with the basal site having the potential to block modification of the regulatory site and thus exert an anti-lipolytic effect on the enzyme [39]. A number of kinases are capable of phosphorylating the basal site *in vitro* [39,41], with the AMP-activated protein kinase being the most likely candidate *in vivo*.

More recently, however, the picture has turned out to be more complex, with both additional phosphorylation sites and additional kinases being shown to be involved. Further detailed analysis has provided evidence that Ser-659 and Ser-660 are also phosphorylated by cAMP-dependent protein kinase *in vitro* and in response to lipolytic stimuli in rat adipocytes [42]. In particular, it was demonstrated, using recombinant HSL in which Ser-563 and Ser-565 had been mutated to alanine, that cAMP-dependent

protein kinase could still phosphorylate and activate the enzyme against triacylglycerol, although the extent of activation was not stated. This is of particular interest in view of the finding, in a separate study, that mutation of Ser-563 to alanine essentially abolished the activity of HSL *in vitro* against triacylglycerol and other substrates [43]. Clearly this apparent discrepancy needs to be resolved.

The role of cAMP in mediating the lipolytic response to β -adrenergic stimuli has been established for many years. More recently, however, evidence has accumulated that stimulation of the cAMP signalling pathway can lead to activation of the MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) pathway [44]. Four potential ERK phosphorylation sites have been identified on HSL. Site-specific mutagenesis eliminated three of them, but ERK activation has been shown to cause phosphorylation of HSL at Ser-600, activating the enzyme approx. 2-fold [35].

Further research is necessary to establish the relative importance of the different phosphorylation sites under physiological conditions. In this regard, generation of site-specific anti-phospho-HSL antibodies would greatly facilitate this work, to allow study of the process in physiological cells such as rat adipocytes, or in an *in vivo* system. Of particular interest will be investigation of possible interactions between the sites and determination of the relative contributions of the individual phosphorylation sites to the various aspects of the response to lipolytic hormones.

The serine/threonine protein phosphatases responsible for dephosphorylation of HSL in rat adipocytes have also been investigated [45], although not in as much detail as the corresponding kinases. Ser-563 is dephosphorylated efficiently by both PP2A (protein phosphatase 2A) and PP2C, with Ser-565 being dephosphorylated predominantly by PP2A, but with PP1 and PP2C also being of potential significance. Rat adipocytes contain relatively little PP2B, and this does not appear to play a significant role in the regulation of HSL, at least in that cell type. In view of the subsequent demonstration of additional phosphorylation sites on HSL, the dephosphorylation of the enzyme requires further detailed investigation.

The observation that catecholamines activate ERK in adipocytes raises at least one very interesting question in that insulin, which exerts a potent anti-lipolytic action, also activates the ERK signalling pathway. Does insulin cause phosphorylation or dephosphorylation of Ser-600? Furthermore, it has been shown that the action of insulin has both cAMP-dependent and cAMP-independent components [46]. It seems well established, at least in adipose tissue, that the cAMP-dependent action is via activation of a cAMP phosphodiesterase (PDE3B), mediated by phosphorylation and activation of that enzyme by protein kinase B [47]. However, other mechanisms may be responsible in other tissues (see below). There is as yet no clear indication of the molecular mechanism responsible for the cAMP-independent component of the anti-lipolytic action of insulin in adipose tissue, although activation of a protein phosphatase remains an obvious possibility [48].

Some evidence has been presented that growth hormone stimulates lipolysis through a JAK/STAT signalling pathway, but no molecular mechanism linking activation of this tyrosine kinase system with the presumed serine phosphorylation of HSL has yet been proposed [49]. Recently, evidence has been presented that the stimulation of lipolysis by ANP (atrial natriuretic peptide) involves increased phosphorylation of HSL [50]. As ANP is known to act via activation of guanylate cyclase and its effects on HSL are blocked by inhibitors of that enzyme, it is tempting to speculate that the activation involves direct phosphorylation of HSL by cGMP-dependent protein kinase, as has been shown

previously *in vitro* [39,51,52]. However, there is currently no evidence in direct support of this.

PERILIPIN AND HSL TRANSLOCATION

An outstanding question for many years was why purified HSL is activated only by approx. 2–3-fold *in vitro* following stoichiometric phosphorylation by cAMP-dependent protein kinase [3,7,37], and yet lipolytic rates can increase up to 100-fold in fat cells in response to lipolytic hormones [46,53]. This was at least partly resolved by the finding that a lipid droplet-associated protein, perilipin, may play a key role in mediating the association of HSL with its lipid substrate in the intact adipocyte (reviewed in [54]).

This hydrophobic protein was identified as a major adipocyte phosphoprotein whose degree of phosphorylation was increased dramatically by lipolytic hormones, an effect antagonized by insulin [55]. Perilipin was subsequently found to be associated with the lipid droplet in fat cells [56] and was originally proposed to be a docking protein to which HSL could bind following lipolytic stimulation of the cell, assisting in allowing access of HSL to its triacylglycerol substrate [56]. Another possibility was that perilipin actually inhibited lipolysis by blocking access of HSL to its lipid substrate, with phosphorylation of perilipin in response to lipolytic stimulation removing the perilipin 'barrier'.

Recently, convincing evidence has accumulated that the role of perilipin is indeed to prevent lipolysis under basal conditions, leading to increased deposition of lipid [57]. This evidence includes the demonstration that ectopic expression of perilipin A in 3T3L1 pre-adipocytes, which normally lack this protein, led to a 6–30-fold increase in the lipid content of the cells over the levels found in control cells, indicating that perilipin functions to inhibit lipolysis as opposed to stimulating it. Of course, the increased lipid accumulation could also have been due to stimulation of lipid synthesis or accumulation, but this was shown not to be the case by the observation that triacsin C, an inhibitor of triacylglycerol synthesis, did not block the effect of perilipin [57]. In addition to the overexpression of perilipin leading to lipid accumulation, a reciprocal effect has been observed, whereby endogenous levels of perilipin in cells are increased by lipid accumulation, with the lipid acting to stabilize newly synthesized perilipin protein [58].

A very striking demonstration of the importance of perilipin in the regulation of lipolysis has come from two studies involving perilipin knockout mice. The first study demonstrated clearly that the knockout mouse is healthy, but leaner (and more muscular) than its wild-type littermates. Its HSL is constitutively active in the fat cell, as judged by elevated rates of lipolysis under 'basal' conditions, and the mouse is able to tolerate a high-fat diet. Crossing the knockout strain with *db/db* mice 'corrects' the obesity of these genetically obese mice by increasing their metabolic rate, as indicated by magnetic resonance imaging [59]. The second study produced broadly similar results, but in addition showed that while basal rates of lipolysis were elevated, there was attenuation of the stimulated rate, indicating that perilipin, which is protective of the lipid droplet under basal conditions, may actually be required for maximal rates of its degradation under conditions of lipolytic stimulation [60].

Under basal conditions HSL is principally a cytosolic protein. However, upon lipolytic stimulation of adipocytes there is a rapid and dramatic redistribution of HSL, with movement of the protein to the surface of the lipid droplet [61–63]. This is consistent with studies reporting that the adipocyte lipid becomes 'activated' following lipolytic stimulation, presumably due to increased association of HSL with the recovered fatcake, although changes in the composition of the lipid may also be involved [64].

The translocation of HSL to the lipid droplet is less pronounced in older rats, perhaps indicating that this is involved in the diminished lipolytic response in older animals [63]. In contrast with the situation with HSL, perilipin is associated principally with the lipid droplet, but upon lipolytic stimulation of rat adipocytes it becomes rapidly phosphorylated and dissociates from the large droplet, presumably allowing increased access of HSL to the lipid [63]. The exact trigger for these movements is not yet clear, and indeed it has yet to be resolved whether perilipin relocates to become a truly cytoplasmic protein (which is perhaps unlikely, in view of its hydrophobic properties [65]) or remains associated with significantly smaller lipid particles which partition away from the surface of the large single droplet. A schematic representation of the events involved in the stimulation of lipolysis is given in Figure 3.

Recent mutagenesis studies have demonstrated that phosphorylation of both HSL [66] and perilipin [67] is necessary for translocation of HSL to the lipid droplet. Furthermore, specific domains have been identified on perilipin which are responsible for inhibition of basal lipolysis and promotion of stimulated lipolysis [68]. An interesting observation is that only catalytically active HSL can be translocated to the lipid droplet, as a mutated form of the protein in which the active-site serine residue was altered to alanine failed to translocate to the droplet in response to lipolytic stimulation [66].

Phosphorylation of perilipin has not been studied in great detail. There are six potential sites for phosphorylation by cAMP-dependent protein kinase, but to date no other kinases have been demonstrated to act on the protein, although the finding that treatment of human adipocytes with ANP causes phosphorylation of perilipin suggests that the protein may be a substrate for cGMP-dependent protein kinase [50]. It has been reported that under basal conditions there is phosphorylation of one or more threonine residues on the protein [65], but neither these sites nor the kinase(s) and phosphatase(s) responsible for their phosphorylation and dephosphorylation have been identified. Work with adipocyte extracts has indicated that PP1 is the major phosphatase in these cells that acts on perilipin after its phosphorylation in response to lipolytic stimulation [69]. However, no detailed analysis of the dephosphorylation of the individual sites has taken place.

As was the case previously with HSL, following the initial reports of the protein being restricted to adipocytes, there is now evidence for a wider distribution of perilipin. In particular, perilipin has been shown to be present on the surface of cholesteryl ester droplets in steroidogenic cells, such as adrenal cortical and Leydig cells [70]. This raises the question of whether perilipin and HSL are always co-expressed in cells. However, there is at present no clear evidence that perilipin is found in other tissues, such as heart and skeletal muscle, in which HSL has been detected.

In addition to perilipin, a number of other lipid-binding proteins have been implicated in the regulation of HSL via interaction with the protein. ADRP (adipose differentiation-related protein) is, despite its name, expressed ubiquitously and found on lipid droplets in a number of cell types [71,72]. It is present in pre-adipocytes, but surprisingly it is not found in mature adipocytes, being replaced by perilipin as the cell matures [71]. In view of its sequence identity with perilipin, it is tempting to speculate that ADRP adopts the role of perilipin in other cell types, but convincing evidence for this is currently lacking. Indeed, arguing against this possibility is the observation that both ADRP and perilipin are expressed in steroidogenic cells. In 1999, a novel docking protein for HSL was reported, termed lipotransin [73]. It has a number of interesting features, including the fact that the interaction between the two proteins is increased by

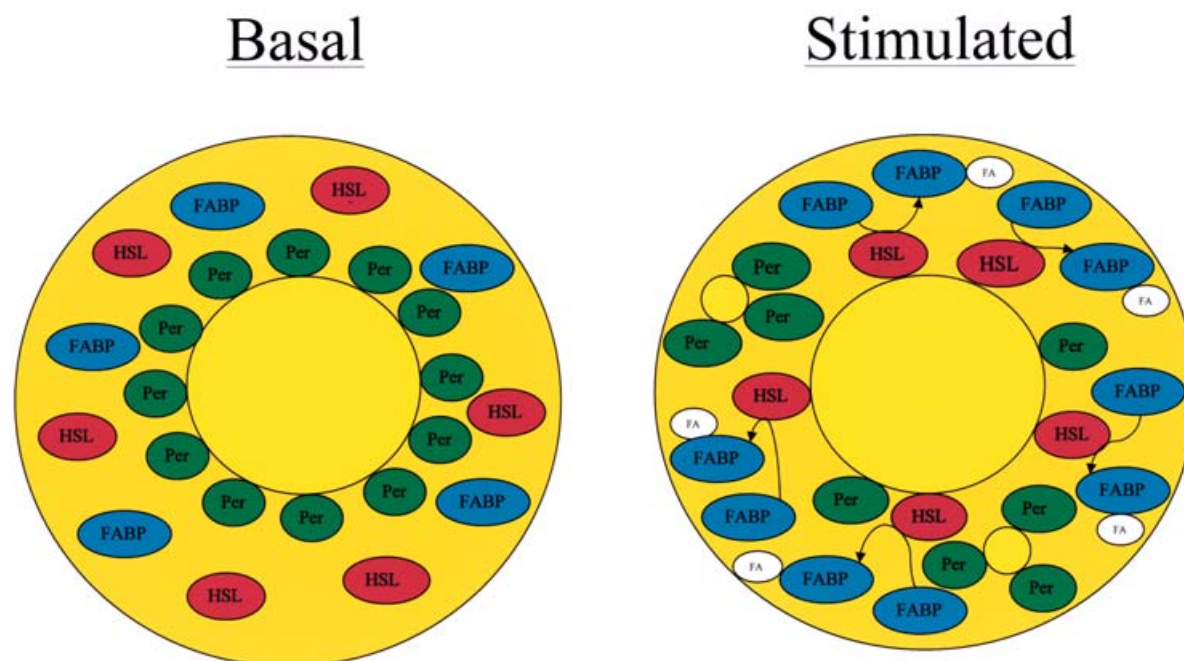


Figure 3 Schematic representation of the activation of lipolysis by lipolytic hormones

Under basal conditions, perilipin (Per) is located on the surface of the single triacylglycerol droplet, with HSL in the cytoplasm. Upon lipolytic stimulation, both perilipin and HSL become multi-phosphorylated, with perilipin being displaced from the droplet, allowing access for HSL. There is also evidence that fatty acids (FA) are removed from HSL by FABPs, preventing accumulation and resultant product inhibition.

phosphorylation by cAMP-dependent protein kinase, and also by pretreatment of cells with insulin. Sequence analysis of lipotransin indicates that it is a member of the AAA superfamily and has an ATPase activity which appears to be necessary for dissociation of the complex formed by lipotransin and HSL. However, despite the many very interesting properties of lipotransin, no subsequent work appears to have been done on this protein. As discussed above, HSL has also been shown to bind to FABP4 [28], which leads to a modest increase in the activity of HSL. This stimulation is dependent on the presence of fatty acids [30] and may be related to the inhibition of HSL by its lipid products observed *in vitro* [74], with FABP preventing the build-up of direct and indirect lipid products of the HSL reaction. A recently described interaction of HSL with steroidogenic acute regulatory protein may have a similar function, this time directing the cholesterol released by HSL from cholesteryl ester stores towards the mitochondria, to serve as precursor for steroid synthesis [75].

In summary, therefore, a current view is that activation of HSL, via phosphorylation, is achieved *in vivo* by a modest increase in the intrinsic catalytic activity of the enzyme and by improved access to its lipid substrate via its translocation to the surface of the lipid droplet. A number of other proteins are likely to play a role in this phenomenon. It is important to emphasize that, although HSL is responsible for the regulatory step in lipolysis, hormonal and neuronal influences can be exerted on the process via these other proteins such as perilipin. These effects can be exerted both chronically, via alterations in the expression levels of the protein, and acutely, via phosphorylation, possibly involving protein kinases and phosphatases which themselves do not act directly on HSL. One example of this is the lipolytic activity of tumour necrosis factor α , which exerts its effects by decreasing the levels of perilipin, an action that can be reversed by overexpression of the protein using recombinant adenovirus [76].

Table 2 Isoforms of HSL

See Figure 2(A) for exon/intron organization of the human HSL gene.

Isoform size (kDa)	Exon usage
84	B (non-coding), 1–9
88	A, 1–9
120	T, 1–9
80 (inactive)	B, 1–5, 7–9

GENETIC AND EXPRESSION STUDIES

The human gene encoding HSL is positioned on chromosome 19q13.3 [17]. Human HSL contains 775 amino acids, with a mass of 84032 Da, and migrates on SDS/PAGE as a polypeptide of molecular mass 88 kDa. The more-studied rat HSL protein contains 768 amino acids and migrates on SDS gels at approx. 84 kDa. The human gene contains nine 'core' exons, which encode regions of the protein that are common to all three known isoforms of HSL. Upstream of exon 1 are several other exons, including exons A, B and T (see Figure 2), which are spliced into exon 1 in a mutually exclusive and tissue-specific manner. Exon B is a non-coding exon and is used in the predominant isoform expressed in adipocytes [77]. Exon A is a coding exon that is expressed in several tissues, including adipose tissue, ovary, adrenal gland and the pancreatic β -cell, giving rise to an HSL protein encompassing an additional 43 amino acids [78]. There is also a testis-specific isoform of HSL, involving a testis-specific T exon that results in the expression of a protein of increased mass (120 kDa) containing an additional 300 amino acids [79]. The functional significance of the differing lengths of the N-terminal domains in the isoforms has yet to be determined. Table 2 shows

the major HSL isoforms and indicates the specific exon usage responsible.

A number of other molecular variants of HSL have been reported. Of particular interest is a truncated form of the enzyme (molecular mass 80 kDa) reported in human adipose tissue [80]. This lacks exon 6, which encompasses the serine residue of the catalytic triad, and as a result is devoid of enzymic activity. No such variant has been observed in other species, although, in immunoblotting experiments, antibodies to HSL often recognize 'additional' proteins of similar molecular mass. A number of obese patients who express this form in addition to the wild-type protein show reduced hormone-stimulated lipolytic rates (compared with those observed in adipocytes from similarly obese subjects expressing only the full-length protein), indicating that the truncated form may act in a dominant-negative fashion, perhaps by translocating to the lipid droplet and restricting access by the active, wild-type enzyme [81]. Evidence against this explanation is of course the observation that inactive HSL with a mutation at the active-site serine does not translocate to the lipid droplet [66].

Several laboratories have succeeded in generating mouse knockouts of HSL, which have provided a number of surprising findings [82–84]. Firstly, male HSL^{-/-} mice are sterile because of oligospermia [82], but the molecular basis underlying this condition has not yet been determined. Secondly, HSL-deficient mice are not obese, although there are some differences in their adipose tissue distribution, adipocyte size and lipid composition within tissues normally expressing HSL. The fatty acid composition of the stored triacylglycerol in HSL^{-/-} mice is altered from that in the wild type, indicating a substrate specificity for HSL that is not apparent from studies with the purified enzyme [85]. Furthermore, the HSL^{-/-} mice show diacylglycerol accumulation in adipocytes, muscle and testis, indicating that HSL is rate-limiting for diacylglycerol hydrolysis [84]. The third major surprise was that the levels of neutral triacylglycerol lipase activity in white adipose tissue extracts from HSL^{-/-} mice were decreased by only approx. 60% compared with those in wild-type animals, although nCEH activity in adipose tissue (white and brown) and testis was undetectable [82,83]. Furthermore, there was still significant lipolytic activity in adipocytes isolated from the knockout mice, and indeed there was lipolytic activity in these animals *in vivo*, as measured by plasma levels of glycerol and non-esterified fatty acids [82]. This residual lipolytic activity remained hormone-sensitive, as indicated by its stimulation by isoprenaline (isoproterenol).

The most likely explanation for this latter finding is the presence of one or more additional intracellular triacylglycerol lipase(s), at least in the knockout mice. This additional lipase activity is also hormone-sensitive, but the molecular species responsible has not yet been reported, and nor indeed has the basis of its hormone sensitivity. Evidence indicates that the additional lipase translocates to the lipid droplet in response to lipolytic stimuli, suggesting that similar regulatory mechanisms may be involved. Interestingly, unlike the situation with HSL, the additional lipase(s) appears to lack enzymic activity against cholesteryl esters [82,86]. This latter observation suggests that nCEH activity may be a better monitor of HSL in adipocyte extracts than the traditional assay using triacylglycerol as substrate.

In view of the crucial role of HSL in different aspects of lipid metabolism, it is perhaps surprising that altered levels of HSL expression or activity, or genetic variation, have not been demonstrated unequivocally as causative features of any major clinical condition. However, there is some evidence that polymorphisms in the HSL gene or its promoter region may be risk factors in a number of conditions.

The possible involvement of HSL in different pathological situations has been reviewed recently [87]. In particular, there are conflicting data on whether altered expression or activity of HSL is involved in familial combined hyperlipidaemia [88,89]. Similar debate surrounds the role of altered levels of HSL in obesity and in insulin resistance, two linked conditions which predispose to Type II diabetes. Elevated levels of plasma non-esterified fatty acids are associated with insulin resistance. A body of evidence indicates that visceral and omental fat make a much greater contribution to this than subcutaneous depots, with visceral fat being more lipolytically active (reviewed in [90]). Furthermore, the fatty acids released from visceral adipose tissue drain directly to the liver via the portal circulation, perhaps leading to an increased risk of insulin resistance in the liver. Interestingly, in work with human pre-adipocytes maintained in culture, cells derived from the omental region demonstrated increased maximal lipolytic rates compared with cells from a subcutaneous source [91]. The increased rate of lipolysis in visceral fat may well be due both to increased lipolytic responsiveness, at least in men [92], involving increased expression of β -adrenergic receptors [93], and to impaired inhibition of lipolysis by insulin [94], and not to any alteration in the HSL polypeptide itself. Indeed, altered levels of perilipin could contribute to any differences. However, there is some evidence from work in rats that HSL is in fact present in higher amounts in omental fat than in subcutaneous fat [95], but this is contradicted by work in humans (control subjects), where HSL expression, as indicated by maximal lipolytic rate, HSL activity and levels of HSL mRNA appear to be higher in adipocytes isolated from subcutaneous fat [96]. However, obesity appears to reduce this increased HSL expression in subcutaneous adipocytes [97], which may relate to the decreased response to lipolytic hormones observed in obesity. Weight reduction in obese women decreased the levels of HSL mRNA in subcutaneous adipose tissue, but no such loss was observed in obese men, indicating a gender difference in the regulation of HSL expression [98]. In a separate study, lipolytic capacity per cell was shown to decrease in obese women (but not men) after weight loss, but no change in HSL expression was observed [99].

A promoter variant of HSL, -60C \rightarrow G, has been implicated in a number of conditions, playing a role in body fat composition in a sex-, race- and insulin-dependent manner [100]. This variant, which exhibits a 40% decrease in promoter activity [101], has been associated with increased insulin sensitivity in women and decreased levels of plasma non-esterified fatty acid in men [102]. In a separate study, it has been shown to be associated with lower fasting insulin levels in healthy young men, consistent with it being protective against insulin resistance [103]. Population studies suggest that a polymorphic marker in the HSL gene is in linkage disequilibrium with an allele that increases susceptibility to abdominal obesity, which is itself a risk factor for Type II diabetes [104], and a genotyping study found that allele 5 of the i6 dinucleotide repeat polymorphism is associated with a decrease in lipolytic rate in subcutaneous fat, which again can contribute to obesity [105]. Differences in the phenotypes of homozygous subjects were observed between non-obese women and men, but both indicated that this polymorphism is a risk factor for obesity [106]. A separate polymorphism (R309C) may also contribute to elevated serum cholesterol levels in Japanese subjects, but this has yet to be confirmed [107].

A number of studies have used cell lines to look both at the effects of altering the expression levels of HSL and also at mechanisms involved in controlling its expression. Using the 3T4-F442A adipogenic cell line, it has been clearly demonstrated that dramatic overexpression of HSL prevents lipid accumulation [108]. Work with the same cell line has demonstrated that both

cAMP and phorbol esters inhibit endogenous HSL gene expression [109]. Insulin was reported to have no effect on HSL expression in that study.

The elevated lipolytic rate in insulin resistance may also be at least partly explained by several recent observations using adipocytes (either rat primary cells or different cell lines) that glucose maintains/induces increased levels of HSL. Work with 3T3-F442A cells showed that incubation in the absence of glucose for 32 h caused decreases in HSL activity and mRNA levels which were reversed by re-administration of glucose for 12 h [110]. Subsequently, primary rat adipocytes, maintained in the presence of high glucose (20 mM), were shown to demonstrate increased rates of lipolysis (both basal and stimulated), associated with an increase (approx. 40%) in the levels of HSL. However, this effect was observed only in the presence of insulin, with neither insulin nor glucose alone exerting this effect [111]. Similar effects were observed in 3T3-L1 adipocytes, where again increased levels of HSL protein and activity were observed in response to high concentrations of glucose (20 mM) and insulin (1 μ M) [112]. A molecular explanation, at least for the effects of glucose, has come from the discovery of a glucose-responsive region within the HSL promoter [113]. Mutations within a consensus E-box in this region lead to a decrease in promoter activity and a loss of responsiveness to glucose.

Recently, a role for leptin has emerged in the control of HSL, both in adipose tissue and in macrophages (see below). Leptin increases glycerol release from adipocytes [114], but, unlike the response most lipolytic stimuli, this is not accompanied by the release of significant amounts of non-esterified fatty acids [115]. The most likely explanation for this is that leptin also increases the expression of several enzymes of fatty acid oxidation, allowing the fatty acids to be oxidized *in situ* within the adipocytes. Consistent with this is the observation that chronic treatment of rats with leptin increases adipocyte lipolysis and fatty acid oxidation, albeit in skeletal muscle [116]. This is not accompanied by any observable increase in HSL expression, indicating either that HSL phosphorylation is increased or else that there is a chronic change in some other protein contributing to the regulation of lipolysis (see above). This suggests that one role of leptin is to regulate intracellular lipid levels via HSL, a process that is not necessarily restricted to adipose tissue and that can contribute to insulin sensitivity.

HSL IN MACROPHAGES

Disturbances in the cholesteryl ester cycle in macrophages are implicated as key events in foam cell formation, a process with major relevance for the development of atherosclerosis. Cholesterol homeostasis is achieved by a balance between the activities of ACAT (acyl-CoA:cholesterol acyltransferase), which catalyses the formation of cholesteryl esters, and the cytosolic neutral nCEH, catalysing the re-mobilization of free cholesterol. Evidence was presented a number of years ago that the nCEH activity in the mouse macrophage cell line WEHI is catalysed by HSL [12]. This was based principally on the ability of antibodies against HSL to immunodeplete nCEH activity from cell extracts and to immunoprecipitate an 84 kDa [32 P]phosphoprotein from extracts treated previously with [γ - 32 P]ATP and cAMP-dependent protein kinase. Phosphorylation of this protein was subsequently demonstrated in intact WEHI cells and in mouse peritoneal macrophages [117]. It has also been demonstrated that anti-HSL antibodies can be used to immunoprecipitate nCEH activity from J774.2 macrophage extracts [118]. Others have provided evidence in support of the presence of HSL in mouse macrophages

(both peritoneal and cell lines), based principally on reverse transcription-PCR [119–121], and certainly overexpression of HSL in the RAW 264.7 mouse macrophage cell line resulted in increased mobilization of cholesteryl esters, a process stimulated by cAMP [122]. Paradoxically, overexpression of HSL in the macrophages of a transgenic mouse did not protect against the development of atherosclerotic lesions; indeed, this harmful process seemed to be increased [123].

Sterol esters appear to down-regulate the expression of HSL in mouse macrophages [124], a phenomenon recently observed also in 3T3-L1 adipocytes [125], while chronic exposure to elevated concentrations of glucose and insulin and/or leptin cause decreased activity of HSL, but increased activity of ACAT, perhaps contributing to increased foam cell formation in hyperinsulinaemia, hyperleptinaemia and hyperglycaemia [112]. Acute treatment of mouse macrophage cell lines with leptin and insulin also influences the activity of nCEH, as measured in anti-HSL immunoprecipitates. Leptin stimulated HSL in these cells in a phosphoinositide 3-kinase-dependent manner, while insulin inhibited HSL [118]. Intriguingly, the effects of insulin were not dependent on phosphoinositide 3-kinase, and indeed did not involve PDE3B, as the J774.2 cells apparently do not express that isoform of phosphodiesterase.

While confirming that HSL is present in mouse macrophages, others have presented evidence that it is lacking in human monocyte-derived macrophages [121]. Of particular interest is the observation that HSL is expressed in monocytes from both the THP-1 cell line and peripheral blood, but is absent from macrophages from the corresponding sources [126]. In the same study, bile salt-stimulated nCEH was found to be expressed in all four cell types, suggesting an important role for that enzyme in cholesterol metabolism in human macrophages [126]. However, as this enzyme is usually exported from the cell, it appears unlikely that it is responsible for the major nCEH activity within the cytoplasm. In contrast with these findings, another group have reported that HSL mRNA *can* be detected in human monocyte/macrophages [127]. An explanation for the different findings is not immediately obvious, although one possible explanation is that the macrophages used in the study in [127] might have contained some non-differentiated monocytes. However, an entirely different approach, using human (and rabbit) arterial macrophage-derived foam cells, has also demonstrated the presence of low levels of HSL mRNA in these cells [128].

A major finding of particular relevance to the possible role of HSL in macrophages is the report that, in HSL null mice, nCEH activity in peritoneal macrophages is apparently normal [82]. In the same study nCEH activity was ablated in white and brown adipose tissue and testes, consistent with it being catalysed by HSL. Similarly, macrophages from a different HSL null mouse line showed unimpaired cholesteryl ester turnover [129]. There are of course several plausible explanations for the observations in macrophages from the HSL knockout mice, including the possibility that HSL is not responsible for this nCEH activity, or that HSL is normally responsible but that, in its absence, there is a compensatory increase in the expression of another enzyme capable of fulfilling this crucial function. In that regard it would be of interest to measure the lipolytic activity against triacylglycerol in the macrophages from the HSL knockout mouse as compared with that in the wild type. Convincing evidence has been presented for the presence of another nCEH activity in human THP-1 monocytes and macrophages [130]. This enzyme is related to the nCEH found in liver, and its overexpression causes cholesteryl ester mobilization from THP-1 cells [131], but the contribution that its activity makes to the total nCEH activity in macrophages has yet to be determined.

HSL IN MUSCLE AND HEART

Following the identification of HSL in muscle and heart, a number of studies have been carried out looking at its regulation in these tissues. HSL in skeletal muscle is activated in response to adrenaline, presumably via increases in cAMP levels [11], and also by contraction [132]. Use of kinase inhibitors has suggested that this is mediated by protein kinase C, partly involving the ERK signalling pathway [133]. Others have also shown that activation of ERK1/2 during contraction coincides with HSL activation, and that adrenaline further stimulates contraction-induced HSL activation [134]. However, the activation of HSL precedes any significant breakdown of lipid, and it has been proposed that regulatory mechanisms other than the phosphorylation of HSL may be involved [135]. As yet, no information is available on the phosphorylation sites on HSL that are involved in these responses. Exercise training does not appear to alter the levels of HSL, but it does reduce sensitivity to insulin [136]. As with the case in macrophages, the effect of insulin does not appear to involve PDE3B (nor other PDE3, 4 or 5 enzymes) [137].

In contrast with muscle, the role and regulation of HSL in the heart seems to have been little studied. One role may be to control the intracellular levels of non-esterified fatty acids as, although they are essential fuels, excess fatty acids can also reduce contractility. Cardiac-specific overexpression of HSL prevented the accumulation of intracellular triacylglycerol following overnight starvation, indicating that HSL can indeed control intracellular lipid metabolism in the heart. Interestingly, altered expression of a number of key genes was observed in hearts from the HSL-overexpressing animals [138].

HSL IN THE PANCREATIC β -CELL

It was first demonstrated in 1999 that HSL is expressed and active in the β -cells of the pancreatic islet [15]. Two isoforms are expressed in the β -cell: the 84 kDa protein seen in adipocytes and other tissues and an 89 kDa protein [15] encoded by exons 1–9 plus exon A, which is spliced to exon 1, introducing an additional 43 amino acids into the polypeptide [78].

The role of HSL in the β -cell has yet to be fully established. Two studies have independently looked at insulin secretion in HSL-knockout mice [139,140]. Both demonstrate features of insulin resistance in peripheral tissues, but differ in the effect of the knockout on insulin secretion from the pancreas. In one study, the glucose-responsiveness of insulin secretion was impaired [139], both *in vivo* and in pancreatic islets isolated from the mice. The islets from the HSL^{-/-} mice were totally unresponsive to glucose, but showed a normal response to depolarizing concentrations of KCl. This work is consistent with a key role for HSL in coupling of glucose metabolism to increased insulin secretion, perhaps by generating a lipid messenger such as diacylglycerol or fatty acyl-CoA from intracellular stores of triacylglycerol [15,139–141]. Also consistent with this role is the increased expression of HSL in response to high concentrations of glucose, with the elevated glucose ensuring an appropriate supply of the lipid mediators [142]. The second study in HSL null mice demonstrated that insulin secretion was essentially unimpaired, with an increase in islet mass being found, presumably as a result of the insulin resistance in peripheral tissues [140]. Possible reasons for the apparent discrepancies between the two studies, such as the genetic background of the mice, are discussed in that paper.

However, recent studies on transgenic mice that overexpress HSL specifically in the β -cell have provided evidence for a role for HSL in mediating the lipotoxicity associated with Type II

diabetes. These mice showed impaired glucose tolerance and had defective glucose-stimulated insulin secretion when fed a high-fat diet, accompanied by lower levels of β -cell triacylglycerols than found in control mice fed the same diet [143]. One proposed mechanism for this is that HSL provides endogenous ligands for lipid-activated transcription factors, including the peroxisome-proliferator-activated receptors. In this regard, it would appear that, in control animals, the accumulation of intracellular triacylglycerols is actually a protective mechanism [144], with the lipotoxicity being manifested when the storage capacity is exceeded or when the lipid stores are mobilized by HSL. Consistent with this is the observation that long-term feeding with a high-fat diet leads to down-regulation of pancreatic HSL levels by as much as 75 %, presumably as a protective mechanism against the generation of these lipid metabolites [145].

FUTURE DIRECTIONS

Despite the major advances in our understanding of HSL, a number of key questions remain. Clearly there is a major need for a three-dimensional structure for the protein, especially with a view to understanding how phosphorylation leads to increases in the intrinsic activity of the enzyme. The exact role and nature of the phosphorylation sites and their respective kinases needs to be re-investigated.

The molecular mechanism underlying the translocation of HSL to the lipid droplet provides a fascinating challenge. What 'kick starts' this process? Is it the displacement of perilipin, allowing access of HSL to the droplet, or is it partial activation of HSL, which causes sufficient lipolysis to result in either displacement of perilipin from the droplet or fragmentation of the large droplet into smaller entities? What are the roles of the different phosphorylation sites in mediating any activation of the enzyme compared with translocation? Do similar translocation events occur in other tissues where HSL is found and where the lipid substrate is present in a different physicochemical structure? Is there any direct interaction of HSL with perilipin? Is there any co-ordinate control of the expression of HSL and the lipid-binding proteins such as perilipin?

What is the nature of the 'other' hormone-sensitive lipase (or lipases!) which is present in the adipocytes of HSL-knockout mice? Is it also expressed in wild-type mice, or only in mice lacking HSL? Is it truly 'hormone-sensitive', directly as a result of its phosphorylation, or is its activity regulated indirectly, perhaps via other proteins of the lipolytic response such as perilipin? Is HSL the key nCEH in macrophages? How are the 'correct' levels of HSL in the β -cell achieved, to allow glucose coupling to insulin secretion without inducing lipotoxicity? All the indications are that HSL still has a few more surprises in store.

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