Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism

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Lipase hormono-sensible de l'adipocyte: un régulateur important du métabolisme lipidique

RÉSUMÉ

Le tissu adipeux joue un rôle important dans le contrôle de la balance énergétique. La mobilisation des triacylglycérols par la lipase hormono-sensible (*EC* 3.1.1.3; LHS) est soumis à un contrôle direct par les hormones et neurotransmetteurs qui modulent les concentrations intracellulaires d'AMP cyclique (AMPc). L'hydrolyse des triacylglycérols par la LHS constitue l'étape limitante de la lipolyse. La LHS est phosphorylée sur le site régulateur (Ser552 dans la LHS humaine) par la protéine kinase dépendante de l'AMPc (*EC* 2.7.1.37) lorsque les concentrations intracellulaires d'AMPc augmentent. Cette phosphorylation conduit à l'activation de l'enzyme. Une deuxième site de phosphorylation (Ser554 dans la LHS humaine) dénommé site basal est la cible de la protéine kinase activée par l'AMP. La phosphorylation du site basal ne conduit pas à l'activation de la LHS et empêche la phosphorylation sur le site régulateur. La phosphorylation et l'activation de la protéine kinase activée par l'AMP constitue donc un mécanisme antilipolytique qui est fonctionnel sur cellules isolées mais dont l'importance physiologique n'est pas connue. Les ADN complémentaires de la LHS de plusieurs espèces ont été clonés et la structure des gènes de LHS de l'homme et de la souris sont connus. Différents domaines fonctionnels de la protéine ont été proposés. Une région d'homologie de séquences en amont de la sérine 424 du site catalytique avec cinq enzymes d'organismes procaryotes et une enzyme humaine a été décrite. La localisation chromosomique du gène de la LHS est connue chez l'homme (chromosome 19, région q13·1→13·2), le porc et la souris. La mise en évidence de marqueurs polymorphiques dans le gène devrait permettre de tester l'hypothèse d'une implication de la LHS dans certaines maladies héréditaires du métabolisme lipidique. L'expression de la LHS varie selon la localisation anatomique du tissu adipeux chez le rat. Cette expression subit également des variations durant la gestation chez le rat et le cycle annuel chez les mammifères hibernants. Chez l'homme, les taux d'ARN messagers de la LHS sont diminués dans le tissu adipeux de certains patients atteints de cancer. L'activité enzymatique totale est diminuée chez les patients atteints d'hyperlipidémie familiale combinée mais pas chez les patients atteints du syndrome métabolique bien que, dans les deux cas, la lipolyse adipocytaire maximale soit diminuée. Les mécanismes moléculaires de contrôle de l'expression de la LHS sont pratiquement inconnus.

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All animals feed and fast intermittently. During evolution it has been necessary to develop precisely-regulated mechanisms to control the storage and release of metabolic fuels. In mammals, short-term fluctuations in energy balance are to some extent buffered by the glycogen stores. However, the capacity to store glycogen is limited. Longer-term imbalances between energy intake and expenditure are translated into changes in the body's store of lipid, mainly in the form of intracellular triacylglycerol (TAG) in adipose tissue. Thus, a highly developed adipose tissue is characteristic of all mammalian species. The vast majority of the body's TAG (>95%) is found in adipose-tissue stores. Lipolysis refers to the hydrolysis of TAG, via di- and monoacylglycerol intermediates, to fatty acids and glycerol. Adipose-tissue lipolysis is the major regulator of the supply of lipid energy because it controls the release of fatty acids into the plasma. The rate-limiting step of adipose-tissue lipolysis is the hydrolysis of TAG by hormone-sensitive lipase (*EC* 3.1.1.3; HSL). Adipose tissue HSL is thus one of the enzymes determining whole-body lipid fuel availability. In the post-absorptive state, HSL activity accounts for most of the detectable lipolysis (Frayn *et al.* 1995).

The present review focuses mainly on recent advances in the understanding of HSL function and regulation in the adipocyte. Cellular aspects of lipid mobilization such as substrate selection have been recently reviewed (Lafontan & Langin, 1995).

MOLECULAR CONTROL OF HORMONE-SENSITIVE LIPASE ACTIVITY

It is generally accepted that lipolysis is controlled mainly by sympathetic nervous system activity and plasma insulin levels. Basically, the lipolytic response of the fat cell depends on the balanced action of stimulatory and inhibitory pathways on HSL activity. The different steps of the lipolytic process leading to the activation of HSL are quite well defined (Fig. 1). The first cellular action of catecholamines and of a number of endocrine and/or paracrine regulators of lipolysis (e.g. adenosine and prostaglandins) is their binding to plasma membrane receptors. The stimulatory effect on lipolysis is strictly connected to the receptor-controlled increment of intracellular cAMP concentrations which in turn promotes activation of cAMP-dependent protein kinase (*EC* 2.7.1.37; cAMP-PK; Honnor *et al.* 1985) which phosphorylates HSL.

The first step leading to activation of the lipolytic cascade, involves the multi-regulated enzyme, adenylate cyclase (*EC* 4.6.1.1), which produces cAMP. Detailed mechanistic considerations have been reviewed recently by Lafontan & Berlan (1993). Catecholamines are the most sophisticated regulators of fat cell function since they operate through five separate adrenergic receptors. They are able to stimulate three subtypes of β-adrenoceptors which are positively coupled to adenylate cyclase by Gs proteins, and an α_2-adrenoceptor negatively coupled to the enzyme by a Gi protein. An important point in the metabolic actions initiated by endocrine and paracrine regulators concerns the functional significance of intracellular cAMP elevations promoted by receptor-mediated adenylate cyclase control. In fat cells, the lipolytic agents promote cAMP increments which largely overcome the concentrations required for maximal activation of cAMP-PK and lipolysis (Fain & Garcia-Sainz, 1983; Honnor *et al.* 1985). Biphasic regulation of lipolysis by catecholamines has been demonstrated clearly in human fat cells (Berlan & Lafontan, 1985; Mauriège *et al.* 1987). The interplay between α_2- and β-adrenoceptors plays a key role in the triggering of cAMP increments in fat cells; important species-specific differences exist.

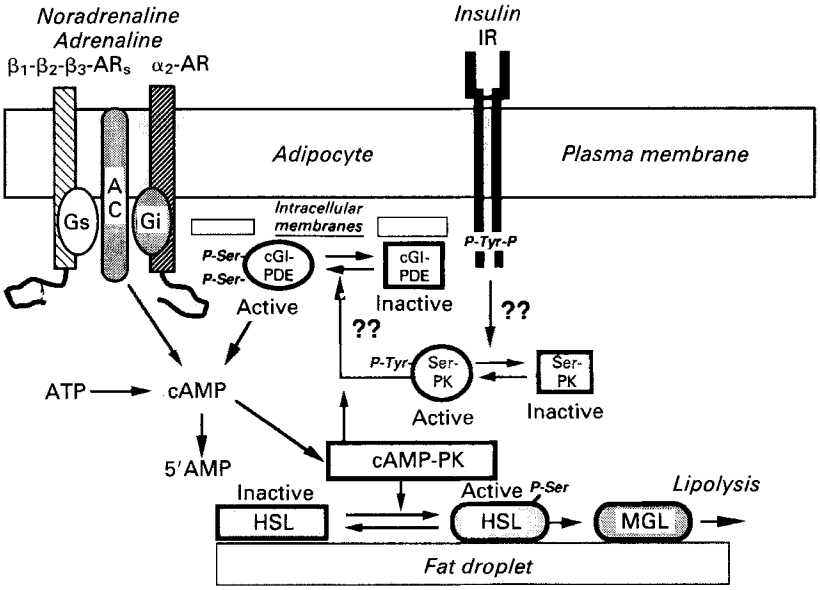


Fig. 1. Adipose-tissue lipolysis showing catecholamine receptors and insulin receptor (IR), G proteins (Gs and Gi), cGMP-inhibited low K_m cAMP phosphodiesterase (*EC* 3.1.4.17; cGI-PDE) and the catalyst moieties of adenylate cyclase (*EC* 4.6.1.1; AC). Three stimulatory β-adrenoceptors (β_1, β_2 and β_3-AR_s), coupled to Gs, and one inhibitory α_2-adrenoceptor (α_2-AR), coupled to Gi, exert antagonistic actions on AC activity, cAMP production, and cAMP-dependent protein kinase (*EC* 2.7.1.37; cAMP-PK) activity. Insulin promotes the degradation of cAMP via phosphorylation and activation of cGI-PDE which is associated with intracellular membranes. Phosphorylation of hormone-sensitive lipase (*EC* 3.1.1.3; HSL) by cAMP-PK is followed by HSL enzymic activation and lipolysis. Monoacylglycerol lipase (*EC* 3.1.1.23; MGL) hydrolyses the breakdown of monoacylglycerol into fatty acid and glycerol. Ser-PK, serine kinase.

Insulin is the physiologically important anti-lipolytic hormone. Initially insulin was thought to cause dephosphorylation of HSL and its deactivation by an effect which could involve phosphatase activation. However, its action is probably linked to a decrease in cellular cAMP levels. Insulin-induced reduction of cAMP could result from the inhibition of adenylate cyclase and/or from a stimulation of cGMP-inhibited low-K_m cAMP-phosphodiesterase (*EC* 3.1.4.17; cGI-PDE). Effects through adenylate cyclase inhibition are still largely questionable and Gi proteins do not play a role in the transduction of the insulin signal in the adipocytes (Wesslau *et al.* 1993). Activation of cGI-PDE by insulin is believed to be the major mechanism whereby insulin reduces cellular cAMP levels. This activation is the result of serine phosphorylation of cGI-PDE (Degerman *et al.* 1990; Smith *et al.* 1991). A synergistic activation and phosphorylation of cGI-PDE was seen in response to insulin and catecholamines (Smith & Manganiello, 1988; Smith *et al.* 1991). Results obtained in intact adipocytes are consistent with the notion that insulin mediates the phosphorylation of serine site(s) on cGI-PDE and promotes its activation (Eriksson *et al.* 1995). The insulin-induced activation–phosphorylation of cGI-PDE is catalysed by a cGI-PDE serine kinase (Lopez-Aparicio *et al.* 1993). The identity of this protein is unknown. Very recently, it was shown that

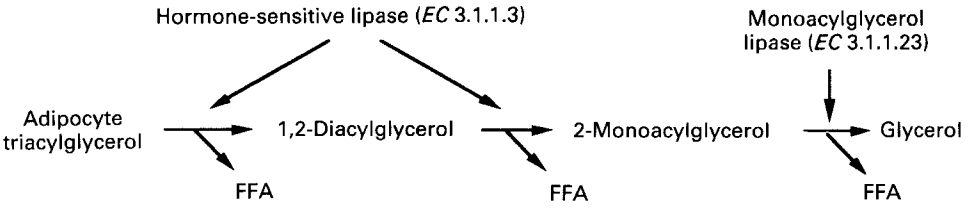


Fig. 2. Hydrolysis of triacylglycerols stored in adipocytes. The breakdown of triacylglycerols into diacylglycerols is the rate-limiting step in adipose tissue lipolysis. FFA, free fatty acids.

phosphatidyl inositol 3-kinase, an important mediator of insulin-dependent metabolic effects, was involved in mediating the anti-lipolytic effect of insulin at a step upstream from the activation of the cGI-PDE serine kinase. Further investigations should lead in the near future to a complete characterization of signal transduction steps involved in insulin-mediated anti-lipolysis.

In vivo, HSL catalyses the hydrolysis of TAG to diacylglycerol, and then to monoacylglycerol (Fig. 2). The hydrolysis of the monoacylglycerol-fatty acid bond is assured by monoacylglycerol lipase (*EC* 3.1.1.23). The abundance of this enzyme, which is not under hormonal control, is sufficient to avoid accumulation of intermediary products of lipolysis (Fredrikson *et al.* 1986). HSL exhibits positional specificity for the 1(3)-ester bond, although this specificity is less pronounced than that for lipoprotein lipase (*EC* 3.1.1.34; LPL) or pancreatic lipase (*EC* 3.1.1.3). TAG are hydrolysed at a much lower rate than diacylglycerol. Therefore, the first step of lipolysis is rate-limiting. Moreover, phosphorylation of HSL by cAMP-PK is paralleled by an enhanced TAG lipase (*EC* 3.1.1.3) activity, whereas the activity against diacylglycerol is unchanged.

The hallmark of HSL, which distinguishes this enzyme from all other known lipases, is the control of its activity through phosphorylation (Fig. 3). A single serine residue (regulatory site) is phosphorylated by cAMP-PK, 1 mol phosphate being incorporated per mol subunit (Strålfors & Belfrage, 1983). The reversible phosphorylation of the regulatory site controls the active state of the enzyme. However, the mechanism of activation is still unclear. HSL activity is increased 2–3-fold by cAMP-PK-mediated phosphorylation *in vitro*, while a more than 20-fold increase in lipolytic rate is measured in intact fat cells in response to hormonal stimulation. The TAG emulsion used as substrate *in vitro* provides a much larger interfacial area than the larger TAG droplet present in the adipocyte (Strålfors *et al.* 1987). Hence, a high basal activity of HSL in the dephosphorylated form might be caused *in vitro* by favourable conditions for enzyme–substrate interaction, phosphorylation promoting a small increase in an already-high submaximal activity. The relationship between the subcellular distribution of HSL and its phosphorylation and activation may be important. HSL has some properties of an intrinsic membrane protein, for example it associates strongly with phospholipids, requires detergents for solubilization and exhibits an amphiphilic character (Holm *et al.* 1986). Redistribution of HSL may be a major event associated with phosphorylation and activation. On lipolytic stimulation, translocation of HSL from the cytosol to a particulate fraction was found in 3T3-L1 adipocytes (Hirsch & Rosen, 1984). This view was recently strengthened. Using mild disruption of rat adipocytes and polyclonal antiserum directed against HSL, a translocation of phosphorylated HSL at the surface of

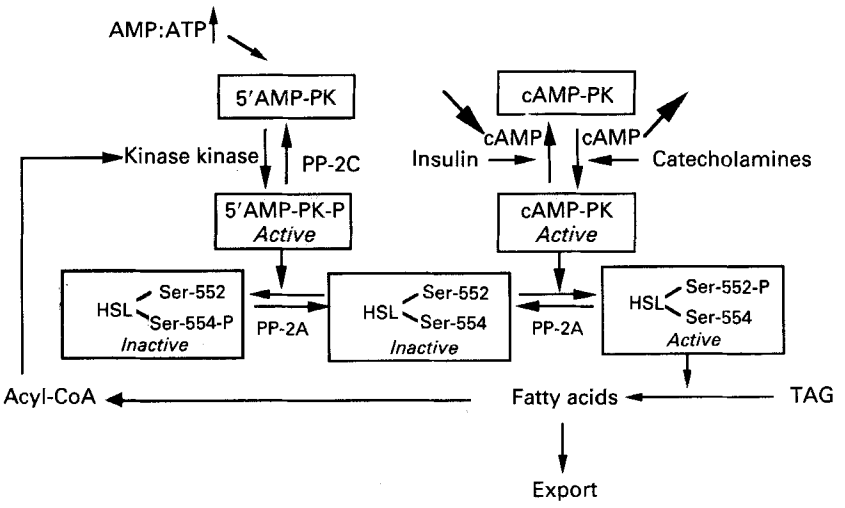


Fig. 3. Short-term regulation of hormone-sensitive lipase (*EC* 3.1.1.3; HSL). The diagram illustrates the phosphorylation of the regulatory site of human HSL (Ser552) by cAMP-dependent protein kinase (*EC* 2.7.1.37; cAMP-PK) and the hypothesis regarding the phosphorylation of the basal site (Ser554) by 5'-AMP-activated protein kinase (5'AMP-PK). PP-2A, PP-2C, protein phosphatases 2A and 2C (*EC* 3.1.3.16); TAG, triacylglycerol.

the lipid droplet was demonstrated (Egan *et al.* 1992). The nature of the binding of the enzyme to the lipid droplet is not known. The primary structure of HSL does not show any highly hydrophobic regions, which could explain the propensity of HSL to bind to lipids. Early reports (Wise & Jungas, 1978) have brought up the question as to whether a protein component located at the surface of the lipid droplet may undergo some kind of 'substrate activation' occurring concomitantly with HSL activation and governing droplet-driven translocation of HSL. It could be speculated that perilipins, specific adipocyte lipid-droplet-associated proteins, are possible candidates as 'docking' proteins for HSL (Greenberg *et al.* 1991). These proteins, probably contribute to the organization of lipid droplets and lipid vacuoles found in mature adipocytes (Hare *et al.* 1994). Perilipins are phosphorylated by cAMP-PK in parallel with activation of lipolysis. This event could represent the 'substrate activation' process. The recent isolation of cDNA for perilipins will allow detailed analysis of their role in fat cells (Greenberg *et al.* 1993).

In addition to the regulatory site, another serine residue (basal site) can be phosphorylated (Fig. 3). Three protein kinases i.e. Ca^{2+}/calmodulin-dependent protein kinase II (*EC* 2.7.1.123), glycogen synthase kinase-4 (*EC* 2.7.1.37) and the 5'-AMP-activated protein kinase have been shown to phosphorylate site 2 *in vitro*. Phosphorylation of site 2 does not directly alter HSL activity, but can exert a regulatory role since phosphorylation of site 1 and site 2 on HSL are mutually exclusive. Using a synthetic peptide, based on the sequence surrounding sites 1 and 2 of HSL, it was shown that phosphorylation of the peptide at site 2 totally prevents the subsequent phosphorylation of site 1 and *vice versa* (Garton *et al.* 1989; Garton & Yeaman, 1990). Evidence for a role of 5'-AMP-activated kinase in adipocytes was provided by use of a cell-permeable precursor of 5-amino-imidazole 4-carboxamide ribonucleoside monophosphate (ZMP)

which mimicks the effect of AMP on allosteric activation of rat liver 5′-AMP-activated kinase. Incubation of isolated adipocytes with the ZMP precursor reduces isoprenaline-induced lipolysis (Sullivan *et al.* 1994; Corton *et al.* 1995). It is not clear at present in which physiological situations the 5′-AMP-activated protein kinase regulates HSL activity. AMP not only allosterically activates the enzyme, but also promotes its phosphorylation and activation by a kinase kinase. Conditions which induce cellular stress cause a large increase in intracellular AMP as well as a depletion of ATP and these changes correlate with activation of the 5′-AMP-activated protein kinase. The physiological targets of this enzyme are, besides HSL, acetyl-CoA carboxylase (*EC* 6.4.1.2) and 3-hydroxy-3-methylglutaryl-CoA reductase (*EC* 1.1.1.34) which catalyse regulated steps in the biosynthesis of fatty acids and isoprenoids and/or steroids respectively. Therefore, in conditions of cellular stress, 5′-AMP-activated protein kinase has a possible protective role through inactivation of key enzymes involved in the synthesis or release of fatty acids and cholesterol (Hardie & McIntosh, 1992). Another possible regulation of HSL by 5′-AMP-activated protein kinase involves fatty acids. The kinase kinase is activated by sub-micromolar concentrations of palmitoyl-CoA. When adipocyte fatty acids released by lipolysis and converted to CoA esters reach a level which triggers activation of the kinase kinase and 5′-AMP-activated protein kinase, phosphorylation of site 2 of HSL could occur, and prevent the cAMP-PK-dependent activation of the enzyme and further release of fatty acids. This kinase cascade could constitute a novel form of feedback regulation of lipolysis. Direct negative feedback of HSL by oleoyl-CoA and oleic acid, in a non-competitive manner, has been demonstrated in *in vitro* assays (Jepson & Yeaman, 1992). Inhibition operates via binding of fatty acids to a specific site on the enzyme protein. This coordinated feedback mechanism whereby elevated levels of fatty acids or fatty acyl-CoA regulate the level of free fatty acids in the cell, via inhibition of their mobilization from fat stores, is attractive, although its demonstration *in vivo* has not yet been proved. The existence of additional processes involved in the control of non-esterified fatty acid disposal (for example, attachment to fatty acid-binding proteins, controlled outflow from fat cell by fatty acid transporter, limited access in cell compartments) complicates the delineation of the limiting steps.

HSL is a good substrate for phosphatases 2A and 2C (*EC* 3.1.3.16; Olsson & Belfrage, 1988). Phosphatases 1 and 2A appear to contribute the major phosphatase activities within rat adipocytes, whereas phosphatase 2C is present at low levels (Wood *et al.* 1993). Phosphatases 2A and C exhibit similar degrees of activity towards the regulatory site, but the basal site is dephosphorylated predominantly by phosphatase 2A. It is considered that the control of HSL phosphorylation–dephosphorylation by insulin and catecholamines occurs predominantly at the level of cAMP concentration rather than at the level of phosphatase activity (Manganiello *et al.* 1992).

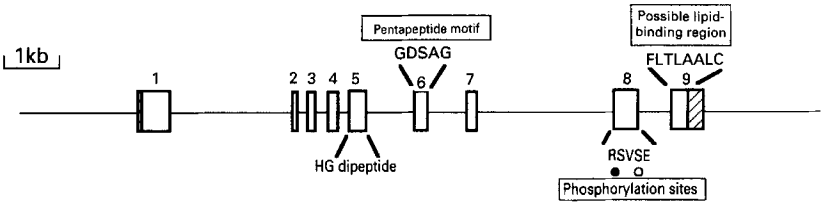
GENOMIC ORGANIZATION AND FUNCTIONAL DOMAINS OF HORMONE-SENSITIVE LIPASE

cDNA for HSL have been obtained from both rat and human adipose tissues (Holm *et al.* 1988; Langin *et al.* 1993). The human HSL gene is composed of nine coding exons spanning eleven kilobases and encodes a protein that is 775 amino acids long (Fig. 4; Langin *et al.* 1993) (J. A. Contreras, T. Østerlund, D. Langin and C. Holm, unpublished results). The organization of the mouse HSL gene is strikingly similar to its human

counterpart (Li *et al.* 1994). Each putative functional region of HSL is encoded by a different exon, suggesting that HSL could be a mosaic protein. Exon 6 encodes a motif for the serine of the catalytic site (Ser^{424}) found in most lipases. Site-directed mutagenesis of the serine residue leads to a complete abolition of both lipase and esterase activity (Holm *et al.* 1994). It has been shown for several lipases that the catalytic site serine is part of a catalytic triad that includes also a histidine and an aspartic acid (Derewenda, 1994). The catalytic triad is responsible for nucleophilic attack on the carbonyl-C of the scissile ester bond, which is the first step of the hydrolysis of ester bonds. X-ray structure and site-directed mutagenesis will be necessary to identify these residues, since HSL does not show homology with other eukaryotic lipases (see below). The phosphorylation site sequence (Met-Arg-Arg-Ser^{552}-Val-Ser^{554}-Glu-Ala-Ala) is encoded by exon 8. Exon 9 encodes a potential lipid-binding domain that shows homology with sequences present in other lipid-binding proteins, including lecithin: cholesterol acyltransferase (*EC* 2.3.1.43), cholesteryl ester transfer protein and pancreatic carboxyl ester lipase (*EC* 3.1.1.1; Au-Young & Fielding, 1992). Overall, the HSL protein is highly conserved between species. Human HSL is 82% identical to both mouse and rat HSL. However, a stretch of amino acids upstream of the phosphorylation site region diverges significantly. Compared with the rat sequence, there is a deletion of six amino acids in mouse HSL and of twelve amino acids in human HSL. The functional importance of this deletion, if any, is presently under investigation.

HSL shares no homology, except for a pentapeptide motif around the catalytic site serine (Gly-Xaa-Ser-Xaa-Gly), with other eukaryotic lipases. In particular, HSL does not belong to the so-called lipase gene family that includes LPL, hepatic lipase (*EC* 3.1.1.3) and pancreatic lipase. However, it does show sequence similarity to five prokaryotic enzymes from distantly related eubacteria (Langin & Holm, 1993). The region of highest similarity is bordered by the pentapeptide motif (Gly-Asp-Ser-Ala-Gly) which is identical in the six proteins and a His-Gly dipeptide which may constitute one of the hydrophobic 'wings' flanking the catalytic site (Fig. 4). The strongest sequence similarity is found with lipase 2 (*EC* 3.1.1.3) of Moraxella TA144, an antarctic bacterium. Since lipase 2 catalyses lipolysis below 4°, the cold adaptability of HSL, an unexpected property for a mammalian lipase, was investigated. HSL retained distinctly more catalytic activity at low temperatures than either LPL or carboxyl ester lipase. The so-called 'cold adaptability' of HSL has recently been discussed (Langin & Holm, 1993; Langin *et al.* 1993). This unexpected property of HSL could be of critical survival value when lipids mobilized at low temperature are the primary energy sources, e.g. in hibernating mammals and poikilotherms. To date, HSL shows sequence similarity with only one eukaryotic protein, a human liver arylacetamide deacetylase (*EC* 3.1.1.1) (Probst *et al.* 1994). This esterase is involved in the metabolic activation of arylamine carcinogens. Interestingly, the first region of homology is bordered by the pentapeptide motif and the His-Gly dipeptide. A second region of homology was found in the C-terminal part of the protein downstream of the phosphorylation site domain. This region is also found in Moraxella TA144 lipase 2 and other prokaryotic enzymes with a lower similarity (Hemilä *et al.* 1994). Therefore, it seems that during the course of evolution regions important for the lipase and/or esterase activity have been conserved whereas the insertion of the regulatory domain of HSL, i.e. the phosphorylation site region, is probably a more recent event.

(a)



(b)

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1   MDLRTMTQSL VTLAEDNIAF FSSQGPGETA QRLSGVFAGV REQALGLEPA LGRLLGVAHL FDLDPETPAN GYRSLVHTAR
81  CCLAHLLHKS RYVASNRRSI FFCTSHNLAE LEAYLAALTQ LRALVYYAQR LLVTNRPGVL FFEGDEGLTA DFLREYVTLH
161 KGCFYGRCLG FQFTPAIRPF LQTISIGLVS FGEHYKRNET GLSVAASSLF TSGRFAIDPE LRGAEFERIT QNLDVHFWKA
241 FWNITEMEVL SSLANMASAT VRVSRLLSLP PEAFEMPLTA DPTLTVTISP PLAHTGPGPV LVRLISYDLR EGQDSEELSS
321 LIKSNGQRSL ELWPRPQQAP RSRSLIVHFH GGGFVAQTSR SHEPYLKSWA QELGAPIISI DYSLAPEAPF PRALEECFFA
401 YCWAIKHCAL LGSTGERICL AGDSAGGNLC FTVALRAAAY GVRVPDGIMA AYPATMLQPA ASPSRLLSLM DPLLPLSVLS
481 KCVSAYAGAK TEDHSNSDQK ALGMMGLVRR DTALLLRDFR LGASSWLNSF LELSGRKSQK MSEPIAEPMR RSVSEAALAQ
561 PQGPLGTDSL KNLTLRDLSL RGNSETSSDT PEMSLSAETL SPSTPSDVNF LLPPEDAGEE AEAKNELSPM DRGLGVRAAF
641 PEGFHPRRSS QGATQMPLYS SPIVKNPFMS PLLAPDSMLK SLPPVHIVAC ALDPMLDDSV MLARRLRNLG QPVTLRVVED
721 LPHGFLTLAA LCRETRQAAE LCVERIRLVL TPPAGPGPSG ETGAAGVDGG CGGRH*  775
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Fig. 4. Genomic organization and amino acid sequence of human hormone-sensitive lipase (*EC* 3.1.1.3). (a) The exon–intron organization of the human hormone-sensitive lipase gene. (□), Exons; (—), introns; (▨), non-coding regions. Different functional regions are encoded by different exons as indicated. (b) The amino acid sequence, indicating the histidine-glycine dipeptide (shown in exon 5) and the catalytic site serine (shown in exon 6), the regulatory phosphorylation site (Ser552) (●) and the basal phosphorylation site (Ser554) (○) and a putative lipid-binding domain (indicated in exon 9).

HORMONE-SENSITIVE LIPASE AND FAMILIAL DISORDERS OF LIPID METABOLISM

HSL is a candidate gene for obesity and related disorders because of its strategic role in the control of lipid metabolism. A precise knowledge of the chromosomal localization of the HSL gene in man and animal models is important for the study of the genetics of adipose tissue metabolism and related disorders. The human HSL locus, designated LIPE, is located on the long arm of chromosome 19 (q13·1→13·2 region; Holm *et al.* 1988; Schonk *et al.* 1990). Several familial disorders, e.g. myotonic dystrophy and malignant hyperthermia, have been mapped to this region of chromosome 19. A refined map of the region ruled out the localization of the HSL gene at the myotonic dystrophy locus (Schonk *et al.* 1990). With respect to malignant hyperthermia, the gene is located at the proper locus very close to the gene for the ryanodine receptor (a Ca^{2+}-release channel of the skeletal muscle sarcoplasmic reticulum; Levitt *et al.* 1995). Since free fatty acid release is increased in muscle homogenates from affected patients, HSL has been proposed as a candidate gene (Levitt *et al.* 1990). Some breeds of swine constitute a good model for the study of malignant hyperthermia and it was shown that a mutation in the ryanodine receptor gene was associated with the disease (Fujii *et al.* 1991). As in man, the HSL gene in pig is located very close to the ryanodine receptor gene on chromosome

6q12 (Gu *et al.* 1992; Chowdhary *et al.* 1994). Therefore, it is still of interest to test the hypothesis that variability in the HSL gene could be linked to, or contribute to, some symptoms of malignant hyperthermia in man and in the pig (Levitt *et al.* 1995).

DNA polymorphisms are of great interest to relate variance in the phenotype and genetic variation. A polymorphic marker, D19S120, was identified within a human genomic clone selected by hybridization to a rat HSL cDNA and a poly(dG-dT) oligonucleotide probe (Levitt *et al.* 1992). D19S120 was subsequently localized to the short arm of chromosome 19 (p13·3 region) by linkage (Jedlicka *et al.* 1994). These findings raised the possibility of the existence of a HSL pseudogene or that the human HSL gene was duplicated on the short arm of chromosome 19. Using cosmid clones from the q13·1→13·2 and p13·3 regions no evidence was found of sequences related to HSL cDNA in the 19p13·3 region. D19S120 was probably identified in a human genomic clone obtained from an artifactual signal due to a reduced stringency used with the rat HSL cDNA probe (Laurell *et al.* 1995). Recently, a high polymorphic dinucleotide repeat was found upstream of exon 8 in the HSL gene. Unlike D19S120, this marker provides a tool for analysing the importance of LIPE in disorders of lipid metabolism (Levitt *et al.* 1995).

The mouse is a valuable animal model to examine the potential role of candidate genes in complex traits, such as non-insulin-dependent diabetes mellitus and obesity, in which multiple genes are involved. LIPE is near the gene cluster containing apolipoproteins CII and E genes on a region of chromosome 7 homologous to human chromosome 19q (Warden *et al.* 1993). Two mouse monogenic obesity loci, Ad (adult obesity and diabetes) and Tub (tubby) map to chromosome 7, but LIPE is clearly distinct from these loci (Warden *et al.* 1993; Wang *et al.* 1994). A multifactorial mouse model has recently been investigated (Warden *et al.* 1995). Four obesity loci were identified. A chromosome 7 locus was shown to affect body fat, total cholesterol and hepatic lipase activity. However, as in the Tub model, LIPE is distinct from this locus. Although it is plausible that HSL may be implicated in some forms of obesity, no obesity-related trait has so far been associated with LIPE.

REGULATION OF HORMONE-SENSITIVE LIPASE EXPRESSION

Acute regulation of HSL by catecholamines and insulin is well documented (Strålfors *et al.* 1987; Frayn *et al.* 1995), but few studies have been devoted to variations in HSL gene expression. Being the rate-limiting enzyme in adipose tissue lipolysis, any variation in the amount of HSL protein would have an impact on the capacity of adipose tissue to mobilize TAG. The recent cloning of HSL cDNA in several species has provided tools that permit accurate mRNA determination and, using recombinant HSL produced in insect cells or HSL–bacterial fusion proteins, large amounts of antibodies against HSL can now be produced (Kraemer *et al.* 1993; Holm *et al.* 1994). The acute regulation of HSL through reversible phosphorylation is studied by measuring glycerol and/or fatty acids released from the adipocyte since there is no satisfactory method to probe directly the proportion of HSL in active form, i.e. HSL phosphorylated at the regulatory site. Measurement of HSL activity using a diacylglycerol analogue as substrate is an indirect measure of total HSL protein. The adaptation of existing methods allows the measurement of total enzyme activity and of HSL mRNA levels in samples of human adipose tissue obtained from small biopsies (Frayn *et al.* 1993).

The lipolytic activity of fat cells has been shown to vary according to the anatomic

location of the fat depot. The understanding of the molecular mechanisms underlying regional variations of lipolysis is important with respect to the consequences of the metabolic disturbances associated with obesity. In particular, accumulation of visceral fat is associated with a higher risk of cardiovascular disease (Björntorp, 1990). In the rat, subcutaneous adipocytes show lower maximal lipolysis rates than adipocytes from internal fat stores (Tavernier *et al.* 1995). The impaired capacity of mobilization of subcutaneous adipose tissue is explained by a lower expression of HSL, i.e. lower activity, protein and mRNA levels. The expression of the three β-adrenoceptors is also impaired in subcutaneous fat depots (Sztalryd & Kraemer, 1994; Tavernier *et al.* 1995). Ageing is accompanied by a decrease in adipose tissue lipolysis in man and in the rat (Lönnqvist *et al.* 1990; Gettys *et al.* 1995), for which a post-receptor defect in the lipolytic cascade has been suggested as an explanation (Lönnqvist *et al.* 1990). However, HSL mRNA levels (Kraemer *et al.* 1991) and activity (D. Langin, unpublished results) did not appear to vary in rat epididymal adipose tissue between 3 weeks and 2 years of age.

Fasting induces an increase in plasma concentrations of free fatty acids in rats and in human subjects. This effect is due to the combination of an acceleration of the rate of lipolysis and a diminished rate of re-esterification (Frayn *et al.* 1995). The acute increase in free fatty acid concentrations is most probably due to an increase in HSL phosphorylation and activation via increased cAMP levels since the plasma concentration of insulin, the main anti-lipolytic hormone promoting cAMP degradation, is decreased. In the rat, regulation of HSL expression occurs after 3 d of starvation. This effect is pre-translational since activity, protein and mRNA levels are increased (Sztalryd & Kraemer, 1994).

In many physiological situations, a coordinated regulation of HSL and LPL is observed in white adipose tissue. HSL is concerned with fat mobilization whereas LPL is responsible for the hydrolysis of TAG contained in lipoprotein particles, thereby governing the input of free fatty acids to be stored as TAG. Pregnancy is a physiological state characterized by changes in maternal adipose tissue mass. Fat accumulates during the early stages of pregnancy and decreases during the later phases. In rat lumbar adipose tissue, LPL activity decreases to one-third that of virgin control animals with less than 10% initial LPL mRNA levels. HSL activity increased maximally 1·5-fold with a fourfold increase in HSL mRNA levels. The HSL:LPL mRNA and activity values are greatly enhanced during late pregnancy. This enhancement coincides with increments in plasma free fatty acid and glycerol levels indicating increased adipose tissue mobilization. These results suggest that changes in gene expression are important for the institution of a catabolic state in late pregnancy (Martin-Hidalgo *et al.* 1994). Another reciprocal regulation of HSL and LPL is shown by hibernating mammals. In the summer and early autumn, hibernators such as the yellow-bellied marmot (*Marmota flaviventris*) dramatically increase food intake and double their spring body mass. Throughout the winter and early spring, marmots fast and body mass declines due to intense adipose tissue mobilization. During the hibernating period there is an increase in plasma free fatty acids suggesting sustained lipolysis. LPL mRNA levels are high during the mass gain phase, whereas HSL mRNA levels are high during the fasting period (Wilson *et al.* 1992). As in pregnancy, these results suggest that the LPL and HSL genes are coordinately regulated to control fat depot mass.

Studies of HSL regulation in human subjects are beginning to emerge. A weight-reduction programme in women with upper-body obesity leads after 5 weeks of

stabilization at the new BMI to an increased efficiency of lipolysis. The *in vitro* sensitivity of β_2-adrenoceptors is increased whereas resting lipolytic rate is decreased. This latter effect is most probably due to a 50% reduction in HSL activity. Changes in circulating levels of catecholamines, insulin and testosterone may play a role in these modifications of adipocyte function (Reynisdottir *et al.* 1995*b*). Altered lipid metabolism in cancer patients is associated with hyperlipidaemia, depletion of fat stores and progressive weight loss. This phenomenon can occur without a decrease in food intake, suggesting that increased lipolysis may be involved. A twofold increase in HSL mRNA levels correlated with increased plasma free fatty acid levels was found in adipose tissue of cancer patients (Thompson *et al.* 1993). This interesting observation needs further support by studying, in parallel with HSL mRNA and activity levels, fat-cell lipolysis in a larger number of cancer patients with controlled energy and protein intake. Some disorders of lipid metabolism such as the insulin-resistance syndrome and familial combined hyperlipidaemia are associated with impaired fat-cell lipolysis (Reynisdottir *et al.* 1994, 1995*a*). Familial combined hyperlipidaemia is the most common familial form of hyperlipidaemia in young survivors of myocardial infarction. The insulin-resistance syndrome has been defined as an association of insulin resistance, elevated triacylglycerolaemia and decreased HDL-cholesterol (Reaven, 1988). These metabolic disturbances are very often found in patients with upper-body obesity, which is also associated with an increased risk of developing non-insulin-dependent diabetes mellitus and coronary heart disease (Frayn & Coppack, 1993). In insulin-resistance syndrome and familial combined hyperlipidaemia, a decrease of maximally-stimulated lipolysis was shown and this defect occurred at a post-receptor level. HSL activity was decreased in patients with familial combined hyperlipidaemia but not in patients with insulin-resistance syndrome (Reynisdottir *et al.* 1995*a*; S. Reynisdottir and D. Langin, unpublished results). These results indicate that multiple mechanisms can account for diminished maximal lipolysis at the level of cAMP-dependent protein kinase–HSL complex. A decrease in HSL protein level explains impaired lipolysis in familial combined hyperlipidaemia whereas a post-translational defect, for example in the mechanism of translocation and/or activation of HSL, could be of importance in the insulin-resistance syndrome.

Few data are available on the factors controlling HSL gene expression. Neutral-TAG lipase activity is greatly enhanced during adipocyte differentiation of 3T3-L1 fibroblasts (Kawamura *et al.* 1981). A marked increase in HSL activity and mRNA levels is also observed during differentiation of human pre-adipocytes (D. Langin, M. Dauzats and M. Lafontan, unpublished results). HSL is generally classified as a late marker of differentiation, along with the fatty acid-binding protein and glucose transporter 4 (Ailhaud *et al.* 1992). Overexpression of HSL in murine 3T3-F442A fibroblasts prevents the accumulation of TAG in pre-adipocytes induced to differentiate. This is accompanied by a slight decrease or no change in LPL expression, an early marker of differentiation, and a marked drop in the expression of late markers of differentiation such as the fatty acid-binding protein and glycerol-3-phosphate dehydrogenase (*EC* 1.1.99.5). Therefore, aberrant expression of HSL before differentiation impairs the appearance of late markers and of a normal adipocyte phenotype (Sztalryd *et al.* 1995). Whether a disruption of cellular lipid pathways is involved remains to be investigated. Although the acute control of HSL activation in response to hormones is well known (Fig. 1), very few data are available concerning the modulation of HSL expression by

hormones and cytokines. An increase in HSL mRNA levels in isolated rat adipocytes in response to sub-micromolar concentrations of the glucocorticoid dexamethasone has been reported after 24 h exposure (Slavin *et al.* 1994). Much interest has been devoted recently to the role of tumour necrosis factor α (TNFα) in obesity. TNFα seems to be involved in the insulin-resistant state of obesity (Spiegelman & Hotamisligil, 1993). TNFα expression in adipose tissue is increased in obese patients. Body-weight reduction in these patients results in a decrease in TNFα mRNA expression associated to an improvement in insulin sensitivity (Hotamisligil *et al.* 1995; Kern *et al.* 1995). TNFα causes an increase in adipose tissue lipolysis (Patton *et al.* 1986; Kawakami *et al.* 1987). Surprisingly, HSL total activity and mRNA levels are decreased after TNFα treatment of 3T3-L1 adipocytes (Sumida *et al.* 1990). These apparently contradictory observations might be explained by the de-differentiating effect of TNFα, which leads in cultured cells to a decrease in expression of several markers including C/EBPα, a transcription factor crucial for the acquisition of the adipocyte phenotype (Ron *et al.* 1992), and by a strong direct lipolytic effect via an unknown pathway.

CONCLUSION AND FUTURE TRENDS

In the present review, we have focused on the role of HSL in the hydrolysis of adipose tissue TAG. The enzyme is expressed in other tissues, most notably steroidogenic tissues such as testes, adrenals and ovaries (Holm *et al.* 1987). The role of HSL in these tissues is not known. HSL could hydrolyse TAG or cholesteryl esters since both classes of molecules are HSL substrates. A possible importance of the cholesteryl esterase activity of HSL in adipose tissue also remains to be investigated.

The accumulation of data derived from molecular biology will facilitate research on different aspects of HSL. The cloning of human, mouse and rat cDNA was a prerequisite for the design of expression vectors. Large amounts of recombinant HSL can now be produced using a baculovirus–insect cell expression system (Holm *et al.* 1994). The functional domains of the protein are being mapped using proteolytic cleavage in combination with site-directed mutagenesis. The availability of the protein in large quantities is also critical for the determination of the three-dimensional structure of HSL. Much has recently been learnt from crystallographic studies of lipase-mediated catalysis (Derewenda, 1994). Of particular importance will be the studies of the relationship between the phosphorylation sites and the catalytic site, since HSL is the only known eukaryotic lipase which is regulated directly through phosphorylation. Establishment of transgenic mouse lines expressing HSL mutants will be very important for studies of structure–function relationships *in vivo*.

The precise chromosomal localization of the HSL locus in several species and the characterization of DNA polymorphisms will facilitate genetic studies and permit the testing of the hypothesis of the involvement of HSL in inherited diseases of lipid metabolism.

New insights into the regulation of HSL expression are also expected. Clinical data are starting to accumulate showing that in certain disorders and physiological states HSL expression varies. The possibility of measuring HSL total activity and mRNA levels in small adipose-tissue biopsies is important in understanding the level of regulation (pre- or post-translational). The combination of lipolysis experiments and HSL studies in man and animal models will lead to new findings in the near future. The study of the

molecular mechanisms underlying the regulation of HSL expression should be facilitated by the use of mouse pre-adipocyte cell lines and human pre-adipocyte primary cultures. The elucidation of HSL genomic organization with the characterization of flanking regions is important for studies of regulatory elements controlling HSL gene transcription. Transgenic mice expressing promoter–reporter gene constructions will be a necessary complement to studies on pre-adipocyte cell lines and *in vitro* characterization of transcription factors.

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