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1 Hormone-sensitive lipase: sixty years later

2

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1 Abbreviations:

2 AICAR, 5-Aminoimidazole-4-carboxamide ribonucleotide; AMPK, AMP-activated kinase;
3 AT, adipose tissue; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; CE,
4 cholesteryl ester; ChREBP, carbohydrate-responsive element-binding protein; DAG,
5 diacylglycerol; DNL, de novo lipogenesis; ELOVL6, Elongation of very long chain fatty acids
6 protein 6; ERK, extracellular signal-regulated kinase; FABP4, fatty acid binding protein 4;
7 FAHFAs, fatty acid esters of hydroxyl fatty acids; HSL, hormone-sensitive lipase; LD, lipid
8 droplet; MAG, monoacylglycerol; MGL, monoacylglycerol lipase; NEFA, non esterified fatty
9 acids; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; PPAR,
10 peroxisome proliferator-activated receptor; PP2A, phosphatase 2A; PP2C, phosphatase 2C;
11 PRIP, phospholipase C-related catalytically inactive protein; RE, retinyl esters; STaR,
12 steroidogenic acute regulatory protein; TAG, triacylglycerols; UCP1, uncoupling protein 1

13

14 Highlights

- 15 - Hormone-sensitive lipase (HSL) is a neutral lipase with broad substrate specificity
- 16 - HSL activity is under hormonal control via protein kinase-dependent phosphorylation
- 17 - Isoforms contain various N-terminal domains, a catalytic domain and a phosphorylation
18 site region
- 19 - The catalytic domain is the only region sharing homology with non-mammalian
20 enzymes
- 21 - Dysfunction of HSL is observed in metabolic diseases

22

23

1 Abstract

2 Hormone-sensitive lipase (HSL) was initially characterized as the hormonally regulated neutral
3 lipase activity responsible for the breakdown of triacylglycerols into fatty acids in adipose
4 tissue. This review aims at providing up-to-date information on structural properties, regulation
5 of expression, activity and function as well as therapeutic potential. The lipase is expressed as
6 different isoforms produced from tissue-specific alternative promoters. All isoforms are
7 composed of an N-terminal domain and a C-terminal catalytic domain within which a
8 regulatory domain containing the phosphorylation sites is embedded. Some isoforms possess
9 additional N-terminal regions. The catalytic domain shares similarities with bacteria, fungus
10 and plant proteins but not with other mammalian lipases. HSL singularity is provided by
11 regulatory and N-terminal domains sharing no homology with other proteins. HSL has a large
12 substrate specificity compared to other neutral lipases. It hydrolyzes acylglycerols, cholesteryl
13 and retinyl esters among other substrates. A novel role of HSL, independent of its enzymatic
14 function, has recently been described in adipocytes. Clinical studies revealed dysregulations of
15 HSL expression and activity in disorders, such as lipodystrophy, obesity, type 2 diabetes and
16 cancer-associated cachexia. Development of specific inhibitors positions HSL as a
17 pharmacological target for the treatment of metabolic complications.

18

19 Keywords

20 Hormone-sensitive lipase; LIPE; lipids; lipolysis; lipodystrophy; obesity

1. Brief historical overview

Lipolysis is defined as the hydrolysis of triacylglycerols (TAG), commonly referred to as fat. The importance of fat degradation for general metabolism has been uncovered between the mid-19th and the early 20th century. Studying food physical and chemical modification in the mammalian intestine, Claude Bernard observed in 1856 fat degradation by the pancreatic juice [1]. It was the first description of gastrointestinal lipolysis allowing catabolism of dietary fat. In 1909, it was found that fat (TAG) could not enter in or emerge from cells in a nonhydrolyzed form, showing the importance of vascular and intracellular lipolysis [2]. In white adipose tissue (AT), intracellular lipolysis corresponds to the breakdown of TAG into non esterified fatty acids (NEFA) and glycerol, for the most part delivered into the blood circulation. The liberation of NEFA plays a crucial role in providing energy-rich substrates to consuming tissues during energy requirement. Studies over the 1960's began to decipher the hormonal regulation of AT lipolysis [3, 4]. Exposure of isolated rat AT to adrenocorticotrophic hormone and epinephrine induced release of NEFA in the culture medium. This effect was inhibited by glucose and insulin. Through these studies, the existence of an intracellular lipase which activity is regulated by these stimuli was inferred. The enzyme was called hormone-sensitive lipase (HSL) [3, 5]. Later, two other intracellular lipases were discovered : monoacylglycerol lipase (MGL) and adipose triglyceride lipase (ATGL) [5-8]. The three neutral lipases, with optimal enzymatic activity around pH 6-7, act sequentially in order to achieve effective intracellular lipolysis [9]. HSL differs from the other two adipose neutral lipases on important points. First, HSL is the only one to be directly regulated by neurohumoral signaling, which confers HSL a pivotal role in basal and stimulated lipolysis [10]. Second, a most surprising feature is the broad substrate range of HSL as it efficiently hydrolyzes tri-, di- and monoacylglycerols, but also cholesterol and retinyl esters among other substrates [11, 12]. The combination of hydrolysis of several

1 substrate classes with a wide tissue expression pattern explains the involvement of HSL in many
2 physiological processes. The major steps in HSL research are summarized in Figure 1. This
3 review aims at describing the current view at HSL detailing structural properties with
4 evolutionary related enzymes, expression profile and regulation, activity and function, and
5 potential as a therapeutic target against metabolic disorders.

6

7 2. LIPE gene organization: transcript and protein diversity

8

9 The human LIPE gene generates several HSL mRNA transcripts and protein isoforms through
10 alternative promoter usage and exon skipping. Following presentation of the general
11 organization of the LIPE gene, we describe alternatively used exons, along with the
12 corresponding promoters and resulting isoforms (Figure 2).

13

14 2.1 LIPE gene

15

16 The LIPE gene encoding human HSL is located on chromosome 19 in the q13.2 region. In this
17 chromosomal region, LIPE is the only lipase encoding gene. Initially, nine exons spanning 11kb
18 transcribed into an approximately 2.8kb mRNA had been described [13]. Subsequently, four
19 alternative exons, called exon B, A, T2 and T1, were found upstream of exon 1. The four exons
20 are alternatively used to produce different 5' termini in LIPE transcripts. Splicing occurs
21 between these upstream exons and exon 1 to generate the different HSL protein isoforms. Use
22 of multiple 5' exons is found in different species. In the mouse, the corresponding four
23 alternative exons upstream of exon 1 have been mapped [14]. The heterogeneity in transcript
24 5' ends is also detected in rat, ewe, chicken and more recently in fish (grass carp) where two
25 isoforms have been found [15-17].

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2.2 Isoform heterogeneity

2.2.1 HSL-exon B isoform

In humans, the most frequent 5'-untranslated region found in adipocyte HSL transcripts corresponds to the non-coding exon B located 1.5kb upstream of exon 1. This transcript encodes a 775 amino acid protein of 88 kDa [13, 18]. The corresponding adipocyte HSL isoforms in rodents, other mammalian species, fish, birds but also invertebrates such as *Drosophila melanogaster* and *Caenorhabditis elegans* range from 82 to 90kDa [19-21]. The overall degree of HSL identity is 72% and 78% at nucleotide and amino acid levels, respectively, showing a high conservation between species. Although initially characterized in AT, HSL-exon B isoform is also expressed in other tissues such as skeletal muscle, heart, intestine, adrenals and liver. Oocyte microinjection of a 21 kb human genomic fragment encompassing 8 kb of 5'-flanking sequence, exon B and exons 1 to 9 results in robust expression of HSL in AT of transgenic mice [22]. The human adipocyte LIPE minimal promoter has been characterized in vitro in differentiated preadipocytes [18]. The first 2.4 kb of the 5'-flanking region contains a strong positive regulatory sequence. The 137 bp upstream of the transcriptional start site do not contain CAAT- and TATA-boxes but other functional cis-acting elements: two GC-boxes binding Sp1-like transcription factors, and one E-box binding basic helix-loop-helix transcription factors [23]. Glucose exposure of mouse and human adipocytes increases HSL gene expression while FA treatment has no impact [23, 24]. The glucose-induced expression is dependent on the presence of the E-box. Further work is required to identify the metabolites and transcription factors involved in the glucose response. Modulation of HSL expression is not limited to glucose, since treatment of human adipocytes with peroxisome proliferator-activated receptor (PPAR) γ agonist also increases HSL gene expression. This effect is mediated

1 through the enhancement of Sp1 binding to the GC-boxes rather than direct DNA-binding of
2 PPAR γ [25].

3

4 2.2.2 HSL-exon A isoform

5 In humans, exon A, mapped 13kb upstream of exon 1, is used in a mutually exclusive way with
6 exon B. Exon A encodes 43 amino acids at the N-terminus resulting in an isoform slightly larger
7 than the HSL-exon B isoform [26-28]. The isoform, also found in mice, is expressed in
8 pancreatic β cells and enterocytes and, to a lesser degree in adipocytes. In pancreatic β cells,
9 several cis elements of the human exon A promoter have been found within 170 bp upstream
10 of the putative transcription start site. As described for exon B, this promoter does not contain
11 classical elements, like TATA-box, but shows putative Sp1 and NF-Y binding domains [26].
12 Two stimuli, glucose and FA-induced TAG accumulation, have been identified to upregulate
13 HSL expression in β cells [29]. Further work is needed to identify the transcription factors
14 involved in these responses.

15

16 2.2.3 HSL-exon T isoforms

17 In humans, two HSL isoforms specific of the testis are produced by exons T1 and T2. Exon T2,
18 located near the 3'end of exon A, is non coding and leads to the production of a 775 amino acid
19 protein that is identical to the adipocyte form of HSL. Exon T1, located approximately 16kb
20 upstream of exon 1, encodes the N-terminus of a second testis-specific large transcript of 3.9
21 kb [30]. This testis isoform, approximately 120-130 kDa, contains an additional N-terminal 313
22 amino acids. A similar larger isoform of HSL is expressed in rodent testis [30]. The long form
23 testicular HSL is restricted to spermiogenesis stages, first in early spermatids and at higher
24 levels in later stages of the seminiferous epithelium cycle, elongating and elongated spermatids
25 [31, 32]. The short form is expressed in spermatogonia, pachytene spermatocytes, Sertoli cells,

1 Leydig cells and peritubular cells. Therefore, whereas exon T1 is exclusively transcribed in
2 haploid germ cells, exon T2 is used in somatic and pre-meiotic germ cells. To date, only the
3 promoter of the larger testis form of HSL has been characterized. The sequence governing in
4 vivo testis-specific expression is located within 95 bp of the 5' region flanking exon T1 [31-
5 33]. A GT-rich region found within the minimal promoter is required as mutation of this region
6 in transgenic mice abolished testis-specific expression of a reporter gene [33]. Gel retardation
7 assays suggest that the nuclear protein binding the GT-rich region belongs to the Zinc finger
8 transcription factor family but the protein has not yet been identified.

9

10 2.2.4 HSL-exon 6 skipping

11 Through alternative splicing, a catalytically inactive truncated exon-B isoform is expressed in
12 human testis, adrenal gland, skeletal muscle and white AT [34]. It results from skipping of exon
13 6 which encodes the catalytic site Serine. In human subcutaneous adipocytes, it represents
14 approximately 20% of HSL transcripts. The truncated isoform of 80 kDa has not been observed
15 so far in other species.

16

17 3. HSL protein structure

18

19 3.1 Catalytic domain

20

21 In humans, the C-terminal catalytic domain is encoded by exons 5, 6, 5' part of exon 7, 3' part
22 of exon 8 and exon 9 (Figure 2) [35, 36]. As in other hydrolases such as lipases, esterases,
23 proteases, peroxidases and dehalogenases, the catalytic domain is structured in an α/β fold
24 architecture [37, 38]. The active site is composed by three amino acids defined as the catalytic
25 triad [39]. Correspondence of critical residues in human and rodent HSL is shown for exon-B

1 isoform in Table 1. The triad usually contains one basic and one acidic residue forming a
2 favorable environment for the attack of the substrate by the nucleophilic residue. In humans,
3 Ser-424, Asp-693 and His-723 were identified as HSL catalytic triad through site-directed
4 mutagenesis, the serine residue catalyzing the nucleophile attack [36]. HSL active site serine
5 residue is positioned within a GXSXG motif found in proteases and lipases such as lipoprotein
6 lipase and pancreatic lipase [40]. Larger patterns have been defined according to the Prosite
7 database. The active site serine is included in the LIPASE_GDXG_SER (PS01174) pattern
8 found in 70 members of the “G-D-X-G” eukaryotic and prokaryotic lipolytic enzyme family.
9 A HGGG motif is included in the LIPASE_GDXG_HIS (PS01173) pattern found in 49
10 members of this family. The HGGG motif located through protein folding close to the catalytic
11 triad, participates in the formation of an oxyanion hole [41]. This oxyanion hole contributes to
12 the stabilization of the hydrolysis reaction. Accordingly, mutation of the last two glycines of
13 the HGGG motif induces a significant loss of HSL enzymatic activity [42]. The catalytic
14 domain is well conserved, the human sequence shares 80% identity with the mouse sequence,
15 and from 31 to 51% with *Danio rerio*, *Drosophila melanogaster* and *Caenorhabditis elegans*
16 sequences.

17

18 3.2 Regulatory domain

19

20 Within the human catalytic domain, the region ranging from 495 to 665 amino acids, encoded
21 by the 3' part of exon 7 and most of exon 8, contains all known phosphorylation sites [36]. This
22 so-called regulatory domain confers control of HSL enzymatic activity by phosphorylation. The
23 human sequence shows 71% identity with the mouse sequence. Its secondary structure is not
24 known. The regulatory domain has no similarities with other human proteins and is not found
25 in other members of the HSL family (see 5.1). It therefore seems to result from late insertion

1 during evolution. However, in sequences of HSL-like proteins, shorter insertions within the
2 catalytic domain are observed. The presence of phosphorylation sites regulating activity has not
3 been shown in these proteins.

4

5 3.3 N-terminal domains

6

7 The N-terminal domain encoded by exons 1 to 4 comprises the first 314 amino acids. While its
8 function has not yet been clearly identified, the direct interaction between HSL exon-B isoform
9 and FABP4 (fatty acid binding protein 4, a carrier protein of FA) has been localized between
10 residues 192 and 200 [43]. Moreover, the N-terminal region seems to play a role in HSL
11 dimerization. HSL isoforms can form homodimers. Of note, both HSL N-terminal (1–300) and
12 C-terminal (300–768) regions are able to interact with full-length HSL suggesting a ‘nose to
13 tail’ interaction between two monomers [44]. This observation leads to the hypothesis that the
14 N-terminal domain is a docking domain for protein-protein interactions. This domain is well
15 conserved among species with 92% identity between human and mouse sequences and a range
16 from 27 to 52% with *Danio rerio*, *Drosophila melanogaster* and *Caenorhabditis elegans*
17 sequences.

18 A hinge region makes the link between the N-terminal and catalytic domains [35]. Typically,
19 hinge regions allow flexibility, allowing the domains to move relative to one another. Further
20 work is needed to determine whether this region is involved in HSL protein/protein interaction,
21 substrate accessibility or enzymatic activities.

22 The function of the additional N-terminal sequences found in HSL-exon A and -exon T1
23 isoforms remains unknown. A proline-rich region, known to be a potential motif for protein-
24 protein interaction involving SH3 domain, has been identified within the HSL-exon T1 N-
25 terminal domain [30]. Thus, additional N-terminal sequences could modulate HSL interactome

1 in a tissue specific manner. As the regulatory domain, HSL N-terminal domains are unique
2 among human proteins.

3

4 4. HSL enzymological properties

5

6 HSL ester hydrolase activity promotes the cleavage of a covalent bond at ester position using
7 one water molecule and producing a carboxylic acid and an alcohol (Figure 3A). This reaction
8 is achieved in two steps. First, HSL cleaves the covalent bond through the nucleophilic attack
9 of the catalytic serine and forms a covalent intermediate stabilized by the oxyanion hole with
10 the carboxylic acid-containing product at the serine position. The first step results in release of
11 the alcohol-containing product. Second, a water molecule displaces the carboxylic acid
12 releasing the second product. An important feature of HSL is the capacity to recognize many
13 substrates. The most documented activities concern the hydrolysis of acylglycerols, cholesteryl
14 esters (CE) and retinyl esters (RE) (Figure 3B) [11, 12].

15

16 4.1. Acylglycerol hydrolysis

17

18 Following esterification on a glycerol backbone, FAs are stored as TAG. Lipolysis is achieved
19 through the breakdown of one TAG into three NEFA and one glycerol molecules. Current
20 knowledge about AT lipolysis postulates sequential action of three lipases, each of them
21 hydrolyzing one ester bond releasing one FA from the glycerol backbone. First, ATGL
22 hydrolyzes TAG into diacylglycerol (DAG). Then HSL breaks DAG into monoacylglycerol
23 (MAG), and finally MGL cleaves MAG into FA and glycerol [45]. HSL, the first AT neutral
24 lipase identified appeared for a long time as the key enzyme catalyzing AT lipolysis.
25 Accordingly, mice and rats treated with a specific HSL inhibitor have decreased plasma NEFA

1 and glycerol levels [46]. However, in HSL deficient mice, a residual TAG hydrolase activity
2 was observed, subsequently leading to the identification of ATGL which catalyzes the initial
3 step in TAG hydrolysis [6-8, 47]. DAG, but not TAG, accumulation was shown in several
4 tissues of HSL knock-out mice, defining HSL as the rate-limiting enzyme for the cellular
5 catabolism of DAG [48]. However, opposite to ATGL which only hydrolyzes TAG among
6 acylglycerols, in vitro studies show the capacity of HSL to hydrolyze TAG, DAG and MAG
7 [11]. The respective specific activities of human HSL against these three substrates have been
8 determined in vitro on triolein (5 ± 2 $\mu\text{mol}/\text{min}/\text{mg}$), diolein (50 ± 5 $\mu\text{mol}/\text{min}/\text{mg}$) and monoolein
9 (25 ± 2 $\mu\text{mol}/\text{min}/\text{mg}$), showing HSL preference for DAG [49]. Supporting the possible
10 involvement of HSL in TAG hydrolysis, ATGL deficient mice have a residual TAG hydrolase
11 activity in contrast to ATGL and HSL double deficient mice [50]. In addition, HSL can
12 compensate up to 50% of the MAG hydrolase activity in AT of MGL deficient mice [51]. Thus,
13 whereas HSL main role in AT is DAG hydrolysis, the lipase can also hydrolyze TAG and MAG.
14 AT TAG of most mammals contain a wide spectrum of FAs differing by chain length and
15 unsaturation degrees. Interestingly, HSL seems to selectively release individual FAs according
16 to their structure. In vitro, the use of a stable lipid emulsion and human recombinant HSL
17 showed HSL preference for 12 to 24 carbon atom FA [52]. Among saturated FA, the relative
18 hydrolysis markedly decreased with increasing chain length. The effect of carbon chain length
19 is more pronounced than that of degree of unsaturation [53]. The FA position on glycerol
20 skeleton is also important since HSL has been shown to preferentially cleave the outer (sn-1
21 and sn-3) positions, with a stereopreference for the hydrolysis of the ester bond at the sn-3
22 position [49, 54]. Accordingly, using diolein racemate as initial substrate, 2,3-sn-DAG is
23 preferentially hydrolyzed while 1,2-sn-DAG accumulates. Furthermore, MAG hydrolysis by
24 HSL seems dependent on the lipid environment. MAG shows more affinity for water than TAG
25 and DAG. MAG hydrolysis by HSL may therefore depend on whether HSL is bound to water-

1 insoluble lipid droplets or released from the oil-water interface. Thus, the release of FA from
2 acylglycerols mediated by HSL is not a random process which physiological implications is not
3 fully understood.

4

5 4.2 Cholesteryl ester hydrolysis

6

7 Cholesterol consists of a tetracyclic cyclopenta[a]phenanthrene structure with an iso-octyl side-
8 chain at carbon 17. Binding of a long-chain FA to the hydroxyl group results in formation of
9 CE. CE are less polar than free cholesterol and are the preferential form for transport in plasma
10 and storage in tissues. Purified HSL exhibits CE hydrolase activity two times more efficient
11 than TAG lipase activity [11]. CE activity of HSL on cholesteryl oleate dispersed in the
12 presence of phosphatidylcholine and gum arabic was 4- to 5-fold higher than on long-chain
13 TAGs. This is a particularity of HSL compared to other lipases hydrolyzing TAG and CE
14 which usually show a relatively higher activity on TAG compared to CE [55]. HSL specific
15 activities on CE and DAG are in the same order of magnitude *in vitro*. The lipase preferentially
16 hydrolyses oxidized CE, however the underlying mechanism leading to this preference remains
17 unclear [56]. HSL is the major neutral CE hydrolases in many tissues [57]. Thus, the lipase,
18 through cholesterol liberation, is involved in the regulation of intracellular cholesterol
19 metabolism, contributing to a variety of pathways in which cells use cholesterol.

20

21 4.3 Retinyl ester hydrolysis

22 Another substrate for HSL are RE which are cleaved by HSL to release retinol, a form of
23 vitamin A. Dibutyryl cAMP treatment of mouse adipocytes inducing HSL enzymatic activation
24 (see 6.1.1) increases retinol accumulation in culture media and decreased cellular RE
25 concentrations [12].

1 While the previously described enzymological studies of HSL highlight its capacity to
2 hydrolyze a range of well-known lipid substrates, it can be speculated that other lipid species
3 are HSL substrates. As a matter of fact, the bioactive lipids, fatty acid esters of hydroxyl fatty
4 acids, FAHFAs, can be esterified to a glycerol backbone, and ceramides can be esterified into
5 acylceramides [58, 59]. As the two lipid species can be stored in lipid droplets, HSL is a
6 potential candidate hydrolase against these lipids.

7

8 5. The HSL family

9 5.1 Catalytic domain

10

11 The HSL family gathers several proteins that display primary structure homology with HSL C-
12 terminal catalytic domain including a catalytic triad composed by serine, aspartic acid and
13 histidine residues and the presence of both GX SXG and HGGG motifs [13, 60]. They also share
14 the α/β fold architecture. This family is largely represented among the Three Domains of Life.
15 In Bacteria, many esterases defined as HSL-like proteins belong to family IV, one of the fifteen
16 families of bacterial esterases [61-63]. Members of HSL family are also found in Archea [64,
17 65]. In Eukaryotes, HSL-like proteins are found in Plants and Fungus [66, 67]. HSL-like
18 proteins have an esterase activity, except a noticeable exception found in plant, *GID1* [67].
19 *GID1* is an intracellular receptor, preferentially located in the nucleus, for gibberellins,
20 hormones involved in plant growth [68]. The lack of *GID1* enzymatic activity results from the
21 absence of well conserved essential amino-acid residues in both the catalytic triad, where
22 histidine is substituted with valine, and the HGGG motif, where the last glycine is substituted
23 with a serine [69]. This observation reveals the importance of these motifs in the hydrolase
24 activity but also highlights for the first time a non-enzymatic function for a HSL-like protein.

25

5.2 Substrate variability and structural variation

1
2
3 Enzymatic activities of several HSL family members have been studied. However, endogenous
4 substrates of HSL-like esterases are often not yet known. As does HSL, some HSL family
5 members hydrolyze TAG to promote bacterial or fungus growth. As an example, inhibition of
6 members of the HSL family reduced growth of *Mycobacterium tuberculosis* [70]. These
7 enzymes display various degrees of affinity and specificity toward FA notably according to
8 chain lengths. Some of the esterases cleave ester bonds on other substrates like tertiary alcohols
9 (linalyl acetate and α -terpinyl acetate), acylated monosaccharides or 4-hydroxyphenylacetate
10 (Figure 3C) [71-73].

11 The substrate variability results from differences in both tertiary protein structure and nature of
12 a few residues localized near the active site. Variations at the level of the binding pocket
13 influence substrate specificity. In the fungus *Rhizomucor miehei*, RmEstA possesses a curved
14 tunnel-shaped binding pocket allowing hydrolysis of longer chain FA (C₆-C₁₆) esters while
15 RmEstB has a pocket in funnel-like shape containing two aromatic residues that prevents long
16 chain access, and thus favors esters of shorter carbon chain length (C₂-C₆) [74]. Specific residues
17 near the active site are important determinants of substrate specificity in other members of the
18 HSL family. As in HSL, the GX SXG motif of many bacterial HSL-like proteins corresponds
19 to GDSAG, and the HGGG motif is followed by a phenylalanine residue [75]. These bacterial
20 esterases preferentially hydrolyze middle-size chain esters (C₄-C₆). However, EstA and PsyEst
21 have the particularity to hydrolyze shorter chain (C₂) [76, 77]. EstA presents a GDSVG motif
22 with a tryptophan residue following the HGGG motif, while in PsyEst, a glycine residue is
23 located very close to the catalytic triad because of loop folding. These differences promote the
24 hydrolysis of shorter chain (C₂). Structural studies revealed that the side chain of valine in
25 GDSVG motif of EstA extends between the active site serine and the oxyanion hole glycine,

1 preventing the stabilization of long or middle-size chains, and that the glycine residue in PsyEst
2 enzyme rigidifies the active site, allowing only short chain ester hydrolysis [76, 77].
3 The study of *Mycobacterium tuberculosis* lipases provides additional information on the
4 importance of residues surrounding the catalytic site. LipN, presents the particularity to have a
5 tryptophan in close proximity of the active site, conferring the capacity to participate in
6 xenobiotic degradation and to promote bacterial drug resistance [72]. Another example is
7 RmEstB that is able to hydrolyze the tertiary alcohol ester linalyl acetate to linalyl alcohol
8 through the presence of GGGW motif close to the active site which facilitates binding of tertiary
9 alcohol esters [78].
10 Finally, beyond the C-terminal domain, the N-terminal section of HSL family members seems
11 to play a role in substrate specificity. Indeed, most of these esterases have a CAP domain, also
12 called LID domain, partly located in the N-terminal section [73]. This domain forms a loop
13 turning in front of the catalytic site and could influence substrate accessibility. While EST2
14 hydrolase activity toward TAG decreases as the acyl chain length increases, EST2 mutant in
15 which N-terminal section is deleted shows a similar esterase activity whatever the length of the
16 acyl chains [73]. As the primary sequence of CAP domain is very different among HSL family
17 members, further work is needed to study its implication on substrate preference.

18

19 5.3 Adaptation to extreme environments

20

21 HSL family members have been found in microorganisms living in extreme
22 environments. *Psychrobacter* sp. TA144 from antarctic sea, and *Psychrobacter*
23 *cryohalolentis* K5T from Siberian permafrost, express the HSL like proteins PsyHSL and
24 PMGL2, respectively [61, 79]. Oppositely, the esterase AFEST expressed by *Archaeoglobus*
25 *fulgidus* is found in oil field waters from Hot North Sea while EST2 from *Alicyclobacillus*

1 acidocaldarius has been discovered in Yellowstone National Park geysers. Besides extreme
2 temperatures, HSL family members are found in other extreme environments such as HmEST
3 expressed by the halophilic archaea *Haloarcula marismortu* found in the saline soil of
4 Yuncheng Salt Lake in China [80]. HSL family therefore gathers psychrophilic, mesophilic,
5 thermophilic and halophilic enzymes. Interestingly, many of them have the capacity to be
6 thermostable and therefore efficient in a large temperature range. For example, the thermophilic
7 enzyme EST2 is active between 30 and 100°C with a peak of activity at 70°C [81, 82]. EstCS1
8 is active between 10 and 80°C with a peak of activity at 50°C [83]. Hosl-1 is an HSL-like
9 enzyme expressed in *Caenorhabditis elegans*, a poikilotherm nematode whose internal
10 temperature varies considerably as a result of its inability to maintain thermal homeostasis.
11 Therefore, Hosl-1 is active upon different temperatures to allow fat mobilization and plays a
12 crucial role at low temperatures to allow accumulation of glycerol through efficient lipolysis
13 and protect nematodes against cold stress [84]. Of note, mammalian HSL is also efficient at
14 different temperatures. Significant hydrolysis activity of human HSL is found between 10°C
15 and 42°C with a peak at 37°C [13, 42].

16 This thermal resilience is complex and results from a combination of several factors. Increased
17 hydrogen bonds and electrostatic interactions within secondary structure of the protein seems
18 necessary for thermal stability [85, 86]. Interdomain hydrophobic interactions between CAP
19 and catalytic domains are also important [87]. Moreover, HSL family members as human HSL
20 are able to form homodimers through hydrophobic interactions and salt bridge formation [88].
21 Dimer formation promotes catalytic activity and favors heat-resistance.

22

23 5.4 Industrial interest of HSL family members

24

25 The industry shows interest in lipases because of their potential use in biotechnology

1 applications. The wide substrate affinity, detailed knowledge about the catalytic domain and
2 efficient enzymatic activity in extreme conditions such as high temperature are attributes
3 making HSL family members attractive for the industry. Importantly, bacterial and fungal HSL
4 can be produced at large scale.

5 The bacterial EST2 lipase has generated a specific interest as it is stable at high temperature
6 and efficient in organic and aqueous solutions. EST2 could be used as a pesticide biosensor in
7 liquid food [89]. Paraxon is an organophosphate pesticide with high poisoning potential due to
8 strong parasympathomimetic activity. As paraxon is a potent inhibitor of EST2 enzymatic
9 activity, it can be detected through a colorimetric assay using 2-naphthyl acetate as substrate.

10 The different organophosphate pesticides have very similar structure [89]. Knowledge about
11 EST2 catalytic domain may allow designing EST2 mutants with specificity for a wide spectrum
12 of pesticides. EST2 has also been proposed to enhance cheese ripening [90, 91]. Through
13 induction of lipolysis, the addition of exogenous lipases has been shown to accelerate ripening
14 and enhance flavours [92]. EST2 which lipolytic activity is preserved during the cheese ripening
15 process proves superior to commercial exogenous lipases [90]. Finally, EST2 could also help
16 for detection of foodborne bacteria as a reporter enzyme that detect DNA hybridization in
17 electrochemical chip systems [93, 94]. To this end, EST2 is conjugated with synthetic
18 oligodeoxynucleotides. In case of bacterial RNA hybridization, immobilization of conjugated
19 lipase on electrochemical chip allows hydrolysis of p-aminophenyl butyrate into p-aminophenol
20 generating an electrical signal. The stability of HmEST lipase activity at high temperature and
21 high salt concentration represents an attractive property to be used in biocatalytic reactions in
22 organic solvents such as biodiesel production [95]. The surprising activity of fungal RmEstB
23 toward tertiary alcohols can be used in the flavor industry as linalyl alcohol is an aromatic
24 compound used as a fragrance [71].

25

6. Physiological roles of HSL

Physiological roles of HSL are summarized in Figure 4.

6.1 White adipose tissue

6.1.1 HSL and lipolysis

AT is a tissue specialized in energy storage and mobilization. The primary function of fat cells is to store TAG in a cytosolic lipid droplet (LD) and, during periods of energy demands such as fasting or physical exercise, to mobilize fat stores through lipolysis to fulfill other organ needs. During lipolysis, HSL translocates at the surface of the LD and participates, along with ATGL and MGL, in TAG hydrolysis. As mentioned above, an important feature of HSL, in comparison with other neutral lipases, is its regulation by hormones through reversible phosphorylation of sites located within the regulatory domain. Pro- and anti-lipolytic hormones, such as catecholamines and insulin respectively, directly control HSL activity through regulation of cAMP intracellular level [45]. HSL phosphorylation by cAMP-dependent protein kinase (PKA) increases its translocation from the cytosol to the LD and, induces hydrolase activity due to conformational changes enhancing exposure of HSL hydrophobic surface and thus lipid substrate binding [96-99]. Ser-649 and Ser-650 are the major PKA phosphorylation sites controlling human HSL translocation and enzymatic activity [100-102]. The catalytic serine (Ser-424) is also required for LD migration as its mutation prevents HSL translocation [102]. Two other activating phosphorylation sites have been described, Ser-552 and Ser-589, phosphorylated by PKA and extracellular signal-regulated kinase (ERK), respectively. However, mutagenesis of Ser-552 to Ala did not cause significant change in activation compared with wild-type HSL whereas Ser-589 phosphorylation leads to a modest increase in lipolysis, suggesting a minor role of these residues in HSL activation [101, 103]. In human adipocytes, natriuretic peptides stimulate lipolysis by increasing the intracellular level of cGMP

1 [104]. cGMP-dependent protein kinase (PKG) phosphorylates the same sites as PKA [105].
2 HSL activity can be inactivated by the phosphorylation of Ser-554 by AMP-activated kinase
3 (AMPK) resulting in vitro in blunting of catecholamine-induced lipolysis [106]. Indeed,
4 treatment of adipocyte with an AMPK activator, AICAR, decreases stimulated lipolysis [107].
5 Stimulation of lipolysis leads to AMPK inactivation through PKA phosphorylation of AMPK
6 at Ser-173, and thus decrease of HSL phosphorylation at Ser-554 [108]. Adipose-specific
7 knockout mice for catalytic subunits of AMPK provide an in vivo confirmation of AMPK role
8 in antilipolysis. Impairment of mouse HSL phosphorylation at Ser-565 results in higher
9 phosphorylation at Ser-563 and Ser-660 PKA sites and increased HSL hydrolase activity (Table
10 1) [109]. Although less documented, an important step in HSL inactivation is phosphatase
11 2A(PP2A)- and 2C-mediated dephosphorylation of activating phosphorylation sites [110].
12 Feeding mice with high fat diet results in an increased expression of alpha-isoform of PP2A
13 regulatory subunit B' (B56alpha), associated with decreased stimulated lipolysis and HSL
14 phosphorylation at Ser-660 [111]. In accordance, in vitro, knock down of B56alpha in adipocyte
15 increases HSL activation [111]. To exert its inhibitory effect, PP2A translocates to the LD with
16 the help of phospholipase C-related catalytically inactive protein (PRIP), a binding partner. In
17 PRIP null mice, translocation of PP2A and phosphatase activity in LD fraction are reduced
18 associated with increased HSL phosphorylation and FA release [112].
19 In adipocyte, in addition to post-translational modifications, protein-protein interactions also
20 contribute to the regulation of HSL activity. First, as mentioned above, HSL is able to form a
21 homodimer, enhancing the hydrolytic efficiency of the enzyme [113]. HSL interacts with
22 different LD-associated proteins, notably perilipins. In the basal state, perilipin 1 reduces the
23 access of endogenous lipases to store lipids. Upon stimulated lipolysis, perilipin 1 is
24 phosphorylated by PKA or PKG, allowing HSL/perilipin 1 interaction and thus HSL
25 translocation at the LD surface [114]. Interaction with vimentin is also required for this

1 translocation [115]. In adipocytes from perilipin 1 or vimentin -null mice, HSL translocation
2 upon lipolytic stimulation is lacking, leading to an alteration of lipolysis [116]. The perilipin
3 1/vimentin/HSL interaction is therefore a key step in the modulation of the lipolytic process.
4 Once on the LD surface, HSL catalyzes the breakdown of DAG into one FA and MAG, two
5 products that inhibit HSL activity. When HSL is activated and carries FA, a direct interaction
6 occurs between HSL and FABP4 [117, 118]. The hydrolytic activity of the enzyme is increased
7 because FABP4 sequesters FA and prevents the inhibition by the released products.
8 HSL activity and translocation are therefore regulated by a combination of post-translational
9 modifications and protein-protein interactions.

10 6.1.2 HSL and retinol metabolism

11 Storage of RE in AT represents 15-20% of the total body pool. HSL participates in the liberation
12 of retinol through RE hydrolase activity [12]. Mice lacking HSL display RE accumulation in
13 white AT resulting in decreased availability in retinoids [119]. In consequence, genes known
14 to be positively regulated by retinoic acid, such as RIP140, a factor promoting conversion from
15 white to brown-like adipocyte, were down-regulated in AT of HSL-null mice [120, 121]. A
16 dietary supplementation in retinoic acid partially restores the altered AT gene expression
17 profile. These findings demonstrate the importance of HSL as an RE hydrolase in AT and
18 suggest that HSL influences lineage commitment of fat cells through this activity.

19

20 6.1.3 HSL non enzymatic function and de novo lipogenesis

21 De novo lipogenesis (DNL) allows the conversion of glucose carbons into FA. The liver and
22 AT have the highest lipogenic capacity in the body. In human AT, this pathway is of a more
23 qualitative than quantitative importance [122]. Indeed, while de novo synthesis of FA does not
24 significantly influence the fat mass in habitual dietary conditions, it allows the synthesis of lipid
25 species involved in the control of insulin sensitivity [123, 124]. The glucose-responsive

1 transcription factor ChREBP is a master regulator of DNL [125]. In adipocyte, DNL induction
2 through ChREBP activation enhances insulin signaling [126, 127]. Our group recently
3 described direct protein-protein interaction between ChREBP and HSL representing a novel
4 crosstalk between glucose and lipid metabolism [127]. HSL prevents ChREBP translocation
5 from the cytosol to the nucleus, thereby influencing DNL gene expression. In adipocytes, a
6 decrease in HSL level results in enhanced ChREBP nuclear translocation and expression of
7 DNL target genes, notably the FA elongase ELOVL6. As ELOVL6 catalyzes a critical step in
8 the synthesis of oleic acid in fat cells, the induction of ELOVL6 results in a higher proportion
9 of oleic acid in phospholipids, promoting plasma membrane fluidity and hence insulin signaling
10 [127, 128]. HSL appears as a new regulator of ChREBP activity through a mechanism
11 independent of HSL enzymatic activities. Indeed, the catalytically inactive isoform of HSL
12 generated by exon 6 skipping exerts the same effect as the full-length exon B HSL isoform. In
13 the liver, DNL induction through ChREBP participates in the deleterious effects associated with
14 steatosis [129, 130]. DNL is therefore considered as an important pathway in the pathogenesis
15 of non-alcoholic fatty liver disease. As the result of the low expression of HSL in hepatocytes,
16 HSL/ChREBP interaction is not detected in the liver [127]. Therefore, HSL represents a
17 modulator of DNL specifically in adipocyte.

18 6.2 Brown adipose tissue: lipolysis and fatty acid oxidation

19 Brown adipose tissue (BAT) is abundant in infants and hibernating mammals, and present in a
20 lesser amount in adult humans [131]. Its main function is to produce heat through non-shivering
21 thermogenesis, a process allowed by the presence of the uncoupling protein, UCP1, located in
22 the mitochondrial inner membrane. UCP1 dissipates the proton electrochemical gradient
23 generated during oxidative phosphorylation as heat instead of ATP synthesis [132].
24 FA are the main substrates fueling thermogenesis, serving both as substrates for β oxidation
25 and UCP1 activators. Indeed, FA fixation on UCP1 changes conformation of the protein,

1 increasing its capacity to catalyze proton leak [133]. A human study using positron emission
2 tomography imaging shows that oral treatment with nicotinic acid, an inhibitor of intracellular
3 lipolysis, decreases BAT thermogenesis in response to cold exposure highlighting the
4 importance of adipocyte lipolysis to supply FA for heat production [134]. The persistence of
5 HSL activity at cold temperature could put the lipase in a central role to ensure the mobilization
6 of FA for the activation of non-shivering thermogenesis in animals [42]. In vitro, inhibition of
7 neutral lipases in brown adipocytes decrease UCP1 activation and thermogenic function with a
8 more pronounced effect for ATGL than HSL inhibitors [135]. ATGL null mice are not able to
9 maintain body temperature after cold exposure and present BAT abnormalities such as
10 hypertrophy, lipid accumulation and inflammation [136, 137]. In HSL null mice, an increase of
11 BAT mass and lipid accumulation in brown adipocytes is also observed but the mice are not
12 cold sensitive [47, 138, 139]. These observations suggest a more important role of ATGL than
13 HSL to support non-shivering thermogenesis. In the fasted state, mice with brown adipocyte
14 specific ATGL deficiency are not cold intolerant contrary to white and brown adipocyte specific
15 ATGL knock out mice, suggesting a more important role of WAT than BAT lipolysis to supply
16 FA [137]. In the fed state, there is no alteration of cold sensitivity in mice with depletion of
17 ATGL in WAT and BAT [137]. Supply of diet-derived FAs is sufficient to support BAT
18 thermogenesis and FA oxidation.

19

20 6.3 Skeletal and cardiac muscles: control of lipid content

21 HSL-exon B isoform is expressed in both skeletal and cardiac muscles to regulate their TAG
22 content. HSL and ATGL are expressed at higher levels in oxidative than in glycolytic fibers in
23 proportion to TAG stores in cytoplasmic LD and are the most important lipases involved in
24 intramyocellular TAG hydrolysis [140, 141]. Indeed, HSL pharmacological inhibition in
25 muscle from ATGL null mice completely abolish TAG hydrolase activity [142]. Both, muscle

1 contraction and catecholamine signaling induce HSL activation and translocation to the LD
2 [143, 144]. Study from human skeletal muscle samples shows that during the initial minutes of
3 a low and moderate aerobic exercise, muscle contraction and HSL activation occur in the
4 apparent absence of an increase in circulating adrenaline level [145]. Muscle contraction
5 increases the intramyocellular calcium level leading to ERK activation, HSL phosphorylation
6 at Ser-600 and increased HSL activity [103, 146]. The contraction-induced HSL activation also
7 involves PKC which phosphorylation sites have not yet been identified [146]. Skeletal muscle
8 contractions increase AMPK activity resulting in phosphorylation of the AMPK target site in
9 HSL, Ser565. The consequence of this phosphorylation step is not well-understood. It is not
10 directly involved in the contraction-induced activation of HSL [143]. If the contraction
11 increases in intensity, increased adrenaline level leads to increased HSL activity through PKA-
12 mediated phosphorylation [147, 148]. However, in rat muscle, inhibition of HSL does not
13 influence the breakdown of intramyocellular TAG in response to contraction [142]. Thus,
14 despite its regulation during muscle contraction, HSL does not seem essential suggesting a total
15 compensation by ATGL.

16 In humans, endurance training does not change the amount of HSL in skeletal muscle but
17 increases ATGL content [149]. HSL and ATGL null mice have a reduced endurance capacity
18 [150, 151]. However, HSL deficiency in mice does not affect intramyocellular TAG in skeletal
19 muscle in contrast to ATGL deficient-mice which exhibit a defective TAG hydrolase activity
20 [152]. These observations are in accordance with the hypothesis that ATGL could play a more
21 important role than HSL in the regulation of lipolysis in muscle. Further work is needed in
22 humans to determine the relative contribution of the two lipases.

23 Concerning the cardiac muscle, there is few information about HSL role and regulation.
24 Cardiomyocytes utilize FA as main energy source [153]. When FA supply is important such as
25 during fasting, cardiomyocytes convert FA into TAG that are stored in LD [154]. This pool can

1 be hydrolyzed by ATGL and HSL to produce energy and lipid second messengers [155]. Heart-
2 specific transgenic HSL overexpression leads to an increased TAG hydrolysis and prevents
3 lipid accumulation during fasting [156]. It also affects the expression levels of genes involved
4 in cardiac metabolism, immune response, cell growth, and cytoskeleton organization. An
5 abnormal TAG accumulation and heart dysfunction is observed in diabetic patients [157, 158].
6 Heart-specific HSL-overexpression in diabetic mice decrease cardiac lipid and lipid peroxide
7 contents [159]. Fibrosis and mortality are also reduced in comparison with diabetic control
8 mice. These observations suggest a protective effect of neutral lipases such as HSL on cardiac
9 steatosis through hydrolyzing toxic lipids and reducing toxic lipid peroxides. However, the
10 model of cardiac HSL overexpression is not physiological as HSL level is markedly increased
11 compared to endogenous expression. HSL depletion induces an increase of cardiac weight only
12 under high fat diet but no studies have been performed to evaluate cardiac function [139]. More
13 thoroughly investigated, ATGL depletion promotes large accumulation of lipids and severe
14 tissue damage leading to premature death [152, 160]. Thus, current data suggest that the lipid
15 content in muscles is chiefly regulated through ATGL.

16

17 6.4 Pancreas: insulin secretion

18

19 HSL-exon A isoform is predominantly expressed in pancreatic β -cells which main function is
20 to produce and secrete insulin in response to glucose [27]. Upon glucose stimulation, there is
21 an induction of HSL gene and protein expression in β -cells along with increased glycerol
22 release from pancreatic islets reflecting lipolytic activity [29, 161]. A defective insulin secretion
23 in response to glucose has been reported in β -cells from HSL null mice [162]. In vivo, this
24 alteration observed in fasted male, but not female, HSL null mice can be rescued by exogenous
25 delivery of NEFA [163]. Treatment of rat islets with a HSL inhibitor supports the role of HSL

1 in controlling insulin secretion [46]. In genetic model with β -cell-specific HSL deficiency,
2 fasting hyperglycemia, alteration of the initial secretory response along with an accumulation
3 of insulin in pancreatic islets is observed in female mice independently of the nutritional state
4 [164]. Collectively, these studies highlight a critical role for HSL in β -cell metabolism through
5 the delivery of lipids involved in insulin secretion. Whether genetic background, indirect action
6 of HSL deficiency in other tissues or the compensatory actions of other lipases explain the sex
7 differences observed between global and β -cell-specific HSL knockout mice remain to be
8 determined. Long-chain acylCoAs, the activated form of FA, and MAG are known to stimulate
9 insulin secretion [165-167]. ATGL and MGL seem to have the same effect, as blocking these
10 lipases specifically in β -cells results in decreased insulin secretion in mice [168, 169].
11 Altogether, the data suggest that FA derived from lipolysis are needed to sustain insulin
12 secretion.

13 HSL may also control insulin secretion independently of DAG hydrolase activity. The secretory
14 granules of islets have been observed to contain numerous cholesterol-rich complexes involved
15 in the granule fusion process [170, 171]. In islets of HSL null mice, CE hydrolase activity is
16 blunted and β -cells are depleted in cholesterol-enriched microdomains [172]. The authors
17 proposed a role for HSL in providing cholesterol for the formation and maintenance of
18 cholesterol-enriched plasma membrane microdomains allowing the clustering of proteins
19 involved in granule fusion process. Further work is needed to support this hypothesis.

20

21 6.5 Macrophage: cholesterol metabolism

22

23 Early studies identified a PKA-activating neutral CE hydrolase in murine macrophages [173,
24 174]. HSL-exon B isoform is expressed in murine macrophages [175] and anti-HSL antibody
25 inhibits the macrophage neutral CE hydrolase activity [176]. In human macrophages, HSL

1 expression is more controversial. While some groups do not report expression of the lipase,
2 others detect low gene expression level in human monocyte-derived macrophages and THP-1
3 human monocyte cell line [177, 178]. Overexpression of HSL increases the hydrolysis of CE
4 stores in both murine and human macrophages in vitro [179]. Because macrophage foam cells
5 loaded with CE are a hallmark of atherosclerosis, HSL activation through PKA could prevent
6 deleterious lipid accumulation and foam cell formation [180]. In vivo studies also provided
7 controversial data. It was first observed that macrophage-specific HSL overexpression in mice
8 increases CE hydrolysis capacity, however this was concomitant with increased incidence and
9 severity of atherosclerosis [181]. As, in this study, cholesterol efflux was not modified, the
10 results could be explained by a defective export competing with efficient reesterification of free
11 cholesterol within macrophages, allowing foam cell formation. In HSL-deficient mice, no
12 alteration of CE hydrolysis activity was first reported in peritoneal macrophages [182].
13 However, aggravated atherosclerosis was later described in HSL null mice [183]. Another
14 group demonstrated a decrease CE hydrolase activity in HSL null mouse macrophages, but a
15 similar CE accumulation in comparison to wild-type mouse macrophages [184]. Whereas these
16 findings do not rule out a role for HSL in macrophage cholesterol metabolism, they suggest the
17 presence of other enzymes with neutral cholesterol ester hydrolase activity such as Nceh1 [183].

18

19 6.6 Liver: cholesteryl and retinyl ester metabolism

20

21 The liver is an important organ for cholesterol metabolism. The pool of cholesterol in the liver
22 is tightly regulated, resulting from uptake of dietary cholesterol, biosynthesis, and, secretion
23 and uptake of cholesterol from plasma lipoproteins. Although expression level of HSL-exon B
24 isoform is low in the liver, a study suggests an involvement of the lipase in hepatic CE
25 hydrolysis [185]. Indeed, HSL null mice display hepatic CE accumulation when fed a high-

1 cholesterol diet. As HSL neutral CE hydrolase activity is present in macrophages, this effect
2 could therefore result from HSL deficiency in liver resident macrophages and not in
3 hepatocytes. However, HSL expression is higher in parenchymal cells, i.e. hepatocytes, than in
4 non-parenchymal cells. In vitro, hepatocytes from HSL null mice show a decreased CE
5 hydrolase activity. Taken together, these findings indicate that HSL is expressed in hepatocytes
6 at low level and may contribute to hepatic CE hydrolase activity. Further studies are needed to
7 decipher the importance of HSL activity in comparison to other liver enzymes notably
8 lysosomal CE hydrolases.

9 Hepatic stellate cells are perisinusoidal resident cells storing vitamin A in LD. Once activated,
10 stellate cells become profibrogenic cells as observed in liver diseases [186]. HSL is expressed
11 in rat and human non-activated stellate cells at higher levels than in hepatocytes [187]. HSL
12 colocalizes with LD. As in adipocytes, the beta-adrenergic agonist, isoproterenol, promotes LD-
13 associated HSL phosphorylation and RE hydrolysis [187]. Hepatic stellate cells
14 transdifferentiation into profibrogenic cells is associated with HSL downregulation. These data
15 suggest a role for HSL in hepatic vitamin A metabolism in healthy conditions.

16

17 6.7 Intestine: cholesterol metabolism

18

19 HSL-exons A and B isoforms are expressed in enterocytes of the small intestine [28]. While
20 DAG lipase activity is found in all intestine segments, it is only impaired in jejunum of HSL
21 null mice [28]. However, CE activity is totally abolished in duodenum, jejunum and ileum. In
22 intestine-specific HSL-null mice, no alteration of gut transit time or TAG absorption and
23 metabolism has been observed [188]. However, there is accumulation of CE in the intestine
24 along with accelerated cholesterol absorption and reduced expression of proteins involved in
25 intestinal cholesterol biosynthesis. As little consequence of intestinal HSL depletion on hepatic

1 and plasma lipid parameters is observed, counter-regulatory mechanisms may contribute to the
2 maintenance of whole body cholesterol homeostasis.

3 4 6.8 Adrenal steroidogenesis

5
6 The adrenal gland is a steroidogenic tissue producing corticosteroid hormones. Cholesterol is
7 the basic substrate for the synthesis of all steroid hormones. HSL is the main neutral CE
8 hydrolase in adrenals [189]. HSL null mice exhibit 98% reduced neutral CE hydrolase activity
9 and lower corticosterone plasma levels in response to adrenocorticotrophic hormone stimulation
10 [190]. The HSL-exon B isoform is mostly expressed in the inner cortex within microsomes and
11 to lesser extent in the cytosol. LIPE gene expression is induced through a pathway involving
12 PKA and steroidogenic factor-1 and through activation of C/EBP α , a transcription factor
13 known to induce the expression of number of genes involved in steroid hormone synthesis
14 [191, 192]. As in adipocytes, HSL activity is induced by phosphorylation and protein-protein
15 interaction in adrenals. Binding of the adrenocorticotrophic hormone to the melanocortin 2
16 receptor stimulates the PKA signaling pathway leading to HSL phosphorylation [193, 194].
17 Furthermore, HSL directly interacts with StAR, a transport protein that
18 regulates cholesterol transfer within the mitochondria, a critical step in steroidogenesis [195].
19 This interaction occurs in the cytosol and increases both hydrolytic activity of HSL and
20 cholesterol movement from stored LD to mitochondria.

21 22 6.9 Testis function

23
24 As described above, two testis-specific isoforms have been described, a short form expressed
25 in diploid cells and a long form expressed in haploid cells. In Leydig cells, an impairment in

1 CE hydrolase activity by the short form could impair steroidogenesis. In guinea pigs, which as
2 humans but unlike rodents, express HSL in Leydig cells, HSL enzymatic activity is positively
3 correlated with serum testosterone levels during development [196, 197]. In these cells, in vitro
4 studies show that HSL promotes steroid biosynthesis through regulation of StAR expression
5 resulting from production of the liver X receptor ligand, oxysterols [193]. Whether the protein-
6 protein interaction between HSL and StAR described in adrenals plays a role has not been
7 investigated.

8 LIPE gene expression increases dramatically during sexual maturation. In rat testis, the steady
9 state level of HSL mRNA is undetectable before 4 weeks of age, and then increases about 25-
10 fold to reach the adult level [198]. Testis from HSL null mice show complete lack of neutral
11 CE hydrolase activity and CE accumulation [47]. The most dramatic feature in HSL null mice
12 is male sterility, associated with reduction and abnormal shape of elongating spermatids,
13 leading to a dramatic reduction in sperm number and motility [47, 199]. Expression of human
14 testicular HSL in HSL null mice rescued testicular abnormalities and restored fertility [197,
15 200]. Additional transgenic mouse models revealed that the testis-specific N-terminal part of
16 HSL is dispensable whereas an intact catalytic function is required for male fertility [201]. HSL
17 plays therefore a critical role in rodent spermatogenesis. However, the mechanisms by which
18 HSL contributes to male fertility or the lipase substrates remain unclear. No signs of infertility
19 has been described in the few men reported with HSL deficiency [202, 203]. To date,
20 assessment of sperm count and proper evaluation of spermatogenesis have not been performed.

21

22 7. HSL involvement in physiopathology

23 Pathophysiological roles of HSL are summarized in Figure 5.

24

25 7.1 Lipodystrophy

1 In humans, the clinical characteristics of the first patients with HSL deficiency have been
2 reported in 2014 [202]. Individuals from the Amish community carry a 19-bp frameshift
3 deletion in exon 9 of the LIPE gene resulting in an aberrant unstable protein,
4 p.(Val767Glyfs*102). In AT of heterozygous carriers, the mutation leads to a 50% decrease of
5 HSL level whereas total lack of the protein is observed in the few homozygous carriers.
6 Whereas heterozygous carrier AT show no overt differences compared with non-carriers,
7 homozygous carriers were characterized by smaller adipocytes, macrophage infiltration and
8 altered gene expression in subcutaneous abdominal AT. AT dysfunction was associated with
9 systemic traits such as hepatic steatosis, dyslipidemia and insulin resistance. Other genetic
10 studies strengthened the link between HSL and lipodystrophy [203, 204]. Nonsense mutations
11 (p.(Ala507fs*563); p.(Glu1035*)) were described, respectively, in Italian and Israel-Arab
12 families. Homozygous carriers showed partial lipodystrophy in adulthood associated with type
13 2 diabetes, hypertriglyceridemia and hepatic steatosis (Figure 5A). At first glance, a
14 lipodystrophic phenotype is unexpected as the mutations result in lower AT lipolytic rate which
15 should result in increased adipose stores.

16 The clinical manifestations of HSL deficiency are consistent with most HSL null mouse studies.
17 First, heterogeneity in white adipocyte size and AT inflammation have been reported [205-207].
18 As in humans, some studies do not describe changes in total fat mass while others show a
19 striking reduction of WAT depots [138, 139, 208, 209]. These differences likely result from
20 studies of mice at various ages. There is progressive emergence late in life of a lipodystrophic
21 phenotype associated with systemic insulin resistance and liver steatosis [207]. Of note, the
22 metabolic phenotype of adipose specific and whole body HSL null mice are similar.
23 Consistently, rescuing adipose HSL expression in HSL null mice abrogates the metabolic
24 abnormalities [210]. These studies highlight the crucial role of adipose HSL on adipocyte
25 biology and systemic metabolism. Mechanistically, treatment of HSL null mice with a PPAR γ

1 agonist partially rescues several metabolic traits suggesting that HSL could directly or indirectly
2 control the production of endogenous PPAR γ ligands [139]. Alternatively, the positive effects
3 obtained with the PPAR γ agonist treatment may result from the direct effect of
4 thiazolidinediones as the treatment has also a favorable action in wild type mice. Besides,
5 identity of endogenous PPAR γ ligands remain elusive despite years of active research [211].
6 The molecular basis for the delayed effect of HSL deficiency on AT development is also not
7 understood and warrants further work.

8

9 7.2 Obesity-related type 2 diabetes

10

11 An alteration of basal, i.e. unstimulated, lipolysis is a consistent feature in obesity and insulin
12 resistance [212]. Enlargement of fat cells may be one of the contributors to the increased basal
13 lipolysis. Basal lipolysis is closely associated with insulin-resistance, independently of body
14 mass index and age [213]. Following bariatric surgery-induced weight loss, obese individuals
15 exhibiting the highest decrease in basal lipolysis display the higher recovery of insulin
16 sensitivity [214]. Several hypothesis connecting basal lipolysis and alteration of insulin
17 sensitivity has been proposed. First, increased NEFA release by AT could promote lipotoxicity
18 in several organs [215]. Second, it could be associated with modifications of the adipocyte
19 secretory profile [216]. Inflammation may not be directly involved, as NEFA from lipolysis are
20 stored in AT macrophages without inducing a pro-inflammatory response [217]. A study in
21 obese female monozygotic twins suggests that HSL is a determinant of basal lipolysis in human
22 AT [218]. Moreover, in obese people, HSL expression is higher in visceral fat depot which is
23 generally considered as the harmful fat depot [219, 220].

24 The association between lipolysis-derived circulating FA and insulin resistance in obese
25 humans is a debated question [221]. Obesity-associated insulin resistance may be observed

1 without increase in plasma NEFA concentration [222]. High plasma NEFA levels are not
2 always associated with a decreased insulin response [223]. As an example, diabetic obese
3 women tend to be less insulin-resistant than men, while NEFA plasma concentrations are
4 significantly higher [221]. Interestingly, in HSL heterozygous mice, a model that do not develop
5 lipodystrophy and is prone to high fat diet-induced obesity, a greater whole body insulin
6 sensitivity without changes in plasma FA levels is observed suggesting that adipose HSL
7 expression is associated with insulin sensitivity independently of the lipolytic role of the
8 enzyme [213]. As mentioned above (see 6.1.3), the recent finding of ChREBP interaction with
9 HSL, independently of its enzymatic activity, revealed a new pathway regulating the insulin
10 sensitivity of AT with beneficial systemic impact [127]. To date, the regulation of the
11 HSL/ChREBP complex in pathological conditions has not been investigated. However,
12 alterations in HSL interactome may contribute to insulin resistance (Figure 5A).

13

14 7.3 Cancers and cancer-associated cachexia

15

16 HSL depletion in a mouse model of pancreatic ductal adenocarcinoma results in AT and
17 pancreatic inflammation, promoting adenocarcinoma development [224]. In patients with
18 pancreatic ductal adenocarcinoma, pancreatic HSL level is reduced and is associated with
19 decreased overall survival. Another example is provided by liposarcoma, an often fatal cancer
20 of fat cells [225]. Mice deficient in both HSL and ATGL but not mice with single lipase
21 knockout develop liposarcoma in BAT at advanced age revealing an epistatic interaction
22 promoting premalignant alterations of the adipocyte. This observation highlights a tumor-
23 suppressive role of HSL in some cancers, but the mechanisms remain to be elucidated.

24 On the other hand, through lipolysis, HSL promotes the development of tumors in adipocyte-
25 rich environment as in breast cancer. Human adipocyte-ovarian cancer cell co-culture results in

1 increased HSL activation and FA released by adipocytes which contribute to tumor growth. In
2 addition, the presence of peritumoral adipocytes increases ATGL and HSL expression in tumor
3 cells, promoting intracellular lipolysis of stored TAG correlated with tumor aggressiveness
4 (Figure 5B) [226, 227].

5 The deleterious effect of lipolysis is also observed in cancer-associated cachexia. Around half
6 of cancer patients develop cachexia, having a dramatic impact as it is associated with poor
7 responses to chemotherapy and survival [228, 229]. Cachexia is a complex syndrome
8 characterized by unintentional loss of AT and skeletal muscle. Several clinical studies have
9 described a reduction of both visceral and subcutaneous AT before any loss of lean mass, an
10 observation that may help in diagnosing early stages of cachexia. The early diminution of AT
11 volume may be a consequence of altered lipolysis [230]. Indeed, adipocytes from cancer
12 patients with cachexia exhibit a 2- to 3-fold higher lipolytic activity in response to
13 catecholamines and natriuretic peptides than adipocytes from cancer patients without cachexia
14 [231]. In parallel, basal lipolysis is decreased. Thus, upon non stimulated conditions, adipocytes
15 preserve their energy store in order to be more effective when energy demand increases [212].
16 HSL expression level is strongly increased in adipocytes from cachectic patients which could
17 explain the higher levels of stimulated lipolysis (Figure 5B) [231]. HSL deficiency prevents AT
18 and skeletal muscle loss in cancer-associated cachexia mouse models [232]. The protective
19 effect is more pronounced with ATGL deficiency indicating that adipose lipolysis is involved.

20

21 8. Therapeutic strategy targeting HSL

22

23 Inhibition of AT lipolysis appears as a promising therapeutic strategy against insulin resistance
24 and dyslipidemic disorders. Series of drugs targeting G-protein-coupled receptors and ATGL
25 have been characterized [233-236]. Lack of effects in humans and side effects have impaired

1 further development. HSL, thanks to its central role in regulating FA metabolism constitutes an
2 interesting pharmacological target. As mentioned above (see 5.1), HSL sequence is not closely
3 related to other human lipases and the work on the bacterial HSL family has provided detailed
4 knowledge on the structure of the catalytic domain, two favorable features for the development
5 of highly specific inhibitors [37, 237]. Different classes of HSL inhibitors have been
6 synthesized. Besides new assays have been set up to screen for HSL inhibitors [234]. Among
7 others, Bayer, Sanofi-Aventis and Ontogene produced isoxazolone, oxadiazolone and
8 pyrrolopyrazinedione inhibitors, respectively [238-240]. Novo Nordisk published work on
9 carbazates and carbamoyltriazoles [241, 242]. Many of these compounds inhibit HSL activity
10 through a pseudo-substrate inhibition mechanism [243]. The enzyme inactivation and
11 reactivation are accompanied with the breakdown of the inhibitor forming two products. The
12 safety assessment of the two breakdown products may prove important. Oxadiazolone
13 inhibitors developed by Sanofi-Aventis, such as compound 7600 also named MmPPOX, were
14 first described as rather specific inhibitors of lipases from the HSL family [70, 244]. The initial
15 conclusions were later challenged as various inhibitors of the oxadiazolone series could also
16 inhibit mammalian gastrointestinal lipases [245]. The existence of multiple targets for this class
17 of HSL inhibitors probably explain why they have not met expectations in humans so far. An
18 inhibitor from Bayer group, BAY59-943, has been thoroughly characterized. This molecule
19 inhibits both murine and human HSL but not other mammalian lipases [246]. In diabetic mice,
20 a treatment with the drug diminishes AT lipolysis and improves glucose and insulin tolerance
21 without weight gain [46, 213]. However, BAY59-943 and other HSL inhibitors have not so far
22 been used in humans. Novo Nordisk synthesized aryl boronic acids acting through reversible
23 hydrolase inhibition of HSL [247]. Oral treatment with these derivatives in mice specifically
24 inhibited HSL as do pseudo-substrate inhibitors [248]. Beside metabolic disorders, HSL
25 inhibitors could be of interest as an early therapeutic strategy to prevent tumor growth or

1 cachexia development in cancer patients. However, if confirmed in humans, the tumor-
2 suppressive role of HSL shall be borne in mind.

3 In the future, therapeutic strategy targeting HSL interactome could emerge. Recent findings
4 linking HSL/ChREBP complex and insulin resistance highlight a new implication of the lipase
5 in type 2 diabetes and a new therapeutic strategies using inhibitors of protein-protein
6 interactions [127]. Inhibition of HSL/ChREBP interaction would promote DNL specifically in
7 adipocyte and thus enhance whole body insulin sensitivity. Protein-protein interactions are
8 central in many biological processes [249]. Over the past decade, the development of protein-
9 protein interaction regulators have been exponentially growing [250, 251]. An in-depth
10 knowledge of the targeted protein complex is necessary. Further work on HSL/ChREBP
11 complex is required to assess the feasibility of the approach.

12

13 Conclusion

14

15 During the last sixty years, the importance of HSL in metabolism gained in consistency. The
16 gene structure along with the variety of HSL mRNA and protein isoforms were characterized.
17 In the various tissues where the lipase is expressed, mechanistic insights in the regulation of
18 HSL expression are often lacking. Indeed, the precise metabolites and transcription factors
19 involved in LIPE gene expression remain poorly described. Concerning the protein structure,
20 the catalytic domain in HSL and other members of the family has been extensively studied.
21 However, black boxes persist such as the structure and function of the N-terminal domains.
22 Through its enzymatic activity, HSL plays crucial roles in physiology. The recent finding of
23 non-catalytic HSL involvement in adipocyte metabolism opens a new research field. A direct
24 link between HSL and human metabolic disorders, e.g. lipodystrophy, obesity, insulin
25 resistance, cancer and cachexia, is well documented. However, the potential of HSL as a

- 1 therapeutic target is not firmly established in humans. The mysteries surrounding HSL pave the
- 2 way for research on this old but rejuvenating protein.

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3

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10 AstraZeneca France.

1 Table 1 Location of critical residues in human, rat and mouse HSL exon B isoform amino acid
2 sequences.

3 Critical residues	Localization in Rat/Mouse sequence	Localization in Human sequence
4 5 Catalytic triad	Ser-423, Asp-703, His-733	Ser-424, Asp-693, His-723
6 GDSAG motif	421-425	422-426
7 HGGG motif	349-352	350 – 353
8 Regulatory Domain*	494-666	495-665
9 PKA phosphorylation sites	Ser-659, Ser-660, Ser-563	Ser649, Ser-650, Ser-552
10 ERK phosphorylation site	Ser-600	Ser-589
11 AMPK phosphorylation site	Ser-565	Ser-554

12 * approximate boundaries

13 AMPK, AMP-activated protein kinase. ERK, extracellular signal-regulated kinases. PKA, protein
14 kinase A. PKG, protein kinase G.

15

16

1 Figure legends

2

3 Figure 1. Number of publications on HSL published per year (Pubmed search August 2020
4 “HSL or hormone-sensitive lipase” in article titles and abstracts). The major steps in HSL
5 research are summarized as blue boxes.

6

7 Figure 2. Structure of the human hormone-sensitive lipase gene and protein. Panel A: Exon
8 organization of human LIPE gene. Nine exons labelled 1 to 9 encode three HSL protein
9 domains; green, N-terminal; blue, catalytic domain; orange, regulatory domain. Four alternative
10 exons are located upstream of exon 1; two are non coding, exons B and T2 and, two are coding,
11 exons T1 and A adding 313 and 43 amino acids (a.a.) to the N-terminal domain respectively.
12 Panel B: mRNA isoforms expressed in human tissues. Five alternative 5' termini in LIPE
13 transcripts are generated from promoter usage and alternative splicing. Another HSL-exon B
14 isoform generated by exon 6 skipping has been characterized. Tissue distribution of HSL
15 mRNA isoforms is indicated with font sizes specifying expression level. Panel C: Human HSL
16 protein structure. The catalytic domain shown in blue shows the α/β fold architecture. It
17 contains the catalytic triad (Ser-424, Asp-693, His-723) and HGGG and GX SXG motifs which
18 form the oxyanion hole. These features shared by all members of the HSL protein family are
19 essential for hydrolase activity. The catalytic site serine is encoded by Exon 6. Therefore, the
20 shorter HSL-exon B isoform generated by exon 6 skipping is catalytically inactive. The
21 regulatory domain in orange contains protein kinases A and G (PKA and PKG) phosphorylation
22 sites shown in red promoting HSL translocation to the lipid droplet and inducing enzymatic
23 activity. It also contains an AMPK phosphorylation site shown in brown exerting an inhibitory
24 effect. The N-terminal (N-ter) domain shown in green with isoform-specific additional regions
25 has no clearly defined function. It may play a role in protein/protein interaction. Structures of

1 regulatory and N-terminal domains are not known. They share no homology with other human
2 proteins. Other proteins of the HSL family do not possess the later domains.

3

4 Figure 3. HSL and HSL protein family member enzymatic activities. Panel A: Two-step
5 hydrolysis reaction. HSL binds to the ester bond of the substrate shown in green. Cleavage
6 occurs following the nucleophilic attack of the catalytic site Serine which leads to the release
7 of an alcohol-containing first product and formation of a covalent intermediate with the
8 carboxylic acid-containing product. The second product is generated after displacement by a
9 water molecule. Panel B: HSL broad substrate specificity illustrated by hydrolysis of
10 acylglycerols, cholesterol and retinyl esters. Acylglycerol hydrolase activity represented here
11 shows diacylglycerol (DAG) hydrolysis. In vitro, HSL also hydrolyses triacylglycerols (TAG)
12 and monoacylglycerol (MAG) through similar reactions. In TAG, HSL preferentially
13 hydrolyses fatty acids at sn-1 and sn-3 positions. Panel C: RmEstB, LipN and Est2 esterases
14 belong to the HSL protein family. Their primary structure shares similarity with the HSL C-
15 terminal catalytic domain. Structural variations allows hydrolysis of various types of substrates
16 such as terpene (linalyl acetate), tertiary alcohol (4-hydroxyphenylacetate) and acylated
17 monosaccharides (α -d-glucose pentaacetate).

18

19 Figure 4. The physiological roles of HSL. HSL function in human tissues according to
20 enzymatic activities. HSL deficiency in mice reveals that HSL is required for spermatogenesis.
21 In humans, the few men with HSL deficiency are fertile. Whether spermatogenesis is normal
22 has not been investigated. FFA, free fatty acids.

23

24 Figure 5. HSL in physiopathology. Panel A: Systemic insulin resistance, dyslipidemia and
25 hepatic steatosis are complications found in conditions of lack and excess of adipose tissue, i.e.

1 lipodystrophy and obesity. In humans, mutations in the LIPE gene leading to HSL deficiency
2 cause a lipodystrophic phenotype associated with metabolic disorders and adipose tissue
3 inflammation suggesting a role of HSL in adipose tissue development and/or maintenance. In
4 obese patients, enlargement of adipocytes results in increased lipolysis and HSL activity in
5 unstimulated conditions. Enhanced basal lipolysis is associated with metabolic complications.
6 HSL has biological functions independent of its enzymatic activity. In adipocytes, de novo
7 lipogenesis (DNL) is closely related to insulin sensitivity. Through direct interaction with the
8 transcription factor carbohydrate-responsive element-binding protein (ChREBP), HSL prevents
9 ChREBP nuclear translocation and de novo lipogenesis (DNL) gene induction. Panel B: In
10 cancer and cancer-associated cachexia, the tumor induces fat mobilization from adipose tissue
11 to deviate metabolic substrates for its own growth. Cancer cells produce stimulatory molecules
12 increasing both HSL expression and activity in peritumoral adipocytes. In breast cancers, it has
13 been shown that tumor cells can store fatty acids (FA) leading to the induction of HSL
14 expression for intracellular lipolysis. Through acylglycerol hydrolase activity, HSL promotes
15 tumor growth, aggressiveness and drug resistance. Mice with both HSL and adipose triglyceride
16 lipase (ATGL) develop brown fat liposarcoma.

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11

Figure 1

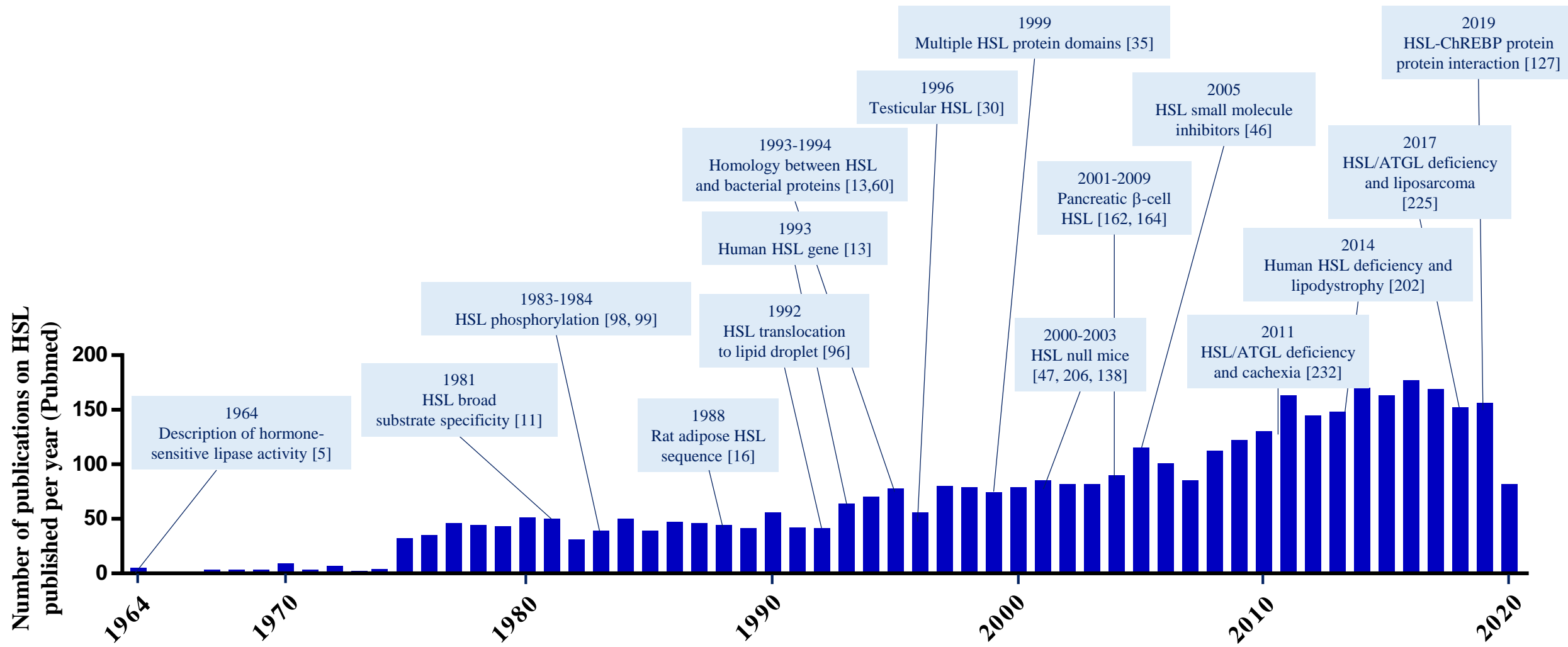
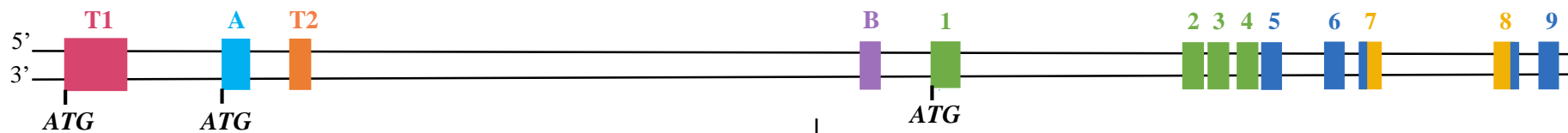


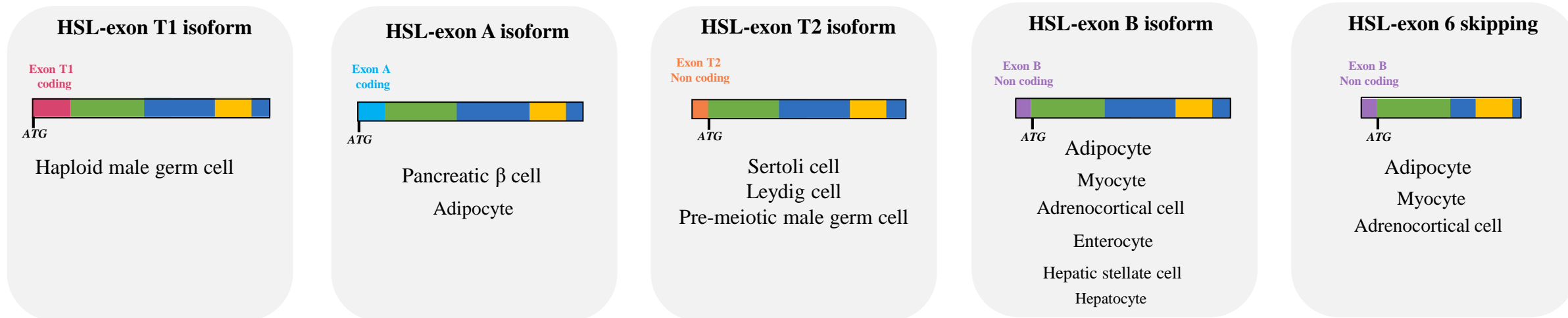
Figure 2

A) DNA



B) Mature mRNA

Transcription – Alternative splicing



C) Protein

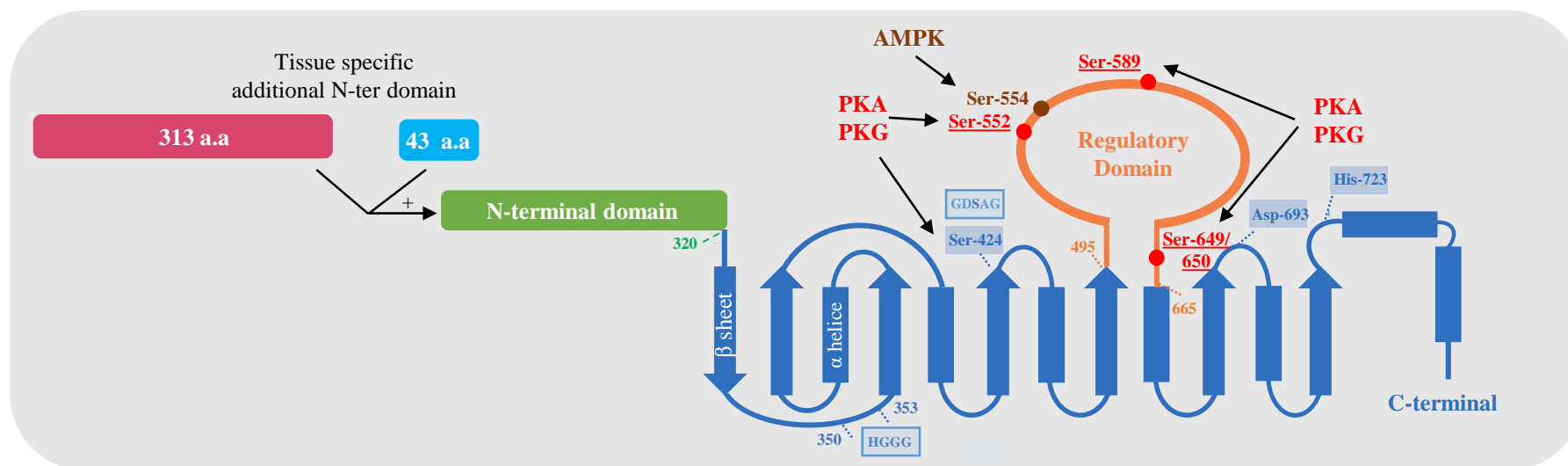
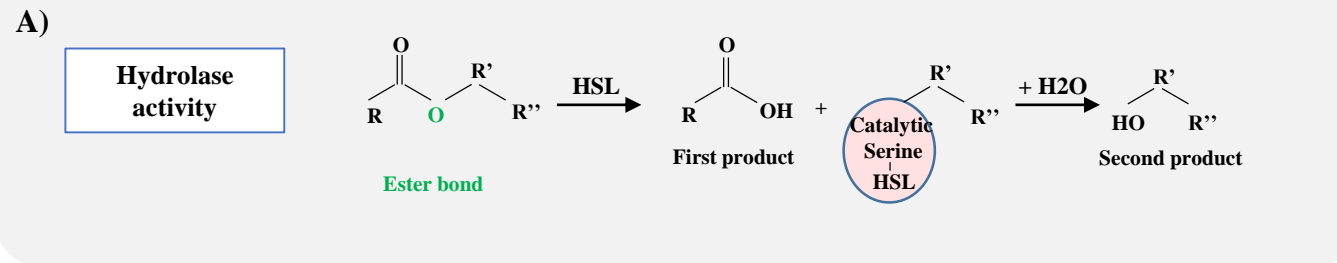
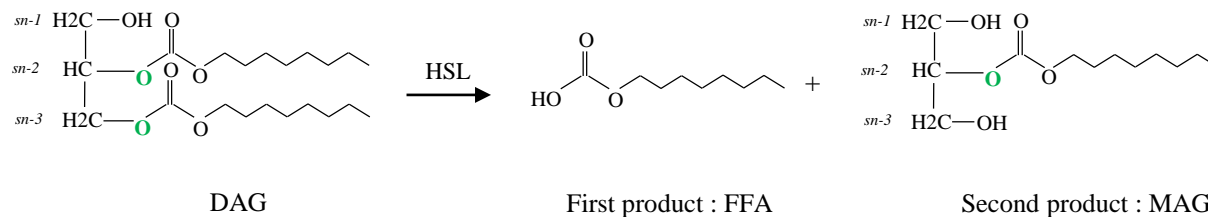


Figure 3

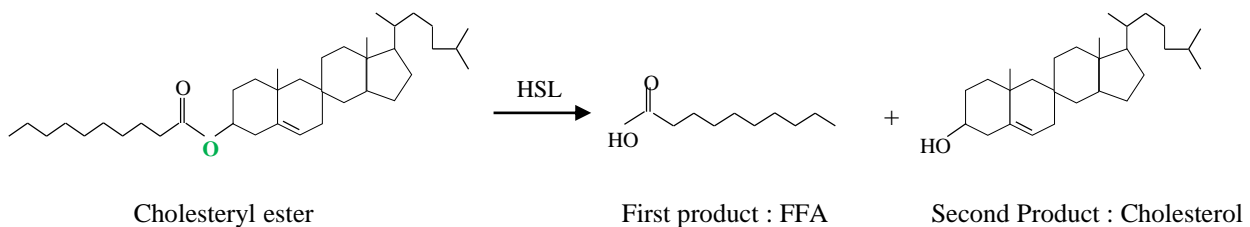


B) HSL activities

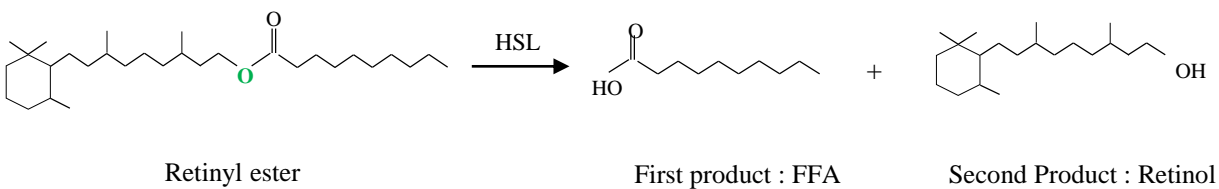
Acylglycerol hydrolase activity



Cholesterol ester hydrolase activity



Retinyl ester hydrolase activity



C) HSL-like protein activities

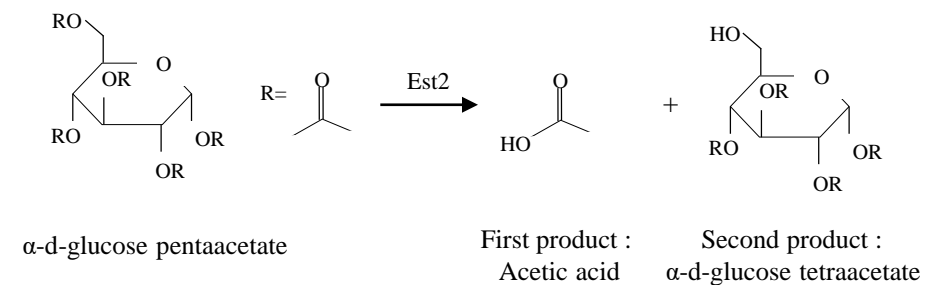
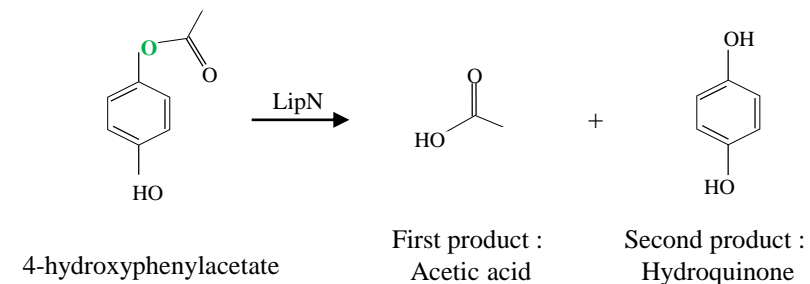
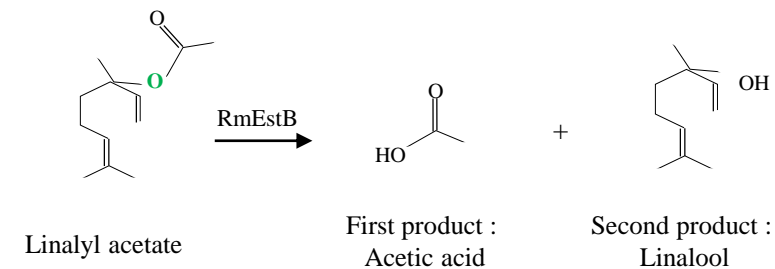
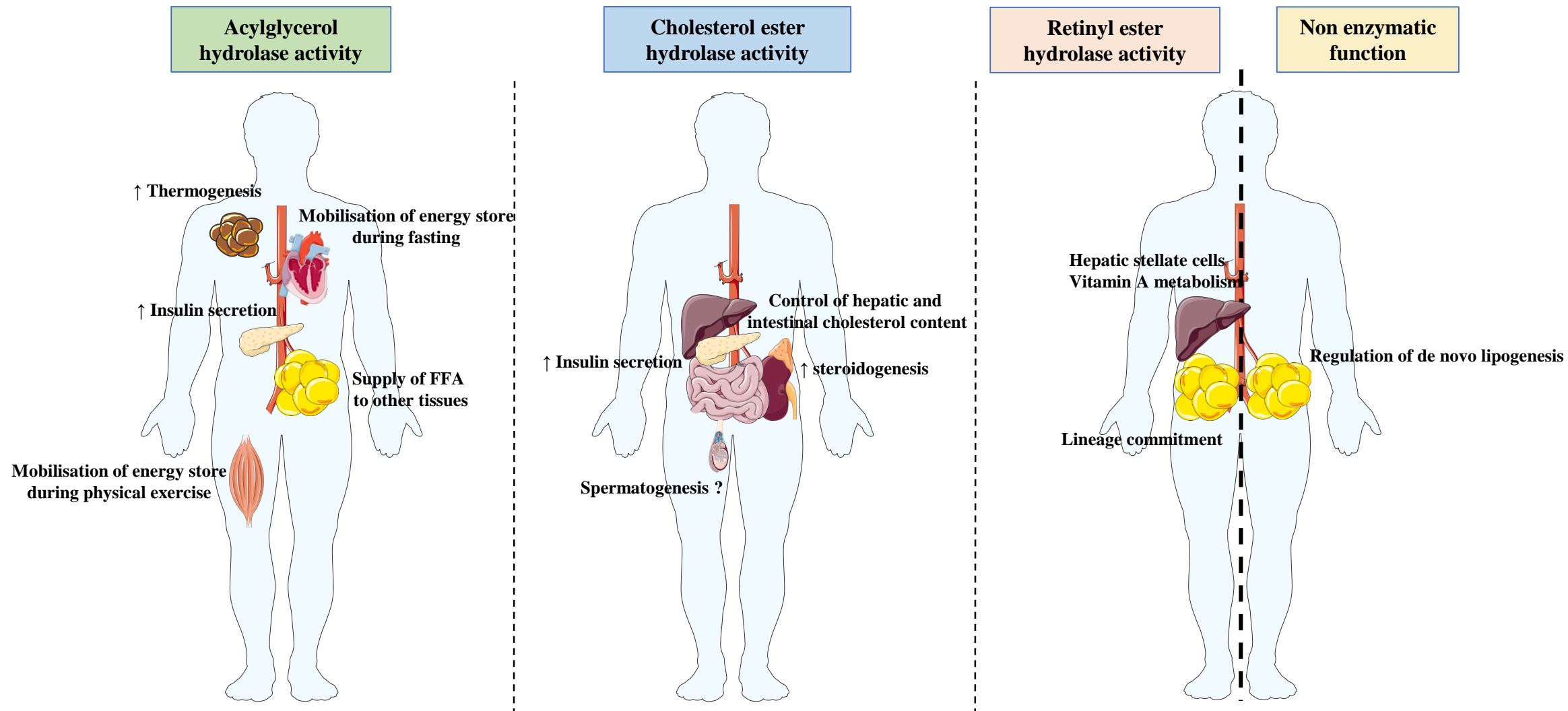


Figure 4



Acylglycerol
hydrolase activity

Cholesterol ester
hydrolase activity

Retinyl ester
hydrolase activity

Non enzymatic
function

↑ Thermogenesis

Mobilisation of energy store
during fasting

↑ Insulin secretion

Supply of FFA
to other tissues

Mobilisation of energy store
during physical exercise

Control of hepatic and
intestinal cholesterol content

↑ Insulin secretion

↑ steroidogenesis

Spermatogenesis ?

Hepatic stellate cells
Vitamin A metabolism

Regulation of de novo lipogenesis

Lineage commitment

Figure 5

