Update on the synergistic effect of HSL and insulin in the treatment of metabolic disorders

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***Abstract*:** Hormone-sensitive lipase (HSL) is one of the three lipases in adipose tissue present during periods of energy demand. HSL is tightly controlled by insulin regulation *via* the central and peripheral systems. The suppressive effects of insulin on HSL are also associated with complex crosstalk with other pathways in the metabolic network. Because impaired insulin action is the driving force behind the pathogenesis of diabetes and other metabolic complications, elucidation of the intricate relationships between HSL and insulin may provide an in-depth understanding of these pandemic diseases and potentially identify strategies to inhibit disease development. Insulin not only differentially regulates HSL isoform transcription but also post-transcriptionally affects HSL phosphorylation by stimulating PKA and endothelin (ET-1), and controls its expression indirectly via regulating the activity of growth hormone (GH). In addition, a rapid elevation of HSL levels was detected after insulin injection in patients, which suggests that the inhibitory effects of insulin on HSL can be overridden by insulin-induced hypoglycemia. Conversely, individuals with hereditary HSL deficiency, and animals with experimental HSL deletion, showed major disruptions in mRNA/protein expression in insulin signaling pathways, ultimately leading to insulin resistance, diabetes, and fatty liver. Notably, HSL inactivation could cause insulin-independent fatty liver, while insulin resistance induced by HSL deficiency may further aggravate disease progression. The common beliefs that HSL is the overall rate-limiting enzyme in lipolysis and that insulin is an inhibitor of HSL have been challenged by recent discoveries; therefore, a renewed examination of their relationships is required. In this review, by analyzing current data related to the role of, and mutual regulation between, HSL and insulin and discussing unanswered questions and disparities in different lines of studies, the authors intend to shed light on our understanding of lipid metabolism and provide a rational basis for future research in drug development.

Keywords: hormone-sensitive lipase, insulin, metabolic disorders, treatment

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A brief history of hormone-sensitive lipase

Basic physiology

Excessive high-calorie intake leads to various health problems, and further elucidation of the process and metabolism of fat accumulation is urgently required. The incidence of many metabolic syndromes is determined by the balance between lipolysis and lipogenesis.[1] Currently, many studies have investigated potential factors that could influence lipolysis, such as age, diet, hormones, genetics, and stress. However, more new technologies and target genes should be delineated, and greater effort should be directed toward exploring new pathways of lipid metabolism.

Hormone-sensitive lipase (HSL), a multifunctional enzyme, participates in fatty acid metabolism.[1] This enzyme hydrolyzes triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), retinyl esters (REs), cholesterol esters (CEs), and other lipids in various tissues.[2]

In addition to adipose tissues, HSL is expressed in several nonadipose tissues, including the heart,[3] skeletal and smooth muscle,[4] adrenal glands,[5] placenta,[6] ovaries,[7] and testis. Furthermore, HSL has been detected in various cell lines, such as intestinal mucosa cells,[8] human bladder cancer cells,[9] and Chinese hamster ovary (CHO) cells.[10] Based on studies performed by Sekiya and colleagues,[11] HSL is also detected in hepatocytes, contributing to the activity of hepatic CE hydrolase. Furthermore, HSL is detected in parenchymal and nonparenchymal cells. Therefore, being a multifunctional lipase, HSL not only plays a role in energy provision but also contributes to various other physiological processes, which require further exploration.

Main functions

Lipolysis requires at least three enzymes: HSL, monoglyceride lipase (MGL), and adipose triacylglycerol lipase (ATGL). HSL participates in the hydrolysis of TAGs to DAGs and DAGs to MAGs.[12] Initially, HSL was thought to be responsible for the first lipolytic step, and adipocyte TAG lipase is now known to be the most important enzyme for mediating lipolysis initiation. Intriguingly, in addition to hydrolyzing MAGs, TAGs, CEs, and REs, HSL could have a broader substrate specificity than the other two enzymes. Furthermore, HSL can catalyze the hydrolysis of other lipid substrates, such as lipoidal esters of steroid hormones[13] and water-soluble butyrate substrates.[14] In addition, the fatty acid hydrolase activity of HSL is 10-fold lower against TAGs than DAGs *in vitro*, suggesting that HSL may be more critical as a DAG hydrolase than a TAG hydrolase.[15] Thus, compared with other lipolytic enzymes, HSL is critically important for lipolysis in the human body because of its ability to hydrolyze DAGs more strongly than TAGs.[16] Therefore, HSL is the main focus of various current explorations.

HSL under the control of insulin

Activation of the HSL enzyme: PKA and H_2O_2

Previous studies have suggested that HSL, induced by catabolic hormones, could be activated by the cyclic AMP (cAMP)-dependent protein kinase (PKA) in adipocytes.[17] Acute insulin treatment could stimulate cAMP phosphodiesterase in an ATP-dependent manner, accelerating cAMP hydrolysis, suppressing PKA activity, and inhibiting PKA-dependent activation of HSL.[18] A study performed by Holm and colleagues also confirmed that insulin could inhibit HSL lipolysis.[19] In addition, Zentella de Piña and colleagues confirmed that H_2O_2 generated by insulin could affect the amplification cascade of lipolysis in adipocytes.[20] Micromolar concentrations of H_2O_2 inhibited cAMP-activation of the type IIβ-PKA holoenzyme, suppressing lipolysis mediated by HSL.

HSL phosphorylation: AMPK and endothelin (ET-1)

An important characteristic of HSL is its reversible phosphorylation, and this mechanism mediates the activation of HSL by lipolytic hormones. Generally, HSL has two phosphorylation sites.[1] Site 1 is called the regulatory site, which is essential for HSL activation. This site is phosphorylated by PKA and glycogen synthase kinase-4.[21] Interestingly, this kinase is controlled hormonally, and specifically by insulin.[22] Site 2 is called the basal site and is phosphorylated by the AMP-activated protein kinase (AMPK) and Ca^{2+}/Calmodulin-dependent kinase II.[22,23] Previously, phosphorylation on site 2 was not believed to have a direct effect on HSL activity[23]; however, a recent study by Daval and colleagues demonstrated an important role for AMPK in HSL phosphorylation.[24] Interestingly, AMPK activity can be inhibited by insulin, and recent evidence has also indicated that AMPK could play key roles in the insulin signaling pathway.[25] Furthermore, crosstalk between the insulin pathway and AMPK activity may exist.[26] In addition to phosphorylation, dephosphorylation by phosphatases could also be essential for HSL activation. Phosphatases are believed to be important for the antilipolytic effect of insulin. However, the dephosphorylation process requires further investigation.

Briançon-Marjollet and colleagues suggested that endothelin-1 (ET-1) secretion could modulate adipocyte metabolism.[27] ET-1-induced lipolysis could be mediated *via* the activation of HSL by Ser^{660} phosphorylation. Furthermore, insulin could exert an inhibitory effect on ET-1. However, the precise mechanisms underlying the regulation of the insulin/ET-1 pathway still require more in-depth research. Importantly, more efforts should be directed toward clarifying the effect of insulin on AMPK, ET-1, and phosphorylation/dephosphorylation of HSL to promote clinical insulin

application for the treatment of diabetes and various other insulin-related clinical problems.

HSL translocation: perilipin, PKA, and PKG

Upon phosphorylation, HSL translocates to lipid droplets to participate in lipolysis. The lipid droplet-associated protein perilipin may be important for mediating the interaction of HSL and its target lipid substrates in adipocytes.[28] An important clue to the HSL translocation process came from analysis of the lipolytic reaction in a perilipin-null mouse.[29,30] Perilipins are the most heavily modified proteins under lipolytic activation in adipocytes.[31] Interestingly, their responses, such as phosphorylation in response to lipolytic agents, markedly parallel those of HSL. These data strongly indicated that, in adipose cells, perilipins are essential for functional lipolytic activation and are strongly associated with HSL translocation and activation.

PKA phosphorylation could mediate a conformational change to expose hydrophobic groups on HSL, facilitating the binding of HSL to its lipid substrates.[32] The phosphorylation of perilipin proteins mediated by PKA is important for the translocation of HSL to lipid droplets, which could enhance lipolysis.[33] Perilipin A is produced from differential splicing as perilipin B.[34] Intriguingly, various studies have also indicated that the translocation of HSL requires PKA-dependent phosphorylation of perilipin A.[30] In addition, perilipin A is phosphorylated by PKA and by the cGMP-dependent protein kinase G (PKG); however, the kinetics of phosphorylation in protein activation has not been elucidated.[35]

The interplay of perilipin and PKA has been found to regulate lipolysis.[36] Significantly, insulin could regulate lipolysis through the spatially compartmentalized modulation of this pathway.[37] However, further studies should investigate the insulin signaling pathways that regulate adipocyte lipolysis, and, more specifically, the activation of HSL. The identification of these distinct pathways will improve the development of treatments that target specific components of the insulin signaling pathway.

Indirect regulation: GH and IGF-1

A recent study performed by Bergan-Roller and colleagues[38] provided novel insights into the various functions of growth hormone (GH) and helped clarify its lipolytic actions. The authors have confirmed that, during feeding, the growth-promoting actions of GH result from GH receptors (GHRs) linked to Akt/PI3K and JAK/STAT pathways that are activated by insulin and IGF-1. During fasting, the lack of insulin and IGF-1 'reprograms' cells such that GHRs linked to Akt/PI3K and JAK/STAT are inactivated and the GHR linked to PKC is activated, followed by the activation of HSL and lipolysis.[38]

However, the mechanisms by which insulin influences various pathways to regulate GH activation still require further research. Insulin and IGF-1 signaling involves various interacting pathways,[39] such as the Akt/PI3K, ERK and JAK/STAT pathways, some of which also affect growth in mammals.[40,41] Recent studies have indicated that when GH is present with IGF-1 and insulin (during feeding), intracellular signaling becomes aligned with growth-promoting processes.[38] Additionally, insulin could degrade cAMP *via* PI3K/Akt and therefore inhibit PKA activation, which results in HSL phosphorylation and activation.[42] In the absence of insulin (during fasting), cAMP is not degraded, and GH signaling shifts away from Akt/PI3K- and JAK/STAT-stimulated growth to PKC-activated lipolysis.[41] Furthermore, in the absence of IGF-1 (during fasting), PTP1B inhibition is lifted, leading to JAK/STAT degradation and thus contributing to the shift away from GH-stimulated lipolysis.

Insulin under the control of HSL

HSL deficiency in humans: clinical evidence of HSL deficiency in patients

Despite our detailed knowledge of the functions of HSL, the exact roles of HSL deficiency in various human diseases are unclear. Interestingly, by first using individuals with a frameshift mutation in the *LIPE* gene encoding HSL, Albert and colleagues found that the lack of HSL could affect lipid metabolism in humans.[43] The mutation results in decreased HSL expression in the adipose tissue of carriers due to decreased enzyme synthesis or increased turnover. The clinical manifestations of patients with defective HSL expression in carriers were found to be less pronounced than those in patients with neutral lipid storage disease with myopathy (NLSDM) (caused by ATGL deficiency in humans).[44] Humans with defective HSL expression are not obese and

develop partial lipodystrophies with age. These findings are critical because they indicate that HSL-mediated lipolysis is also involved in cellular signaling processes in humans.[45]

The results of Albert and colleagues also suggested that the absence of HSL was associated with the risk of type 2 diabetes mellitus (T2DM).[43,46] Both homozygous and heterozygous individuals with the mutation had an increased risk of developing T2DM. These results indicate that HSL might significantly affect insulin function. In addition, individuals homozygous for the mutation had small adipocytes and increased inflammation. Furthermore, their results suggested that HSL activation could be a potential method for treating glucose intolerance and dyslipidemia in patients with T2DM.

HSL inactivation: experimental data regarding the discrepancies between mouse models and the human phenotype

Interestingly, Xia and colleagues demonstrated that HSL-deficient patients and HSL knockout mice both develop partial lipodystrophy.[47] This finding could indicate that mechanistically, the pathogenesis and progression of hepatic steatosis in HSL-deficient patients may be similar to that of HSL-deficient mice. In addition to common findings, some notable differences were also reported between mice and humans with defects in HSL-mediated lipolysis.

First, unlike male HSL-deficient mice, male homozygous carriers of HSL-deficient mutations have offspring. The mechanism regarding these differences in fertility is not yet clear, but this finding may imply that there are species differences in the role of HSL in spermatogenesis.[45]

Second, homozygous carriers have decreased plasma high-density lipoprotein (HDL), increased plasma triglyceride (TG), and increased liver fat, despite decreased lipolytic rates. These findings were completely different from the phenotypes of HSL-deficient mice, where lipolytic defects resulted in decreased plasma TG, increased plasma HDL, and decreased liver fat.[45] Intriguingly, fatty liver in HSL-deficient mice was also reported to be age dependent. Young HSL-deficient mice showed decreased liver fat,[48–50] while old HSL mice showed increased liver fat.[51,52]

Third, the differences in glucose metabolism between humans and mice with HSL deficiency were the most significant. HSL-deficient mice are nondiabetic; however, intriguingly, all four homozygous carriers investigated by Albert and colleagues developed diabetes. The authors suggested that partial lipodystrophy in these patients may cause insulin resistance (IR) and T2DM. However, a larger study group is needed to further confirm the role of HSL in the pathogenesis of diabetes.

Synergistic effect of HSL and insulin: schematic depiction of links between FFAs, lipotoxicity, T2DM, IR, inflammation, fatty liver, NAFLD, obesity, etc

Plasma free fatty acids (FFAs) can be reabsorbed into the blood in various organs. If these molecules are not oxidized, they will accumulate in triglycerides and promote cellular lipotoxicity and mitochondrial dysfunction.[53] FFAs are also implicated in the etiology of obesity-induced IR.[54] Conversely, IR plays a key role in the lipid hydrolysis of adipose tissue, which can induce the transport of excess FFAs and accelerate the development of adipose toxicity. In humans, a short-term increase in FFAs could result in hepatic IR.[55] In addition, FFAs can interact with the insulin signaling pathway, thus promoting the occurrence of IR.[56] Circulating FFAs, the main source of hepatic fat accumulation in nonalcoholic fatty liver disease (NAFLD), are derived mainly from lipid hydrolysis of adipose tissue and partly from excess lipoproteins. During fasting, plasma FFA concentrations are increased, but, after feeding, plasma FFA concentrations are decreased due to the antilipolytic effect of insulin.

The excessive consumption of storage capacity is usually accompanied by gradual changes in endocrine function, and the accumulation of the generated ectopic fat might lead to lipotoxicity.[57] Intriguingly, lipotoxicity could also promote IR and inflammation in the liver.[58] At present, lipotoxicity is thought to be a contributing factor in the progression from simple steatosis to nonalcoholic steatohepatitis (NASH).[59] In addition, lipotoxicity damages insulin signals, causes oxidative damage and promotes inflammation and fibrosis.[60]

In the case of IR, because of the decrease in insulin sensitivity in peripheral tissues, increased levels of insulin are needed to metabolize glucose and inhibit the production of glucose in the liver. In the case of IR, the pancreas is stimulated to

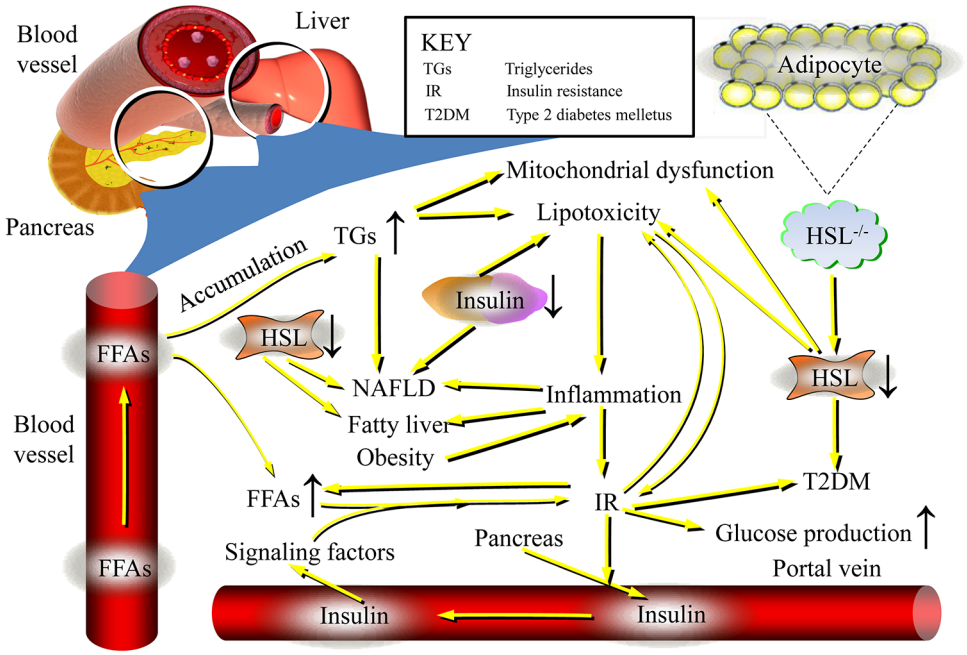

Figure 1. Schematic depiction of the synergistic effect of HSL and insulin in regulating various metabolic disorders in the human body, including lipotoxicity, T2DM, IR, inflammation, fatty liver, NAFLD, obesity, etc.
HSL, hormone-sensitive lipase; IR, insulin resistance; NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

increase insulin secretion in the portal vein, resulting in higher levels of insulin in the liver than in the periphery. A high concentration of plasma insulin is recognized as a biomarker of hepatic IR.[61] Furthermore, obesity may lead to IR via promoting inflammation. In addition to the influence of abnormalities in lipid metabolism, inflammation could also enhance IR, as previously mentioned. Obesity leads to lipid accumulation, activating the signaling pathways of nuclear factor-kappa B (NF-κB) and c-Jun N-terminal kinase (JNK), thus increasing the production of proinflammatory cytokines, such as interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α).[62]

As mentioned above, HSL and insulin could play a joint role in regulating various metabolic disorders in the human body, including lipotoxicity, T2DM, IR, inflammation, fatty liver, NAFLD, obesity, etc. A schematic depiction of the links between these factors is shown in Figure 1.

Implications in treatment of metabolic disorders: HSL might be a treatment target

Diabetes

The absence of HSL was strongly associated with an increased risk of T2DM. As mentioned above, the results of Albert and colleagues suggested that HSL activation could be an important approach for treating glucose intolerance and dyslipidemia in patients with metabolic syndrome or T2DM.[43] Thus, in future research, therapeutic strategies activating HSL function *via* activating the HSL enzyme or promoting HSL phosphorylation or HSL translocation might be promising, and the ensuing modification of relevant molecules, including PKA, AMPK, ET-1, perilipin and PKG, could be used to identify effective activators or agonists of HSL. Regrettably, however, no specific HSL activator has been found, indicating further research is urgently needed.

Obesity

Since HSL is responsible for the release of FFAs from stored triacylglycerols in adipose tissues, influencing the regulation of HSL can be effective for preventing or treating obesity if caloric restriction is ensured at the same time.[63] The roles of HSL in human obesity have been gradually revealed. The importance of HSL expression is well established, although discrepancies certainly exist. Thus, HSL mRNA expression in subcutaneous abdominal adipose tissue in obesity has been reported to be increased,[64] reduced,[65,66] or not affected.[67,68] However, irrespective of gender,

the majority of studies have found the corresponding HSL protein levels to be reduced in obesity.[64,65,69,70] Similarly, in the obese state, IR is associated with a reduction in HSL mRNA and protein in subcutaneous abdominal adipose tissue.[71] In visceral adipose tissue, HSL mRNA levels have consistently been found to be upregulated in obesity,[64,66,67,68] but protein levels seem to be unaffected,[68] or possibly reduced.[64] Overall, therapeutic strategies targeting HSL might have potential for obesity treatment.

Fatty liver

People with hereditary deficiency of HSL have been reported to develop fatty liver.[47] Xia and colleagues suggested that adipose tissue deficiency of HSL can cause age-dependent hepatic steatosis, [47] and adipose tissue is a potential target for treating hepatic steatosis in HSL deficiency. The authors suggested that strategies for fatty liver treatment related to HSL deficiency should focus on adipose tissue. However, this result should be interpreted with caution given the small number of patients included. Because HSL could be important for liver function, identification of more HSL activators will promote the development of stratification strategies in which patients are treated based on their HSL expression status. However, unlike various other targets, HSL has not been validated in epidemiological studies or meta-analyses. Thus, more efforts should be directed toward exploring HSL-related drugs.

Pancreatic diseases

Lipids were also shown to be required for the normal function of pancreatic β-cells.[72,73] Thus, a lipid-derived factor may play important roles in insulin secretion. Production of such a factor may require the action of a lipase, such as HSL, which mobilizes a potential lipid coupling factor from complex lipids.[74] Thus, HSL deficiency might lead to pancreatic disorders. In addition, pancreatic HSL could exert an important role in mediating pancreatic inflammation and tumorigenesis.[75] Uhlen and colleagues detected strong HSL expression in pancreatic islets and pancreatic intraepithelial (PanIN) lesions and confirmed that reduced expression of LIPE (the gene encoding HSL) in pancreatic tissue of patients with pancreatic ductal adenocarcinoma (PDAC) is associated with decreased overall survival.[76] These findings emphasize the need for caution in targeting HSL for pancreatic tumors or various other pancreatic disorders. However, an increase in the level and activity of HSL has been implicated in the pathogenesis of cachexia,[77,78] and pharmacological inhibitors of HSL have been proposed for the treatment of cancer-associated cachexia.[78] Thus, whether activating HSL would promote the deterioration of the condition of cancer patients and disrupt the normal environment of the normal human body is unclear. These issues could be obstacles for promoting the development of effective HSL activators, which may be a major challenge.

Skeletal muscle dysfunction

Skeletal muscle IR is linked to the accumulation of lipotoxic lipid species. Several studies have shown the detrimental role of DAGs in cultured myotubes,[79,80] as well as *in vivo* in mouse and human skeletal muscle.[81–84] Reduced HSL activity in skeletal muscle is causally linked to IR *in vitro*. Badin and colleageus also showed that HSL knockout mice could exhibit defective skeletal muscle insulin signaling and DAG accumulation compared with wild-type mice.[85] Thus, HSL could be important for normal insulin function in skeletal muscle, as well as normal functions of skeletal muscle during exercise.

Conclusion

Overall, the regulation of HSL expression and activities, especially the crosstalk between insulin and HSL, could be have major implications in future drug development. Various unanswered questions regarding the mechanistic signaling pathways of the mutual regulation between HSL and insulin, which involve many key regulators of metabolism of the human body, need to be answered, and various molecules in these pathways could also be treatment targets. Currently, extensive data from various studies support the protective effect of HSL activators. However, research results indicating that HSL is the main factor of metabolic disorders are rarely reported. More research is needed before these data can be used and explored by the pharmaceutical industry. More efforts should be directed toward clarifying the role of mutual regulation between HSL and insulin to address unanswered questions and disparities in different studies.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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