thematic review

Hepatic lipase: structure/function relationship, synthesis, and regulation

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Abstract Hepatic lipase (HL) is a lipolytic enzyme, synthesized by hepatocytes and found localized at the surface of liver sinusoid capillaries. In humans, the enzyme is mostly bound onto heparan-sulfate proteoglycans at the surface of hepatocytes and also of sinusoid endothelial cells. HL shares a number of functional domains with lipoprotein lipase and with other members of the lipase gene family. It is a secreted glycoprotein, and remodelling of the *N*-linked oligosaccharides appears to be crucial for the secretion process, rather than for the acquisition of the catalytic activity. HL is also present in adrenals and ovaries, where it might promote delivery of lipoprotein cholesterol for steroidogenesis. However, evidence of a local synthesis is still controversial. HL activity is fairly regulated according to the cell cholesterol content and to the hormonal status. Coordinate regulations have been reported for both HL and the scavenger-receptor B-I, suggesting complementary roles in cholesterol metabolism. However, genetic variants largely contribute to HL variability and their possible impact in the development of a dyslipidemic phenotype, or in a context of insulin-resistance, is discussed.—Perret, B., L. Mabile, L. Martinez, F. Tercé, R. Barbaras, and X. Collet. **Hepatic lipase: structure/function relationship, synthesis, and regulation. *J. Lipid Res.* 2002.** 43: **1163–1169.**

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Hepatic lipase (HL) is a lipolytic enzyme synthesized mostly by hepatocytes and found localized at the surface of liver sinusoidal capillaries. It can be considered as a lipase of the vascular compartment, together with lipoprotein lipase (LPL), with which it shares number of structural and functional similarities. HL exerts both triglyceride lipase and phospholipase A1 activities, and is involved at different steps of lipoprotein metabolism. During recent years, cellular and molecular biology approaches, as well as studies in transgenic animals, have been useful in elucidating the enzyme structure and functional domains, its metabolic roles, and the mechanisms regulating its expression. This article will focus on the structure/function relationship of HL, its synthesis, and the regulation of its expression in liver and steroidogenic tissues. The metabolic and patho-physiological roles of HL are developed in a joint review.

STRUCTURE/FUNCTION RELATIONSHIP

The HL gene is located on chromosome 15 (q15–q22) in humans and on chromosome 9 in mice (1–3). It spans over 60 kb with eight introns and nine exons accounting for 1.6 kb (**Fig. 1A**). The organization of introns and exons is relatively close to that of the LPL gene and the exons are all of average size (118–234 bp) (1). Two transcription start sites were described 43 or 77 nucleotides upstream of the translation initiation codon (1, 2). The 5′-flanking region of the HL gene spans between nucleotides −1550 and +129 (2, 4). DNA^{ase} footprint analysis in presence of hepatic nuclear factors revealed eight zones of interaction in the proximal promoter (Nt −281 to Nt +129) (Fig. 1A). For instance, the A zone (between Nt −28 and Nt −75) contains an AGGTTAATTATTAAT motif frequently found in liver-expressed genes, and binds to the positive transcriptional factor HNF1 (5) (Fig. 1B). In contrast, the domains E2, E3, and E4, present in the first exon, contain a negative regulatory element (5). In addition, the 5′ nontranscribed region contains sequences corresponding to regulatory responsive elements (6) (see section 4).

Interest for HL modulations was renewed by the discovery of functional genetic variants of HL, with high frequencies among populations (7). The proximal promoter

Abbreviations: CE, cholesteryl esters; CETP, cholesterylester transfer protein; EL, endothelial lipase; HL, hepatic lipase; LPL, lipoprotein lipase; PL, pancreatic lipase; SR- BI, scavenger receptor BI; TG, triglycerides.

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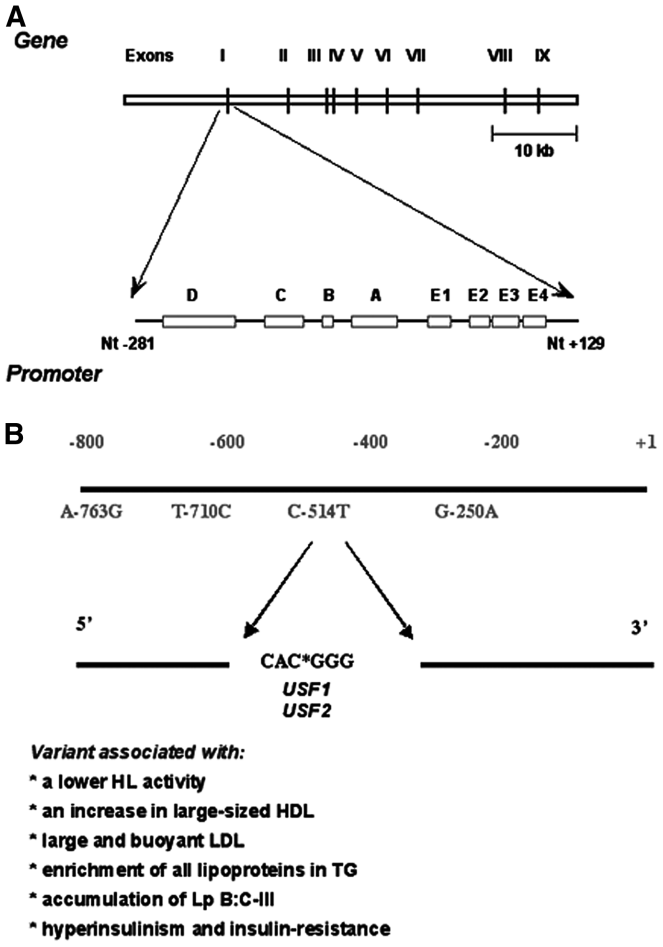

Fig. 1. A: Genomic organization of hepatic lipase (HL). The human gene of HL is localized on chromosome 15. It spans 60 kb and consists of nine exons and eight introns. The lower part of the figure is an enlarged view of the proximal promoter, with interaction regions as defined by DNase footprinting. Zone A binds positive transcription factors specific for liver expression. The E2 and E4 zones contain a negative regulatory element. A disubstitution within the E2 zone causes an overexpression of the gene (×12). B: Polymorphisms of the HL promoter. The delineated four polymorphic sites in the HL promoter are in complete linkage disequilibrium, defining a single variant. The variant allele frequency is about 0.2 among Caucasians, but is higher in African Americans. The variant is associated with a lower HL activity and its metabolic implications are summarized.

of HL gene contains four polymorphic sites (Fig. 1B) in complete linkage desequilibrium: G-250A, C-514T, T-710C, and A-763G (7, 8), according to the nomenclature of Ameis et al. (2). The minor allele frequency ranges between 0.15 and 0.21 in Caucasians, but is about 50% in African Americans (9). It is associated with a low post-heparin HL activity (9, 10), with high HDL-cholesterol (HDL-C) and large HDL-particle size, and with the presence of large and buoyant LDL, all features that would confer a favorable phenotype. However, enrichment of all particles in TG is also observed. Functionally, the variant allele, as compared with wild-type, drives a decreased transcriptional activity of a promoter/reporter construct in murine hepatoma cells (11).

Rat and human cDNAs (1,569 bp) display strong homologies (12, 13) and contain two polyadenylation sites. However, only one mRNA of 1.7 kb has been detected in hepatic cells. The human HL protein consists of 499 amino acids including a leader-peptide of 22 residues (12) so that the secreted protein contains only 477 residues. The amino acid sequence reveals the presence of several functional domains; for instance, two hydrophobic segments of 10 amino acids involved in interactions with lipids. Each one segment contains a Serine residue (Ser146 and Ser267). In humans, Ser146 is at the center of a Gly-X-Ser-X-Gly consensus, which is part of a classical Ser-Asp-His catalytic triad found in the endothelial lipase, LPL, pancreatic lipase, and other esterases (13, 14) (**Table 1**). Site-directed mutagenesis showed that in the rat Ser147 is essential for the catalytic activity (15). At the center of the protein, 10 cysteine residues are present and the distances between them are conserved in the structures of LPL and pancreatic lipase, suggesting that the formation of disulphide bridges is important for the conformation and catalytic activity of these lipases. Human HL also contains four putative heparin-binding sites, with sequences like BBBXXB or BBXB (Table 1), where B is a basic residue, and which are likely involved in the binding to cell-surface heparan sulfates (13, 16). Such consensus sequences were found in other proteins of the lipoprotein metabolism, like apoB-100, apoE endothelial lipase, and LPL. The fourth motif (Lys337-Arg443), located in the carboxy-terminus of human HL, is absent in murine HL (17) and in other members of the lipase gene family. It is noteworthy that, in mice, HL is primarily free in the circulation and not bound to the cell surfaces (18). However, conflicting results have been reported concerning the affinity of the mouse HL for heparin-like polysaccharides (19), as well as regarding the role of the fourth C-terminal cluster in the binding of HL onto liver heparan-sulphates (17).

SYNTHESIS, SECRETION, AND TISSUE SPECIFICITY

HL is a secreted glycoprotein and synthesized in the endoplasmic reticulum (ER). An NH_2-terminal leader peptide is lost after crossing through the ER membrane. Kinetics of endogenously labeled enzyme and use of glycosidases or inhibitors have demonstrated that HL is first synthesized as a high mannose form (52–55 kDa in the rat), and further acquires sialic acid-containing complex oligosaccharides during transit through the Golgi cisternae (20). The mature HL (57–59 kDa, in the rat) is then rapidly secreted. The residence half-time of HL in hepatocyte is about 60 min (20). The rat enzyme contains two *N*-glycosylation sites. Mutation of either one impairs HL processing rather than the enzyme activity itself (21). Trimming of the glucose residues off the high-mannose form appears to be a determining step for rat HL secretion (22). The human HL presents four glycosylation sites, which are all utilized at positions 20, 56, 340, and 375, and a molecular mass around 65 kDa (23, 24). Site-directed mutagenesis indicated that Asn 56, a conserved

TABLE 1. Structure similarities between hepatic lipase, other lipases, and apolipoproteins

	Active Site		
Lipases	Serine	Aspartate	Histidine
hHL	HLIGY**S**$_{146}$LGAHVSG	RITGL**D**$_{172}$AAGP	CS**H**$_{257}$ERS
hEL	HLIGY**S**$_{151}$LGAHVAG	RITGL**D**$_{175}$PAGP	CE**H**$_{256}$ERS
hLPL	HLLGY**S**$_{132}$LGAHAAG	RITGL**D**$_{156}$PAGP	CS**H**$_{241}$ERS
hPL	HVIGH**S**$_{153}$LGAHAAG	RITGL**D**$_{177}$PAEP	CN**H**$_{263}$LRS
	Heparin Binding Domains		
Consensus	XBBBXXBX	XBBXBX	
hApoE	$_{144}$LRKRLLRD		
hApoB	$_{3364}$TRKRGLKL	$_{84}$LKKTKL	
		$_{2081}$VRKYRA	
		$_{2119}$TKKYRIT	
rHL	$_{162}$GKRKIGRI	$_{294}$LKKTGR	
hHL	$_{163}$HKIGRITG	$_{294}$CKKGRC	
hLPL	$_{145}$TNKKVNRI	$_{278}$CRKNRC	
hEL		$_{293}$CRKNRC	

Active Site: Homology of the amino acids of the catalytic triad between human (h) hepatic lipase (HL), endothelial lipase (EL), lipoprotein lipase (LPL) and pancreatic lipase (PL).

Heparin Binding Domains: Sequence similarities among basic regions in heparin binding domains of human (h) or rat (r) apoB, apoE, HL, LPL and EL.

site in the NH_2-terminus, is crucial for the secretion of active HL (23). Moreover, besides remodeling of the N-linked oligosaccharides, association of HL with calnexin, a chaperone protein, may increase the efficiency of HL export from the ER (25). Different physico-chemical techniques have beeen used to determine the sub-unit structure of secreted active HL, all indicating a homodimer structure (26).

Some mutations, described in the rare cases of familial HL-deficiency, are associated either with a loss of enzymatic activity, or with an impaired cellular processing. For instance, in a Canadian pedigree, the affected patients are compound heterozygotes, bearing both Ser267Phe and Thr383Met substitutions (27). The first mutation results in an inactive lipase and the second one in an impaired secretion.

Earlier reports had described the binding of HL to heparin-sensitive sites of liver non-parenchymal cells, leading to the assumption that the enzyme was essentially present at the vascular endothelium (28). However, HL immunolocalization experiments in rats or, in rabbits, overexpressing human HL have revealed that it is concentrated at the surface of hepatic sinusoids, and mostly located in the microvilii of parenchymal cells, with lesser amounts found at the sinusoid endothelium (29, 30). This predominant situation of the enzyme at the surface of hepatocytes is in good agreement with its role in the catabolism of lipoproteins by liver parenchymal cells. Finally, a heparin-releasable 70 kDa protein was identified in liver perfusates as a potential HL binding site (31).

A lipase activity has been reported in rat adrenals and ovaries, sharing common properties with HL in terms of salt resistance and immuno-reactivity toward monoclonal antibodies raised against rat HL (32). In adrenals, HL activity has been localized in blood vessels and could be induced by corticotrophin (33). In ovaries, HL has been mostly found in corpora lutea, while very little activity was detected in pre-ovulatory follicles (34). HL activity was strongly correlated with the plasma levels of progesterone (34). However, neither adrenal nor ovarian cells appeared to synthesize HL, as illustrated by the inability to detect mRNA transcripts by Northern blot, or to immuno-isolate labeled newly synthesized HL (35). Thus, so far, the leading opinion is that the enzyme is primarily synthesized in hepatocytes, is further transported in the circulation, and can accumulate at heparin-sensitive sites in ovarian or adrenal blood vessels. Using a sensitive RT-PCR assay, Jansen and co-workers (36, 37) have described the presence, in rat adrenals and ovaries, of a truncated mRNA, deleted of the first two exons. Accordingly, these steroidogenic cells were found to synthesize a 45 kDa catalytically inactive HL-like protein that remained intra-cellular (36, 37). The ovarian transcript and the corresponding protein synthesis can be induced by gonadotrophin treatment, but this transient induction preceded the appearance of HL-activity (38). This and the fact that those shorter transcripts in adrenals are 40× less abundant than full-length mRNA in the liver, questions the physiological relevance of those truncated forms (36). As regards human ovarian cells, our group has recently detected HL transcripts and measured a HL-secreted activity in luteinizing hormone-stimulated human pre-ovulatory granulosa cells isolated during an in-vitro fertilization protocole (L. Mabile, unpublished observations).

REGULATION OF HEPATIC LIPASE EXPRESSION

Several putative regulatory elements have been identified in the rat HL promoter, allowing the definition of partners potentially involved in the regulation of the enzyme expression. Among them, responsive elements for cholesterol (SRE), estrogens (ERE), thyroid hormones (TRE), and glucocorticoids (GRE), and for cAMP (6). In addition, another motif possibly responsive for glucose and/or insulin (proximal E-box), has been since described in humans (11) (Fig. 1B). The major regulators of HL activity are summarized in **Tables 2** and **3**.

HL regulation and cell cholesterol status

Several experimental evidences suggest that HL is regulated as a function of the cholesterol demand, as are various proteins of cell sterol homeostasis. In cultured hepatoma cells, an inverse relation has been described between the cell cholesterol content and the levels of HL mRNA and activity (39). Accordingly, incubation with Mevinolin, a blocker of cholesterol synthesis, induced a stimulation of both HL and HMG-CoA reductase transcripts, an effect reversed by mevalonate (40). The SRE-binding proteins are supposed to be involved in this regulatory process. Concordant arguments support the view that HL and the scavenger receptor BI (SR-BI) would

TABLE 2. Physiological and pharmacological regulators of HL activity in liver

Regulatory Agents	HL Activity	References
Sex steroids		
Estrogens	−	
Androgens	+	(39, 49–51)
Glucocorticoids		
Corticotrophin	−[a]	(54–56)
Corticosteroids (dexamethasone, triamcinolone)	−	
Thyroid hormones		
Thyroxin (T4)	+	
Triiodo-thyronin (T3)	+	57, 58
Catecholamines (adrenalin)	−	(65)
Leptin	−[b]	(66)
Heparin	++	(71)
Diet		
Cholesterol	−	
Saturated fatty acids	−	(72–74)
N-3 fatty acids	−	
Drugs		
Fibrates	+	(67, 75)
Statins	−	
Genetic variant −514T	−	(11)

[a] Opposite (increased) effect in adrenal cells.
[b] Reported for mRNA levels; not determined for HL activity.

exert coordinate functions in cell cholesterol supply. Indeed, both proteins display comparable tissue distributions, being particularly expressed in the liver and steroidogenic organs (41). The multi-ligand receptor SR-BI has been involved in the selective uptake of HDL-cholesteryl esters (HDL-CE). On the other hand, studies carried out in liver perfusions or in cell cultures have established a role for HL in promoting the uptake of HDL-CE, acting in cooperation with SR-BI (42, 43). The hypothesis of synergistic roles is also supported by common and reciprocal regulation for HL and SR-BI in liver and in steroidogenic tissues (Table 3). In mice, for instance, invalidation of the HL gene, or its in vivo inhibition by antibodies resulted in a depletion of the adrenal cholesterol stores, and in the compensatory up-regulation of SR-BI mRNA and protein (×3–5) (44). In rats, corticotrophin treatment led to a 10-fold stimulation of adrenal HL activity, SR-BI transcripts, and protein, as well as to an increased uptake of HDL-CE by the adrenal gland (44). As regards ovaries, hCG priming in rats resulted in a stimulated expression of both HL (38) and of SR-BI (45). In a recent paper, Wade et al. (46) reported that gonadotrophin-primed $HL^{-/-}$ mice had smaller ovaries, fewer ovulations, and corpora lutea, and produced less progesterone than did wild-type littermates. This suggests that HL might be required for optimal progesterone synthesis and maturation of pre-ovulatory follicles. Stimulated human pre-ovulatory granulosa cells can take up HDL-CE by selective uptake and utilize it for progesterone synthesis through a cAMP dependent process (47). It is noteworthy that cAMP mediates the effects of extra-cellular gonadotrophins, and that a cAMP-response element has been identified around Nt −534 in the human HL promoter (6). Finally, granulosa cells can also utilize HDL-free cholesterol for steroid production, a process that might be stimulated by HL (48).

TABLE 3. Common and symmetrical regulations of hepatic lipase and of the Scavenger Receptor class B-type I in response to cholesterol and hormones

	Cell Types	SR-BI	HL
Chol.rich-diet	hepatocytes	−	−
Estrogens	hepatocytes	−	−
$HL^{-/-}$	hepatocytes	++	KO
ACTH	adrenal cells	+	+
HCG	ovarian cells	+	+

Hormonal regulation of HL

Clinical studies have long established that HL activity, as measured in post-heparin plasma, is fairly modulated according to the hormonal status. The effects of sex steroids have been particularly studied in various patho-physiological conditions and upon replacement therapy. Schematically, native or alkylated estrogens (ethinyl-estradiol) depress HL activity, whereas progestagens with androgen property (norgestrel) or anabolic steroids (stanozolol, oxandrolone) increase it (49, 50). In agreement, HL activity is much lower in pre-menopausal women than in men, but it increases after menopause. Only a few experimental evidences confirm these bio-clinical observations. In rats, administration of high doses of ethinyl-estradiol (20 μg/day) led to reciprocal changes in the levels of liver HL- and LDL-receptor transcripts (51), and we reported comparable data in cultured hepatoma cells (39). Moreover, cotransfection experiments using estrogen-receptor and HL-promoter expression constructs indicated that 17β-estradiol represses HL transcription, possibly through an AP-1 pathway (52). In hepatocytes, the expression of SR-BI is also down-regulated by estrogens (53). It thus appears that sex steroids have opposite effects, on the one hand, on the HL/SR-BI pair which promotes delivery of HDL-cholesterol, and, on the other hand, on the LDL-receptor expression.

Glucocorticoids have also been involved in the regulation of HL. In rats, corticotrophin-induced hypercorticism resulted in a decrease of hepatocyte HL-activity (54). These data were confirmed by the negative effects of dexamethazone and triamcinolone on HL mRNA and activity (55). However, prolonged administration of a corticotrophin dramatically increased HL-like activity in adrenals (33). Thus, corticotrophin and/or corticosteroids can alter the enzyme distribution, favoring expression in adrenals at the expense of the liver. In humans, corticotrophin but not glucocorticoid treatment significantly decreased HL activity (56).

HL activity also varies in parallel with the levels of thyroid hormones, as observed in hypo- or hyperthyroid states, or following thyroxin administration (57), but the underlying mechanisms are still poorly understood. In cultured cells, HL activity increased moderately in response to physiological concentrations of triiodo-thyronine, but no modifications in HL-mRNA or protein were recorded (58). A few reports have also described the modulation of HL activity by growth hormones and parathormone.

Insulin has been shown for long to actively regulate HL transcription during development when its secretion

reaches maximal rates. In adult humans, HL responsiveness to insulin is still controversial. In most situations, HL activity was positively associated with insulin levels. For instance, in insulin-dependent diabetes post-heparin HL activity was found to be 50% reduced (59) while it increased after intraperitoneal insulin administration (60). Conversely, in normoglycemics as well as in type 2 diabetes, high insulin levels were shown to be associated with high HL activity and a low HDL2-cholesterol (61). HL would be responsible for the inverse relation between insulin and HDL. In agreement, increased HL activity has been reported in Type 2 diabetes (62). However, at a variance with those data, HL activity fell following provoked hyperinsulinemia in normoglycemics as well as in Type 2 diabetics (63). In rats, insulin stimulated HL activity secreted from hepatocytes (64), but whether this involves transcriptional or post-transcriptional mechanisms was not elucidated.

Catecholamines are responsible for the changes in HL expression associated with feeding and fasting. Adrenaline, for instance, has been shown to reduce HL secretion from hepatocytes, mostly in a post-translational way (65).

Finally, a recent work on leptin-deficient mice (ob/ob) has reported dysregulations of genes involved in HDL metabolism and reverse cholesterol transport, including marked decreases in mRNAs encoding HL. Accordingly, leptin administration in those mice reversed the transcriptional profile and dramatically up-regulated the HL mRNAs (66). It is noteworthy that leptin also decreased transcription of sterol regulatory element binding protein, SREBP-1. Thus, it is speculated that leptin could alter the expression of many SREBP-target genes (66). This might also concern HL, although the involvement of SREBP in HL regulation has not yet been formally documented.

HL polymorphisms

Apart from hormonal modulations, HL activity is largely influenced by genetic factors. According to recent studies (67), the polymorphism C-514T in the promoter region would explain up to 38% of the variability of the HL activity. Functionally, the variant allele, as compared with wild-type, drives a decreased transcriptional activity of a promoter/reporter construct in murine hepatoma cells (11) and it has been recently associated with fasting hyperinsulinemia and insulin resistance (68). It is noteworthy that the −514 site is at the center of a CAC*GGG sequence, almost analogous to the CACGTG motif characteristic of an E-box onto which can bind the upstream stimulatory factors USF1/2 (Fig. 1B). The latter are transcription factors involved in the regulation of glucose and lipid metabolism in the liver. For instance, USFs are part of the insulin response complexes that interact with the fatty acid synthase gene (69). It is tempting to speculate that the C*–514T substitution would disrupt the E-box analogous sequence and impair the stimulatory regulation exerted by insulin. Interestingly, it has been recently reported that USF proteins can bind to the −514 region, and that the affinity is 4-fold reduced by the C-514T substitution (70).

HL and heparin

The most spectacular stimulations of HL-activity have been recorded with heparin, both in vivo and in cultured cells. Not only does heparin remove HL from cell surfaces, but, in prolounged incubations, it stimulates by 5–8-fold the recovery of HL activity (39). Heparin might stabilize the enzyme, which thus escapes from re-endocytosis and secondary degradation. Moreover, heparin stimulates HL transcription and secretion of the mature enzyme (71).

HL and nutrients

Although it is well known that nutrients are involved in modulating the metabolism of lipoproteins, these aspects have been poorly investigated as regards HL. In rats, HL activity has been found to be inhibited by diets rich in saturated fats (72) or enriched in fish oil (73). Cholesterol-enriched diets reduced HL activity and mRNA expression by 30% (74), providing an additional argument in favor of a feedback control of HL by cholesterol.

HL and drugs

Among the pharmacological hypolipidemic molecules, fibrates have been shown to increase HL activity in normolipemic as well as in hypertriglyceridemic subjects (75), but no peroxisome proliferator recognition element has been so far identified in the HL promoter. It should be remembered that fibrates also increase HDL-levels and HDL-particles size, providing a situation where those parameters are not unequivocally inversely related to HL. In contrast, statins have been reported to decrease HL activity in dyslipidemic patients. This effect was shown to be allele-specific, the −514CC carriers displaying the most important decline in HL activity in response to lovastatin treatment (67).

CONCLUDING REMARKS

In recent years, much progress has been made in our understanding of the metabolic roles of HL and also of the mechanisms regulating its expression. Moreover, the impact of the promoter polymorphism has been described in detail demonstrating its importance in HL variability. The possible relationship between this polymorphism and hormonal or pharmacological regulations opens up new perspectives.

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