Hepatic lipase: a marker for cardiovascular disease risk and response to therapy

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Purpose of review

Hepatic lipase plays a key role in the metabolism of pro-atherogenic and anti-atherogenic lipoproteins affecting their plasma level as well as their physico–chemical properties. However, controversial evidence exists concerning whether hepatic lipase is pro or anti-atherogenic. The goal of this review is to summarize recent evidence that connects the enzyme to cardiovascular disease. The potential impact of genetic determinants of hepatic lipase activity in modulating both the development of coronary and carotid atherosclerosis will be discussed based on hepatic lipase proposed roles in lipoprotein metabolism.

Recent findings

Twenty to 30% of individual variation of hepatic lipase activity is accounted for by the presence of a common polymorphism in the promoter region (–514 *C* to *T*) of the hepatic lipase gene (*LIPC*). This polymorphism, via its impact on hepatic lipase synthesis and activity, appears to contribute to (1) individual susceptibility to cardiovascular disease: the presence of the *T* allele (low hepatic lipase activity) may carry a marginally increased risk of atherosclerosis; (2) carotid plaque composition and individual susceptibility to cerebrovascular events: the presence of the *C* allele (high hepatic lipase activity) is associated with increased carotid intima-media thickness and abundance of macrophages in the carotid plaque (unstable plaque); and (3) response of cardiovascular disease patients to lipid-lowering therapy: patients with the *CC* genotype have the greatest clinical benefit from intensive lipid-lowering therapy.

Summary

Convincing evidence shows that hepatic lipase plays a key role in remnant lipoprotein catabolism as well as in remodeling of LDL and HDL particles. The anti or pro-atherogenic role of hepatic lipase is likely to be modulated by the concurrent presence of other lipid abnormalities (i.e. increased LDL cholesterol levels) as well as by the genetic regulation of other enzymes involved in lipoprotein metabolism. Characterization of patients by their *LIPC* genotype will contribute to a better definition of individual risk of coronary and cerebrovascular events, specifically in patients with qualitative (small, atherogenic LDL and low HDL_2 cholesterol) rather than quantitative lipid abnormalities for whom the routine lipid profile may underestimate the risk of coronary and cerebrovascular disease.

Keywords

hepatic lipase gene polymorphism, LDL density, HDL cholesterol, cerebrovascular disease, lipid-lowering therapy

Curr Opin Lipidol 14:179–189. © 2003 Lippincott Williams & Wilkins.

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Current Opinion in Lipidology 2003, 14:179–189

Abbreviations

CAC	coronary artery calcification
CFR	coronary flow reserve
CHD	coronary heart disease
IMT	intima-media thickness
LIPC	hepatic lipase gene
REGRESS	Regression Growth Evaluation Statin Study
USF	upstream stimulatory factor

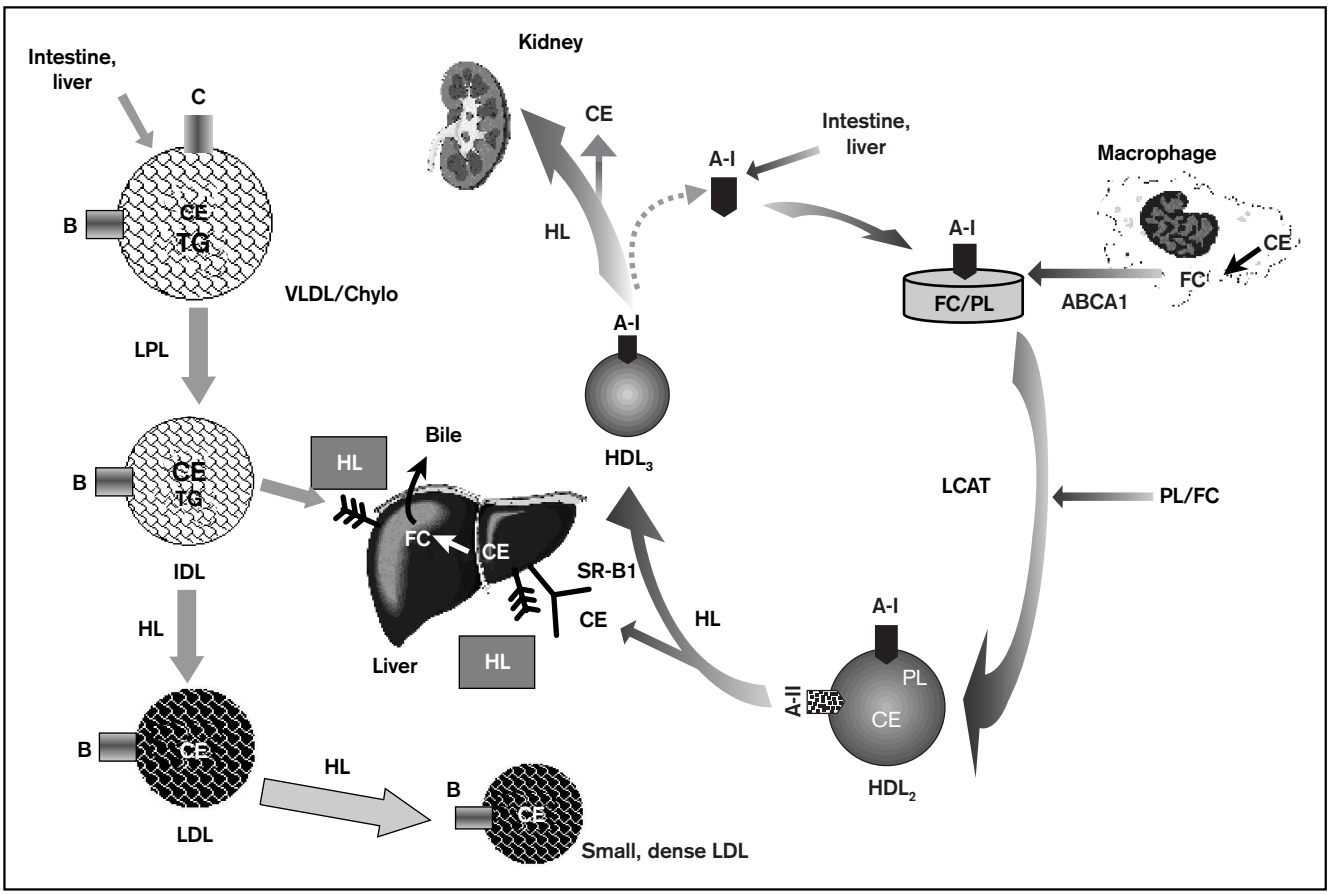
0957-9672

Introduction

Hepatic lipase is a glycoprotein that catalyzes the hydrolysis of lipoprotein triacylglycerols and phospholipids. The majority of hepatic lipase is synthesized and secreted by the liver and is bound to heparan sulfate proteoglycans on the surface of sinusoidal endothelial cells and external surfaces of microvilli of parenchymal cells in the space of Disse [1], promoting the uptake of HDL and apolipoprotein-B-containing remnant particles [2]. Its catalytic activity contributes to the remodeling of LDL and high-density HDL resulting in smaller, denser particles. In addition to its lipolytic activity, hepatic lipase participates, with surface proteoglycans, the scavenger receptor B1 (SR-B1) and the LDL receptor-like-protein, as a ligand in promoting hepatic uptake of lipoproteins, including triglycerides-rich lipoprotein remnants, LDL and HDL particles (Figure 1). Hepatic lipase may therefore contribute to reverse cholesterol transport and the process of atherosclerosis. Recent *in-vivo* and *in-vitro* studies suggest alternative pathways, both through its catalytic activity and independently, by which hepatic lipase may modulate the development of cardiovascular and cerebrovascular disease [2,3••]. Despite extensive research during the past decade on both humans and genetically modified animal models, the exact nature of the relation between hepatic lipase and atherosclerosis remains controversial [3••].

DOI: 10.1097/01.mol.0000064055.08840.77

Figure 1. Central role of hepatic lipase in lipid metabolism



Hepatic lipase influences different lipoprotein pathways (1) by hydrolyzing phospholipids and triglycerides (TG) carried in large HDL_2, intermediate density lipoprotein (IDL) and large LDL particles, resulting in smaller HDL_3 and small, dense LDL lipoproteins, respectively; or (2) by bridging the lipoproteins (HDL and TG-rich lipoprotein remnants/IDL) to specific receptors or heparan sulphates on the hepatocytes. Apo B, apolipoprotein B; C, apolipoprotein C; A-I, apolipoprotein A I; CE, cholesteryl-esters; VLDL, very low density lipoprotein; Chylo; Chylomicrons; HL, hepatic lipase, catalytic activity; A, apolipoprotein A; FC, free cholesterol; PL, phospholipid; ABCA1, ATP-binding cassette transporter A1; LPL, lipoprotein lipase; HL*, hepatic lipase, ligand activity; SR-B1, scavenger receptor B1; LCAT, lecithin–cholesterol acyltransferase.

Postheparin plasma hepatic lipase activity varies widely (five to eight-fold) in normal individuals. The activity is about twice as high in men as in women. In addition, significant racial variation in hepatic lipase activity is observed, indicating an important role of genetic factors. It is estimated that 30–45% of this variability is genetically determined [4]. A genetic background that significantly modulates hepatic lipase activity *in vivo* may potentially impact on the risk of coronary heart disease (CHD) and possibly affect individual CHD response to lipid-lowering therapy. The ability to find significant genetic markers of individual risk of CHD as well as CHD response to therapy, bears relevant clinical implications as a substantial number of patients with CHD disease on lipid-lowering therapy still experience either no benefit or even disease progression and cardiovascular events.

The focus of this review will be on recent clinical and experimental evidence supporting a role of genetic determinants of hepatic lipase activity in modulating both the development of coronary and carotid atherosclerosis, and the disease response to different lipid-lowering strategies.

Common polymorphisms in the hepatic lipase gene promoter: association with hepatic lipase activity and HDL and LDL size and density

It is estimated that 30–45% of variability in hepatic lipase activity is genetically determined [4]. Four common polymorphisms – *G-250A*, *C-514T*, *T-710C*, and *A-763G* – in the hepatic lipase gene (*LIPC*) promoter were reported [5]. The *C-514T* polymorphism is also referred to as *C-480T* by some investigators. These polymorphisms are in

almost complete linkage disequilibrium and therefore define two haplotypes. The frequency of the *T* allele at –514 was found to range between 0.15 and 0.21 among Caucasians [6,7], 0.45 and 0.53 among African Americans [6,8•], and is 0.47 among Japanese Americans [6]. The *–514T* allele is associated with a 30–40% decrease in post-heparin plasma hepatic lipase activity in men [6,7] and in premenopausal women [9,10,11••]. The transcriptional activity of the promoter with *T* at –514 is approximately 70% of that with *C*, as determined by transient transfection of the mouse hepatocytic cell line ML12 [12]. The molecular mechanism responsible for the decreased hepatic lipase activity associated with the *–514T* allele has not been fully elucidated. The –514 *C* to *T* substitution disrupts one of two potential upstream stimulatory factor 1 (USF1)-binding sites present in the proximal promoter region of *LIPC*. The presence of *T* at –514 resulted in a 50% decrease in binding of USF1, a ubiquitous transcription factor, to its recognition motif. Activation of the *–514T –250A* allele of the *LIPC* promoter by co-transfection of HepG2 cells with USF1 was about half of that of wild type [13•].

The –514 *C* to *T* polymorphism of the *LIPC* promoter is significantly associated with LDL and HDL size and density distribution rather than their plasma concentrations. These effects impact on the overall atherogenicity of the lipoprotein phenotype. The *–514T* allele was observed to be associated with increased concentrations of cholesterol carried in the large, buoyant HDL_2 particles (but not necessarily HDL_3 cholesterol or total HDL cholesterol) [6,14]. Recently, this observation was confirmed in a large population of African Americans from the Coronary Artery Risk Development in Young Adults (CARDIA) Study [8•]. Carriers of the *CT* and *TT* genotypes had approximately 13 and 27% higher mean HDL_2 cholesterol, respectively, than *CC* carriers; mean HDL_3 cholesterol was similar among the three genotypes. Furthermore, the effect of the –514 *C* to *T* polymorphism on HDL subclass distribution was also reported by Carr *et al.* [11••] in healthy premenopausal women. Interestingly, a significant effect of the *Taq1B* polymorphism in the cholesteryl ester transfer protein (CETP) gene on HDL_3 cholesterol but not on total or HDL_2 cholesterol was observed in this study. Grundy and colleagues [15] investigated whether increased hepatic lipase activity actually causes a decrease in HDL_2 cholesterol levels by treating 20 men with the synthetic anabolic steroid stanozolol, which increased hepatic lipase activity by approximately two-fold. This treatment markedly reduced the levels of large HDL_2 particles. This result supports the hypothesis that the *LIPC* promoter polymorphism exerts its effect on HDL particles by modulating the levels of hepatic lipase. In addition, both catalysis and ligand activities of hepatic lipase play a major role in promoting the scavenger receptor B1-mediated uptake of HDL-cholesteryl ester [16]. Thus, the *LIPC* polymorphism, by affecting hepatic lipase synthesis and activity, may significantly contribute to the process of reverse cholesterol transport modulating both HDL subclass distribution and HDL catabolism. Interestingly, dietary fat intake has recently been shown to significantly modify the association between the *LIPC* –514 *C* to *T* polymorphism and HDL cholesterol concentrations [17•]. In the Framingham Study, the *T* allele was correlated with higher HDL cholesterol concentrations only in individuals who usually consumed a low-fat diet. In contrast, the *TT* genotype was associated with lower HDL cholesterol levels in individuals who usually consumed a high-fat diet. Similar results were observed when HDL_2 cholesterol was the variable. This gene–diet interaction was observed for saturated and monounsaturated fat, but not for polyunsaturated fat. While the mechanism for this interaction is unclear, *TT* subjects may have an impaired adaptation to diets high in animal fats, which might result in increased cardiovascular risk. This gene–diet interaction may, at least partly, contribute to explain some of the controversial results on the association between *LIPC* promoter polymorphisms and CHD risk, as detailed in the following section.

As mentioned above, hepatic lipase also catalyzes the hydrolysis of triglycerides and phospholipids in intermediate density lipoproteins and LDL. LDL size and buoyancy are inversely associated with hepatic lipase activity [18,19]. The prevalence of small, dense LDL, such as found with increased plasma levels of hepatic lipase activity, is associated with a three to six-fold increase in CHD [20]. The *–514T* allele of the *LIPC* promoter is associated with both lower hepatic lipase activity and prevalence of large, buoyant LDL particles [6]. This inverse relationship between hepatic lipase activity and LDL buoyancy is observed in premenopausal women as well [9]. Interestingly, in all these studies hepatic lipase activity is consistently not associated with LDL cholesterol levels. When factors affecting activity are modified by either lifestyle or pharmacological intervention, changes in activity induce significant modifications in LDL particle size and density [21,22]. These data strongly support a role for hepatic lipase in the remodeling and catabolism of LDL, which is associated with its lipolytic activity. The *–514T* allele of *LIPC* is therefore associated with a lipoprotein profile (large, buoyant LDL and higher levels of HDL_2 cholesterol), which apparently bears a lower atherogenic potential than the profile commonly found in carriers of the *CC* genotype (smaller, more atherogenic LDL particles and lower levels of HDL_2 cholesterol).

A significantly higher frequency of the *–514T* allele was observed among Americans of African, Japanese, and

Hispanic descent. This may partially account for the lower hepatic lipase activity observed among African Americans, as well as for the larger, more buoyant LDL particles and higher HDL cholesterol (possibly HDL_2 cholesterol) levels found among all the above-mentioned ethnic groups [23].

Hepatic lipase as a marker for coronary artery disease risk

As indicated above, high hepatic lipase activity would be predicted to result in an increased prevalence of atherogenic, small, dense LDL particles and an increased risk of CHD. The effects of hepatic lipase on LDL size and density support the observations that subjects with small, dense LDL and high hepatic lipase activity have an increased CHD risk. This is seen in men versus premenopausal women and in centrally obese versus lean individuals. However, hepatic lipase has other and potentially anti-atherogenic functions involving the uptake of HDL cholesterol by the liver as part of the reverse cholesterol transport pathway.

Hepatic lipase gene promoter polymorphism and cardiovascular disease risk: are they associated?

Several groups have investigated the association of hepatic lipase activity and the *LIPC* promoter polymorphism with CHD risk [3••]. Apparently conflicting results were obtained. Against the evidence of high levels of hepatic lipase being pro-atherogenic is the high incidence of CHD in patients with *LIPC* mutations leading to complete hepatic lipase deficiency [4]. A possible explanation for their increased CHD risk is that defects in remnant lipoprotein metabolism may be predominant in those individuals [24].

Jansen and colleagues [25] found that the *–514T* allele, associated with lower hepatic lipase activity, was more common in 782 male patients from the Regression Growth Evaluation Statin Study (REGRESS) with angiographically documented CHD than in 316 asymptomatic controls. However, the CHD population contained only half of the expected number of *–514T* homozygotes (based on the Hardy–Weinberg equilibrium), casting some doubts on the association between *T* allele and increased risk of CHD. In addition, in this study, hepatic lipase activity in CHD patients was not different from age-matched controls. Tahvanainen and co-workers [26] compared 395 Finnish men with documented CHD (coronary bypass surgery; the Lopid Coronary Angiography Trial – LOCAT) with 194 healthy younger university students and found no difference in frequency of the *–514T* allele. Shohet *et al.* [27] supported these results by demonstrating that, although the *–514T* allele was associated with a 15–29% reduction in hepatic lipase activity, its frequency was the same in men with CHD ($n = 317$) and men without CHD ($n = 74$). Low hepatic lipase activity has been reported in patients with clinically overt CHD [28•]. In a population of 200 men undergoing elective coronary angiography, the extent of CHD was inversely correlated with hepatic lipase activity ($r = -0.19$; $P < 0.01$), indicating that a small percentage, approximately 4%, of the variance in CHD, could be explained by different levels of hepatic lipase activity. However, 45% of these men were on lipid-lowering medications and this may have influenced the association as lipid-lowering therapy is reported to significantly reduce hepatic lipase activity [22,29]. Similar results were recently published by Herbison *et al.* [30] who showed that, in males, the *T* allele was associated with higher HDL cholesterol and higher triacylglycerol levels and its frequency was higher in patients with established CHD than in controls (*T* allele frequencies 0.231 versus 0.177, respectively). No mention of lipid-lowering treatment was provided in this study.

Polymorphisms of the *LIPC* promoter seem to be associated with the presence of early markers of coronary artery disease, such as coronary artery calcification (CAC), a measure of sub-clinical coronary artery disease, and impaired coronary flow reserve (CFR), a marker of vascular dysfunction before the appearance of angiographic lesions. The potential association between *LIPC* polymorphisms and CAC was recently studied by Hokanson *et al.* [31•]. In this study, the *LIPC* –514 *C* to *T* promoter polymorphism was associated, in a dose-dependent manner, with a greater than two-fold increased presence of CAC among patients with type 1 diabetes. This relation was independent of HDL cholesterol levels. A potential limitation of this study is represented by the remarkable importance of other genetic factors in the variation of coronary artery calcification as measured by CAC quantity; 43.5% of the variation in CAC quantity is attributable to genetic factors after adjusting for age, sex, lipids, blood pressure, fasting glucose and smoking [32]. The presence of the *–514T* allele in healthy mildly hypercholesterolemic young men appears to modify coronary reactivity and is associated with impaired CFR [33•]. Abnormalities in CFR may indicate vascular dysfunction before the appearance of angiographic lesions.

The hepatic lipase gene promoter polymorphism and cardiovascular disease risk: pathophysiological links

Despite the anti-atherogenic effects of the *LIPC –514T* allele on HDL_2 cholesterol (increased), and on LDL size and density (large, buoyant particles) the results available suggest that this allele is associated with more subclinical atherosclerosis and to either no benefit or even a slightly increased risk of angiographically diagnosed CHD. A possible explanation for the discrepancy between the results is that the relationship between

hepatic lipase and atherosclerosis could be modulated by other factors such as the concentration of plasma LDL (Figure 2). High hepatic lipase activity may result in increased risk of CHD in individuals who have high concentration of LDL particles, which become small, dense LDL upon the action of the enzyme. The presence of high concentrations of small, dense LDL may override the potential beneficial effect of having high hepatic lipase on reverse cholesterol transport. However, among individuals with low levels of LDL, having high levels of hepatic lipase could be anti-atherogenic, as reverse cholesterol transport and triglycerides-rich lipoprotein remnant catabolism may be enhanced. Conversely, low hepatic lipase activity could be anti-atherogenic in individuals who have high LDL by limiting the formation of large quantities of small, dense LDL. However, low hepatic lipase may be atherogenic among subjects with low LDL, as reverse cholesterol transport is attenuated and triacylglycerol-rich lipoprotein remnant catabolism may be impaired [24]. Therefore, high or low hepatic lipase may be either atherogenic or anti-atherogenic depending on the concentration of plasma LDL particles. We propose that the concentration of small dense LDL is a primary determinant of the atherogenicity of hepatic lipase. Two common syndromes in which elevated hepatic lipase is associated with increased numbers of small, dense LDL particles are type 2 diabetes and familial combined hyperlipidemia.

While the role of hepatic lipase in lipoprotein metabolism is considered to be the major mechanism by which it may affect the risk of CHD, recent evidence suggests that the *LIPC* promoter variant and hepatic lipase activity may also interfere with components of glucose homeostasis. Pihlajamaki *et al.* [34] studied healthy subjects and members of families with familial combined hyperlipidemia in a Finnish population. They showed that the *LIPC –514T* allele was associated with insulin resistance and high triglyceride levels in both groups. In support of this association between *LIPC* promoter variants and glucose metabolism, Jansen and co-workers [35] showed, in healthy young males from the European Atherosclerosis Research Study (EARS-II), that interaction of the *LIPC T* allele with common variants of the apolipoprotein C-III gene (–482 *C>T*) results in significantly increased glucose and insulin response during oral glucose tolerance testing. Although little is known about the physiological relevance of these findings, the link between *LIPC* variants and glucose homeostasis represents an exciting new area of future research and may become a novel pathophysiological mechanism linking hepatic lipase to CHD.

Figure 2. Atherogenicity of hepatic lipase activity varies with the concentration of plasma LDL

Risk of atherosclerosis	HL activity	LDL-C levels	Pathophysiology of CHD risk
Higher	↑	↑	Elevated concentrations of small, dense LDL
	↓	↑	Increased LDL-C (large, buoyant LDL); impaired reverse cholesterol transport, and, possibly, remnants catabolism
Lower	↓	↓	Impaired reverse cholesterol transport, and, possibly, remnants catabolism
	↑	↓	Increased reverse cholesterol transport. low levels of LDL-C (but small, dense LDL)

Recently, an exciting report provided evidence for a new pathway by which hepatic lipase may modulate atherosclerosis. Gonzales-Navarro *et al.* [36••] suggested that hepatic lipase might be directly implicated in the development of the atherosclerotic lesion by showing the presence and synthesis of hepatic lipase in mouse as well as human macrophages. These findings raise the possibility that hepatic lipase may be produced by macrophages residing in the arterial lesion, thereby promoting foam cell formation by facilitating lipoprotein retention and modulating their atherogenicity in the subendothelial space.

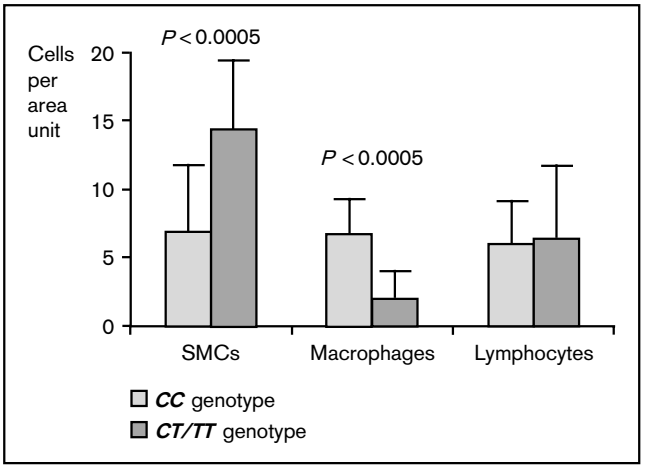
Hepatic lipase as a marker for carotid artery disease and risk of cerebrovascular events

Among lipoprotein risk factors for coronary artery disease, variations in plasma HDL cholesterol appear to be the strongest predictors of extracranial atherosclerosis progression [37]. Moreover, Liu *et al.* [38•] recently showed a potentially critical role for small, dense LDL particles in the progression of carotid intima-media thickness (IMT), a prelude to overt atherosclerosis [38•]. The association between LDL size and carotid IMT was independent of clinical, lipoprotein and antioxidant variables in a multivariate analysis. It is interesting to point out that both HDL cholesterol levels and LDL size and density are strongly modulated by hepatic lipase activity and *LIPC* promoter variants. Early stages of the atherosclerotic process are difficult to identify as the initial disease is asymptomatic. Carotid IMT measured by high-resolution B-mode ultrasound has been demonstrated to correlate well with pathologically and clinically defined atherosclerosis as well as with an increased risk of myocardial infarction and ischemic stroke [39]. The potential impact of the *LIPC –514 C* to *T* polymorphism on carotid IMT was investigated by Rundek *et al.* [40•] in a multiethnic population. The

presence of the *C* allele was associated in a dose-dependent manner with increasing carotid IMT, and the *CC* genotype was associated with a 13% ($P = 0.025$) higher IMT than other genotypes. This association was independent of ethnicity and environmental exposures. The *CC* genotype is associated with higher hepatic lipase activity and prevalence of small, dense LDL particles, which are highly susceptible to oxidation. An increased oxidative stress may, at least partially, account for the increased carotid IMT seen in carriers of the *CC* genotype.

As carotid IMT is a good early marker of atherosclerosis and risk of cerebrovascular ischemic events, the results by Rundek *et al.* [40•] would suggest that patients with the *CC* genotype and higher hepatic lipase activity might be exposed later in life to a higher risk of cerebrovascular ischemic events. The potential association between *LIPC –514 C* to *T* polymorphism and unstable carotid plaque is strongly supported by the results of a recent study by Faggin *et al.* [41••]. The presence of unstable plaque in the carotid arteries has recently been proposed as a risk factor for ischemic stroke [42]. Faggin and co-workers [41••] investigated the potential association between *LIPC C* to *T* polymorphism and prevalence of inflammatory cells in the carotid plaque of 68 patients with severe carotid artery stenosis undergoing carotid endarterectomy. In this population, a strong association was observed between the *CC* genotype of the *LIPC* promoter and features of the unstable atherosclerotic plaque, namely an abundance of macrophages with fewer smooth muscle cells (Figure 3). Interestingly, carriers of the *CC* genotype had significantly smaller, denser LDL particles. However, LDL and HDL cholesterol, triglycerides, lipoprotein (a) [Lp(a)], systolic and diastolic blood pressure, glucose levels, fibrinogen, plasminogen and homocysteine were not associated with different *LIPC* genotypes. Despite a similar CHD risk profile (except for the presence of dense LDL), patients with the *CC* genotype and the unstable carotid plaque had a significantly higher incidence of cerebrovascular ischemic events prior to carotid surgery than *CT/TT* patients ($P = 0.002$). In a multiple regression analysis, the *LIPC* polymorphism was a strong predictor of carotid plaque composition and specifically of the abundance of macrophages in the plaque: about 40% of the variance in the number of macrophages in the plaque was accounted for by the different *LIPC* genotypes (Figure 4). None of the quantitative lipid variables [i.e. LDL and HDL cholesterol, Lp(a), triacylgycerol] was associated with carotid plaque composition, while a strong inverse

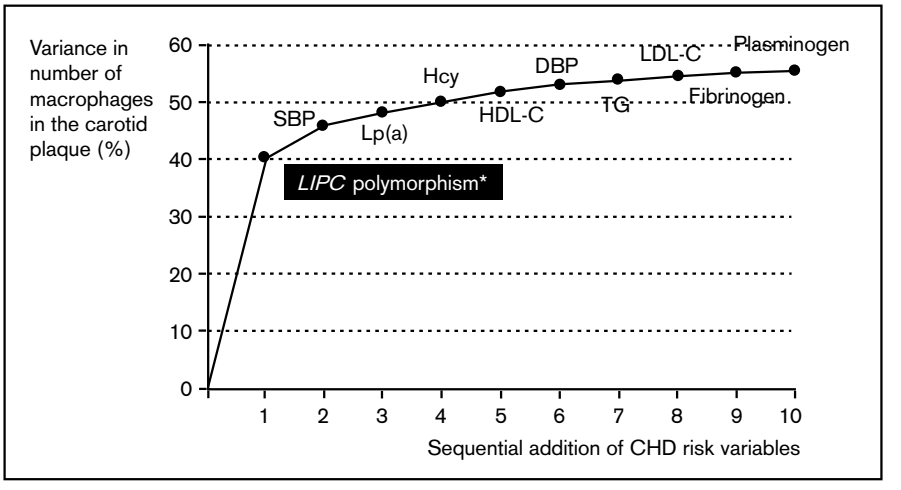
Figure 3. Cell composition of carotid atherosclerotic plaque and hepatic gene promoter –514 *C* to *T* polymorphism



SMC, smooth muscle cell. Reproduced with permission [41••].

Figure 4. Changes in the number of macrophages in the carotid plaque and CHD risk factors

The results of a multiple linear regression analysis show the percent variance of changes in the number of macrophages in the carotid plaque accounted for by changes in each of the variables sequentially added. SBP, systolic blood; pressure; Lp(a), lipoprotein (a); Hcy, homocysteine; HDL-C; HDL cholesterol; DBP, diastolic blood pressure; TG, triacylglycerol; LDL-C, LDL cholesterol; CHD, coronary heart disease. *$P < 0.001$. Reproduced with permission [41••].



relationship was found between LDL buoyancy and number of macrophages in the plaque ($r = -0.639$, $P < 0.001$). Although total HDL cholesterol was not associated with the abundance of macrophages in the plaque, a possible effect of HDL subpopulations, specifically HDL_2 particles, on plaque composition cannot be ruled out, which may be clinically relevant as the *LIPC* polymorphism affects also HDL_2 cholesterol levels.

There is clear preliminary evidence that higher hepatic lipase activity, as seen in carriers of the *LIPC CC* genotype, possibly by its association with small, dense LDL particles and low HDL_2 cholesterol, may significantly affect both early and advanced stages of the carotid atherosclerotic process. In addition, by affecting plaque composition and stability, *LIPC* polymorphisms significantly contribute to define the genetic susceptibility to ischemic cerebrovascular events.

Genetic modulation of cardiovascular disease response to lipid-lowering therapy: relevance of hepatic lipase gene polymorphisms

Treatment with hydroxyamethylglutaryl-coenzyme-A-reductase inhibitors (statins) is associated with a significant reduction in CHD mortality and in the number of patients experiencing heart attacks or ischemic strokes or undergoing a revascularization procedure. However, a substantial number of patients receiving lipid-lowering treatment for both primary and secondary CHD prevention still experience either no benefit or even CHD progression resulting eventually in myocardial infarction and other cardiovascular events. Both lipoprotein metabolism and atherogenesis are modulated by genetic and environmental factors that interact to determine individual responsiveness to lipid-lowering intervention. Genetic polymorphisms that may underlie the differences in response to lipid-lowering therapy include those in genes encoding cholesteryl ester transfer protein (*CETP*, *TaqIB*), stromelysin-1 (a matrix metalloproteinase), beta-fibrinogen, apolipoprotein E4, angiotensin converting enzyme (*ACE*), lipoprotein lipase (*LPL*), and hepatic lipase (–514 *C* to *T*) [43•].

The potential involvement of the *LIPC* polymorphism in modulating response of CHD patients to therapy is supported by a recently elucidated pathophysiological pathway of CHD regression upon intensive lipid-lowering treatment [22]. Based on the available evidence, at least part of the cardiovascular benefits observed in the recent CHD lipid treatment prevention trials that are unaccounted for by the decrease in LDL cholesterol, might be explained by a pharmacological effect on LDL size and density possibly mediated by changes in hepatic lipase activity. In patients originally studied in the Familial Atherosclerosis Treatment Study [44] with premature coronary artery disease and elevated apolipoprotein B levels, intensive lipid-lowering therapy was associated with a significant decrease in hepatic lipase activity, which was strongly associated ($r = -0.80$, $P < 0.001$) with increased LDL particle buoyancy. Independently of variation in LDL cholesterol levels and changes in other CHD risk variables, hepatic lipase-mediated changes in LDL buoyancy were the strongest predictors of CHD benefits in these patients, accounting for 38% of the variance of changes in coronary disease severity ($P < 0.01$) [22]. Evidence from these studies suggests that regression of coronary atherosclerosis results from at least two independent effects of lipid-lowering therapy on lipoprotein metabolism: the well known one leading to changes in LDL-C and apolipoprotein B levels, and an independent pathway of hepatic lipase-mediated improvements in LDL buoyancy (Figure 5). The presence of these two independent effects of lipid-lowering therapy, on both LDL cholesterol and on hepatic lipase activity and LDL size, was also observed in males with familial hypercholesterolemia receiving atorvastatin for 12 weeks [29].

The common *LIPC* –514 *C* to *T* polymorphism significantly affects hepatic lipase activity, thereby modulating a critical step of this recently highlighted pathway leading to CHD improvement with lipid-lowering therapy. A recent study [45•] by our group showed that, in men with established CHD and dyslipidemia, the *LIPC* –514 *C* to *T* polymorphism significantly predicts coronary stenosis regression during intensive lipid-lowering treatment. This association appears to be mediated by the modulating effect of the *LIPC* polymorphism on specific drug-induced changes in the metabolism of both LDL and HDL. The *LIPC* polymorphism has no significant impact on the lipoprotein pathway leading to changes in LDL cholesterol and apolipoprotein B levels (Figure 5). Homozygous *CC* patients exhibited a greater decrease in hepatic lipase activity and a greater increase in LDL buoyancy and HDL_2 cholesterol with lipid-lowering therapy than both *CT* and *TT* carriers. Therefore, patients with the *CC* genotype that started with small, dense LDL, lower HDL_2 cholesterol at baseline, in addition to decreasing LDL cholesterol and apolipoprotein B concentrations (quantitative lipoprotein changes), normalized their HDL_2 cholesterol levels and LDL buoyancy (qualitative lipoprotein changes) (Figure 5). The combination of quantitative and qualitative lipoprotein changes seen in the *CC* patients may account for the significantly greater angiographic regression of coronary stenosis ($P < 0.001$) seen in this group as compared with *CT* and *TT* individuals (Figure 6). Possible mechanisms accounting for the greater benefit seen in carriers of the *CC* genotype include (1) a reduced

Figure 5. Effect of intensive lipid-lowering treatment on lipoprotein metabolism

Impact on the pathophysiology of coronary artery disease regression of the hepatic lipase gene, *LIPC*, –514 *C* to *T* promoter polymorphism. VLDL, very low density lipoprotein; LDL-C, LDL cholesterol; Apo B, apolipoprotein B. Adapted with permission [45•].

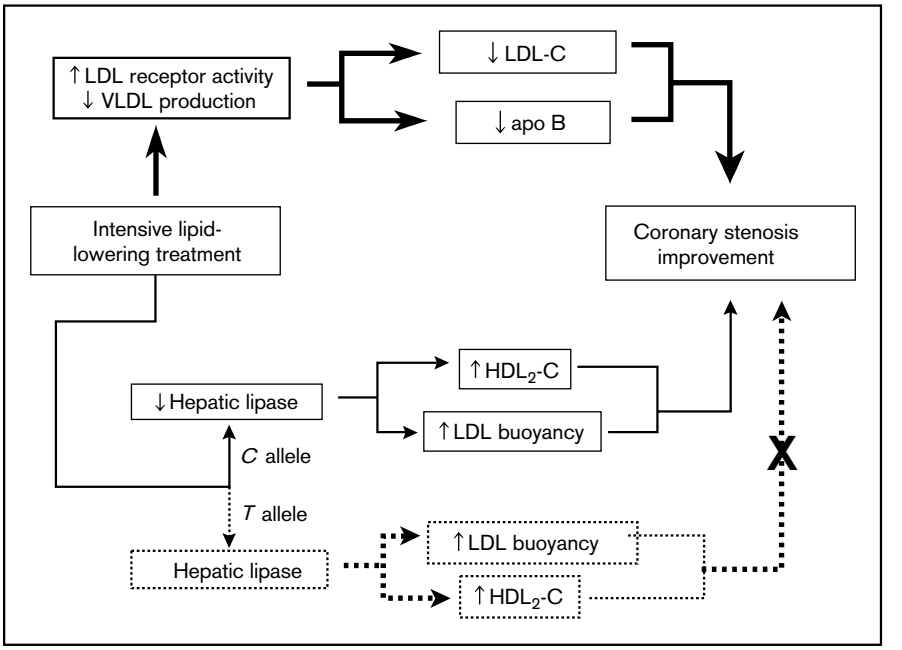
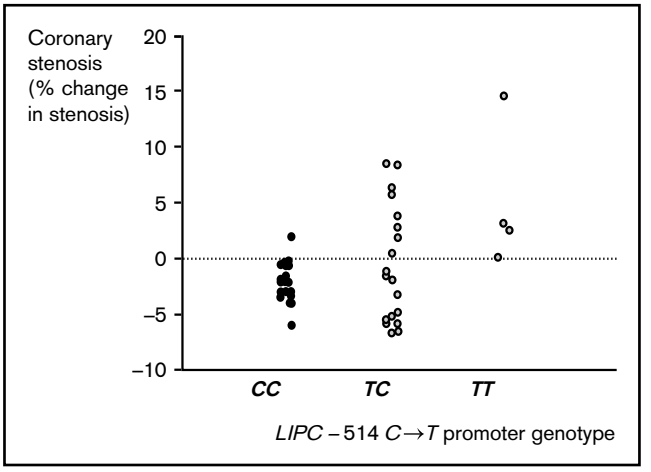


Figure 6. Change in coronary disease severity with intensive lipid-lowering therapy



Results in patients with different hepatic lipase gene (*LIPC*) genotypes (–514 *C* to *T* polymorphism). $\chi^2 = 16.43$; $P < 0.001$. Reproduced with permission [45•].

atherogenicity of their LDLs that, by shifting toward larger and more buoyant particles, decrease their susceptibility to oxidative modification in the subendothelial space, and (2) a normalization of their HDL_2 cholesterol levels which may reflect a more efficient reverse cholesterol transport, a key pathway to reduce CHD risk and progression.

A significant modulation of the *LIPC* –514 *C* to *T* polymorphism on changes in hepatic lipase activity during lipid-lowering therapy was confirmed by a recent study on normolipidemic and mildly hyperlipidemic men with CHD enrolled in the REGRESS trial [46]. As in the Familial Atherosclerosis Treatment Study, the greater decrease in hepatic lipase activity was observed in carriers of the *CC* genotype with triacylglycerol levels greater than 2 mmol/L. Although LDL density was not measured in this study, both the *CC* genotype and increased triacylglycerol levels are commonly associated with the presence of small, dense LDL particles. It would be interesting to analyze in REGRESS if carriers of the *CC* genotype who presented the greater decrease in hepatic lipase activity also had a significantly better clinical outcome with lipid-lowering therapy.

No data are currently available on the potential modulating effect of the *LIPC* polymorphism on carotid atherosclerosis response to lipid-lowering therapy, and specifically on the potential *LIPC* modulation of the effect of drug treatment on plaque composition and plaque stability. Studies investigating this topic may bear potentially critical information to improve current cerebrovascular disease prevention strategies, particularly in patients at high risk, whose lipoprotein abnormalities are mainly qualitative (small, dense LDL and low HDL_2 cholesterol) rather than quantitative (high LDL cholesterol).

Conclusion

Although the role of hepatic lipase as an anti or pro-atherogenic factor is still controversial, there is convincing evidence that the enzyme plays a key role in remnant lipoprotein catabolism as well as in remodeling of LDL and HDL particles. The anti or pro-atherogenic role is likely to be modulated by the concurrent presence of other lipid abnormalities (i.e. increased LDL cholesterol levels) as well as by the genetic regulation of other enzymes involved in lipoprotein metabolism (i.e. phospholipid transfer protein, cholesteryl ester transfer protein and lipoprotein lipase). Twenty to 30% of the individual variation of hepatic lipase activity itself is accounted for by the presence of a common polymorphism in the promoter region (–514 *C* to *T*) of *LIPC*. This polymorphism, via its impact on hepatic lipase synthesis and activity, appears to contribute to (1) individual susceptibility to CHD; (2) carotid plaque composition and individual susceptibility to cerebrovascular events; and (3) response of CHD patients to lipid-lowering therapy.

As regards the first point, conflicting evidence suggests that the presence of the *T* allele (low hepatic lipase activity) may carry a marginally increased risk of atherosclerosis despite the presence of high HDL cholesterol. This apparent discrepancy might be accounted for by a defective reverse cholesterol transport due to a decreased hepatic lipase-mediated HDL catabolism. An impaired triacylglycerol-rich lipoprotein remnant catabolism may also play a role. Secondly, concerning carotid plaque composition and individual susceptibility to cerebrovascular events, preliminary evidence shows that hepatic lipase activity is associated with carotid atherosclerosis at both early and advanced stages. The presence of the *CC* genotype (higher hepatic lipase activity) is associated with increased carotid IMT and abundance of macrophages in the carotid plaque (unstable plaque). These patients have a significantly higher incidence of cerebrovascular events. Finally, patients with higher hepatic lipase activity (*CC* genotype) and small, dense LDL particles, have the greatest clinical benefit from intensive lipid-lowering therapy. *LIPC* contribution to CHD response to therapy is explained by its modulation of the pharmacological effect on hepatic lipase activity and LDL density, both crucial steps in stenosis regression.

Although larger, epidemiological studies are needed to further support these data, the importance of the *LIPC* promoter polymorphism as a genetic marker for both coronary and carotid atherosclerosis, as well as CHD response to therapy, is emphasized by the high frequency of this polymorphism ranging from 20 to 47% depending upon the ethnic group studied. Screening for these variants in the *LIPC* promoter region will contribute to a better characterization of individual risk of coronary and cerebrovascular events, specifically in patients with qualitative (small, atherogenic LDL and low HDL_2 cholesterol), rather than quantitative, lipid abnormalities for whom the routine lipid profile may underestimate the risk of coronary and cerebrovascular disease. Characterization of patients by their *LIPC* genotype may also contribute to identifying those who will benefit most from lipid-lowering strategies, as well as patients who appear to be resistant to CHD regression and for whom a more aggressive LDL cholesterol-targeted and overall risk-reducing approach might be warranted.

Acknowledgements

NIH Grants HL-30086 and HL-64322 contributed to some of the work described in this paper. A portion of the work on which this review is based was performed in the University of Washington General Clinical Research Center (NIH grant RR37), and at the Department of Medical and Surgical Sciences, University of Padova.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Perret B, Mabile L, Martinez L, *et al.* Hepatic lipase: structure/function relationship, synthesis, and regulation. J Lipid Res 2002; 43:1163–1169.

2 Deeb SS, Carr M, Zambon A, Brunzell JD. Hepatic lipase: genetics and role in lipoprotein metabolism and cardiovascular disease. Heart Disease (in press).

3 •• Jansen H, Verhoeven AJM, Sijbrands JG. Hepatic lipase: a pro- or anti-atherogenic protein? J Lipid Res 2002; 43:1352–1362.
This is an excellent, comprehensive review on the controversial evidence linking hepatic lipase to CHD. The potential pro or anti-atherogenic effects of both hepatic lipase catalytic and ligand activity are discussed. The atherogenicity of hepatic lipase activity is likely to be modulated by the concurrent presence of different lipoprotein abnormalities as well as other CHD risk factors.

4 Brunzell J, Deeb S. Familial lipoprotein lipase deficiency, apo CII deficiency, and hepatic lipase deficiency. In: Scriver C, Beaudet A, Sly W, Vale D, editors. The metabolic and molecular basis of inherited disease. New York: McGraw-Hill Book Co; 2001. pp. 2789–2816.

5 Guerra R, Wang S, Grundy S, Cohen J. A hepatic lipase (LIPC) allele associated with high plasma concentrations of high density lipoprotein cholesterol. Proc Natl Acad Sci U S A 1997; 94:4532–4537.

6 Zambon A, Deeb S, Hokanson J, *et al.* Common variants in the promoter of the hepatic lipase gene are associated with lower levels of hepatic lipase activity, buoyant LDL and higher HDL2 cholesterol. Arterioscler Thromb Vasc Biol 1998; 18:1723–1729.

7 Jansen H, Verhoeven AJ, Weeks L, *et al.* Common C-to-T substitution at position −480 of the hepatic lipase promoter associated with a lowered lipase activity in coronary artery disease patients. Arterioscler Thromb Vasc Biol 1997; 17:2837–2842.

8 • Juo SH, Han Z, Smith JD, *et al.* Promoter polymorphisms of hepatic lipase gene influence HDL(2) but not HDL(3) in African American men: CARDIA study. J Lipid Res 2001; 42:258–264.
The authors, reporting longitudinal data from a large sample of African American men, confirm that the genetic effect of the *LIPC* promoter haplotype on HDL cholesterol levels is primarily due to the effect on HDL_2 cholesterol levels as previously reported in a population of Caucasian men. See Ref. [6].

9 Carr MC, Hokanson JE, Deeb SS, *et al.* A hepatic lipase gene promoter polymorphism attenuates the increase in hepatic lipase activity with increasing intra-abdominal fat in women. Arterioscler Thromb Vasc Biol 1999; 19:2701–2707.

10 Carr MC, Hokanson JE, Zambon A, *et al.* The contribution of intraabdominal fat to gender differences in hepatic lipase activity and low/high density lipoprotein heterogeneity. J Clin Endocrinol Metab 2001; 86:2831–2837.

11 Carr MC, Ayyobi AF, Murdoch SJ, *et al.* Contribution of hepatic lipase, •• lipoprotein lipase, and cholesteryl ester transfer protein to LDL and HDL heterogeneity in healthy women. Artertioscler Thromb Vasc Biol 2002; 22:667–673.

This study provided a comprehensive analysis aimed to determine the relative contribution of proposed biochemical factors and genetic markers modulating the metabolism of small, dense LDL and HDL subpopulations.

12 Deeb SS, Peng R. The C-514T polymorphism in the human hepatic lipase gene promoter diminishes its activity. J Lipid Res 2000; 41:155–158.

13 Botma GJ, Verhoeven AJ, Jansen H. Hepatic lipase promoter activity is • reduced by the C-480T and G-216A substitutions present in the common LIPC gene variant, and is increased by Upstream Stimulatory Factor. Atherosclerosis 2001; 154:625–632.

This interesting study demonstrated that the *C-480T LIPC* promoter variant is functional and affects the binding affinity of the USF to this locus.

14 Couture P, Otvos JD, Cupples LA, *et al.* Association of the C-514T polymorphism in the hepatic lipase gene with variations in lipoprotein subclass profiles: the Framingham Offspring Study. Artertioscler Thromb Vasc Biol 2000; 20:815–822.

15 Grundy SM, Vega GL, Otvos JD, *et al.* Hepatic lipase activity influences high density lipoprotein subclass distribution in normotriglyceridemic men: genetic and pharmacological evidence. J Lipid Res 1999; 40:229–234.

16 Lambert G, Chase MB, Dugi K, *et al.* Hepatic lipase promotes the selective uptake of high density lipoprotein–cholesteryl esters via the scavenger receptor B1. J Lipid Res 1999; 40:1294–1303.

17 Ordovas JM, Corella D, Demissie S, *et al.* Dietary fat intake determines the • effect of a common polymorphism in the hepatic lipase gene promoter on high-density lipoprotein metabolism: evidence of a strong dose effect in this gene–nutrient interaction in the Framingham Study. Circulation 2002; 106:2315–2321.

The authors provide novel and intriguing evidence, from the Framingham population, that dietary fat intake modifies the effect of the *LIPC* –514 *C/T* polymorphism on both HDL cholesterol levels and HDL particle subclass distribution. Data from this study on gene–nutrient interaction may contribute to explain the controversial observation on the increased CHD risk in TT patients.

18 Zambon A, Austin MA, Brown BG, *et al.* Effect of hepatic lipase on LDL in normal men and those with coronary heart disease. Arterioscler Thromb 1993; 13:147–153.

19 Watson T, Caslake M, Freeman D, *et al.* Determinants of LDL subfraction distribution and concentrations in young normolipidemic subjects. Arterioscler Thromb 1994; 14:902–910.

20 Austin MA, Breslow JL, Hennekens CH, *et al.* Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988; 260:1917–1921.

21 Purnell JQ, Kahn SE, Albers JJ, *et al.* Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men. J Clin Endocrinol Metab 2000; 85:977–982.

22 Zambon A, Hokanson JE, Brown BG, Brunzell JD. Evidence for a new pathophysiological mechanism for coronary artery disease regression: hepatic lipase mediated changes in LDL density. Circulation 1999; 99:1959–1964.

23 Haffner SM, d'Agostino Jr R, Goff D, *et al.* LDL size in African Americans, Hispanics, and non-Hispanic whites: the insulin resistance atherosclerosis study. Arterioscler Thromb Vasc Biol 1999; 19:2234–2240.

24 Zambon A, Deeb SS, Bensadoun A, *et al.* In vivo evidence of a role for hepatic lipase in human apoB-containing lipoprotein metabolism, independent of its lipolytic activity. J Lipid Res 2000; 41:2094–2099.

25 Jansen H, Verhoeven AJ, Weeks L, *et al.* Common C-to-T substitution at position −480 of the hepatic lipase promoter associated with a lowered lipase activity in coronary artery disease patients. Arterioscler Thromb Vasc Biol 1997; 17:2837–2842.

26 Tahvanainen E, Syvanne M, Frick MH, *et al.* Association of variation in hepatic lipase activity with promoter variation in the hepatic lipase gene. The LOCAT Study Investigators. J Clin Invest 1998; 101:956–960.

27 Shohet RV, Vega GL, Anwar A, *et al.* Hepatic lipase (LIPC) promoter polymorphism in men with coronary artery disease: allele frequency and effects on hepatic lipase activity and plasma HDL-C concentrations. Arterioscler Thromb Vasc Biol 1999; 19:1975–1978.

28 Dugi K, Brandauer K, Schmidt N, *et al.* Low hepatic lipase activity is a novel • risk factor for coronary artery disease. Circulation 2001; 104:3057–3062.

This study provided novel evidence that low hepatic lipase activity may be associated with CHD and calcified lesions in the coronary arteries in 200 men undergoing coronary angiography.

29 Hoogerbrugge N, Jansen H. Atorvastatin increases low-density lipoprotein size and enhances high-density lipoprotein cholesterol concentration in male but not in female patients with familial hypercholesterolemia. Atherosclerosis 1999; 146:167–174.

30 Herbison C, Mamotte C, Burke V, *et al.* Hepatic lipase gene −514 C/T polymorphism and premature coronary heart disease. J Cardiovasc Risk 2002; 9:105–113.

31 Hokanson J, Cheng S, Snell-Bergeon J, *et al.* A common promoter • polymorphism in the hepatic lipase gene (LIPC −480 C>T) is associated with an increase in coronary calcification in type 1 diabetes. Diabetes 2002; 51:1208–1213.

The *LIPC* –480 *C>T* (same as –514 *C>T*) polymorphism was associated with subclinical CHD in type 1 diabetics and the *–480T* allele was correlated with the extent of coronary calcification. It will be interesting to elucidate if this common polymorphism also predicts CHD progression in this population of type 1 diabetics.

32 Peyser P, Bielak L, Chu J, *et al.* Heritability of coronary artery calcium quantity measured by electron beam computed tomography in asymptomatic adults. Circulation 2002; 106:304–308.

33 Fan Y, Laaksonen F, Janatuinen T, *et al.* Hepatic lipase gene variation is • related to coronary reactivity in healthy young men. Eur J Clin Invest 2001; 31:574–580.

This interesting study provided the first evidence that the –480 *C* to *T* polymorphism of the hepatic lipase gene may modify coronary reactivity and be a significant determinant of the early pathogenesis of coronary dysfunction in healthy young men.

34 Pihlajamaki J, Karjalainen L, Karhapaa P, *et al.* G −250A Substitution in promoter of hepatic lipase gene is associated with dyslipidemia and insulin resistance in healthy control subjects and in members of families with familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 2000; 20:1789–1795.

35 Jansen H, Waterworth DM, Nicaud V, *et al.* Interaction of the common apolipoprotein C-III (APO C3 −482C>T) and hepatic lipase (LIPC −514C>T) promoter variants affects glucose tolerance in young adults. European Atherosclerosis Research Study II (EARS-II). Ann Hum Genet 2001; 65:237–243.

36 Gonzales-Navarro H, Nong Z, Freeman L, *et al.* Identification of mouse and •• human macrophages as a site of synthesis of hepatic lipase. J Lipid Res 2002; 43:671–675.

This outstanding report provided the first evidence that hepatic lipase is synthesized *de novo* by mouse and human macrophages and raises the possibility that the enzyme, locally produced by macrophages in the arterial lesion, may play a direct role in the pathogenesis of atherosclerosis.

37 Crouse III JR, Tang R, Espeland MA, *et al.* Association of extracranial carotid atherosclerosis progression with coronary status and risk factors in patients with and without coronary artery disease. Circulation 2002; 106:2061–2066.

38 Liu M-L, Ylitalo K, Nuotio I, *et al.* Association between carotid intima-media • thickness and low-density lipoprotein size and susceptibility of low-density lipoprotein to oxidation in asymptomatic members of familial combined hyperlipidemia. Stroke 2002; 33:1255–1260.

This study provided convincing evidence that small, dense LDL, a peculiar feature of the lipoprotein phenotype associated with high hepatic lipase activity, is associated with increased carotid IMT independently of clinical, lipid and antioxidant variables.

39 O'Leary D, Polak J, Kronmal R, *et al.* Carotid artery intima and intima-media thickness as risk factor for mycardial infarction and stroke in older adults; for the Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999; 340:14–22.

40 Rundek T, Elkind M, Pittman J, *et al.* Carotid intima-media thickness is • associated with allelic variants of stromelysin-1, interleukin-6 and hepatic lipase genes: the Northern Manhattan Prospective Cohort Study. Stroke 2002; 33:1420–1423.

This report provides evidence that the presence of the *C* allele at position –480 of the hepatic lipase gene promoter is associated with a significantly greater carotid IMT, a marker of early atherosclerosis. This association was independent of race-ethnicity and may be explained by the presence of higher hepatic lipase activity and small, dense LDL particles in carriers of the *C* allele.

41 Faggin E, Zambon A, Puato M, *et al.* Association between the CC genotype •• of the hepatic lipase gene promoter and unstable carotid plaque in patients with cerebrovascular events. J Am Coll Cardiol 2002; 40:1059–1066.

This compelling study demonstrated that the *LIPC* –514 *C* to *T* polymorphism, by modulating LDL density, significantly affects the number of macrophages in the carotid plaque and thereby plaque stability. It is also significantly associated with the occurrence of cerebrovascular events in patients with carotid artery stenosis.

42 Park AE, McCarthy WJ, Pearce WH, *et al.* Carotid plaque morphology correlates with presenting symptomatology. J Vasc Surg 1998; 27:872—878.

43 Maitland-van der Zee A-H, Klungel OH, Stricker BHCh, *et al.* Genetic • polymorphisms: importance for response to HMG-CoA reductase inhibitors. Atherosclerosis 2002; 163:213–222.
A comprehensive review that highlights the complex genetic background behind individual susceptibility to coronary artery disease response following lipid-lowering therapy.

44 Brown G, Albers JJ, Fisher LD, *et al.* Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high levels of apolipoprotein B. N Engl J Med 1990; 323:1289–1298.

45 Zambon A, Deeb SS, Brown BG, *et al.* A common hepatic lipase gene • promoter variant determines clinical response to intensive lipid-lowering treatment. Circulation 2001; 103:792–798.
In middle-aged men with established CHD and dyslipidemia, the hepatic lipase gene –514 *C* to *T* polymorphism significantly predicts changes in coronary stenosis, with lipid-lowering treatment, which appear to involve a hepatic lipase-associated effect on LDL metabolism. This study identified a gene polymorphism that strongly influences the lipid and clinical response to lipid-lowering drugs.

46 Jansen H. Effect of statin treatment on hepatic lipase activity is dependent on promoter (LIPC −514 C>T) variant and triglyceride levels. Circulation 2001; 104:11–81.