

Evidence for a New Pathophysiological Mechanism for Coronary Artery Disease Regression

Hepatic Lipase-Mediated Changes in LDL Density

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Background—Small, dense LDL particles are associated with coronary artery disease (CAD) and predict angiographic changes in response to lipid-lowering therapy. Intensive lipid-lowering therapy in the Familial Atherosclerosis Treatment Study (FATS) resulted in significant improvement in CAD. This study examines the relationship among LDL density, hepatic lipase (HL), and CAD progression, identifying a new biological mechanism for the favorable effects of lipid-altering therapy.

Methods and Results—Eighty-eight of the subjects in FATS with documented coronary disease, apolipoprotein B levels ≥ 125 mg/dL, and family history of CAD were selected for this study. They were randomly assigned to receive lovastatin (40 mg/d) and colestipol (30 g/d), niacin (4 g/d) and colestipol, or conventional therapy with placebo alone or with colestipol in those with elevated LDL cholesterol levels. Plasma hepatic lipase (HL), lipoprotein lipase, and LDL density were measured when subjects were and were not receiving lipid-lowering therapy. LDL buoyancy increased with lovastatin-colestipol therapy (7.7%; $P < 0.01$) and niacin-colestipol therapy (10.3%; $P < 0.01$), whereas HL decreased in both groups (-14% [$P < 0.01$] and -17% [$P < 0.01$] with lovastatin-colestipol and niacin-colestipol, respectively). Changes in LDL buoyancy and HL activity were associated with changes in disease severity ($P < 0.001$). In a multivariate analysis, an increase in LDL buoyancy was most strongly associated with CAD regression, accounting for 37% of the variance of change in coronary stenosis ($P < 0.01$), followed by reduction in apolipoprotein B1 (5% of variance; $P < 0.05$).

Conclusions—These studies support the hypothesis that therapy-associated changes in HL alter LDL density, which favorably influences CAD progression. This is a new and potentially clinically relevant mechanism linking lipid-altering therapy to CAD improvement. (*Circulation*. 1999;99:1959-1964.)

Key Words: stenosis ■ lipoproteins ■ lipids ■ atherosclerosis

Elevated LDL cholesterol (LDL-C) is associated with increased risk of coronary artery disease (CAD).^{1,2} Numerous primary and secondary CAD prevention trials have convincingly demonstrated that LDL-C reduction is associated with a decrease in clinical cardiac events.³ Emerging evidence also shows that lowering LDL-C is probably only part of the coronary heart risk story.⁴ In particular, LDL subclasses, characterized by different size, density, and lipid composition, have important clinical significance for CAD.⁵ Both cross-sectional⁶⁻¹⁰ and prospective studies¹¹⁻¹³ demonstrate an association between LDL size^{6-9,11-14} or density^{9,10} and CAD. Recent clinical trials indicated that (1) subjects with small, dense LDL at baseline are more responsive to pharmacologically induced CAD improvement than individuals with large, buoyant LDL particles^{15,16} and (2) the on-treatment LDL density is inversely related to progression of coronary artery lesions.¹⁷

In cross-sectional studies, high hepatic lipase (HL) activity is associated with an increase in small, dense LDL particles

and a decrease in HDL₂ cholesterol (HDL₂-C).¹⁸⁻²¹ No convincing epidemiological data are available on the association between HL and CAD. Furthermore, the effect of intensive lipid-lowering therapy on HL activity and its bearing on LDL density are not known.

The present study investigated the effect of intensive lipid-lowering therapy on changes in LDL density and CAD regression in the Familial Atherosclerosis Treatment Study (FATS).²² Moreover, the pathophysiological mechanism linking therapy to LDL density has been studied, with focus on the effect of intensive treatment on HL activity.

Methods

Patients

Of 1198 men ≤ 62 years old with CAD who were screened for inclusion in FATS,²² 42% had apolipoprotein B (apoB) levels ≥ 125 mg/dL and would qualify for the FATS protocol. Eighty-eight sequential subjects of the 120 who completed the FATS protocol had HL measured and were included in the present study. Details of

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TABLE 1. Clinical and Lipid Parameters

	Lovastatin-Colestipol			Niacin-Colestipol			Conventional Therapy		
	Baseline	Treatment	Follow-Up	Baseline	Treatment	Follow-Up	Baseline	Treatment	Follow-Up
CHOL, mmol/L	7.07±1.4	4.60±1.1†	7.48±1.4	7.10±1.1	5.49±0.9†	7.70±1.5	6.91±1.0	6.62±1.0	7.21±1.5
TG	2.24±1.2	2.02±1.1*	2.34±1.4	2.23±1.4	1.49±0.9†	2.32±1.7	2.65±1.8	2.82±1.7	2.82±2.3
LDL-C	5.02±1.2	2.64±0.9†	5.23±1.4	4.93±1.1	3.38±0.9†	5.35±1.4	4.57±1.3	4.15±1.3†	4.69±1.7
HDL-C	0.94±0.2	1.09±0.2†	1.05±0.2	1.06±0.2	1.50±0.3†	1.22±0.3	0.99±0.2	1.04±0.2*	1.02±0.2
HDL ₂ -C	0.07±0.05	0.17±0.08†	0.11±0.09*	0.12±0.10	0.36±0.20†	0.19±0.13*	0.11±0.11	0.12±0.06	0.12±0.07
HDL ₃ -C	0.90±0.14	0.91±0.12	0.94±0.18	0.98±0.17	1.11±0.18*	1.04±0.17	0.89±0.17	0.90±0.15	0.90±0.15
ApoB, mg/dL	158±28	99±23†	157±33	157±27	111±23†	163±41	150±25	142±25†	147±39
ApoAI, mg/dL	128±11	139±16†	128±15	135±11	155±22†	141±17	134±18	131±22	124±18
ApoAII, mg/dL	28±5	27±4	28±5	30±5	29±5	32±5	30±6	30±5	29±5

CHOL indicates total cholesterol.

* $P < 0.05$; † $P < 0.01$ vs baseline.

Values are mean±SD.

randomization and therapies have been published previously.²² Subjects received an American Heart Association step II diet and were randomly assigned to lovastatin (40 mg daily) and colestipol (30 g daily) or niacin (4 g daily) and colestipol, defined as intensive lipid-lowering therapy, or conventional treatment by diet alone (placebo) or diet and colestipol if the subject's LDL-C was ≥ 90 th percentile for his or her age. Blood samples were obtained for lipid analysis at baseline, during 2.5 years of therapy, and 6 weeks after treatment, as described previously.²² At the 2.5-year time point and 6 weeks after treatment, samples for HL activity and LDL buoyancy were obtained. Samples for LDL buoyancy were available from 83 of 88 subjects.

Blood Collection

Blood was collected in EDTA after a 12- to 14-hour fast for LDL buoyancy and plasma lipid measurements. Ten minutes after an intravenous heparin bolus (60 IU/kg), blood was collected in lithium-heparin for HL activity. Blood was immediately centrifuged at 4°C and stored at -70°C.

Lipid and Lipoprotein Determinations

Plasma, LDL, HDL, HDL₂, and HDL₃-C, triglycerides (TG), apoB, apoA-I, and apoA-II were measured as previously described.²² Lipoprotein(a) levels were determined with a double monoclonal antibody-based ELISA.²³

Density Gradient Ultracentrifugation

Lipoprotein particles were separated by flotation rate²⁴ optimized for apoB-containing lipoproteins²⁵ on the basis of strategies previously described.²⁶ A gradient of 1 mL of plasma adjusted to a density of 1.08 g/mL (total volume, 5 mL) and 12 mL of a 1.006 g/mL concentration of NaCl was formed in a Sorvall TV-865B tube (DuPont) and centrifuged at 65 000 rpm for 90 minutes at 10°C. Tubes were fractionated, and cholesterol was measured in 38 fractions by enzymatic kit (Sigma Chemical Co). LDL relative flotation (LDL-Rf) was calculated as the fraction of the major peak of LDL divided by the total number of fractions. The coefficient of variation of LDL-Rf is 0.2%.¹⁸ All samples were assayed within 600 days. LDL-Rf and LDL size are strongly correlated,⁵ and LDL-Rf is different in subjects classified by LDL subclass phenotype.²⁷ The physical principles behind this density gradient ultracentrifugation are similar to the analytical ultracentrifugation.²⁸

Post-Heparin Plasma Lipase Activity

Lipolytic activity was measured in plasma as previously described.²⁹ Glycerol tri[1-¹⁴C]oleate and lecithin were incubated with postheparin plasma for 60 minutes at 37°C, and liberated C¹⁴ free fatty acids were extracted and counted. HL activity, in nanomoles of fatty acids

released per minute per milliliter of plasma ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$), is defined as the activity after incubation with a monoclonal antibody that inhibits lipoprotein lipase (LPL).³⁰ A bovine milk LPL standard was included to adjust for interassay variation. A human postheparin control sample included in each assay had a coefficient of variation of 13.3% and no significant change over 600 days. All samples were assayed within this period of time.

Coronary Angiography

Quantitative coronary angiography was performed, and angiograms were analyzed as previously described.^{22,31} For each arterial lesion, lumen diameter was measured at the point of greatest narrowing (minimum diameter) and at a nearby point of normal diameter. In each subject, we obtained an estimate of percentage proximal disease severity (%Sprox) by averaging the severity of the worst lesion found in each of the 9 standard proximal coronary segments.²² Disease changes ($\Delta\%$ Sprox) were calculated as the difference between %Sprox at baseline and after treatment.

Statistical Analyses

Values are mean±SD. On- and off-treatment effects within the same group were analyzed with the paired Student *t* test. Analyses between different treatment groups used the unpaired Student *t* test. Either the Wilcoxon signed rank or Mann-Whitney rank sum test was used when data were not normally distributed.

Relationships between quantitative variables were tested by linear regression. Changes in LDL-Rf were linearly adjusted to the mean change in TG levels.

Multiple regression of changes in coronary stenosis (dependent variable) used a step-up procedure of risk variables that maximizes the predictive value of the model (R^2).³² We calculated changes in blood pressure, body weight, lipids, and apolipoproteins by subtracting baseline values from on-treatment values. Significance was assumed at $P=0.05$.

Results

Of the 88 sequential FATS subjects studied, 31 received lovastatin and colestipol, 26 niacin and colestipol, and 31 conventional therapy (18 as diet alone and 13 as diet and colestipol) (Table 1). These treatment groups were not statistically different from the treatment groups of the original FATS cohort,²² which indicates that this subset is representative of the larger group initially studied. Moreover, lipid parameters measured at baseline were not significantly different from follow-up values, with the exception of HDL₂-C (Table 1).

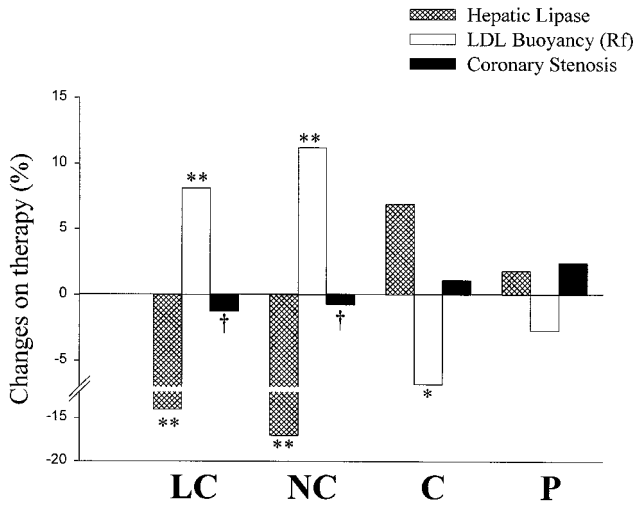


Figure 1. Percentage changes in LDL buoyancy, HL activity, and coronary stenosis on therapy compared with off therapy in the 4 treatment groups. LC indicates lovastatin-colestipol; NC, niacin-colestipol; C, colestipol; and P, placebo. HL activity and LDL buoyancy were compared on and off therapy (* $P < 0.05$; ** $P < 0.01$); changes in coronary stenosis in LC and NC groups were compared with conventional therapy (C+P; † $P \leq 0.01$).

Percent coronary stenosis decreased during intensive lipid-lowering therapy with lovastatin-colestipol (-1.25%) and niacin-colestipol (-0.7%), whereas an increase in stenosis was seen with placebo and with placebo and colestipol (combined mean, 1.87%) (Figure 1). Coronary stenosis was significantly decreased in subjects taking lovastatin-colestipol ($P < 0.01$) or niacin-colestipol ($P = 0.01$) compared with subjects receiving conventional treatment (whole group: placebo plus colestipol), as previously reported.²²

HL activity significantly decreased by 14% in the lovastatin-colestipol group (from 206 ± 72 to 178 ± 62 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$) and by 17% in the niacin-colestipol group (from 224 ± 75 to 185 ± 82 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$) (Table 2; Figure 1). In patients taking lovastatin-colestipol and niacin-colestipol, LDL buoyancy significantly increased by 7.7% (from 0.261 ± 0.04 to 0.281 ± 0.03 Rf) and 10.3% (from 0.252 ± 0.04 to 0.278 ± 0.03 Rf), respectively. On the other hand, patients receiving colestipol had a 6.8% decrease in LDL buoyancy (from 0.267 ± 0.04 to 0.249 ± 0.03 Rf; $P < 0.05$) associated with a 6.1% increase in HL activity (from 214 ± 68 to 227 ± 65 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$). In subjects receiving placebo, no significant changes in HL activity or LDL buoyancy were observed. LPL activity did not change in any of the groups studied (Table 2).

TABLE 2. Plasma Lipase Activity and LDL Buoyancy

	Lovastatin-Colestipol		Niacin-Colestipol		Colestipol		Placebo	
	Off Rx	On Rx	Off Rx	On Rx	Off Rx	On Rx	Off Rx	On Rx
LDL buoyancy (Rf)	0.261 ± 0.04	$0.281 \pm 0.03 \dagger$	0.252 ± 0.04	$0.278 \pm 0.03 \dagger$	0.267 ± 0.04	$0.249 \pm 0.03^*$	0.250 ± 0.04	0.242 ± 0.03
HL, $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$	206 ± 72	$178 \pm 62 \dagger$	224 ± 75	$185 \pm 82 \dagger$	214 ± 68	227 ± 65	222 ± 60	226 ± 59
LPL, $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$	207 ± 90	214 ± 94	215 ± 76	232 ± 88	247 ± 91	219 ± 59	188 ± 53	182 ± 64

Rx indicates treatment. Values are mean \pm SD.

* $P < 0.05$; † $P < 0.01$ vs Off Rx.

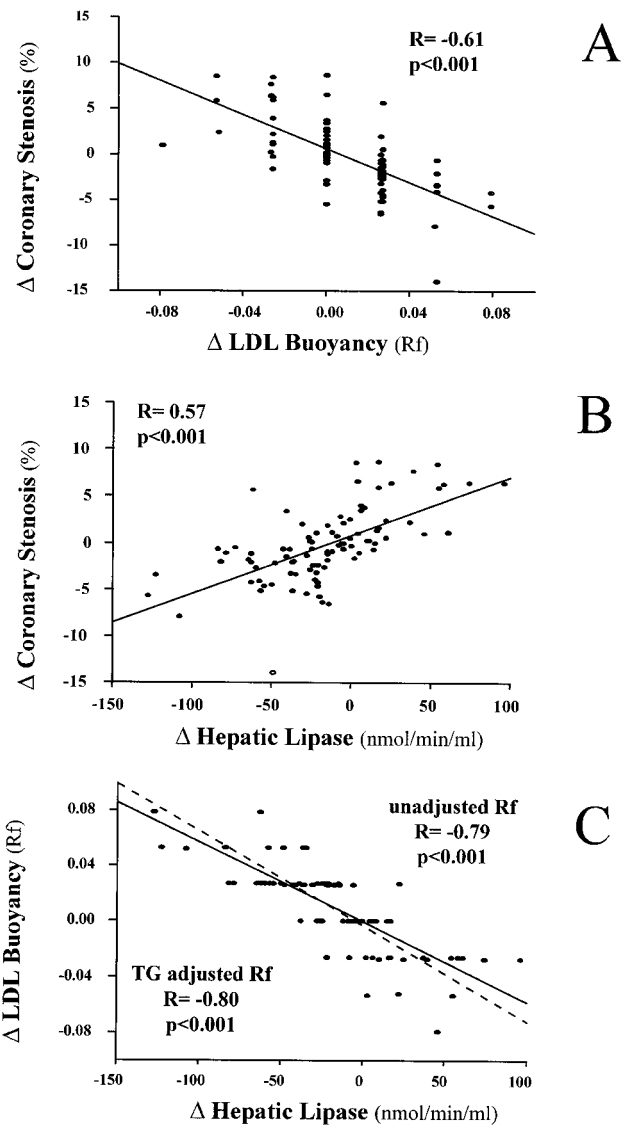


Figure 2. A, Linear regression analysis of changes in LDL buoyancy (LDL-Rf indicates relative flotation) and changes in percentage coronary stenosis. B, Linear regression analysis of changes in HL activity and changes in percentage coronary stenosis. C, Linear regression analysis of changes in HL activity and changes in LDL buoyancy (symbols and solid regression line). Dashed line represents regression line of linear regression analysis with TG-adjusted changes in LDL buoyancy. Symbols for this analysis are omitted.

Changes in LDL buoyancy were associated with changes in coronary stenosis severity ($r = -0.61$, $P < 0.001$) in the whole population (Figure 2A). In subjects receiving intensive lipid-lowering therapy, LDL buoyancy off therapy

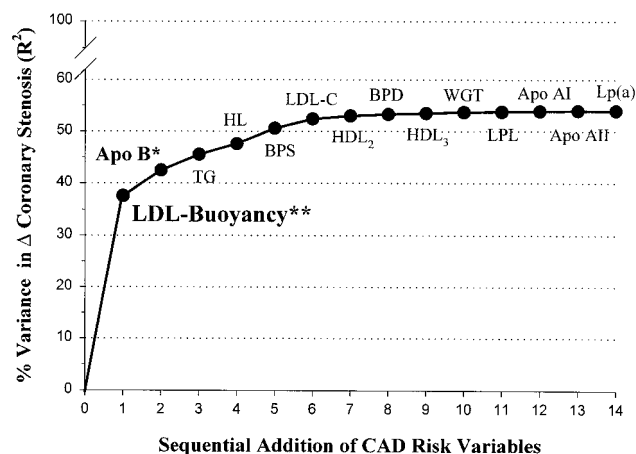


Figure 3. Results of multiple linear regression analysis by best-subset technique, showing the percent variance of changes in coronary stenosis accounted for by changes in each of the variables sequentially added (see “Statistical Analyses” in the Methods section). BPS indicates systolic blood pressure; WGT, body weight; and BPD, diastolic blood pressure. * $P < 0.05$, ** $P < 0.01$.

was significantly associated with changes in disease severity ($r = 0.29$, $P < 0.05$; $n = 54$) such that denser LDL particles off therapy were associated with better treatment outcome.

Changes in HL activity and changes in percentage coronary stenosis with therapy were highly associated ($r = 0.57$; $P < 0.001$; Figure 2B). Cross-sectional analysis showed that HL activity and LDL buoyancy were significantly correlated both off treatment ($r = -0.25$, $P < 0.05$) and with lipid-lowering therapy ($r = -0.31$, $P < 0.01$). In addition, a decrease in HL activity was strongly associated with a corresponding increase in LDL buoyancy ($r = -0.79$, $P < 0.001$; Figure 2C). This association remained after adjustment for changes in LDL buoyancy by changes in plasma TG ($r = -0.80$, $P < 0.001$; Figure 2C). A decrease in HL activity was also associated with an increase in HDL₂-C ($r = -0.50$, $P < 0.001$).

More than 50% of the changes in coronary stenosis could be explained by changes in variables measured in this study (Figure 3). Changes in LDL buoyancy with drug therapy were the best correlates of changes in coronary stenosis, accounting for 37% of the variance of changes in disease severity ($P < 0.01$), with changes in apoB levels accounting for an additional 5% of the variance ($P < 0.05$). Changes in plasma TG explained an additional 3.0% of the variance ($P = 0.08$), with an additional 8.5% explained by the changes in the remaining variables.

Discussion

This analysis of FATS examines the strengths of LDL density and HL activity relative to other common risk factors as correlates of coronary disease progression. Two strong associations have emerged. These appear promising in that they lead toward new and clinically relevant therapeutic approaches.

First, drug-induced increases in LDL buoyancy are strongly associated with improvement in coronary stenosis over a 2.5-year period. By multivariate analysis, this association of disease change with changes in LDL buoyancy is

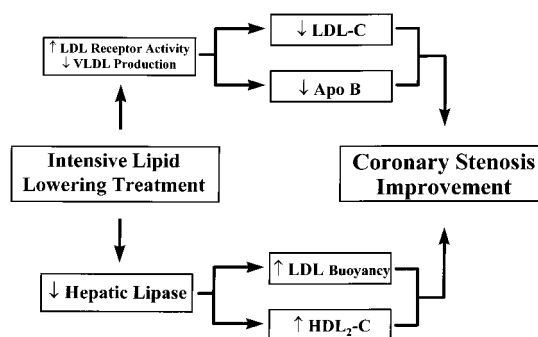


Figure 4. Effect of intensive lipid-lowering treatments on lipoprotein metabolism in FATS.

considerably stronger than that with changes in LDL-C or apoB levels. This dominant association is independent of changes in other risk variables. Thus, changes in coronary disease depend not only on quantitative changes in the number of LDL particles (ie, decrease in apoB and LDL-C levels) but, more importantly, on concomitant changes in lipoprotein composition that influence LDL buoyancy (Figure 4). This first finding is consistent with published evidence associating LDL density with clinical coronary disease risk,^{7–14} with angiographic rate of atherosclerosis progression,^{15,16} or with subgroups of patients more likely to benefit from certain lipid-altering therapies.^{16,17} In many of these reports, as in the present report, the risk attributable to LDL density was independent of other lipoprotein levels or their changes^{7,10,13,15}; in others, the LDL density effects were significant by univariate analysis but were not statistically independent of TG⁹ and HDL-C levels⁸ or of total cholesterol and HDL-C levels¹² or their ratio.¹¹ These reports highlight an issue that confounds statistical attempts to rank lipoprotein particles according to their atherogenic potential: the web of metabolic interrelationships among the plasma lipoproteins, especially the TG-rich particles, HDL-C, and LDL size and density.^{33,34} These metabolic interrelationships violate the fundamental assumption of multivariate analysis that selected independent variables are indeed independent. In this setting, statistical analyses can provide important clues to identify the truly atherogenic particles, but well-designed experimental and clinical studies are required to confirm the atherogenic potential of particles implicated by multivariate analysis. In this regard, small, dense LDL have been found to penetrate the arterial wall more readily than buoyant LDL,³⁵ to bind more avidly to arterial wall proteoglycans,³⁶ and to be more easily modified in an oxidizing environment.³⁷ Thus, a good case can be made for the primary atherogenicity of small, dense LDL. Indeed, therapy-induced changes in LDL-C levels and in LDL buoyancy appear to be largely independent and may even be directionally opposite responses (ie, the colestipol effect in the present study). In this regard, a reasonable explanation for the recent controversy regarding the degree of LDL-C reduction as a determinant of clinical benefit^{38,39} may lie in the unmonitored and variable effects of LDL density and its response to therapy among phenotypically diverse individuals in these study populations.

A second key finding is that HL activity falls significantly in response to intensive lipid-lowering therapy. Furthermore,

there is an exceptionally strong ($r=-0.80$) inverse association between these changes in HL activity and changes in LDL buoyancy. Such evidence generates the hypothesis that the favorable effects on coronary disease severity attributable to increased LDL buoyancy are mediated by a pharmacological reduction in HL activity. HL is responsible for the lipolysis of both VLDL remnant particles⁴⁰ and large, buoyant LDL, as well as the conversion of larger HDL₂ to smaller HDL₃ particles.^{40,41} As yet, the mechanisms for this newly observed therapeutic reduction in HL activity are unclear. However, one consequence of such reduction (namely, an increase in LDL buoyancy) is not unexpected. Studies in subjects with normal lipid levels show that HL levels are inversely correlated with LDL size and buoyancy^{18,19} and that men have twice the HL levels of women and have smaller, more dense LDL particles.²⁰ A similar cross-sectional relationship between HL activity and LDL density exists among CAD patients.¹⁸ Genetic deficiency of HL is associated with large, buoyant, LDL-like particles.²⁵ Recently a polymorphism in the promoter region of the HL gene has been reported that accounts substantially for observed variations in HDL-C^{42,43} and HL activity.^{44,45} We have reported that this polymorphism accounts for 20% of the variation in HL activity among normal subjects and for 32% among coronary disease patients and contributes to the modulation of the LDL buoyancy in these 2 groups.⁴⁵ The present report clearly documents that therapeutic interventions associated with a reduction in HL activity improve LDL buoyancy.

There is some disagreement whether HL activity is proatherogenic or antiatherogenic.⁴⁶ Although the rare individual with familial HL deficiency develops CAD,⁴⁷ the present findings support a proatherogenic role for the enzyme HL.

The clinical implications of these findings apply at least to the large percentage (42%) of the coronary disease population who meet FATS lipid entry requirements on routine catheterization laboratory screening.²² First, LDL density appears to be a realistic and rewarding additional therapeutic target for coronary disease prevention. This is particularly true for individuals with borderline-high LDL-C, mildly elevated TG, and borderline-low HDL-C, a lipid phenotype often associated with small, dense LDL.³³ As this report indicates, the risk for progressive coronary disease in such individuals, underestimated by the standard lipid measurements, can be reduced substantially by regimens that effectively increase LDL buoyancy. Second, HL activity takes on new importance as a potential therapeutic target by which LDL density and coronary disease risk may be favorably affected. HL activity may be inhibited directly at its site of action or indirectly by modulation of the gene promoter region.

In conclusion, these findings add compelling evidence for the role of increased LDL buoyancy for prevention of coronary disease progression and, by implication, of clinical events.^{48,49} They also identify HL as a potential key mediator of beneficial therapeutic effects on lipoprotein composition and coronary risk. These insights may help to improve substantially on the 20% to 35% cardiovascular risk reduction seen with treatment strategies focused on LDL-C lowering.⁵⁰⁻⁵⁴

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