Elevated LDL Triglyceride Concentrations in Subjects Heterozygous for the Hepatic Lipase S267F Variant

Robert A. Hegele, W. Carl Brekenridge, Diane W. Cox, Graham F. Maguire, J. Alick Little, Philip W. Connelly

Abstract—Although naturally occurring loss-of-function mutations in human hepatic lipase (HL) have been described, the biochemical phenotype of heterozygous HL deficiency remains ill defined. This may be due to the relatively small numbers of heterozygous adult carriers of HL mutations in index kindreds. We have identified several new heterozygotes for the catalytically inactive, nonsecreted HL variant S267F in the kindred that was originally ascertained because of hypertriglyceridemia due to the mutant, secreted, circulating apolipoprotein (apo) CII variant apo CII-T. Pairwise comparisons with family controls showed that only the plasma low density lipoprotein triglycerides (LDL TGs) were higher in 11 simple heterozygotes for HL S267F (P=0.002). In contrast, both plasma total TGs and LDL TGs were significantly higher in 12 simple heterozygotes for apo CII-T than in family-matched control subjects (P=0.005 and 0.009, respectively). These findings suggest that the TG content of LDL is increased by heterozygosity for 2 different mutations that affect different proteins involved in lipolysis. However, the mechanisms underlying this compositional change in LDL appear to be different for the 2 mutations, because the total TGs are also elevated in subjects heterozygous for apo CII-T but not in subjects heterozygous for HL S267F. (Arterioscler Thromb Vasc Biol. 1998;18:1212-1216.)

Key Words: genetics ■ lipoproteins ■ LDL subclasses ■ lipolysis ■ lipoprotein lipase

Hepatic lipase (HL; triacylglycerol lipase; EC 3.1.1.3) is a 60-kDa endothelial enzyme that is synthesized by hepatic parenchymal cells and that has phospholipase as well as tri-, di-, and monoglyceride hydrolase activity. In vitro and in vivo experiments have implicated a complex role for HL in human lipoprotein metabolism is unclear. Most patients with HL deficiency due to HL mutations have been ascertained on the basis of dyslipidemia. However, among carriers of HL mutations associated with loss of function, such as R186H, S267F, L334F, and T383M, there are disparities in both the biochemical phenotype and the association with atherosclerosis susceptibility. For example, in a Canadian family, compound heterozygotes for HL S267F and T383M had (1) combined hyperlipidemia, (2) TG enrichment of LDL and HDL, (3) β-migrating VLDL, (4) impaired chylomicron remnant metabolism, and (5) early coronary heart disease. However, the limited number of subjects in this family made it difficult to conclude whether simple heterozygotes had a discrete phenotype. In a Finnish family, compound heterozygotes for L334F and T383M did not have β-migrating VLDL but did have TG enrichment of LDL and HDL. Also, Finnish heterozygotes for an HL allele that carried R186H and L334F appeared to have TG enrichment of LDL and HDL. Homozygosity for a splice-site mutation in HL intron I was associated with hypertriglyceridemia and coronary heart disease; however, there was no obvious biochemical phenotype among heterozygotes.

The disparity among the results of studies of phenotype in heterozygotes for loss-of-function mutations in HL has many possible explanations. First, only a small number of heterozygotes have been identified, limiting the power for statistical analyses. Second, the variable impairment of in vitro HL function attributable to the different HL mutations may be reflected as clinical heterogeneity. Third, there could be underlying hyperlipidemias independent of any HL abnormality in the families studied. Such independent hyperlipidemias might have contributed to the initial ascertainment of the families and might produce a phenotype that is unrelated to, or at least modulates, the phenotype due to HL deficiency.
Finally, heterozygosity for a loss-of-function mutation may not affect biochemical phenotypes in the presence of the other, functionally normal \( \text{HL} \) allele. It may be possible to resolve such disparities by studying larger kindreds with \( \text{HL} \) mutations.

Several subjects in the first kindred ever reported with a mutation in a plasma apolipoprotein, namely apo CII-T, were noted to have depressed postheparin \( \text{HL} \) activity. In particular, 5 of 8 extensively studied apo CII-T homozygotes had low but detectable \( \text{HL} \) activity. This finding suggested the possibility that these subjects were heterozygous for a loss-of-function mutation in \( \text{HL} \). In a screening experiment, we identified the presence of the \( \text{HL} \) S267F mutation in this kindred and subsequently screened a large number of family members to identify several new heterozygous carriers of \( \text{HL} \) S267F to better characterize the biochemical phenotype of these subjects.

### Methods

#### Kindred C2T

This kindred designated C2T was ascertained as we previously reported. A total of >400 family members were identified; height, weight, and succinct medical histories were obtained in the field. A priori exclusion criteria (in decreasing order of effect on total sample size) included (1) an inadequate blood sample available for all determinations, due to a nonfasting state, small quantity, or no DNA (in >200 subjects); (2) age <18 years (in 14 subjects); and (3) homozygosity for the apo CII-T allele (in 6 subjects). After these exclusions, 84 subjects remained; fasting blood samples from these subjects were used for lipoprotein analyses, isoelectric focusing of apolipoproteins, and DNA analysis.

#### Biochemical and Genetic Determinations

Assays of plasma lipids and lipoproteins and electrophoretic analysis of the apolipoproteins of chylomicrons and VLDL were performed by using established procedures19–21 in each of the family members tested. DNA was extracted as described.22 Screening for known mutations in \( \text{HL} \), namely ~480T, V73M, R186H, N193S, S267F, L334F, and T383M, was performed as described[14–17,23] in 3 subjects from C2T who were known to be deficient in \( \text{HL} \) activity in postheparin plasma. Genotypes of \( \text{HL} \) S267F were determined by using polymerase chain reaction amplification and digestion with \( \text{Hin} \)I as described.14

#### Statistical Analyses

We wanted to test the hypothesis that within the nuclear families, subjects who were heterozygotes for either mutation had different biochemical phenotypes than did subjects within the nuclear families who did not carry the mutations. Thus, biochemical traits for heterozygotes for either \( \text{HL} \) S267F or apo CII-T were compared with matched control subjects from these nuclear families. Because of the small numbers of subjects and the nonnormal distribution of the biochemical variables, nonparametric analysis was carried out using the Kruskal-Wallis \( \chi^2 \) approximation test of significance of the Wilcoxon rank sums.23 By convention, a value of \( P<0.05 \) was taken as the nominal level of significance for a difference in the pairwise comparisons.

### Results

#### Screening for Mutant \( \text{HL} \)

All \( \text{HL} \) mutations that were screened for were absent from \( \text{HL} \)-deficient family members, except for \( \text{HL} \) S267F. A total of 16 heterozygotes for this mutation were identified; 1 of these was a homozygote for apo CII-T who was profoundly chylomicromeric and was thus excluded from the subsequent analyses. This subject was known to have had low but detectable \( \text{HL} \) activity in postheparin plasma.

#### Composition of the Study Sample

The 84 adult subjects from the complex C2T kindred who had sufficient plasma and cellular material available for all analyses and who were not homozygous for apo CII-T could be placed into 22 nuclear families. Each subject studied was between 4 and 7 generations removed from a common ancestral husband-wife pair. At least 1 subject with \( \text{HL} \) S267F was found in 8 of the 22 nuclear families. At least 1 subject with apo CII-T was found in 17 of the 22 nuclear families. Three of the nuclear families had compound heterozygotes for \( \text{HL} \) S267F and apo CII-T. At least 1 subject without either mutant allele was found in each of the nuclear families in which simple heterozygotes had been found. Thus, there was an adequate number of genotypically normal subjects from within each nuclear family to serve as controls for simple heterozygotes in pairwise analyses of biochemical traits.

### Association of Genotype With Variation in Biochemical Variables

Table 1 shows the results of nonparametric statistical comparisons of the biochemical traits of 11 adult family members who were heterozygous for \( \text{HL} \) S267F and nuclear family control subjects. Notably, there was no nominally significant difference among the genotypic classes in mean plasma concentrations of total, VLDL, LDL, or HDL cholesterol and no significant difference in plasma TGs. Only the TG content of LDL was significantly different between genotypic classes

Table 2 shows the results of nonparametric statistical comparisons of the biochemical traits of 12 adult family members who were heterozygous for apo CII-T and nuclear family control subjects. Notably, there was no nominally significant difference among the genotypic classes in mean plasma concentrations of total, VLDL, LDL, or HDL cholesterol. In contrast to the \( \text{HL} \) S267F heterozygotes, both the TG content of LDL and the concentration of total plasma TG

<table>
<thead>
<tr>
<th>Biochemical Variables</th>
<th>Wilcoxon Score</th>
<th>Kruskal-Wallis ( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>366.5</td>
<td>0.02</td>
<td>0.87</td>
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<tr>
<td>TGs</td>
<td>431.0</td>
<td>1.71</td>
<td>0.19</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>423.0</td>
<td>2.02</td>
<td>0.16</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>342.5</td>
<td>0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>386.5</td>
<td>0.39</td>
<td>0.53</td>
</tr>
<tr>
<td>LDL TGs</td>
<td>451.0</td>
<td>9.32</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL TGs</td>
<td>387.5</td>
<td>2.69</td>
<td>0.10</td>
</tr>
</tbody>
</table>
TABLE 2. Summary of Nonparametric Pairwise Comparisons of Biochemical Traits in Apo CII-T Heterozygotes (n=12) and Matched Family Controls (n=31)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Wilcoxon Score</th>
<th>Kruskal-Wallis χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>406.5</td>
<td>1.02</td>
<td>0.31</td>
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<tr>
<td>TGs</td>
<td>556.0</td>
<td>2.77</td>
<td>0.005</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>472.0</td>
<td>1.91</td>
<td>0.17</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>386.0</td>
<td>0.06</td>
<td>0.91</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>316.5</td>
<td>2.76</td>
<td>0.10</td>
</tr>
<tr>
<td>LDL TGs</td>
<td>495.5</td>
<td>6.80</td>
<td>0.009</td>
</tr>
<tr>
<td>HDL TGs</td>
<td>459.5</td>
<td>3.00</td>
<td>0.08</td>
</tr>
</tbody>
</table>

were significantly different in apo CII-T heterozygotes compared with control subjects (P=0.009 and 0.005, respectively). The mean ± SD for LDL TGs in 12 apo CII-T subjects and 31 matched family controls were, respectively, 43.7 ± 20.8 and 29.3 ± 10.5 mmol/L. In contrast to simple heterozygotes for HL S267F, the mean ± SD for total plasma TGs in 12 apo CII-T subjects and family matched controls were, respectively, 329.1 ± 536.7 and 150.0 ± 234.6 mmol/L. Pairwise comparisons of 4 compound heterozygotes demonstrated no significant differences in any biochemical trait compared with family matched control subjects (data not shown).

Discussion

In the current study of an extended family C2T that was originally ascertained due to hypertriglyceridemia, we observed significant elevations of LDL TGs in subjects who were simple heterozygotes for either the rare loss-of-function HL S267F variant or the nonfunctional, circulating apo CII-T variant. It was notable that the elevations of LDL TGs in simple heterozygotes for HL S267F occurred in the absence of significant elevations in plasma TG, whereas the elevations of LDL TGs in simple heterozygotes for apo CII-T occurred in the presence of significant elevations in plasma TG. This raises the possibility that the LDL TGs were elevated through different mechanisms in heterozygotes for each variant.

The lipolytic process is mediated by endothelial cell–bound lipoprotein lipase (LPL), for which apo CII is a cofactor, and by HL on the surface of the hepatic endothelium. Although it has greater affinity for the TG-rich lipoproteins, LPL has also been shown to be able to hydrolyze the TG component of LDL.25,26 It is thus possible that impairment of LPL activity due to defective apo CII-T might affect hydrolysis not only of TG-rich lipoproteins, and thus total plasma TG concentrations, but also the TG component of LDL. In contrast, HL has greater affinity in vitro for smaller-size IDL, LDL, and HDL particles than for TG-rich lipoproteins.27,28 HL in vitro can convert larger, TG-rich LDL into smaller LDL.27,28 Thus, the relative lack of affinity of HL for TG-rich lipoproteins, compared with LPL, might explain how a genetic mutation affecting HL activity, namely HL S267F, might affect LDL TGs but not total plasma TGs.

Both HL S267F and apo CII-T have abnormal functions,18,20 and each has previously been shown to contribute to abnormal lipoprotein phenotypes. For example, compound heterozygosity for HL S267F and T383M alleles produces elevated plasma concentrations of cholesterol and TGs, with TG enrichment of LDL and HDL, and the notable presence of β-migrating VLDL.14 Also, homozygosity for apo CII-T produces hyperlipoproteinemia type I, with complete absence of LPL activity, hyperchylomicronemia, and pancreatitis.18 Furthermore, the presence of a single APOE E4 allele occurring in individuals with a single, mutant apo CII-T allele has been associated with higher plasma concentrations of cholesterol, TGs, and VLDL cholesterol when compared with relatives who carried neither or only 1 variant allele.21 The data from the current report suggest that although routine biochemical tests might have been adequate to detect the association of apo CII-T with elevated plasma TGs, only the more labor-intensive procedure of ultracentrifugation of plasma would have permitted detection of the more subtle finding of increased LDL TGs for both of these mutations.

Although it is possible that the phenotypic association with HL variation was due to linkage disequilibrium with another causative variant, it is unlikely, considering that the HL S267F mutation has a known functional impact.29 Heterozygotes for HL S267F have a 50% to 70% decrease in postheparin HL activity compared with normal control subjects,31 consistent with in vitro findings that HL S267F is associated with a complete absence of detectable activity and a virtual absence of secreted mass.29 Heterozygotes for other HL mutations that cause reduced HL mass and postheparin plasma HL activity, such as R186H and L334F, also appear to have TG-enriched LDL particles.

Although it is possible that the phenotypic association with the APOC2 variation was due to linkage disequilibrium with another causative variant, it is unlikely, considering that the apo CII-T mutation has a known functional impact.18 Heterozygotes for apo CII-T have an ≈50% decrease in apo CII mass and capacity to activate LPL.21 The association of heterozygosity for apo CII-T with higher total TGs and LDL TGs suggests that impairment of this pathway can affect lipoprotein quantity and composition. The absence of associations of lipoprotein phenotypes with compound heterozygosity for both mutations was likely due to small numbers.

A possible pathophysiologic role of TG-enriched LDL is not well defined. In the absence of a major lipolytic deficiency, LDL TG content is directly related to plasma TG concentration30 and to body mass.31 Also, LDL TGs increase on a fat-free, high-carbohydrate diet.32 Although the initial TG content of an LDL particle may affect its capacity for lipid exchange,33 it is not clear that this characteristic has any pathological consequence. Occasional reports have suggested that LDL TGs are better discriminators of myocardial infarction than is LDL cholesterol.34 Smaller, dense LDL particles are consistently related to atherosclerosis risk.35–37 However, LDL size and buoyancy are not related to particle TG content, despite higher total plasma TGs in subjects with a preponderance of small, dense LDL.38

In vivo studies indicate that the LDL TG content depends on HL activity. For example, immune inactivation of HL resulted in an increase in LDL TGs.39 In a study of young men, there was an inverse relationship between HL activity and the plasma level of LDL TGs.40 These findings are consistent with our observation that diminished HL activity...
resulting from the presence of HL S267F is associated with an increase in plasma LDL TGs. HL may influence LDL lipid composition by affecting the surface lipid component.41 Subjects who have a preponderance of small, dense LDL also have higher HL activity than do subjects with more buoyant LDL,38 although this would appear to be unrelated to LDL particle TG content.38

In summary, we found that plasma LDL TGs were significantly higher, by ≈40%, in both HL S267F and apo CII-T simple heterozygotes than in relatives who had neither mutation. However, plasma LDL TGs were not significantly higher in compound heterozygotes than in simple heterozygotes. This suggests that the TG content of LDL is increased by heterozygosity for at least 2 different mutations affecting the lipolytic system, including simple heterozygosity for the catalytically inactive HL S267F variant. Furthermore, the association of TG-enriched LDLs with elevated total plasma TG concentrations in heterozygotes for apo CII-T but not in heterozygotes for HL S267F suggests that there are different reasons for TG enrichment from the 2 different mutations. However, in spite of these associations, the relationship, if any, between TG-enriched LDLs and pathological end points remains unclear. The tenuous nature of such a relationship may be a factor that has complicated the study of the association of HL activity, lipoprotein metabolism, and atherosclerosis.

Acknowledgments

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References


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