Low Hepatic Lipase Activity Is a Novel Risk Factor for Coronary Artery Disease

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Background—The crucial function of hepatic lipase (HL) in lipid metabolism has been well established, but the relationship between HL activity and coronary artery disease (CAD) is disputed.

Methods and Results—We measured HL activity in the postheparin plasma of 200 consecutive men undergoing elective coronary angiography and determined the degree of CAD with the extent score, which has been shown to be better correlated with known risk factors than other measures of CAD extent. We found a significant inverse correlation between HL activity and the extent of CAD ($r = –0.19, P < 0.01$). This association was mainly due to patients with HDL levels $> 0.96$ mmol/L ($n = 94, r = –0.30, P < 0.005$). HL activity was lower in 173 patients with CAD than in 40 controls with normal angiograms (286 ± 106 versus 338 ± 108 mmol · mL$^{-1}$ · min$^{-1}, P < 0.01$). To correct for potential confounding factors, we performed multivariate analyses that confirmed the independent association of HL activity with CAD extent. In addition, the presence of the T allele at position –514 in the HL promoter, which leads to a reduced HL promoter activity, was associated with lower HL activity ($r = 0.30, P < 0.001$) and higher CAD extent (42.2 ± 20.8 versus 35.3 ± 23.6 [extent score], $P < 0.05$). In patients with heterozygous familial hypercholesterolemia, calcified lesions in ECG-gated spiral computed tomography were higher in patients with low HL activity (6.3 ± 6.8 versus 1.5 ± 3.1, $P = 0.01$).

Conclusions—Our data show that low HL activity is associated with CAD. Therefore, HL might be useful for CAD risk estimation and might be a target for pharmacological intervention. (Circulation. 2001;104:3057-3062.)

Key Words: angiography ▪ atherosclerosis ▪ coronary disease ▪ lipoproteins

Low plasma levels of HDL cholesterol are an established risk factor for coronary artery disease (CAD), and the gene locus for hepatic lipase (HL) accounts for 25% of genetic variation in HDL levels. HL plays a crucial role in lipid metabolism by hydrolyzing triglycerides and phospholipids in circulating lipoproteins. It has not been determined, however, whether HL acts in a proatherogenic or antiatherogenic fashion. Arguments for a proatherogenic role include the inverse relationship of HL activity with the plasma levels of antiatherogenic HDL cholesterol and the positive association of HL activity with the plasma levels of small dense LDL, which have been suggested to be proatherogenic. In addition, apolipoprotein E–deficient mice develop less atherosclerosis if they are also made HL deficient. Arguments for an antiatherogenic role of HL include that HL transgenic mice have 42% less deposition of aortic cholesterol than their nontransgenic littermates and that some patients with genetic HL deficiency have developed premature CAD.

Methods

Patients

Two hundred consecutive male patients undergoing elective coronary angiography at the University Hospital, Heidelberg, were studied. To obtain a control group, HL activity was determined in 23 patients with completely normal coronary angiograms in spite of suspected CAD. For the ECG-gated spiral computed tomography study, 47 patients (30 males; mean age 37.1 ± 7.1 years) with known heterozygous familial hypercholesterolemia were recruited. For all patients, treatment with subcutaneous or intravenous heparin was an exclusion criterion. The studies were approved by the Review Committee of Heidelberg University, and each patient gave informed consent.

Coronary Angiography

Examiners of coronary angiograms were blinded to the lipase results. The extent of CAD was determined by the “extent score,” which has been shown to correlate better with risk factors of CAD than other scores.
ECG-Gated Spiral Computed Tomography of the Heart

The extent of coronary artery calcification was determined by ECG-triggered spiral computed tomography on a SOMATOM Plus 4 VZ (Siemens) and measured analogous to the method of Agatston et al.9

Analysis of Lipids/Lipoproteins

Total cholesterol, HDL cholesterol, and triglyceride concentrations were determined enzymatically in a Synchron LX-20 (Beckman Coulter GmbH). LDL and VLDL particles were separated by ultracentrifugation in a Beckman LM-8 ultracentrifuge in 100-kL volumes by use of a VT-51.2 rotor (Beckman).

Hepatic Lipase

After an overnight fast, venous blood samples were drawn into EDTA tubes before and 10 minutes after intravenous injection of 60 IU of heparin (Braun Melsungen AG) per kilogram of body weight. The samples were immediately chilled to 4°C, centrifuged, separated into aliquots, and stored at −80°C until assayed. Postheparin HL activity was determined with a triolein/phosphatidylcholine emulsion as described previously.10 Selective measurement of HL was based on the inactivation of LPL by 1.0 mol/L NaCl. The samples were measured in duplicate, and postheparin plasma from pooled normal controls was used to correct for interassay variation. The intra-assay coefficient was 7.3%. Correction with pooled postheparin samples resulted in a reduction of the interassay coefficient from >25% to 12.9%. Five patients were excluded from the analysis because very low activities for HL and LPL in postheparin plasma indicated insufficient heparin delivery.

In 150 patients, HL activity was determined by a novel, commercially available (Wak-Chemie Medical GmbH) fluorometric assay, based on the method of Duque et al.11 The results correlated well with the conventional method (r=0.70, P<0.0001). The −514C/T polymorphism was determined as described previously.12

Statistical Analysis

Statistical analyses were performed with SPSS for Windows, release 10.0.7 (SPSS Inc). Two-tailed bivariate correlations were determined by the Pearson coefficient for parameters with normal distribution and with the Spearman coefficient for parameters with other distributions. Depending on the distribution of data, comparisons between 2 sets of patients were performed by the t test or the Mann-Whitney U test, and comparisons between quartiles were performed by ANOVA or Kruskal-Wallis H test.

Results

In the 200 men undergoing coronary angiography, the values for extent score ranged from 0 to 95, with a mean of 37.6±22.9 and a median of 39.4. As expected, there was a significant inverse correlation between HDL cholesterol levels and the extent score (r = −0.17, P<0.05). Eighty-nine patients were taking lipid-lowering drugs. In those not taking such medication, we found a significant association of LDL cholesterol levels with the extent score (r = 0.25, P<0.05), in addition to an inverse association of HDL cholesterol with CAD extent (r = −0.22, P<0.05). There was a trend for greater CAD extent with higher lipoprotein(a) values (r = 0.13, P=0.08). There were no significant correlations of the concentrations of total cholesterol, plasma triglycerides, or VLDL cholesterol with CAD extent.

HL activity in postheparin plasma (n=195) ranged from 60 to 590 nmol·mL⁻¹·min⁻¹, with a mean of 287±107 and a median of 281 nmol·mL⁻¹·min⁻¹. HL fluorometric activity in postheparin plasma (n=150) ranged from 3.3 to 50.4 pmol·mL⁻¹·min⁻¹, with a mean of 15.3±8.7 and a median of 15.5 pmol·mL⁻¹·min⁻¹. As expected, there was an inverse relation of HL activity with HDL cholesterol levels (HL fluorometric activity: r = −0.24, P<0.005; HL activity: r = −0.13, P=0.08). There were no significant correlations between HL activities and plasma cholesterol, plasma triglycerides, VLDL cholesterol, or lipoprotein(a).

As shown in Figure 1A, we found a significant inverse relation between HL activity and the extent score (r=−0.19, P<0.01). Figure 1B illustrates the association of HL activity with CAD extent, when HL activity is expressed in quartiles. Here, lower HL activity was consistently associated with greater CAD extent (HL >358 nmol·mL⁻¹·min⁻¹: extent score 32.6±21.4; HL 283 to 358 nmol·mL⁻¹·min⁻¹: 35.1±20.8; HL 213 to 282 nmol·mL⁻¹·min⁻¹: 38.3±23.8; HL <213 nmol·mL⁻¹·min⁻¹: 45.1±24.0; P<0.05 for trend). In 150 patients, we determined HL activity in postheparin plasma with a novel fluorometric assay. Patients with HL fluorometric activity of less than the median had significantly greater CAD extent than patients
with HL activity higher than the median (41.0±22.8 versus 33.5±22.8, \( P < 0.05 \); Figure 1C).

Subgroup analysis revealed that the association of low HL activity with CAD extent was mainly due to patients with normal HDL cholesterol levels. Although there was no significant association of HL activity with CAD extent in the patients with HDL cholesterol levels less than the median of 0.96 mmol/L (37 mg/dL), there were highly significant associations of HL activity (n=94, \( r = -0.30, P < 0.005 \)) and HL fluorometric activity (n=77, \( r = -0.32, P < 0.005 \)) with CAD extent in the patients with HDL cholesterol levels above the median.

Because these results are mainly based on patients with CAD, we recruited 23 additional patients with normal angiograms. Compared with patients without any signs of CAD (extent score of 0, n=40), patients with documented CAD (extent score >0, n=173) had significantly lower HL activity (286±106 versus 338±108 nmol · mL\(^{-1} \) · min\(^{-1} \), \( P < 0.01 \); Figure 1D).

To confirm the association of low HL activity with greater CAD extent, we investigated a different group of patients. We measured calcified coronary lesions with ECG-gated spiral computed tomography in 47 patients with heterozygous familial hypercholesterolemia. There was an inverse association of HL fluorometric activity with coronary lesions (\( r = -0.17 \)), and patients with HL activity less than the median had a considerably greater lesion score (5.3±6.7 versus 2.6±4.6, \( P = 0.10 \)). Similar to the patients analyzed by angiograms, this association was particularly strong in individuals with HDL cholesterol levels >0.96 mmol/L (37 mg/dL; n=39; 6.3±6.8 versus 1.5±3.1, \( P = 0.01 \); Figure 2).

As expected, there was a significant association of the lesion score with age (\( r = 0.48, P = 0.001 \)), but the association of HL activity with coronary lesions was not attenuated by age. Multivariate analysis confirmed the independent association of low HL activity with calcified coronary lesions (data not shown).

We also analyzed potential confounding factors. The Table illustrates the distribution of the conventional risk factors age, body mass index (BMI), hypertension, smoking, diabetes mellitus, and HDL cholesterol over the HL activity quartiles. There were no significant differences between the highest and lowest HL activity quartiles for age, BMI, or hypertension. In agreement with a recent report,\(^{13} \) there was a trend toward a positive association of smoking with HL activity. As expected, HDL cholesterol levels were lower in patients with higher HL activity. There was an overrepresentation of patients with diabetes mellitus in the lowest HL activity quartile. We also measured insulin levels. As expected, insulin levels were correlated to BMI (\( r = 0.37, P < 0.001 \)), plasma triglycerides (\( r = 0.23, P < 0.01 \)), and VLDL cholesterol (\( r = 0.23, P < 0.01 \)). Interestingly, there were also positive associations of insulin levels with CAD extent (\( r = 0.15, P < 0.05 \)) and HL activity (\( r = 0.16, P < 0.05 \)). In multivariate analysis, the insulin level was the first parameter to be omitted, whereas HL activity was retained in the model.

Because confounding factors could influence the relation of postheparin HL activity and CAD extent, we used 2 methods to investigate the independent relation of HL activity and CAD. First, we performed a multivariate analysis. Here, the association of HL activity with the extent score was statistically significant in all models (\( P < 0.01 \)). In multivariate analysis that included the conventional risk factors (age, BMI, presence of diabetes, pack-years of smoking, and hypertension), HL activity and age were the only parameters included in the forward model, whereas HL activity, age, and BMI were retained in the backward model. Inclusion of lipid parameters into partial regression analysis did not change the

### Conventional Risk Factors and CAD Extent Score in HL Activity Quartiles

<table>
<thead>
<tr>
<th>Variables</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>( P )</th>
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<tr>
<td>Age, y</td>
<td>61.2±11.1</td>
<td>63.0±8.5</td>
<td>62.9±8.8</td>
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<tr>
<td>BMI, kg/m(^{2})</td>
<td>27.2±3.7</td>
<td>27.4±3.1</td>
<td>27.5±3.2</td>
<td>28.1±3.4</td>
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<tr>
<td>Hypertension</td>
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<td>52%</td>
<td>53%</td>
<td>48%</td>
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<tr>
<td>Pack-years</td>
<td>14.5±18.7</td>
<td>17.7±24.4</td>
<td>15.8±20.2</td>
<td>24.3±24.5</td>
<td>0.06</td>
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<tr>
<td>Diabetes, %</td>
<td>29</td>
<td>12</td>
<td>13</td>
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<tr>
<td>HDL-C, mmol/L</td>
<td>1.05±0.28</td>
<td>0.96±0.23</td>
<td>1.07±0.31</td>
<td>0.96±0.32</td>
<td>0.03</td>
</tr>
<tr>
<td>CAD extent score</td>
<td>44.7±24.1</td>
<td>38.7±24.1</td>
<td>35.9±20.4</td>
<td>32.1±21.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

HDL-C indicates HDL cholesterol.

Results are given in percent or as mean±SD as indicated. The \( P \) value represents the comparison of lowest vs highest quartile.

Quartiles were defined as follows: quartile 1, <213 nmol · mL\(^{-1} \) · min\(^{-1} \); quartile 2, 213 to 282 nmol · mL\(^{-1} \) · min\(^{-1} \); quartile 3, 283 to 358 nmol · mL\(^{-1} \) · min\(^{-1} \); and quartile 4, >358 nmol · mL\(^{-1} \) · min\(^{-1} \).
significant inverse association of HL activity with CAD extent. In models that integrated lipid parameters, HL activity, age, and HDL cholesterol were included in the forward and backward models (HL activity, \( P < 0.005 \)).

As a second method to avoid potential confounding factors, we investigated the \(-514C/T\) polymorphism in the HL promoter, which has been shown to be associated with lower HL activity in vivo\(^{12-16} \) and a lower HL promoter activity in vitro in 2 of the 3 studies published to date.\(^{17-19} \) In the present study, the presence of the rare T allele was strongly associated with lower HL activity in postheparin plasma \(( r = -0.30, P < 0.001 \)).\(^{20-21} \) Compared with patients with the wild-type allele \(( n = 131, \text{HL activity} 304 \pm 103 \text{nmol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \))\(^{20-21} \), HL activity was reduced by 11% in heterozygotes for the T allele \(( n = 53, \text{HL activity} 272 \pm 101 \text{nmol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \), \( P < 0.05 \))\(^{20-21} \) and by 45% in homozygotes \(( n = 11, \text{HL activity} 158 \pm 73 \text{nmol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \), \( P < 0.001 \); Figure 3A)\(^{20-21} \). In the 47 patients with familial hypercholesterolemia, the presence of the T allele was also associated with lower HL activity (fluorometric HL activity: \( n = 13, 11.4 \pm 7.0 \text{ pmol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \) versus \( 13.6 \pm 5.6 \text{ pmol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \)), but because of the low number of patients, the difference was not significant.

There was no significantly different distribution of potential confounding factors such as age, BMI, hypertension, smoking, insulin, LDL, HDL, or lipoprotein(a) between the promoter genotypes. In the presence of the T allele, however, CAD extent was significantly greater \((42.2 \pm 20.8 \text{ versus } 35.3 \pm 23.6, P < 0.05 \); Figure 3B)\(^{20-21} \).

**Discussion**

Our data demonstrate that lower HL activity is associated with the presence of CAD and a higher extent of CAD both in men without defined lipoprotein disorders and in patients with heterozygous familial hypercholesterolemia. The correlation coefficient of \(-0.19\) of HL activity with CAD extent in the overall group is relatively low but typical of individual risk factors in the context of a multifactorial disease. In the initial report about the CAD extent score, for instance, the conventional risk factors family history, hypertension, diabetes, smoking, HDL cholesterol, and LDL cholesterol had coefficients ranging from 0.06 to 0.16. The highest correlation was seen for age, with \( r = 0.30 \).\(^{\#} \) In the present study, the association of low HL activity with CAD extent was mainly due to patients with normal HDL cholesterol levels, and in this group, correlation coefficients were \(-0.30\) and \(-0.32\), respectively.

We used multivariate analysis and determination of a polymorphism in the HL promoter, associated with genetically lower HL activity, to show that this association is independent of other factors. The only conventional risk factors that had a significant uneven distribution between HL activity quartiles were HDL cholesterol and presence of diabetes. The observation of higher HDL cholesterol associated with low HL activity strengthens our results, because higher HDL levels are likely to decrease the observed link between low HL activity and CAD extent. The uneven distribution of diabetes was due to a high number of diabetic patients in the lowest HL activity quartile. In multivariate analysis that included diabetes and HL activity, diabetes was removed from the models, whereas HL activity remained significant (data not shown). To ensure that the observed association of HL activity with CAD was not influenced by the presence of diabetes, we performed a subgroup analysis in the patients without diabetes. In this group of 162 patients, the inverse relation of HL activity with CAD extent remained unchanged \((r = -0.18, P < 0.05)\).

It has been suggested that HMG-CoA reductase inhibitors, or statins, lower HL activity.\(^{20,21} \) The effect of statins on HL activity has been evaluated in 8 clinical studies, 6 of which failed to find a significant effect.\(^{22-25} \) HL activity was similar in patients taking statins and those not taking statins \((274 \pm 107 \text{ and } 286 \pm 100 \text{ nmol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \), respectively; \( P = 0.42)\). In addition, inclusion of statin treatment in multivariate analysis did not change the association of HL activity with CAD extent. Therefore, it is highly unlikely that the association of lower HL activity with CAD extent was influenced by statin treatment.

The effect of the \(-514C/T\) polymorphism on the presence of CAD is controversial. Jansen et al.\(^{15} \) observed a higher frequency of the T allele in patients with CAD, but these results could not be confirmed by other groups.\(^{12,16} \) We are first to investigate the association of the polymorphism with CAD extent. The CAD extent score is likely to be a more sensitive parameter than the mere presence or absence of CAD and may explain why some studies of the \(-514C/T\) polymorphism have failed to find an association with CAD.

Investigations of the association of a polymorphism with a complex disease such as CAD are most reliable when the sample size is large. Recently, a study was presented (R.V. Andersen, MD, unpublished data, 2001) of more than 10 000 subjects of the Copenhagen City Heart Study. Homozygous presence of the rare T allele in the HL promoter was associated with an OR of 1.7 for the presence of CAD, in spite of higher HDL cholesterol levels, which supports our findings. Because the promoter polymorphism is only one parameter influencing HL activity, direct determination of HL activity in postheparin plasma, as performed in the
The present study, is probably the most sensitive and most reliable parameter in the investigation of an association of HL activity with CAD risk.

Our results support an antiatherogenic role for HL and are in accordance with a much smaller study in 20 patients that suggested that low HL activity is associated with the presence of CAD and with our own data in 15 patients with homozygous familial hypercholesterolemia, in which low HL activity was also associated with higher CAD extent as measured by electron beam tomography.

The association of low HL activity with CAD extent is somewhat surprising, because low HL activity leads to higher plasma levels of antiatherogenic HDL cholesterol, a relation that we could also confirm in the present study. It has recently been shown, however, that HL executes crucial functions in the process of reverse cholesterol transport from the periphery, eg, the vessel wall, to the liver. Thus, HL increases the formation of nascent, pre-β HDL particles that mediate cholesterol efflux. HL also enhances the selective cholesteryl ester uptake from HDL to the liver via the recently described HL receptor SR-B1. The flux of cholesterol through the system of reverse cholesterol transport may therefore be a more important determinant of atherothrombotic disease than a steady-state HDL cholesterol level. This hypothesis may be especially relevant to patients with normal HDL cholesterol levels, in which the association of low HL activity with CAD was strongest. An increased rate of reverse cholesterol transport may be insufficient to overcome the negative effects of low HDL cholesterol, including reduced anti-inflammatory and antithrombogenic properties of HDL.

Overt atherosclerotic disease in the presence of very high levels of HDL cholesterol has recently been observed in mice transgenic for lecithin:cholesterol acyltransferase (LCAT) and, more importantly, in patients with combined deficiency of cholesteryl ester transfer protein (CETP) and HL, who demonstrated significantly more CAD than patients with CETP deficiency alone. In men with average HDL cholesterol levels, mutations in the gene for CETP were associated with a relative risk of CAD of 1.68, despite higher HDL cholesterol levels. In another study, a common polymorphism in the CETP gene was associated with higher HDL cholesterol and also a 1.4- to 2.1-fold increased relative risk of CAD in women. Therefore, in subjects with normal HDL cholesterol levels, analysis of CETP and HL, as conducted in the present study, may help to determine CAD risk.

In summary, our data suggest that low HL activity is a novel independent risk factor for CAD. Analysis of HL may therefore help in the estimation of CAD risk, and augmentation of HL activity could become an attractive target for pharmacological intervention in the prevention and treatment of CAD.

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