Dose-Dependent Acceleration of High-Density Lipoprotein Catabolism by Endothelial Lipase

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Background—Factors that regulate the metabolism of HDL and apolipoprotein A-I (apoA-I) are incompletely understood. Overexpression of endothelial lipase (EL) markedly reduces plasma levels of HDL cholesterol and apoA-I in mice, but the mechanisms of this effect remain unknown.

Methods and Results—We used different doses of a recombinant adenoviral vector to overexpress human EL in mice and studied the effects on plasma phospholipase activity, plasma lipids, HDL particle size, HDL turnover, and tissue sites of HDL degradation in mice. Overexpression of EL was associated with a significant dose-dependent increase in postheparin plasma phospholipase activity. Plasma phospholipid, HDL cholesterol, and apoA-I levels were markedly decreased, even at the lowest dose of vector. Kinetic studies demonstrated a significant dose-dependent increase in the fractional catabolic rate of HDL-apolipoprotein in EL-overexpressing mice. The postheparin plasma phospholipase activity was significantly positively correlated with HDL-apolipoprotein fractional catabolic rate. The uptake of apoA-I by the kidney and the liver was significantly increased by 2.5-fold and 3-fold, respectively, in mice overexpressing EL.

Conclusions—Expression of EL in mice results in a dose-dependent increase in postheparin plasma phospholipase activity, catabolic rate of HDL-apolipoprotein, and uptake of apoA-I in both kidney and liver. (Circulation. 2003;108:2121-2126.)

Key Words: lipids ■ lipoproteins ■ apolipoproteins ■ enzymes

Plasma concentrations of HDL cholesterol (HDL-C) and its major protein apolipoprotein A-I (apoA-I) are inversely correlated with risk of atherosclerotic cardiovascular disease.1 HDL-C levels vary 10-fold within the human population, and at least 50% of this variation is genetically determined.2 However, the genetic and metabolic factors that regulate HDL metabolism remain incompletely understood. Hepatic lipase (HL) has been recognized as one factor that influences HDL metabolism. In humans, high plasma HL activity is associated with reduced HDL-C levels and smaller HDL particles,3 and genetic HL deficiency is associated with modestly elevated HDL-C levels and larger HDL particles.4,5 A common single nucleotide polymorphism in the HL promoter has been associated with lower levels of HL activity and increased levels of HDL-C, especially HDL<sub>2b</sub>.6-8 Overexpression of HL in transgenic mice,9-11 transgenic rabbits,12 and mice after adenoviral-mediated gene transfer13-15 resulted in reduced levels of HDL-C. Relatively little data are available regarding the effects of HL overexpression on the turnover of HDL. One study using gene transfer of HL in HL-deficient mice demonstrated increased fractional catabolic rates of HDL-cholesteryl ester and apoA-I.16 HL has both triglyceride lipase and phospholipase activity, although it is conventionally measured based on its triglyceride lipase activity. Some data suggest that triglyceride-rich HDLs are particularly good substrates for HL,17 emphasizing the importance of the ability of HL to hydrolyze HDL triglycerides.

Endothelial lipase (EL) is a more recently discovered member of the same gene family as HL.18,19 In contrast to HL, EL has relatively less triglyceride lipase activity and substantially more phospholipase activity and can hydrolyze HDL phospholipids ex vivo.20 Recent publications highlighted the physiological role of EL in HDL metabolism. Hepatic overexpression of EL in mice using adenoviral gene transfer markedly reduced HDL-C and apoA-I levels.18 Transgenic overexpression of EL under the control of the endogenous promoter resulted in modestly reduced HDL levels.21 Antibody inhibition studies in wild-type, apoA-I transgenic, and HL knockout mice demonstrated that inhibition of mouse EL results in significantly increased HDL-C and apoA-I levels.22 In the EL knockout mouse model, HDL-C levels were significantly increased.21,23 These loss-of-function experi-
ments indicate that EL regulates HDL metabolism and increases the importance of understanding the underlying mechanism.

We hypothesized that EL hydrolyzes HDL phospholipids in vivo, thus generating smaller phospholipid-depleted HDL particles that are more rapidly catabolized. We used gene transfer of EL in mice to test this hypothesis in a dose-dependent fashion. After administration of different doses of EL-expressing vector, plasma phospholipase activity, plasma lipids, HDL size and composition, and HDL turnover were studied. We found that overexpression of EL has a profound dose-dependent effect on HDL metabolism that is highly correlated with its ability to generate phospholipase activity in vivo.

Methods

Animals

C57BL/6 and apoA-I transgenic mice were obtained from Jackson Laboratory (Bar Harbor, Me) and kept on a chow diet. Mice were handled according to the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee.

In Vivo HDL Metabolic Studies

A recombinant adenoviral vector encoding human EL (AdhEL) was constructed as previously described.19 Mouse HDL (1.063<d<1.25) was isolated from 200 µL of pooled wild-type mouse plasma. Human HDL, was isolated from human donors by sequential ultracentrifugation (1.25<d<1.21). Human HDL was passed 2 times over a heparin-Sepharose column for removal of apoE and extensively dialyzed. Dialyzed mouse HDL and human HDL, were labeled with 125I by the iodine monochloride method as described.20 Wild-type mice (n=4 per group) were injected with the following doses of AdhEL: 1×109, 3×109, 1×1010, and 0 particles. The total dose of recombinant adenovirus was adjusted to 1×1010 particles by the addition of control adenovirus without a transgene (Adnull). Postheparin plasma from mice injected with Adnull or AdhEL, were assigned at

\[ P' = \frac{x}{n} \]

Statistical Analysis

Data were analyzed using ANOVA. Experimental groups were compared using the Student Newman-Keuls test. Statistical significance for all comparisons was assigned at P<0.05. Graphs and data represent mean±SEM.

Results

Injection of wild-type mice with 3 different doses of AdhEL resulted in a dose-dependent expression of EL protein as
assessed by Western blotting of postheparin plasma (Figure 1). Expression of EL resulted in a significant and dose-dependent increase in postheparin plasma phospholipase activity, as follows: 9779±733, 6501±1299, 3963±796, and 952±258 nmol/mL per h for the high, middle, and low doses and controls, respectively. The postheparin phospholipase activity was highly correlated with levels of EL protein (r=0.98, P<0.01).

The 3 different doses of injected vector all markedly decreased plasma concentrations of phospholipids, HDL-C, and apoA-I levels (Table 1). Plasma phospholipid concentrations were decreased by 86%, 81%, and 76% at day 5, and HDL-C was decreased by 96%, 95%, and 94% for the AdhEL injected doses of 1×10^{11}, 3×10^{10}, and 1×10^{10} particles, respectively. ApoA-I levels were decreased by 95%, 93%, and 91% at day 7 after virus injection. Analysis of lipoprotein distribution by FPLC confirmed our results, demonstrating that HDL-C was dramatically reduced even in mice injected with the lowest dose of AdhEL (data not shown).

HDL isolated from EL-expressing C57BL/6 mice showed a significant decrease in the percentage of esterified cholesterol, free cholesterol, and phospholipids, whereas the triglyceride content was not changed and the percentage of protein increased (Table 2).

In a separate experiment, HDL isolated from human apoA-I transgenic mice 13 days after AdhEL or control vector injection was separated by gel-filtration chromatography to assess the size of particles. The chromatographic profile of HDL from EL-overexpressing mouse was markedly different compared with HDL from control mice (Figure 2). In proportion to the total area under the curve of the profile, the main large HDL peak (fractions 40 to 50) almost completely disappeared, whereas the 2 minor peaks (fractions 51 to 56 and 60 to 66, respectively) increased. The decreased HDL size of EL-injected mice was also demonstrated using nondenaturing gradient gel electrophoresis. The 2 HDL major bands corresponding to large (10.4 to 12.2 nm) and medium (8.1 to 10.4 nm) HDL were undetectable in the AdhEL group compared with the Adnull group. The small HDL (diameter <8.1 nm) and lipopoor apoA-I or pre-β HDL (diameter <7.1 nm) were still detectable (data not shown).

To determine whether the catabolic rate of mouse HDL-apolipoproteins was increased by EL overexpression, kinetic studies were performed using [125I]-labeled mouse HDL. A dose-dependent relationship between the disappearance rate of [125I]HDL and the AdhEL injected dose was observed (Figure 3). Compared with controls, a 2-fold (P<0.01), 1.7-fold (P<0.01), and 1.5-fold (P<0.01) increase in the HDL-apolipoprotein FCR was observed in mice that received AdhEL at the dose of 1×10^{11}, 3×10^{10}, and 1×10^{10} particles, respectively. The postheparin plasma phospholipase activity, measured in individual mice, was strongly correlated with the FCR of HDL-apolipoprotein (Figure 4), suggesting that the catabolic rate of HDL protein is directly related to the level of EL-induced phospholipase activity in the plasma.

As a control for the reduced HDL pool size in EL-expressing mice, we performed another experiment using apoA-I–deficient mice. Wild-type mice were injected with 1×10^{11} particles of AdhEL or Adnull, and apoA-I–deficient mice were injected with 1×10^{11} particles of AdhEL. [125I]HDL was injected 13 days after virus injection. The mean HDL-C levels in EL-expressing mice were 18% and in apoA-I deficient mice 34% of that in control mice (44±9 mg/dL). As above, HDL-apolipoprotein was catabolized much faster in EL-expressing mice compared with control mice; in addition, the catabolism of HDL in EL-expressing mice was much faster than in apoA-I–deficient mice injected with the control vector (Figure 5). Thus, it is unlikely that the reduction in HDL pool size was the sole cause of the rapid turnover of HDL-apolipoprotein in EL-expressing mice and likely that EL expression itself contributed to the rapid HDL catabolism.

The sites of HDL-apolipoprotein catabolism were assessed using [125I]-tyramine-cellobiose–labeled HDL. EL overexpression markedly increased the uptake of HDL-apolipoproteins by both liver and kidney (Figure 6). An increase of 158% and 189% was observed for the hepatic (P<0.01) and renal (P<0.01) uptake, respectively. In con-

### Table 1. Plasma Lipids (mg/dL) in C57BL/6 Mice After Injection of Different Doses of AdhEL or Adnull

<table>
<thead>
<tr>
<th></th>
<th>AdhEL 1×10^{11}</th>
<th>AdhEL 3×10^{10}</th>
<th>AdhEL 1×10^{10}</th>
<th>Adnull 1×10^{11}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 5</td>
<td>Day 7</td>
<td>Day 0</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>88±2</td>
<td>2±1</td>
<td>15±2</td>
<td>80±9</td>
</tr>
<tr>
<td>HDL-C</td>
<td>66±8</td>
<td>3±1</td>
<td>7±1</td>
<td>64±3</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>162±8</td>
<td>23±3</td>
<td>41±4</td>
<td>161±19</td>
</tr>
<tr>
<td>Non-HDL-C</td>
<td>20±5</td>
<td>1±2</td>
<td>9±3</td>
<td>21±9</td>
</tr>
<tr>
<td>apoA-I</td>
<td>160±5</td>
<td>NA</td>
<td>8±1</td>
<td>158±5</td>
</tr>
</tbody>
</table>

NA indicates not assayed.
TABLE 2. Composition of HDL (1.063<d<1.25) Isolated From Wild-Type Mice 20 Days After Injection of AdhEL or Adnull  

<table>
<thead>
<tr>
<th></th>
<th>Phospholipids</th>
<th>Triglycerides</th>
<th>Cholesterol Ester</th>
<th>Free Cholesterol</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adnull</td>
<td>7.4%</td>
<td>2.4%</td>
<td>5.5%</td>
<td>0.8%</td>
<td>83.9%</td>
</tr>
<tr>
<td>AdhEL</td>
<td>1.9%</td>
<td>2.4%</td>
<td>1.2%</td>
<td>0.0%</td>
<td>94.5%</td>
</tr>
</tbody>
</table>

Discussion

We previously reported that overexpression of EL resulted in a dramatic decrease in HDL-C and apoA-I levels in mice. In the present study, we show that EL expression generates phospholipase activity in vivo, thus generating smaller phospholipid-depleted HDL particles that are more rapidly catabolized in a dose-dependent fashion. Doses of vector as low as 1×10^10 particles (=5×10^6 pfu) significantly increased postheparin plasma phospholipase activity and markedly reduced plasma phospholipid, HDL-C, and apoA-I concentrations. This dose is an order of magnitude less than that generally used for adenovirus studies in mice. Our data suggest that even modest increases in EL expression have profound effects on HDL metabolism.

Overexpression of EL resulted in major changes in the structure of HDL. EL-modified HDL was smaller and had a decreased content of phospholipid and free and esterified cholesterol. The major peak of large HDL found in control human apoA-I transgenic mouse plasma was dramatically reduced in plasma from EL-expressing mice, whereas small HDL subfractions were relatively increased by EL expression. These data indicate that the hydrolysis of HDL phospholipids induced by EL overexpression resulted in depletion of phospholipids and cholesterol, leading to the production of smaller HDL particles.

To determine the effects of EL expression on catabolism of HDL-apolipoproteins, we performed turnover studies using radiolabeled HDL. The FCR of HDL-apolipoprotein was markedly increased in EL-overexpressing mice compared with control mice. A clear dose-dependence of HDL-apolipoprotein FCR on dose of vector injected was observed. The increase in HDL-apolipoprotein FCR was positively correlated with the level of postheparin plasma phospholipase activity, indicating that the greater the hydrolysis of HDL phospholipids, the faster HDL-apolipoproteins are catabolized.

The decrease in plasma apoA-I levels is disproportionate to the increase in the FCR of mature HDL apolipoprotein. Therefore, it seems that the marked reduction in plasma apoA-I levels may not be able to be accounted for simply by increased catabolism of mature HDL. Importantly, our tracer was labeled as whole-particle mature HDL and therefore cannot be considered a tracer for lipid-poor apoA-I. In fact, calculated production rates based on this tracer likely reflect production of apoA-I into the mature HDL pool. Expression of EL may result in hydrolysis of nascent HDL phospholipid.
ids, resulting in reduced maturation of lipid-poor apoA-I to mature HDL, thus reducing the effective production rate of apoA-I into the mature HDL pool. Although our primary conclusion from these studies is that there is a dose-dependent acceleration of HDL catabolism induced by EL expression, our data suggest another, more complex effect on HDL metabolism, ie, reduction of the maturation of nascent HDL to mature HDL.

Overexpression of EL resulted in a 2-fold increase in uptake of HDL-apolipoprotein by the kidney. Given our demonstration that EL overexpression generated small HDL particles, the increased kidney uptake is in keeping with the hypothesis of increased renal filtration and reuptake of lipid-poor apoA-I.29 We hypothesize that the phospholipase activity of EL depletes the HDL particle of phospholipid, thus destabilizing apoA-I and promoting its dissociation, resulting in a lipid-poor form of apoA-I that is rapidly catabolized.

Interestingly, hepatic expression of EL also resulted in significantly increased catabolism of HDL-apolipoprotein in the liver. Barrans et al30 demonstrated that incubation of HL with triglyceride-rich HDL2 particles resulted in the generation of HDL2 remnants that are rapidly taken up by hepatocytes, possibly via the ectopic β-chain of ATP synthase,31 a recently described apoA-I receptor mediating hepatic HDL endocytosis. The importance of HL-mediated hydrolysis of HDL triglyceride is generally accepted, and triglyceride-rich HDLs are particularly good substrates for HL.17,32,33 The specific role of the phospholipase activity of HL in its effect on HDL metabolism is not known. Our studies suggest some important potential differences between HL and EL in their effects on HDL metabolism. HL may act primarily on the triglyceride-enriched HDL particle, resulting in reduction in size and increased uptake of cholesteryl ester as well as remnant HDL2 particles, whereas EL may act primarily on HDL phospholipids, resulting in dissociation of apolipoproteins and subsequent catabolism.

Changes in EL expression in vivo have significant effects on HDL metabolism. The physiological importance of EL in HDL metabolism was demonstrated by loss-of-function studies. Inhibition of mouse EL activity using specific antimirine EL antibodies resulted in increased HDL-C levels.22 In the EL knockout mouse model, HDL-C was significantly increased.21,23 Several common single-nucleotide polymorphisms and rare variants in the human EL gene were described in persons with high HDL-C levels.34 One of these common EL single nucleotide polymorphisms, 584C/T, was found to be associated with increased HDL-C and apoA-I levels in humans on univariate analysis.35 Thus, variation in EL mass or activity may be an important determinant of HDL-C and apoA-I levels.

In summary, hepatic expression of EL, even at relatively low levels, resulted in substantial dose-dependent increases in...
postheparin plasma phospholipase activity associated with dose-dependent reductions in plasma phospholipids, HDL-C, and apoA-I levels, a shift to smaller lipid-poor HDL particles, and accelerated catabolism of HDL-apolipoproteins by both the kidney and the liver. These data support the concept that EL plays an important physiological role in the metabolism of HDL and apoA-I.

Acknowledgments
This work was supported by grant HL55323 (to Dr Rader) from the National Heart Lung and Blood Institute. Dr Maugeais was supported by a grant from ARCOL (France) and from the American Heart Association, Pennsylvania Delaware Affiliate. Dr Tietge was supported by a grant from the Deutsche Forschungsgemeinschaft and the National Heart Lung and Blood Institute. Dr Maugeais was supported by a grant from the American Heart Association, Pennsylvania Delaware Affiliate. Dr Rader is an Established Investigator of the American Heart Association, a recipient of a Burroughs Wellcome Foundation Clinical Scientist Award in Translational Research, and a recipient of a Doris Duke Charitable Foundation Distinguished Clinical Scientist Award. The authors are indebted to Anna Liljestrom, Linda Morrell, and Anthony Secreto for expert technical assistance.

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_Circulation_. 2003;108:2121-2126; originally published online September 29, 2003;
doi: 10.1161/01.CIR.0000092889.24713.DC
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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