High-Density Lipoprotein Restores Endothelial Function in Hypercholesterolemic Men

Lukas E. Spieker, MD; Isabella Sudano, MD*; David Hürlimann, MD*; Peter G. Lerch, PhD; Markus G. Lang, PhD; Christian Binggeli, MD; Roberto Corti, MD; Frank Ruschitzka, MD; Thomas F. Lüscher, MD; Georg Noll, MD

Background—Hypercholesterolemia is a risk factor for atherosclerosis-causing endothelial dysfunction, an early event in the disease process. In contrast, high-density lipoprotein (HDL) cholesterol inversely correlates with morbidity and mortality representing a protective effect. Therefore, we investigated the effects of reconstituted HDL on endothelial function in hypercholesterolemic men.

Methods and Results—Endothelium-dependent and -independent vasodilation to intraarterial acetylcholine and sodium nitroprusside (SNP), respectively, was measured by forearm venous occlusion plethysmography in healthy normo- and hypercholesterolemic men. In hypercholesterolemics, the effects of reconstituted HDL (rHDL; 80 mg/kg IV over 4 hours) on acetylcholine- and SNP-induced changes in forearm blood flow were assessed in the presence or absence of the nitric oxide (NO) synthase inhibitor L-NMMA. Hypercholesterolemics showed reduced vasodilation to acetylcholine but not to SNP compared with normocholesterolemics (P < 0.0001). rHDL infusion increased plasma HDL cholesterol from 1.3 ± 0.1 to 2.2 ± 0.1 mmol/L (P < 0.0001, n = 18) and significantly enhanced the acetylcholine-induced increase in forearm blood flow without affecting that induced by SNP. rHDL infusion also improved flow-mediated dilation of the brachial artery (to 4.5 ± 0.9% from 2.7 ± 0.6%, P = 0.02). NO synthase inhibition prevented the improvement in acetylcholine-induced vasodilation while leaving the response to SNP unchanged. Albumin infusion in an equivalent protein dose had no effect on vasomotion or lipid levels.

Conclusions—In hypercholesterolemic patients, intravenous rHDL infusion rapidly normalizes endothelium-dependent vasodilation by increasing NO bioavailability. This may in part explain the protective effect of HDL from coronary heart disease and illustrates the potential therapeutic benefit of increasing HDL in patients at risk from atherosclerosis. (Circulation. 2002;105:1399-1402.)

Key Words: atherosclerosis ■ cholesterol ■ apolipoproteins ■ nitric oxide ■ acetylcholine

Hypercholesterolemia is a risk factor for the development of atherosclerotic vascular disease. Elevated low-density lipoprotein (LDL) cholesterol plasma levels cause functional injury to the endothelium before morphological lesions develop. Currently, reducing LDL plasma levels and, in turn, the influx of cholesterol into the arterial wall is the primary strategy of reducing atherosclerotic vascular disease. In contrast, high-density lipoprotein (HDL) cholesterol prevents coronary artery disease. As HDL removes cholesterol from peripheral cells, therapeutic strategies aimed at enhancing cholesterol efflux from the arterial wall may thus be of additional benefit for patients with atherosclerosis. Therefore, we investigated the effects of reconstituted HDL on endothelial function in hypercholesterolemic patients.

Methods

Study Patients
Healthy hypercholesterolemic men with plasma LDL cholesterol levels > 4.0 mmol/L and healthy normocholesterolemic with LDL cholesterol < 3.5 mmol/L were included in the study. Exclusion criteria were arterial hypertension (> 140/90 mm Hg), diabetes mellitus, smoking, and a history of cardiovascular disease. All participants gave written informed consent. The study protocol was approved by the local research ethics committee of the University Hospital Zürich.

Protocol
Forearm blood flow (FBF) was measured simultaneously in both arms by venous occlusion plethysmography (EC4; Hokanson Inc). For assessment of endothelium-dependent and -independent vasodilation, acetylcholine (ACh; Miochol E, Ciba Vision) and
TABLE 1. Clinical Characteristics and Laboratory Data

<table>
<thead>
<tr>
<th></th>
<th>Normocholesterolemics</th>
<th>Hypercholesterolemics</th>
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<tbody>
<tr>
<td></td>
<td>Plethysmography Study (n=8)</td>
<td>Plethysmography Study (n=12)</td>
</tr>
<tr>
<td>Age, years</td>
<td>50±10</td>
<td>56±9</td>
</tr>
<tr>
<td>BMI, kg · m⁻²</td>
<td>22.9±1.7</td>
<td>26.7±3.0*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>118±9</td>
<td>128±12</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>70±5</td>
<td>78±14</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>56±6</td>
<td>68±13</td>
</tr>
<tr>
<td>Basal FBF, mL · 100 mL FAV⁻¹ · min⁻¹</td>
<td>2.6±0.7</td>
<td>2.9±1.1</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.8±0.7</td>
<td>7.2±0.9†</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.6±0.5</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>2.8±0.7</td>
<td>5.1±0.7†</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.8±0.2</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.8±0.8</td>
<td>5.0±0.4</td>
</tr>
<tr>
<td>ALAT, U/L</td>
<td>24±8</td>
<td>33±14</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. ALAT indicates alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FAV, forearm volume; FBF, forearm blood flow; FMD, flow-mediated dilation; HDL, high-density lipoprotein cholesterol; HR, heart rate; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

*P<0.01, †P<0.001 vs normocholesterolemics. There were no statistically significant differences between the hypercholesterolemic study groups.

sodium nitroprusside (SNP; Nipruss, Schwarz Pharma) were infused into the brachial artery in increasing concentrations of 0.15, 0.45, 1.5, 4.5, and 15 μg · 100 mL forearm volume (FAV)⁻¹ · min⁻¹ (in NaCl 0.9%; for 5 minutes each) and 1.3, and 10 μg · 100 mL FAV⁻¹ · min⁻¹ (in glucose 5%; for 3 minutes each). A 30-minute rest period was allowed between the intraarterial drug infusions.

In hypercholesterolemic patients, reconstituted HDL particles (rHDL; lot 7.955.004.9 and 7.955.003.9; ZLB Central Laboratory, Blood Transfusion Service, Swiss Red Cross, Berne, Switzerland; n=7), containing apolipoprotein A-I and phosphatidylcholine in a molar ratio of 1:150 but no cholesterol, or albumin (5%; also from ZLB Central Laboratory; n=5), respectively, was infused intravenously into the non-plethysmography arm in an equivalent protein dose of 80 mg · kg⁻¹ over 4 hours. Then, infusions of ACh and SNP were repeated. In further 5 hypercholesterolemic patients, the effects of intravenous rHDL infusion on ACh- and SNP-induced vasodilation were assessed during intraarterial coinfusion of N⁶-monomethyl-L-arginine (L-NMMA, 4 μmol/min; Clinalfa), an inhibitor of NO synthase.

Additionally, flow-mediated vasodilation (FMD) and nitroglycerine (0.4 mg, Nitrolingual Spray, Pohl-Boskamp, Germany)-induced vasodilation of the brachial artery were ultrasonographically measured in 6 hypercholesterolemic men before and after intravenous infusion of rHDL, as described previously, using a 10-MHz linear array transducer. Values of vasodilatation represent

TABLE 2. Characteristics of Hypercholesterolemic Study Subjects and Effects of Intravenous Infusions

<table>
<thead>
<tr>
<th></th>
<th>Plethysmography Study</th>
<th>FMD Study</th>
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<tbody>
<tr>
<td></td>
<td>rHDL (80 mg/kg, n=7)</td>
<td>Albumin (80 mg/kg, n=5)</td>
</tr>
<tr>
<td>Age, years</td>
<td>55±3</td>
<td>...</td>
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<tr>
<td>BMI, m · kg⁻²</td>
<td>27.4±1.2</td>
<td>26.0±1.1</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>123±2</td>
<td>121±11</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>61±3</td>
<td>62±4</td>
</tr>
<tr>
<td>HR, min⁻¹</td>
<td>64±3</td>
<td>60±5</td>
</tr>
<tr>
<td>Basal FBF, mL · 100 mL FAV⁻¹ · min⁻¹</td>
<td>2.9±0.5</td>
<td>3.2±0.5</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.7±0.3</td>
<td>6.9±0.3</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.2±0.1</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>5.2±0.3</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.5±0.2</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>66±14</td>
<td>76±15</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. BMI indicates body mass index; DBP, diastolic blood pressure; FAV, forearm volume; FBF, forearm blood flow; FMD, flow-mediated vasodilation; HDL, high-density lipoprotein cholesterol; HR, heart rate; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; and TG, triglycerides.

*P<0.05, †P<0.001 vs corresponding preinfusion value.
the maximal increase in brachial end-diastolic vessel diameter and are expressed as percent dilation from baseline.

Biochemical Analyses
Blood samples were collected immediately before and after the intravenous infusions. Total cholesterol, HDL cholesterol, triglycerides, liver transaminases, and insulin were measured with standard laboratory methods (University Hospital). LDL cholesterol was calculated using the Friedewald formula.

Statistical Analysis
Results are presented as mean±SEM. Average values of forearm blood flow were obtained from at least 3 consecutive recordings during the last minute of drug infusion. They are expressed as the percentage ratio of the infused/noninfused arm.1 Plethysmographic measurements were compared using 2-way ANOVA for repeated measures. Comparisons of clinical data were made with 2-tailed paired or unpaired t test (StatView 4.5, Abacus Concepts, USA). Statistical significance was accepted at P<0.05.

Results
Patients’ characteristics are shown in Table 1. The vasodilator response to ACh (P<0.0001), but not to SNP (P=0.63), was significantly reduced in hypercholesterolemics compared with normocholesterolemics (Figure). Intravenous cholesterol-free rHDL infusion significantly increased plasma HDL cholesterol levels, but did not affect triglyceride or insulin plasma levels (Table 2). There was a trend toward lowering LDL cholesterol levels (5.1±0.2 to 4.6±0.2 mmol/L, P=0.07) when all rHDL infusions were pooled.

Endothelium-dependent vasodilation to ACh was significantly enhanced by rHDL infusion (P=0.017, Figure). Endothelium-independent vasodilation to SNP was not altered (P=0.31, not shown). Correspondingly, FMD of the brachial artery was improved after rHDL infusion (2.7±0.6% versus 4.5±0.9%, P=0.02), whereas the vasodilator response to nitroglycerine remained unchanged (10.9±0.8% versus 13.3±2.3%, NS).

In hypercholesterolemics, L-NMMA led to a significant decrease in basal FBF (~23±3%, P<0.02) but did not reduce the vasodilator responses to ACh (P=0.81, Figure) or SNP (P=0.84). Under these conditions, the improvement in ACh-induced vasodilation induced by rHDL was prevented (P=0.99; Figure). The response to SNP remained unaffected by L-NMMA (P=0.65). The vasoconstrictor response to L-NMMA before and after rHDL was equal (21±3% versus 23±4% decrease in FBF, P=0.83).

Albumin infusion neither affected the responses to ACh (P=0.99) nor SNP (P=0.80) nor did it affect any laboratory parameter (Table 2). No adverse event occurred. Both in the HDL and the albumin group, basal FBF showed a trend to increase over the about 6 hours duration of the experiments (P=0.59 and P=0.51, respectively).

Discussion
This study provides the first evidence that increasing HDL plasma levels normalize impaired endothelial function in hypercholesterolemic patients. Indeed, endothelium-dependent vasodilation to ACh was reduced in hypercholes-
teroleemics compared with normocholesterolemic controls, although endothelium-independent vasodilation to SNP was similar. In hypercholesterolemicsm, intravenous administration of reconstituted HDL specifically restored vasodilation to ACh and FMD of the brachial artery, a receptor-independent marker of NO-dependent vasodilation.

Plasma HDL cholesterol increased to high physiological levels after intravenous infusion of rHDL (consisting of reconstituted apolipoprotein A-I and phosphatidylcholine but no cholesterol). LDL cholesterol tended to decrease while other lipid parameters remained stable, as previously demonstrated.5 Reverse cholesterol transport by the infused rHDL particles may therefore account for the observed improvement in endothelial function, as exogenous HDL administration enhances fecal cholesterol excretion.6,7 Indeed, HDL experimentally reduces established atherosclerotic lesions.5,9

Decreased LDL oxidation with reduced NO scavenging possibly contributes to the improvement in endothelial function after rHDL infusion.10–13 rHDL as used in the present study renders LDL resistant to oxidation within the arterial wall and reduces the ability of LDL ability to induce monocyte chemotactic activity.14 Indeed, the normalization of endothelial function by rHDL was prevented by L-NMMA, identifying restored NO bioavailability as the responsible mechanism.

Because NO maintains vasodilation, inhibits platelet aggregation, leukocyte adhesion, and proliferation of vascular smooth muscle cells, these findings may explain how HDL protects from the development of atherosclerosis and its complications. In addition, this study illustrates the potential of therapeutic strategies aiming at increasing HDL plasma levels in patients at risk for atherosclerosis, as the presence of endothelial dysfunction is an adverse prognostic parameter.15

Acknowledgments

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References

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