Increased Cholesterol Efflux Potential of Sera From ApoA-I_{Milano} Carriers and Transgenic Mice

Guido Franceschini, Laura Calabresi, Giulia Chiesa, Cinzia Parolini, Cesare R. Sirtori, Monica Canavesi, Franco Bernini

Abstract—The ability of HDL to remove cholesterol from peripheral cells and drive it to the liver for excretion is believed to explain most of the strong inverse correlation between plasma HDL cholesterol levels and coronary heart disease. Carriers of the ApoA-I_{Milano} (A-I_{M}) mutant have a severe hypoalphalipoproteinemia but are not at increased risk for premature of coronary heart disease. To explain this apparent paradox, we compared the capacity of serum from A-I_{M} and control subjects to extract cholesterol from Fu5AH cells. Because the A-I_{M} carriers are all heterozygotes for the mutation, we also compared the cholesterol efflux capacity of serum from transgenic mice expressing A-I_{M} or wild-type ApoA-I (A-I_{WT}), in the absence of murine ApoA-I. In the whole series of human or mouse sera, cholesterol efflux was significantly correlated with several HDL-related parameters; after adjustment for concomitant variables, the only parameter that remained significantly correlated with cholesterol efflux was the serum ApoA-I concentration ($r^2=0.85$ in humans and 0.84 in mice). The same was true when samples from control subjects, A-I_{M} carriers, A-I_{WT} or A-I_{M} mice were analyzed separately. Cholesterol efflux to sera from the A-I_{M} carriers was only reduced slightly compared with control sera (25.0±4.2% versus 30.4±3.3%), although there was a large reduction (~45%) in the serum ApoA-I concentration in the former. Cholesterol efflux was also lower to sera from A-I_{M} than A-I_{WT} mice (15.6±3.8% versus 30.1±7.1%), but less than expected from the 70% reduction in serum ApoA-I concentration. A relative efflux potential of serum was calculated in each group as the slope of the regression line fitting cholesterol efflux to ApoA-I concentration. Therefore, the relative efflux potential reflects the relative efficiency of ApoA-I in determining cell cholesterol efflux. The relative efflux potential of mouse and human sera was in the following order: A-I_{M} mice> A-I_{M} carriers> A-I_{WT} mice=control subjects, suggesting a gene–dosage effect of the A-I_{M} mutation on the efficiency of serum to extract cholesterol from cells. The high efficiency of A-I_{M}-containing HDL for cell cholesterol uptake would result in an improved reverse cholesterol transport in the A-I_{M} carriers, possibly explaining the low susceptibility to atherosclerosis development. (Arterioscler Thromb Vasc Biol. 1999;19:1257-1262.)

Key Words: reverse cholesterol transport ■ ApoA-I ■ cholesterol efflux ■ HDL ■ transgenic mice

Numerous case–control and prospective studies have shown a strong inverse relation between plasma HDL cholesterol (HDL cholesterol) concentrations and the risk of coronary heart disease.1 The mechanism(s) underlying this negative association are not fully understood, and several biological explanations have been proposed.2 Most prominent seems the function of HDL as vehicle of cholesterol in the reverse cholesterol transport (RCT).3,4 The process by which excess cholesterol in peripheral tissues, including the arterial wall, is transported to the liver for excretion.

The first step in RCT is the efflux of unesterified cholesterol from peripheral cells to either free apolipoproteins5 or lipoprotein acceptors6 present in the extracellular space. Several serum factors, including the acceptor structure/composition, and the concentration of lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and other lipoproteins are known to modulate cell cholesterol efflux.7 Therefore, the use of whole serum instead of isolated lipoprotein fractions would provide an ideal tool to evaluate the individual capacity to promote cell cholesterol efflux.8 This approach has the advantage that all of the potential factors affecting efflux are present in the experimental setting, and therefore seems particularly suitable to correlate the efficiency of the earliest step in RCT to atherosclerosis susceptibility in subjects,9,10 or animal models at different risks of cardiovascular disease.11,12

ApoA-I_{Milano} (A-I_{M}) is the first described mutant of human apolipoproteins.13 Thirty-eight heterozygous carriers have been identified, up to now,14 who represent the largest group of individuals with low plasma HDL levels because of a single defect in the ApoA-I gene. The plasma concentration of major factors involved in RCT, ie, HDL, ApoA-I, LpA-I,
LCAT, and CETP is remarkably reduced in A-I₉ carriers compared with controls.⁵⁻⁷ The severe hypoalphalipoproteinemia and the partial LCAT and CETP deficiencies suggest a defective RCT, but the carriers do not suffer from premature coronary heart disease.¹⁴ To investigate the mechanism(s) behind this apparent paradox, we compared in the present study the cholesterol efflux potential of sera from A-I₉ carriers and control subjects. Because of the limited number of carriers and the difficulty in discerning the impact of the mutation in these heterozygous subjects, we also evaluated the cholesterol efflux potential of sera from transgenic mice expressing the human A-I₉ variant or wild-type ApoA-I, in the absence of murine ApoA-I. A-I₉ transgenic mice share with human carriers low plasma HDL levels, increased triglycerides, defective cholesterol esterification, and structural abnormalities in HDL particles,¹⁸⁻¹⁹ and therefore are a suitable model for studying the impact of the mutation in a homozygous condition.

Methods

Subjects

Nineteen adult heterozygous A-I₉ carriers (9 females and 10 males), belonging to the previously described A-I₉ kindred,¹⁴ volunteered for this study. Nineteen age- and sex-matched noncarriers were selected among close relatives in the same families. All participating subjects were healthy, were taking no medications, and consumed a typical Mediterranean diet. All subjects were fully informed of the methods and end points of the study, which were approved by the Internal Review Board.

After an overnight fast, blood was collected both into empty plastic tubes and into tubes containing Na₂EDTA (final concentration, 1 mg/mL). Serum and plasma were prepared by low-speed centrifugation at 4°C. Serum aliquots were added with Na₂EDTA (1 mg/mL) and solid NaBr (final concentration, 5.1 mol/L) and kept at 4°C for HDL subfraction analysis by rate-zonal ultracentrifugation.²⁰ Serum aliquots for cholesterol efflux were immediately frozen and stored at −80°C until assayed.

Transgenic Mice

The generation of transgenic mice expressing human ApoA-I (A-I₉) and the A-I₉ variant has been previously described.¹⁸ These mice express A-I₉ or A-I₉ together with human ApoA-II, and do not express murine ApoA-I because of gene targeting.²¹ Twenty-one A-I₉ mice and 21 A-I₉ mice, of similar age (16 to 24 weeks) and of both sexes, were used for the cholesterol efflux experiments. Blood was collected after an overnight fast from the retroorbital plexus. Serum was prepared by low-speed centrifugation at 4°C and assayed on the same day for human apolipoprotein and lipid/lipoprotein levels. Serum aliquots for cholesterol efflux were immediately frozen and stored at −80°C until assayed.

Cell Cholesterol Efflux

The cholesterol efflux potential of serum was assayed as described by de la Llera Moya et al.²² by incubating diluted serum with [³H]cholesterol-labeled Fu5AH rat hepatoma cells for 4 hours at 37°C. Cells were seeded in Corning 24-well (15.5 mm/well) plates, using 20 000 cells per well, and grown in DMEM with 5% FCS for 2 days. Lipids were radiolabeled by adding 2 μCi/mL [1,2-³H]cholesterol (Amersham) to 25% FCS in DMEM. Cells were grown in the presence of radiolabeled cholesterol for 2 additional days to obtain confluent monolayers. The labeling medium was replaced with DMEM containing 1% essential fatty acid–free albumin for 18 to 20 hours to allow equilibration of the label. Cells were then washed 2 times with PBS and incubated for 4 hours with control medium or serum diluted in DMEM with essential fatty acid–free albumin 1%. All efflux assays were performed by using the same dilution (5%) for mouse and human sera. At the end of this period, the medium was removed, collected into tubes, and centrifuged for 5 minutes at 2000 rpm to remove any floating cells. An aliquot of the medium was then counted for [³H]cholesterol radioactivity (Formula 989, Packard). Cellular lipids were extracted with 2-propanol by overnight incubation at room temperature and radioactivity was measured in an aliquot of the extract (Insta-Fluor, Packard). Cholesterol efflux was calculated as the percentage of total label in each well released to the medium. Individual efflux values were calculated as averages of 3 determinations in different wells, normalized to the cholesterol efflux obtained with a pool of normolipidemic sera tested in each experiment.

Analyses

Serum total and unesterified cholesterol (TC and UC), triglycerides (TG), and phospholipid (PL) levels were determined with standard enzymatic techniques by using a Roche diagnostics Cobas automate. The CE mass was calculated as (TC−UC)×1.68. The protein (P) content in lipoprotein fractions was determined by the method of Lowry et al.²² The lipoprotein surface/core ratio was calculated as (FC+PL+PY)/(TG+CE). HDL cholesterol levels in human sera were measured after precipitation of the ApoB-containing lipoproteins by dextran sulfate-MgCl₂.²³ HDL cholesterol was measured in mouse sera after precipitation of ApoB-containing lipoproteins with polyethylene glycol (20%, wt/vol) in 0.2 mol/L glycine (pH 10).²⁴ ApoA-I, ApoA-II, and ApoB levels were determined by immuno-turbidimetry,²⁵ using the Cobas analyzer with commercially available polyclonal antibodies (Boehringer Mannheim). The anti-A-I antibody recognizes all forms of A-I₉ (monomer, homodimer, and heterodimer) as wild-type ApoA-I²⁶; therefore, the ApoA-I concentration determined in the sera of A-I₉ carriers, who are heterozygotes for the mutation, is the sum of mutant and wild-type ApoA-I, and the ApoA-I concentration in the sera of A-I₉ mice, who are homozygotes for the mutation, is the concentration of mutant ApoA-I.

Human plasma lipoproteins were separated by sequential ultracentrifugation,²⁷ using a Beckman TL 100 ultracentrifuge equipped with a TL 100.3 rotor (Beckman Instruments). HDL subfractions were isolated by rate-zonal ultracentrifugation in a swinging bucket rotor²⁸; 2 fractions, designated as HDL₂ and HDL₃, were collected, and the total cholesterol content measured by enzyme methods.

Statistical Analyses

Quantitative variables are expressed as mean±SD values. Differences among the groups were evaluated by 1-way ANOVA, with post hoc evaluation by the Neuman–Keuls test. Statistical significance was defined as P<0.05. Simple and multivariate regression analyses were performed and the significance of the correlations was determined by the F parameter. In the forward stepwise regression, the independent parameters were included 1 at a time starting with the parameter that had the highest correlation with the dependent variable, fractional cholesterol efflux. Additional parameters were included only if a significant increase in goodness of fit was achieved. Logarithmic transformation was performed on individual data when values were not normally distributed.

Results

Cholesterol Efflux to Human Sera

The fasting serum lipid and lipoprotein concentrations in the examined A-I₉ carriers and control subjects are given in Table 1. The A-I₉ carriers had significantly lower serum total and LDL cholesterol, and phospholipid concentrations than controls; although there was a trend for A-I₉ carriers to have greater serum triglycerides and VLDL cholesterol levels, the difference in average values between carriers and controls did not reach statistical significance (P=0.15 and 0.16, respectively). The unesterified/esterified cholesterol ratio was significantly higher in the A-I₉ carriers (0.45±0.09 versus 0.34±0.05), mostly because of the lower plasma LCAT concentration.²⁹ As expected,³ serum HDL cholesterol, HDL₂ cholesterol, HDL₃ cholesterol, HDL phospholipid, ApoA-I, and ApoA-II levels were much lower in the A-I₉ carriers than in controls.
TABLE 1. Serum Lipid/Lipoprotein Levels in A-I\textsubscript{M} Carriers and Controls

<table>
<thead>
<tr>
<th></th>
<th>A-I\textsubscript{M} Carriers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>9/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.7±18.4</td>
<td>45.4±16.4</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>24.2±3.2</td>
<td>24.0±2.6</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>181.1±42.8*</td>
<td>231.2±35.4</td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dL)</td>
<td>36.9±19.2</td>
<td>28.8±14.7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>126.2±30.6*</td>
<td>154.2±28.0</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>18.1±10.5*</td>
<td>48.2±11.8</td>
</tr>
<tr>
<td>HDL\textsubscript{2} cholesterol (mg/dL)</td>
<td>3.2±2.0*</td>
<td>12.8±6.6</td>
</tr>
<tr>
<td>HDL\textsubscript{3} cholesterol (mg/dL)</td>
<td>15.1±8.5*</td>
<td>35.4±7.9</td>
</tr>
<tr>
<td>Phospholipids (mg/dL)</td>
<td>222.2±62.9*</td>
<td>259.8±40.4</td>
</tr>
<tr>
<td>HDL phospholipids (mg/dL)</td>
<td>69.9±32.2*</td>
<td>104.9±34.0</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>184.3±96.2</td>
<td>144.0±73.5</td>
</tr>
<tr>
<td>ApoA-I (mg/dL)</td>
<td>76.8±33.4*</td>
<td>138.8±28.9</td>
</tr>
<tr>
<td>ApoA-II (mg/dL)</td>
<td>17.5±9.0*</td>
<td>37.4±6.4</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>120.4±39.9</td>
<td>126.0±28.3</td>
</tr>
<tr>
<td>HDL phospholipids/ApoA-I</td>
<td>0.95±0.27</td>
<td>0.75±0.18</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD values. *Significantly different from controls.

Table 1: Serum Lipid/Lipoprotein Levels in A-I\textsubscript{M} Carriers and Controls

Carriers than controls. The ApoA-I/ApoA-II ratio was significantly higher in the A-I\textsubscript{M} carriers than in controls (4.65±1.20 versus 3.70±0.65), consistent with the reported preferential reduction of LpA-I:A-II particles in the former.\textsuperscript{17}

HDL particles from A-I\textsubscript{M} carriers were enriched in triglycerides and phospholipids, and depleted in cholesteryl esters compared with control HDL (Table 2); the surface/core ratio was significantly higher in A-I\textsubscript{M} HDL than in control HDL (4.29±1.26 versus 3.25±0.49), indicative of a prevalence of small particles in the serum of A-I\textsubscript{M} carriers.\textsuperscript{28}

Cholesterol efflux data, expressed as percent efflux from Fu5AH cells during a 4-hour incubation, obtained with sera from A-I\textsubscript{M} carriers and controls, are shown in Figure 1. The average efflux value was 18% lower in A-I\textsubscript{M} carriers (25.0±4.2%) than in controls (30.4±3.3; P<0.001). Both female and male subjects participated in this study; a significantly higher cholesterol efflux to sera from female than male subjects was found among A-I\textsubscript{M} carriers (27.0±4.0% versus 23.1±3.6%) but not controls (30.6±3.2% versus 30.1±3.6%). The trend toward higher efflux with A-I\textsubscript{M} female serum was paralleled by a trend for higher HDL cholesterol levels in female than male serum (20.6±9.9 and 15.9±10.9 mg/dL, respectively).

TABLE 2. Serum Levels of HDL Components in A-I\textsubscript{M} Carriers and Controls

<table>
<thead>
<tr>
<th></th>
<th>A-I\textsubscript{M} Carriers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unesterified cholesterol</td>
<td>4.6±2.3* (2.1±0.4)</td>
<td>7.0±3.3  (1.8±0.6)</td>
</tr>
<tr>
<td>Cholesterol esters</td>
<td>22.7±14.1* (10.2±3.1)</td>
<td>69.2±16.2 (18.4±1.6)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>19.4±8.2 (9.7±3.4)</td>
<td>19.5±6.7 (5.4±2.2)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>69.9±32.2* (33.4±5.0)</td>
<td>104.9±34.0 (27.3±4.0)</td>
</tr>
<tr>
<td>Proteins</td>
<td>94.3±40.0* (44.6±5.6)</td>
<td>176.3±33.7 (47.1±3.7)</td>
</tr>
</tbody>
</table>

Data are mean±SD values and are expressed as milligrams per deciliter (percentages within parentheses). *Significantly different from controls.

Table 2: Serum Levels of HDL Components in A-I\textsubscript{M} Carriers and Controls

Cholesterol efflux in the whole series of sera correlated with several serum parameters, most of which are related to HDL (Table 3). Stepwise regression analysis was then performed to identify which of the correlated parameters best predicted cholesterol efflux to serum. In the whole series, the serum ApoA-I concentration was the strongest predictor of cholesterol efflux (r\textsuperscript{2}=0.85); HDL triglycerides, HDL phospholipids, and HDL unesterified cholesterol made small additions to this correlation, the 4 variables explaining 90% of the variation in cholesterol efflux. A separate stepwise analysis was also performed with data from control and A-I\textsubscript{M} samples. Serum ApoA-I concentration was again the strongest predictor of cholesterol efflux to control sera (r\textsuperscript{2}=0.63), with serum esterified cholesterol adding significantly to this prediction.

TABLE 3. Univariate Correlations Between Cholesterol Efflux and Lipid/Lipoprotein Concentrations in the Examined Sera

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Whole Series</th>
<th>A-I\textsubscript{M} Carriers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.590</td>
<td>0.298</td>
<td>0.520</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td>0.659</td>
<td>0.366</td>
<td>0.516</td>
</tr>
<tr>
<td>Unesterified/esterified cholesterol</td>
<td>−0.514</td>
<td>−0.257</td>
<td>−0.029</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.430</td>
<td>0.154</td>
<td>0.615</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.874</td>
<td>0.928</td>
<td>0.791</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>0.793</td>
<td>0.707</td>
<td>0.697</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.384</td>
<td>0.198</td>
<td>0.333</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.752</td>
<td>0.805</td>
<td>0.519</td>
</tr>
<tr>
<td>HDL-unesterified cholesterol</td>
<td>0.606</td>
<td>0.779</td>
<td>0.305</td>
</tr>
<tr>
<td>HDL esterified cholesterol</td>
<td>0.739</td>
<td>0.790</td>
<td>0.531</td>
</tr>
<tr>
<td>HDL unesterified/esterified cholesterol</td>
<td>−0.328</td>
<td>−0.406</td>
<td>0.083</td>
</tr>
<tr>
<td>HDL triglycerides</td>
<td>0.495</td>
<td>0.653</td>
<td>0.040</td>
</tr>
<tr>
<td>HDL phospholipids</td>
<td>0.744</td>
<td>0.789</td>
<td>0.476</td>
</tr>
<tr>
<td>HDL proteins</td>
<td>0.832</td>
<td>0.866</td>
<td>0.560</td>
</tr>
<tr>
<td>HDL\textsubscript{2} cholesterol</td>
<td>0.570</td>
<td>0.681</td>
<td>0.323</td>
</tr>
<tr>
<td>HDL\textsubscript{3} cholesterol</td>
<td>0.773</td>
<td>0.813</td>
<td>0.513</td>
</tr>
</tbody>
</table>

Parameters that are not significantly correlated with cholesterol efflux in the whole series are not reported. Nonsignificant correlation coefficients in separate groups of A-I\textsubscript{M} carriers and controls are italicized.
The A-I M mice had significantly higher relative efficiency of ApoA-I in determining cell cholesterol efflux. REP was 50% higher in A-IM carriers (0.116) than control subjects, separately. Therefore, REP reflects the impact of the A-IM mutation on lipoprotein metabolism in the A-I M carriers to atherosclerotic vascular disease may be related parameters, confirming that HDL particles are the largest predictor of the ability of serum to extract cholesterol from cells. They also provide indirect evidence of a similar efficiency of human ApoA-I in promoting cell cholesterol efflux when the protein is expressed in either human or murine background. Indeed a strong positive correlation (r=0.87) was found between cholesterol efflux and serum ApoA-I concentration, when data from control subjects and A-IWT mice were analyzed together.

**Discussion**

Cholesterol efflux from peripheral cells to HDL acceptors is the first step in RCT, the pathway believed to explain the protective role of HDL against the development of vascular lesions. To investigate whether the low susceptibility of the A-I M carriers to atherosclerotic vascular disease may be explained by improved cholesterol efflux capacity, we examined the ability of serum from these subjects and from transgenic mice expressing the A-IM mutant to extract cholesterol from cultured cells. The present results indicate that in a series of samples from humans and mice expressing wild-type or mutant ApoA-I, the concentration of ApoA-I is the largest predictor of the ability of serum to extract cholesterol from cells. They also provide indirect evidence of a similar efficiency of human ApoA-I in promoting cell cholesterol efflux when the protein is expressed in humans or mice, and of an improved efficiency of the A-IM mutant versus wild-type ApoA-I in determining cell cholesterol efflux both in humans and mice.

The correlation analyses on the whole series of human data and on data from separate groups indicate that cholesterol efflux to serum is positively correlated with several HDL-related parameters, confirming that HDL particles are the largest predictor of the ability of serum to extract cholesterol from cells. They also provide indirect evidence of a similar efficiency of human ApoA-I in promoting cell cholesterol efflux when the protein is expressed in either human or murine background. Indeed a strong positive correlation (r=0.87) was found between cholesterol efflux and serum ApoA-I concentration, when data from control subjects and A-IWT mice were analyzed together.

**Discussion**

Cholesterol efflux from peripheral cells to HDL acceptors is the first step in RCT, the pathway believed to explain the protective role of HDL against the development of vascular lesions. To investigate whether the low susceptibility of the A-I M carriers to atherosclerotic vascular disease may be explained by improved cholesterol efflux capacity, we examined the ability of serum from these subjects and from transgenic mice expressing the A-IM mutant to extract cholesterol from cultured cells. The present results indicate that in a series of samples from humans and mice expressing wild-type or mutant ApoA-I, the concentration of ApoA-I is the largest predictor of the ability of serum to extract cholesterol from cells. They also provide indirect evidence of a similar efficiency of human ApoA-I in promoting cell cholesterol efflux when the protein is expressed in humans or mice, and of an improved efficiency of the A-IM mutant versus wild-type ApoA-I in determining cell cholesterol efflux both in humans and mice.

The correlation analyses on the whole series of human data and on data from separate groups indicate that cholesterol efflux to serum is positively correlated with several HDL-related parameters, confirming that HDL particles are the largest predictor of the ability of serum to extract cholesterol from cells. They also provide indirect evidence of a similar efficiency of human ApoA-I in promoting cell cholesterol efflux when the protein is expressed in either human or murine background. Indeed a strong positive correlation (r=0.87) was found between cholesterol efflux and serum ApoA-I concentration, when data from control subjects and A-IWT mice were analyzed together.
major factors responsible for cholesterol efflux from cells to serum. By multivariate correlation analysis, the ApoA-I concentration in serum was the largest predictor of cholesterol efflux. A strong positive correlation between serum ApoA-I levels and cholesterol efflux was also found when data from 2 animal species expressing human ApoA-I, as control subjects and A-IWT mice, were combined. Thus, it follows that human ApoA-I determines the cholesterol efflux potential of serum, even in species with a widely different genetic background.

Recent cholesterol efflux studies with human samples, and with sera from transgenic mice and rats expressing human ApoA-I, suggest that the major serum factor involved in the regulation of cell cholesterol efflux is the HDL phospholipids content. Phospholipids may facilitate the interaction of HDL acceptors with cells through the scavenger receptor BI, therefore improving cholesterol desorption from the cell membrane. This is particularly true for FuSAH cells, which display a high level of scavenger receptor BI expression. Indeed, in the present study, HDL phospholipid was strongly correlated with efflux, and contributed to the variability in cholesterol efflux among the various serum samples. However, the serum ApoA-I concentration was the strongest predictor of cholesterol efflux to serum. The reasons for this discrepancy are not immediately clear, and may relate to the different level of ApoA-I expression in the various transgenic lines. In transgenic animals expressing high levels of human ApoA-I, as those used in previous studies, the cholesterol efflux to serum became less efficient as the concentration of ApoA-I increased, because of a marked decrease in the HDL phospholipid/ApoA-I ratio, and to the appearance of poorly effective lipid-free ApoA-I in serum. This is not the case in the present study. The A-IWT and A-IWT mice have low-normal serum ApoA-I levels, no lipid-free ApoA-I in serum, and a similar HDL phospholipid/ApoA-I ratio. The distinct physicochemical properties of acceptor particles containing wild-type or mutant ApoA-I may also contribute to the discrepancy between the present and previous findings, by affecting the interaction of HDL with scavenger receptor BI.

A major observation in this study was that, although there was a large reduction in serum ApoA-I and HDL concentrations, the cholesterol efflux to sera from the A-IWT carriers was only slightly reduced compared with control sera. The explanation for this apparent paradox came from the estimation of the efficiency of A-IWT in determining cell cholesterol efflux, obtained by calculating the serum REP as the slope of the linear regression line between cholesterol efflux and serum ApoA-I concentration. The REP, which provides an indirect estimation of the relative efficiency of acceptor particles to extract cholesterol from cells, was higher in A-IWT carriers than control subjects, suggesting a relative abundance of more efficient acceptors in the sera from A-IWT carriers. Previous extensive studies on the characterization of HDL particles in these subjects demonstrate that A-IWT HDL are smaller in size than control HDL, a characteristic that has been associated with improved efficiency for cell cholesterol uptake. Moreover, efflux studies with reconstituted HDL show that particles containing a recombiant form of the disulfide-linked A-IWT dimer are more efficient than A-IWT-containing particles in removing cholesterol from cultured cells, possibly because of the unique conformation of the mutant ApoA-I on the surface of HDL. A direct effect of the mutation on the efficiency of HDL acceptors in cell cholesterol uptake is demonstrated by the control subjects=A-IWT mice carriers<A-IWT mice gradient in the REP (Figure 2). Indeed, after adjustment for concomitant variables, a highly significant (P=0.009) gene–doseage effect of the A-IWT mutation on REP was found.

Cholesterol efflux from cells to serum acceptors is only the first step in RCT, a pathway that involves many other processes. Therefore, variations in the serum capacity to extract cholesterol from cells would only partially contribute to the efficiency of RCT in vivo, and to the individual cardiovascular risk. Nevertheless, it is noteworthy that the A-IWT carriers, who are not at increased risk despite the severe hypoalphalipoproteinemia, display an improved serum capacity for cell cholesterol uptake. This finding enhances our understanding of the impact of the A-IWT mutation on RCT, and provides an explanation for the apparent protection of A-IWT carriers against atherosclerotic vascular disease. It also supports the concept that cholesterol efflux is a major determinant of RCT in vivo, and may contribute significantly to the determination of individual cardiovascular risk.

Acknowledgments

This work was supported in part by the Istituto Superiore di Sanità, Roma, Italy (ISS project “Gene Therapy”). The authors are indebted to E.M. Rubin, University of California at Berkeley, for the substantial help in generating transgenic mice.

References

apoB-poprotein A-II transgenic mice consistent with the latter being less effective for reverse cholesterol transport. *Biochemistry.* 1997;36:2243–2249.


Increased Cholesterol Efflux Potential of Sera From ApoA-I_{Milano} Carriers and Transgenic Mice
Guido Franceschini, Laura Calabresi, Giulia Chiesa, Cinzia Parolini, Cesare R. Sirtori, Monica Canavesi and Franco Bernini

doi: 10.1161/01.ATV.19.5.1257
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/5/1257

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/