The Ability to Promote Efflux Via ABCA1 Determines the Capacity of Serum Specimens With Similar High-Density Lipoprotein Cholesterol to Remove Cholesterol From Macrophages

Margarita de la Llera-Moya, Denise Drazul-Schrader, Bela F. Asztalos, Marina Cuchel, Daniel J. Rader, George H. Rothblat

Objective—We measured efflux from macrophages to apolipoprotein B-depleted serum from 263 specimens and found instances in which serum having similar high-density lipoprotein cholesterol (HDL-C) differed in their efflux capacity. Thus, we wanted to elucidate why efflux capacity could be independent of total HDL-C or apolipoprotein A-I (apoA-I).

Methods and Results—To understand why sera with similar HDL-C or apoA-I could differ in total efflux capacity, we assessed their ability to promote efflux via the pathways expressed in cAMP-treated J774 macrophages. Briefly, macrophages were preincubated with probucol to block ABCA1, with BLT-1 to block SR-BI, and with both inhibitors to measure residual efflux. ABCG1 efflux was measured with transfected BHK-1 cells. We used apolipoprotein B-depleted serum from specimens with similar HDL-C values at the 25th and 75th percentiles. Specimens in each group were classified as having high or low efflux based on total efflux being above or below the group average. We found that independently of HDL-C, sera with higher efflux capacity had a significant increase in ABCA1-mediated efflux, which was significantly correlated to the concentration of preβ-1 HDL. The same result was obtained when these sera were similarly analyzed based on similar apoA-I.

Conclusion—Sera with similar HDL-C or apoA-I differ in their ability to promote macrophage efflux because of differences in the concentration of preβ-1 HDL.

Key Words: ABCA1 ■ apolipoprotein A-I ■ cholesterol efflux ■ high-density lipoprotein cholesterol ■ macrophages

Epidemiological and interventional studies1–4 demonstrate an inverse relationship between high-density lipoprotein cholesterol (HDL-C) levels and coronary heart disease, which is an observation also supported by animal studies.5 Thus, high HDL-C levels are thought to independently reduce coronary heart disease risk. Although HDL has been shown to have both antioxidant and antiinflammatory properties,6 its beneficial antiatherogenic effect is likely attributable to its central role in reverse cholesterol transport, ie, the transport of cholesterol from peripheral tissues to the liver for excretion to reduce its accumulation in tissue cells such as vessel wall macrophages.7 Because both HDL metabolism and cholesterol transport are complex processes, it has been difficult to obtain in vivo evidence that modulating HDL levels can affect removal of cholesterol from macrophage foam cells in the vessel wall and reduce atherosclerotic lesions. Overexpression of apolipoprotein (apo) A-I in mice can reduce progression of atherosclerotic lesions,8 and infusion of apoA-I/phospholipid complexes in humans promotes lesion regression and increases fecal excretion of bile acids.9 However, results of studies in subjects with monogenic disorders of HDL metabolism10,11 and post hoc analyses of epidemiological studies raise questions regarding the mechanism underlying the association between HDL-C levels and coronary heart disease.12

We recently demonstrated that in healthy individuals having a wide range of HDL-C and apoA-I levels, the capacity of serum HDL to promote cholesterol efflux from macrophages in vitro is negatively correlated with measures of carotid intima thickness independently of HDL-C and apoA-I levels, suggesting that measures of HDL function may be additional predictors of cardiovascular risk.13 HDL exists as a heterogeneous population of particles differing in size and composition.6 In addition, efflux of cellular cholesterol is mediated by a number of pathways, including aqueous diffusion, ABCA1, ABCG1, and SR-BI, with different HDL particles best-suited to promote efflux via each of these pathways.14 Thus, the efficiency of an individual serum to accept cellular cholesterol depends on the distribution of HDL particles and the cholesterol transporters expressed in the cell being used as a cholesterol donor. Because HDL subfractions differ in their...
ability to remove cholesterol from macrophages, the fact that individuals with similar HDL-C may have different distribution of HDL particles provides a rationale for the increased predictive value of measures of HDL function we observed.

In this study, we took advantage of the efflux data previously generated and identified subjects having similar HDL-C but significantly different total macrophage efflux. We assumed that differences in total efflux resulted from differences in the levels of functional HDL particles. We then used a published inhibitor-based assay to measure the relative contribution of different pathways to the total efflux capacity of a given serum as an indication of the relative concentration of HDL particles present. Our results show that subjects with similar HDL-C but higher total macrophage efflux capacity have significantly higher ABCA1-mediated efflux, and this efflux is associated with the level of preβ-1 HDL in serum. The same results were obtained with a subset of the same sera chosen to have similar apoA-I.

Subjects and Methods
As previously described, efflux was measured using serum aliquots specifically collected for this purpose obtained from well-characterized subjects participating in a prospective, observational study to investigate the effect of HDL-C on markers of oxidative stress and inflammation and their relationship to subclinical atherosclerosis. By design, the subjects enrolled were healthy nonsmokers with no clinically evident coronary heart disease, a broad range of HDL-C levels, and no use of drugs known to significantly affect HDL levels. The study was approved by the University of Pennsylvania Institutional Review Board and all subjects gave their informed consent for participation. Approximately equal numbers of males and females were recruited, but the experiments reported here were performed with serum from female donors because more serum aliquots were available. These were selected to have similar HDL-C by choosing sera with HDL-C within a 6% range (HDL-C ±6%), which is <7% biological variation for HDL-C and apoA-I. In addition, to confirm our results, a few critical experiments were repeated using serum from male donors also chosen to have similar HDL-C.

Serum Lipid Parameters
Blood was taken after a 12-hour fast, and several plasma and serum aliquots were prepared and frozen (−70°C) for future studies. EDTA plasma aliquots were used for lipid and lipoprotein analyses performed in a Centers for Disease Control-standardized lipid laboratory as previously described. The distribution of apoA-I containing HDL particles in apoB-depleted serum was measured using immunoblotting and image analysis after separation of the various particles with non-denaturing, 2-dimensional gel electrophoresis as described. To obtain particle mass, the percent distribution of HDL particles was applied to the total apoA-I concentration. Levels of preβ-1 HDL were also assayed using a commercial enzyme-linked immunoassay (preβ-1 HDL ELISA; Daiichi). We obtained a significant correlation between 2-dimensional gel and enzyme-linked immunoassay preβ-1 HDL values for all available values ($r^2=0.357$; $P=0.0069$; $n=19$).

Assay of Cellular Cholesterol Efflux
J774 cells, maintained in RPMI plus 10% fetal bovine serum and antibiotics in 5% CO2, were plated in 24 multi-well plates (70,000 cells/well) and labeled for 24 hours in the presence of ACAT inhibitor ($2 \mu$g/mL, CP113 818; a gift from Pfizer) using 0.5 mL/well of 2 μCi/mL [1,2-3H] cholesterol (Perkin Elmer) in RPMI plus 1% fetal bovine serum. To upregulate ABCA1 in J774 cells, we incubated an additional 16 hours with 0.5 mL/well medium containing 0.3 mmol/L Cpt-cAMP (Sigma) and 0.2% bovine serum albumin in RPMI. The relative contribution of various efflux pathways was measured by 2-hour pretreatment of replicate wells of cAMP-treated J774 cells with RPMI-0.2% bovine serum albumin alone or this medium plus 20 μmol/L probucol, 1 μmol/L BLT-1, or both to specifically inhibit ABCA1 and SR-BI. The contribution of ABCG1 to total efflux capacity was directly measured by 4-hour incubation of ABCG1-transfected BHK 1 cells with the same specimens used with J774 cells. In BHK-1 cells transfected with ABCG1, receptor expression is regulated by mifepristone; the difference between cells treated overnight with mifepristone (10 mmol/L) and untreated cells represents ABCG1 efflux. Sera for these studies were aliquots stored at −70°C, used after a single thaw, and chosen to have similar HDL-C (HDL-C ±6%) at either the 25th (low HDL =45; $n=22$) or 75th (high HDL =73; $n=18$) percentile of the HDL-C distribution. Efflux was the fraction of total cellular cholesterol released in 4 hours to apoB-depleted serum, obtained after removal of apoB lipoproteins with polyethylene glycol (MW 8000; Sigma), and diluted to 2.8% (equivalent to 2% serum) in MEM-HEPES (0.5 mL/well). We have routinely used this dilution to promote release of radioactive cholesterol to the medium in 4 hours, which is well above background. Supplementary Figure I (available online at http://atvb.ahajournals.org) shows the dependence of the various receptor-mediated efflux pathways expressed in J774 cells in the dose of apoB-depleted serum. In this Figure, ABCA1 efflux is the difference between cAMP-treated and control J774 cells and SR-BI is the efflux measured from Fu5AH cells, in which SR-BI is the major efflux pathway and ABCG1 efflux is the difference between mifepristone treated and untreated transfected BHK-1 cells. SR-BI and ABCG1 efflux show linear dose-dependence; the dose-dependence for ABCA1-mediated efflux fits a nonlinear regression that tends to plateau but a concentration of 2.8% apoB-depleted serum is below this point (GraphPad Prism 5; GraphPad Software). To further validate our macrophage model, we measured ABCA1 efflux using BHK-1 cells transfected with ABCA1, in which receptor expression is regulated with mifepristone, and found that the dose-dependence of ABCA1 efflux measured with BHK-1 cells was identical to that measured with J774 cells (Supplementary Figure I). In every experiment, we monitored upregulation of ABCA1 in J774 cells as increased efflux to apoA-1 (20 μg/mL) from cells treated with cAMP compared to untreated cells (11±4-fold stimulation; $n=10$ experiments) and used an aliquot of a standard serum pool at 2% to monitor interassay variability in total efflux from cAMP J774 cells and ABCG1 efflux from BHK-1 cells. Transfected BHK-1 cells were a kind gift from Dr Jack Oram (University of Washington, Seattle, Wash). All cellular incubations were performed at 37°C, and expression of cholesterol transporters was confirmed by Western blot in initial experiments. Sera from subjects with similar high or low HDL-C were defined as having low or high efflux capacity based on total J774 efflux below or above the average efflux for each group. The same analysis was performed using a subset of these specimens chosen to have similar apoA-I at either the 25th (low apoA-I 127 mg/dL; $n=11$) or 75th (high apoA-I=163 mg/dL; $n=10$) percentile of the apoA-I distribution.

Statistical Analysis
Results are reported as mean±SEM. Linear regression was used to establish associations between parameters. Differences between means were established with unpaired t-tests. These analyses were performed using GraphPad Prism 5 (GraphPad Software) and statistical significance was assumed for $P<0.05$.

Results
Figure I summarizes efflux from J774 macrophages to apoB-depleted serum isolated from a human serum pool. Although the total cholesterol released was only slightly increased by cAMP, this treatment significantly changed the relative contribution of different pathways. Compared to control J774 cells, cAMP increased ABCA1-mediated efflux ($−cAMP=1.76±0.524$, $P=0.00086$), but the contribution of SR-BI was decreased ($cAMP=3.07±0.111$, $P=0.0003$). The cAMP treatment
also slightly increased in the residual efflux measured in cells treated with both probucol and BLT-1. The major contribution to this efflux is aqueous diffusion, which represents \( \approx 50\% \) of the total efflux in control J774 cells; however, after cAMP treatment this residual efflux may also include other inhibitor-resistant pathways such as ABCG1, which can be detected by Western blot (data not shown). Figure 1 also shows a low level of ABCA1 efflux in control J774 cells. In agreement with Favari et al.,\(^{20}\) this efflux is probucol-sensitive as confirmed by \( > 90\% \) decrease in efflux to 20 \( \mu \)g/ml apoA-I from both control and cAMP-treated J774 cells (Supplementary Figure II, available online at http://atvb.ahajournal.org). Because we cannot specifically measure ABCG1 efflux in cAMP J774 cells, we used transfected BHK-1 cells. In this model, ABCG1 expression is directly by measuring efflux from transfected BHK-1 cells untreated and treated with mifepristone to induce receptor expression. We defined low and high efflux capacity based on total J774 efflux below or above the average of all sera with similar HDL-C and asked if these specimens differed in their ability to promote efflux via individual pathways.

The results of this analysis are shown in Tables 1 (low HDL-C sera) and 2 (high HDL-C sera). As can be seen, the most dramatic difference we found was that specimens with high efflux capacity had significantly increased ability to promote efflux via ABCA1. We also found a significant but less pronounced increase in the ability of high efflux capacity serum to promote residual or inhibitor-resistant efflux.

**Table 1. Average Efflux (% per 4 Hours) for Serum Specimens From Females With HDL-C of 48±6%**

<table>
<thead>
<tr>
<th>Efflux Pathway</th>
<th>Cell Model</th>
<th>Efflux Below Mean (n=11)</th>
<th>Efflux Above Mean (n=11)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total J774</td>
<td>J774+cAMP</td>
<td>12.7±0.3</td>
<td>16.6±0.3</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>Uninhibited efflux</td>
<td>J774+cAMP</td>
<td>4.8±0.2</td>
<td>5.4±0.2</td>
<td>0.0111</td>
</tr>
<tr>
<td>SR-BI</td>
<td>J774+cAMP</td>
<td>0.80±0.3</td>
<td>1.2±0.3</td>
<td>( NS )</td>
</tr>
<tr>
<td>ABCA1</td>
<td>J774+cAMP</td>
<td>7.6±0.3</td>
<td>10.7±0.4</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>ABCG1</td>
<td>G1-BHK</td>
<td>2.9±0.5</td>
<td>3.4±0.2</td>
<td>( NS )</td>
</tr>
</tbody>
</table>

Efflux values of the serum apoB-depleted serum are given as mean±SEM. Mean total J774 efflux for all sera analyzed (\( n=22 \))=14.7% per 4 hr (11.0–18.7). Average total HDL-C in each group was 48, which represents the 25th percentile for the range of HDL-C values in this set of serum specimens from all female donors (\( n=130 \)). NS indicates, not significant.

HDL-C=45–51.
Table 2. Average Efflux (% per 4 Hours) for Serum Specimens From Females With HDL-C of 73 ± 6

<table>
<thead>
<tr>
<th>Efflux Pathway</th>
<th>Cell Model</th>
<th>Efflux Below</th>
<th>Efflux Above</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total J774</td>
<td>J774 + cAMP</td>
<td>11.7 ± 0.4</td>
<td>15.0 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Uninhibited efflux</td>
<td>J774 + cAMP</td>
<td>4.6 ± 0.2</td>
<td>5.7 ± 0.3</td>
<td>0.0102</td>
</tr>
<tr>
<td>SR-BI</td>
<td>J774 + cAMP</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>ABCA1</td>
<td>J774 + cAMP</td>
<td>6.2 ± 0.5</td>
<td>8.5 ± 0.5</td>
<td>0.0072</td>
</tr>
<tr>
<td>ABCG1</td>
<td>G1-BHK</td>
<td>2.7 ± 0.3</td>
<td>2.6 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Efflux values of the serum apoB-depleted serum are given as mean ± SEM. Mean total J774 efflux for all sera analyzed (n = 18) = 13.4% per 4 hr (10.2–17.6). Average total HDL-C in each group was 73, which represents the 75th percentile for the range of HDL-C values in this set of serum specimens from all female donors (n = 130).

HDL-C = 69–77.

formed: 1 with low apoA-I (average = 127 mg/dL; 25th percentile; range, 119–135; n = 11) and 1 with high apoA-I (average = 163 mg/dL; 75th percentile; range, 153–173; n = 10). Sera with similar low levels of apoA-I (127 mg/dL) and total macrophage efflux above the average for the group had higher ABCA1 efflux when compared to sera with total efflux below the mean for the group (9.98 ± 0.320% per 4 hours, n = 5 vs 7.72 ± 0.533% per 4 hours, n = 6; P = 0.0073). Likewise, sera with high efflux capacity and similar high apoA-I levels (163 mg/dL) also had higher ABCA1 efflux (10.74 ± 0.855% per 4 hours, n = 4 vs 7.25 ± 0.581% per 4 hours, n = 6; P = 0.0004). There were no significant differences in the efflux mediated by any other pathway. Thus, independently of HDL-C or apoA-I level, the increased efficiency of a given apoB-depleted serum to remove cholesterol from macrophages is attributable to the fact that it can more efficiently promote cholesterol efflux via ABCA1.

Because the differences in efflux shown in Tables 1 and 2 were not related to serum HDL-C, we used 2-dimensional gel electrophoresis to measure preβ-1 HDL levels.14 Figure 4 shows that there is a significant correlation between ABCA1 efflux and the level of preβ-1 HDL in all the specimens analyzed using 2-dimensional gels (n = 29). The r² value (r² = 0.425) obtained suggests that 43% of the variability in ABCA1 efflux can be explained by the level of preβ-1 HDL in these sera. There were also significant associations between ABCA1 efflux and the preβ-1 HDL levels in sera having similar low or high HDL-C (low HDL-C: r² = 0.220, 0.425; high HDL-C: r² = 0.535, P = 0.0111, n = 11).

Although we did not have preβ-1 HDL values for all the sera chosen on the basis of similar apoA-I, the ABCA1 efflux mediated by all the specimens in this smaller subset was also significantly associated with the serum level of preβ-1 HDL (r² = 0.357, P = 0.040, n = 12).

Although we had focused these studies on the apoB-depleted serum from female donors, confirmatory experiments were performed with apoB-depleted serum from healthy males who participated in the same clinical study and have a similar demographic profile (data not shown). We found that whether the specimens from male donors had similar low HDL-C (HDL-C = 38 mg/dL; n = 14) or similar high HDL-C (HDL-C = 63 mg/dL; n = 16), sera with higher capacity to promote efflux from J774 macrophages had significantly higher ABCA1 efflux (Supplementary Tables II and III).

To better-link high efflux capacity to increased ABCA1 and preβ-1 HDL, we calculated the average preβ-1 HDL concentration for all sera with high vs low efflux capacity. As expected, high efflux sera had higher preβ-1 HDL. However, the average values were not significantly different (high = 25.3 ± 3.5 mg/dL, n = 15 vs low = 19.2 ± 2.6 mg/dL, n = 14), possibly because of the low number of sera in each group. To increase the statistical power of this analysis, we obtained a commercially available enzyme-linked immunosassay to establish preβ-1 HDL in all the specimens from female and male donors for whom we had ABCA1 efflux (Figure 5; n = 63). Increasing the number of data points allowed us to establish a significant difference in the preβ-1 HDL values measured in specimens with high vs low ABCA1 efflux (high = 27 ± 1.1 mg/mL, n = 33 vs low = 22 ± 1.7 mg/mL, n = 29; P = 0.017).

Discussion

Studies have shown that measures of HDL subclasses in different populations, such as participants in the VA-HIT study,22 postmenopausal women,23 and Finnish families with low HDL,24 are better predictors of coronary artery disease.
than measures of HDL-related parameters in serum. We recently reported\cite{11} that the efflux capacity of the apoB-depleted serum isolated from >200 healthy individuals is negatively associated to carotid intima thickness independently of serum HDL-C and apoA-I. Others have shown that enhanced ABCA1 efflux from J774 cells to serum from type IV hypertriglyceridemic subjects was associated with the serum preβ-1 HDL\cite{25} and that in patients with diabetic nephropathy, defects in ABCA1 efflux are related to decreased levels of preβ-1 HDL.\cite{26} In addition, depletion of preβ-1 HDL by chymase treatment of HDL\textsubscript{3} impaired ABCA1 but not SR-BI–mediated efflux from J774 macrophages.\cite{27} Thus, there is increasing evidence that measures of HDL function, such as in vitro assay of cholesterol efflux, may be useful adjuncts to the traditional assays of serum HDL function, such as in vitro assay of cholesterol efflux, may be useful adjuncts to the traditional assays of serum HDL-C and apoA-I when evaluating cardiovascular risk.

The experiment results we now report agree with the concept that measures of HDL parameters in serum do not always reflect HDL function. We measured cholesterol efflux to the apoB-depleted serum, isolated from human specimens by removing apoB lipoproteins,\cite{18} and used cAMP-treated J774 macrophages.\cite{27} Thus, there is increasing evidence that measures of HDL-C and apoA-I when evaluating cardiovascular risk. The experiments reported in this article were performed with apoB-depleted serum from female donors; however, comparable results were obtained in a small study using apoB-depleted serum from healthy male donors who participated in the same clinical study and have a similar demographic profile. Thus, for specimens with similar low or high HDL-C, a higher capacity to promote efflux from J774 macrophages was attributable to significantly higher ABCA1 efflux (Supplementary Tables II and III).

Figure 5. Correlation of ABCA1 efflux to preβ-1 HDL levels in serum with high or low efflux capacity. ABCA1 efflux was measured as in Figure 4 using apoB-depleted serum from male and female donors. Levels of preβ-1 HDL in all these specimens (n=63) were measured using a commercial enzyme-linked immunoassay (see Methods) and are reported as ng/mL per aliquot assayed. These values were significantly correlated to ABCA1-mediated efflux ($\tau^2=0.102\; P=0.011\; n=63$). White squares, specimens with high efflux; black squares, specimens with low efflux.

and 2 and Figure 4 indicate why neither measures of HDL-C nor measures of apoA-I predict the capacity of individual sera to promote efflux from macrophages. Thus, when we used a published method\cite{15} to establish the relative contribution of various efflux pathways with serum samples having either similar low HDL-C (Table 1) or high HDL-C (Table 2), serum having a higher capacity to promote efflux of cholesterol from J774 macrophages could more efficiently promote efflux via ABCA1, which in turn was significantly associated with the concentration of preβ-1 HDL (Figure 4). Figure 5 shows that when we measured preβ-1 HDL in all sera for which we had ABCA1 efflux (n=63), higher levels of preβ-1 HDL resulted in higher ABCA1 efflux.

Because total macrophage efflux may also be independent of apoA-I levels (Figure 3), we analyzed a subset of the same specimens based on similar high or low apoA-I. As for HDL-C, regardless of apoA-I levels, specimens with higher efflux showed a higher capacity to promote efflux via ABCA1, and this was associated with the levels of serum preβ-1 HDL.

It is thought that the activity of phospholipid transfer protein, hepatic triglyceride lipase, and cholesteryl ester transfer protein can increase serum preβ-1 HDL levels, whereas lecithin-cholesterol acyltransferase activity has the opposite effect.\cite{24,26,28} Our database includes only cholesteryl ester transfer protein mass, and the level of cholesteryl ester transfer protein in sera we identified as having higher ABCA1 efflux and preβ-1 HDL was not different than that in low efflux sera. This could be either because the number of sera in each group is too small or because measures of cholesteryl ester transfer protein activity would be a better indicator.

It has been reported that modifications of HDL particles affect their functionality by either changing their specificity toward receptors\cite{29,30} or their ability to promote efflux. Thus, Francis et al.\cite{31,32} have shown that oxidative tyrosylation of HDL promotes formation of AI–AII heterodimers that can enhance efflux. In contrast, oxidation of apoA-I by myeloperoxidase can significantly impair ABCA1-mediated efflux.\cite{33} Because we used serum specimens from a healthy normolipidemic population, we believe it is unlikely that our results were significantly affected by the presence of abnormal HDL.

In summary, our experiments show for the first time to our knowledge that the apoB-depleted serum from individuals with similar levels of HDL-C or apoA-I may have different capacities to remove cholesterol from macrophages. In our cell model, specimens from subjects with either similar HDL-C or similar apo A-I that have higher efflux capacity can better-promote efflux via ABCA1, and this is related, in
large part, to the serum concentration of preβ-1 HDL. These novel results add to the increasing evidence that measures of HDL function, such as its efflux capacity, can be useful when assessing an individual’s coronary heart disease risk.

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Disclosures

None.

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### Supplementary Table I. Baseline Characteristics of Female Study Subjects

Mean + SD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Subjects (n=130)</th>
<th>Subjects with Low HDL-C (n=22)</th>
<th>Subjects with High HDL-C (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>55±8</td>
<td>54±7</td>
<td>54±7</td>
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<tr>
<td>Race (%AA/W)</td>
<td>23/75</td>
<td>32/68</td>
<td>33/61</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5±5.3</td>
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<td>28.8±5.6</td>
</tr>
<tr>
<td>Syst BP (mm Hg)</td>
<td>122±15</td>
<td>122±12</td>
<td>121±17</td>
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<tr>
<td>Diast BP (mm Hg)</td>
<td>74±9</td>
<td>76±8</td>
<td>76±12</td>
</tr>
<tr>
<td>HDL-C</td>
<td>63±17</td>
<td>48</td>
<td>73</td>
</tr>
</tbody>
</table>

SD, Standard Deviation; yrs, years; AA, African American; W, White; BMI, body mass index; Syst BP, systolic blood pressure; Diast BP, Diastolic blood pressure; HDL-C, High density lipoprotein cholesterol.
### Supplementary Table II. Average Efflux (% per 4h) For Serum Specimens From Males With HDL-C=38±6% (HDL C=36 to 40)

<table>
<thead>
<tr>
<th>Efflux Pathway</th>
<th>Cell Model</th>
<th>Efflux Below Mean (n=7)</th>
<th>Efflux Above Mean (n=7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>J774+ c-AMP</td>
<td>9.16±0.316</td>
<td>13.30±0.512</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Uninhibited Efflux</td>
<td>J774+ c-AMP</td>
<td>3.91±0.137</td>
<td>4.81±0.257</td>
<td>0.009</td>
</tr>
<tr>
<td>SR-BI</td>
<td>J774+ c-AMP</td>
<td>0.48±0.100</td>
<td>0.32±0.156</td>
<td>NS</td>
</tr>
<tr>
<td>ABCA-1</td>
<td>J774+ c-AMP</td>
<td>4.79±0.325</td>
<td>7.71±0.436</td>
<td>0.0002</td>
</tr>
<tr>
<td>ABCG-1</td>
<td>G1-BHK</td>
<td>4.10±0.335</td>
<td>4.23±0.417</td>
<td>NS</td>
</tr>
</tbody>
</table>

Efflux values to the serum HDL fraction are given as Mean ± SEM. Mean total J774 efflux for all sera (n=14) was 11.23%/4h (7.66-15.57). Average total HDL-C in each group was 38 which represents the 25th percentile for the range of HDL-C values in this set of serum specimens from male donors (n=138).
### Supplementary Table III. Average Efflux (% per 4h) For Serum Specimens From Males With HDL-C = 62±6% (HDL C=59 to 66)

<table>
<thead>
<tr>
<th>Efflux Pathway</th>
<th>Cell Model</th>
<th>Efflux Below Mean (n=8)</th>
<th>Efflux Above Mean (n=8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>J774+ c-AMP</td>
<td>12.24±0.527</td>
<td>16.68±0.886</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ubinhibited Efflux</td>
<td>J774+ c-AMP</td>
<td>3.83±0.345</td>
<td>5.50±0.303</td>
<td>0.003</td>
</tr>
<tr>
<td>SR-BI</td>
<td>J774+ c-AMP</td>
<td>1.08±0.254</td>
<td>1.70±0.367</td>
<td>NS</td>
</tr>
<tr>
<td>ABCA-1</td>
<td>J774+ c-AMP</td>
<td>6.85±0.417</td>
<td>10.30±0.539</td>
<td>0.0002</td>
</tr>
<tr>
<td>ABCG-1</td>
<td>G1-BHK</td>
<td>2.50±0.407</td>
<td>2.04±0.244</td>
<td>NS</td>
</tr>
</tbody>
</table>

Efflux values to the serum HDL fraction are given as Mean ± SEM. Average total J774 efflux for all sera (n=16) = 14.46 (9.90-20.08). Average total HDL-C in each group was 62 which represents the 75th percentile for the range of HDL-C values in this set of serum specimens from male donors (n=138).
Supplementary Figure I. Dependence of Efflux on the Concentration of Apo B-depleted Serum. The dependence of receptor–mediated efflux on the dose of apo B-depleted serum was measured as described in methods. The following cells were used: J774 Cells + c-AMP (ABCA1), A1-BHK-1 Cells ± Mifepristone (ABCA1), Fu5AH Cells (SR-BI) and G1-BHK-1 Cells ± Mifepristone (ABCG1)
Supplementary Figure II

Supplementary Figure II. Probucol Sensitivity of Efflux to apo A-I. Efflux from J774 + c-AMP macrophages to 20µg/ml was measured before and after 2h pretreatment with 20µM Probucol as described in methods. Data shown is the average of 2 experiments.
Supplementary Figure III

**Supplementary Figure III. Correlation Between Cholesterol Efflux and HDL Parameters.** Cholesterol efflux from AMP treated J774 macrophages to the isolated HDL fraction obtained from serum specimens from 127 female donors was correlated to HDL parameters measured in serum. Panel A shows a significant correlation between efflux (%/4h) and HDL-C (r²=0.355, p<0.001, n=127). Panel B shows a significant correlation between efflux (%/4h) and apo A-I (r²=0.339, p<0.001, n=130).