A Comparison of the Theoretical Relationship between HDL Size and the Ratio of HDL Cholesterol to Apolipoprotein A-I with Experimental Results from the Women’s Health Study

Norman A. Mazer,1* Franco Giulianini,2 Nina P. Paynter,2 Paul Jordan,3 and Samia Mora2,4*

BACKGROUND: HDL size and composition vary among individuals and may be associated with cardiovascular disease and diabetes. We investigated the theoretical relationship between HDL size and composition using an updated version of the spherical model of lipoprotein structure proposed by Shen et al. (Proc Natl Acad Sci U S A 1977;74:837–41.) and compared its predictions with experimental data from the Women’s Health Study (WHS).

METHODS: The Shen model was updated to predict the relationship between HDL diameter and the ratio of HDL-cholesterol (HDL-C) to apolipoprotein A-I (ApoA-I) plasma concentrations (HDL-C/ApoA-I ratio). In the WHS (n = 26,772), nuclear magnetic resonance spectroscopy (NMR) was used to measure the mean HDL diameter (d_{mean,NMR}) and particle concentration (HDL-P); HDL-C and ApoA-I (mg/dL) were measured by standardized assays.

RESULTS: The updated Shen model predicts a quasilinear increase of HDL diameter with the HDL-C/ApoA-I ratio, consistent with the d_{mean,NMR} values from WHS, which ranged between 8.0 and 10.8 nm and correlated positively with the HDL-C/ApoA-I ratio (r = 0.608, P < 2.2 × 10⁻¹⁶). The WHS data were further described by a linear regression equation: d_{WHS} = 4.66 nm + 12.31(HDL-C/Apo-I), where d_{WHS} is expressed in nanometers. The validity of this equation for estimating HDL size was assessed with data from cholesteryl ester transfer protein deficiency and pharmacologic inhibition. We also illustrate how HDL-P can be estimated from the HDL size and ApoA-I concentration.

CONCLUSIONS: This study provides a large-scale experimental examination of the updated Shen model. The results offer new insights into HDL structure, composition and remodeling and suggest that the HDL-C/ApoA-I ratio might be a readily available biomarker for estimating HDL size and HDL-P.

© 2013 American Association for Clinical Chemistry

α-HDL particles are spherical in shape and contain >90% of the plasma concentrations of HDL cholesterol (HDL-C)5 and apolipoprotein A-I (ApoA-I) (1–3), their major protein constituent (4). These particles are heterogeneous in size and composition, with a number of discrete HDL subclasses identified by different separation and analysis methods (5–7). The subclasses result from HDL remodeling mechanisms that involve particle fusion, lipid transfer, lipolysis, and esterification (2, 3, 8) and typically range in size from about 7.5 to 11.5 nm (5–7). α-HDL particles are believed to collectively play an important role in reverse cholesterol transport and in other processes that may protect individuals from cardiovascular disease (CVD) (2, 9). Current methods for determining HDL size require advanced lipoprotein testing and are not yet routinely used for clinical evaluation (5, 10). Despite considerable progress, the interrelationships between HDL size, composition, and function remain incompletely understood.

1 Clinical Pharmacology and 3 Biostatistics, F. Hoffmann-La Roche Ltd, Basel, Switzerland; 2 Division of Preventive Medicine and 4 Division of Cardiovascular Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA.

* Address correspondence to: N.A.M. at F. Hoffmann-La Roche Ltd, Bldg. 670, Rm. 309.5, 4070 Basel, Switzerland, CH-4070. E-mail norman.mazer@roche.com. S.M. at Brigham and Women’s Hospital, 900 Commonwealth Ave., Third Floor, Boston, MA 02215. E-mail smora@partners.org.

Preliminary versions of this work were presented orally at the KinMet Symposium, Chicago, April 27, 2011, and in poster format at the 79th European Atherosclerosis Society Congress, Gothenburg, Sweden, June 26–29, 2011.

Received October 4, 2012; accepted February 7, 2013.
Previously published online at DOI: 10.1373/clinchem.2012.196349

Nonstandard abbreviations: HDL-C, HDL cholesterol; Apo-A-I, apolipoprotein A-I; CVD, cardiovascular disease; CE, cholesterol ester; TG, triglyceride; NMR, nuclear magnetic resonance; d, HDL particle diameter; (d_{mean,NMR}), mean d determined by NMR; WHS, Women’s Health Study; CETP, cholesterol ester transfer protein; HDL-P, concentration of HDL particles; IQR, interquartile range; TG/CEcore, lipid core ratio; F_ApoA-I, ApoA-I fraction in the HDL proteome; d_{meas}, estimated HDL size from Eq. 1; d_{mean}, measured mean HDL size; EPIC, European Prospective Investigation of Cancer.
In 1977, Shen et al. (11) proposed a simple quantitative model of lipoprotein structure based on an analysis of the size and composition of α-HDL, LDL, VLDL, and chylomicron particles. According to their model, these lipoproteins and their subclasses have a spherical lipid core containing cholesterol esters (CEs) and triglycerides (TGs), covered by a surface monolayer of phospholipids, unesterified cholesterol, and apolipoprotein. The lipid core radius varies among lipoproteins, whereas the monolayer thickness is approximately constant and equal to 20.2 Å (11). Shen’s elegant model used geometric and thermodynamic concepts to link lipoprotein size and composition. Although the qualitative aspects of this model have been generally accepted, the quantitative aspects have not been fully examined, particularly in regard to HDL.

In the present study we updated Shen’s model to investigate the theoretical relationship between HDL size and the corresponding ratio of HDL-C/ApoA-I concentrations. A mathematical derivation of the updated model and its predictions is given in the Data Supplement that accompanies the online version of this report at http://www.clinchem.org/content/vol59/issue6. The primary objective of our study was to compare these theoretical predictions with experimental data on the relationship between HDL size [determined by nuclear magnetic resonance (NMR) spectroscopy (12)] and HDL-C/ApoA-I ratio, as observed in the Women’s Health Study (WHS) (13). A secondary objective was to obtain a simple equation for estimating HDL size from the HDL-C/ApoA-I ratio on the basis of a linear regression analysis of the WHS data. We have assessed the validity of this equation by comparing its predictions with experimental data on HDL size from patients with cholesteryl ester transfer protein (CETP) deficiency or treated with CETP inhibitors. We have shown how HDL size can be combined with ApoA-I concentrations to estimate the concentration of HDL particles (HDL-P), and we briefly discuss the relevance of HDL size and HDL-P as biomarkers of CVD and diabetes.

Materials and Methods

UPDATE OF SHEN’S MODEL

Shen’s model was updated to investigate the theoretical relationship between HDL size and the HDL-C/ApoA-I ratio and to provide estimates of HDL-P. Details are provided in the online Supplemental Data. Nonlinear algebraic equations from the updated Shen model were programed and solved using the Berkeley–Madonna program version 8.3.18 (http://www.berkeleymadonna.com); tabular output was exported to Microsoft Excel 2010 for graphical representation.

WHS POPULATION

Study participants were drawn from the WHS, a randomized, double-blind, placebo-controlled trial of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer in women (13). At the time of enrollment, 28 345 individuals gave written informed consent and provided a baseline blood sample. Of these, 98.5% (n = 27 909) had lipoprotein particle analysis. Individuals with missing (n = 222) or anomalous (n = 4) baseline data on HDL-C and/or ApoA-I and those taking lipid-lowering treatments (n = 891) or whose medication status was unknown (n = 20) were excluded, leaving a total of 26 772 for the present analysis. The study was approved by the institutional review board of the Brigham and Women’s Hospital.

LABORATORY MEASUREMENTS

As reported previously (13), baseline EDTA plasma samples were shipped under dry ice to LipoScience Inc. for analysis by proton NMR spectroscopy. The measured HDL diameter (d_{mean,NMR}) is the mean of the calibrated diameters of individual HDL subclasses that contribute to the NMR spectra, weighted by their respective methyl NMR signals (7, 12). The d_{mean,NMR} values are reported to a precision of 0.1 nm (by rounding); the estimated measurement CV is 0.6% (12). The NMR method also provides a measure of the total HDL-P, CV <1.5% (12). In the current implementation of the NMR method (LipoProfile-3 algorithm), 26 subclasses were used to determine the mean HDL size and the total HDL-P (5, 12).

Baseline plasma samples were further assayed for HDL-C (mg/dL) using a direct enzymatic colorimetric assay (Hitachi 917 analyzer, Roche Diagnostics; CV, <3%) (14) and for ApoA-I (mg/dL) by an immunoturbidimetric assay (DiaSorin; CV, 3%) (assays performed at Clinical Chemistry Laboratory, Boston Children’s Hospital, Boston, MA; data owned by WHS).

The HDL-C/ApoA-I ratio was computed from the respective mass concentrations. From the CVs and expected correlation between the HDL-C and ApoA-I concentrations, the measurement CV of the HDL-C/ApoA-I ratio was estimated to be approximately 2%–4%.

STATISTICAL ANALYSIS AND GRAPHICAL DISPLAY OF WHS DATA

Descriptive statistics of d_{mean,NMR}, HDL-C, ApoA-I, and the HDL-C/ApoA-I ratio were computed on the entire WHS population along with the Pearson product–moment correlation matrix and the partial correlation matrix. As a consequence of rounding to the nearest 0.1 nm, d_{mean,NMR} becomes a discrete variable, whereas the HDL-C/ApoA-I ratio is a continuous variable. For each discrete value of d_{mean,NMR}, the mean, SD, SE, CV, median, and interquartile range (IQR) of the HDL-C/ApoA-I ratios were computed.
Linear regression was performed with HDL-C/ApoA-I (x variable) as the dependent variable and \( d_{\text{mean,NMR}} \) (y variable) as the independent variable. This approach corresponds to an “errors in variables” regression in the limit, where the measurement error in the x variable is much greater than the measurement error in the y variable (15), consistent with our estimates of the measurement CVs for the HDL-C/ApoA-I ratios and \( d_{\text{mean,NMR}} \) values. The slope and intercept of the x vs y regression line were then transformed to express the relationship as y vs x. The experimental relationship between \( d_{\text{mean,NMR}} \) vs the HDL-C/ApoA-I ratio was displayed graphically using a combination scatter plot/heat map. The heat map divides the plot region into a grid of rectangles (width, 0.025; height, 0.1 nm) that are assigned a color on a white-to-red gradient on the basis of the number of individual data points they contain. Statistical analyses were done using R version 2.10.0 (R Foundation for Statistical Computing).

**Estimation of HDL Size in States of CETP Deficiency and Inhibition**

Clinical data on HDL-C, ApoA-I, and HDL size (where available) in individuals with CETP deficiency and during pharmacological inhibition of CETP activity with torcetrapib, dalcetrapib, and anacetrapib were taken from the literature (16–19). In the report by Ballantyne et al. (18), NMR lipoprotein profiles were reported in terms of the concentrations of small, medium, and large HDL particles. The corresponding mean HDL sizes obtained in that study were kindly provided to the authors by Dr. David Kallend, from data on file at F. Hoffmann-La Roche Ltd (David Kallend, personal communication, December 21, 2012). The HDL-C/ApoA-I ratio was derived from the mean values of HDL-C and ApoA-I reported in these studies, and the apparent HDL diameter was computed using the linear regression equation derived from the WHS data. It can be shown that the HDL-C/ApoA-I ratio and the HDL size computed in this way correspond to mean values, weighted by the ApoA-I concentrations of each individual.

**Results**

**Theoretical Relationship Between HDL Size and HDL-C/ApoA-I Ratio**

As derived in the online Supplemental Data (sections 1 and 2), the updated Shen model predicts that for a homogeneous system of HDL particles with fixed values of the lipid core ratio (\( TG/CE_{\text{core}} \)) and ApoA-I fraction in the HDL proteome (\( F_{\text{ApoA-I}} \)), the particle diameter (\( d \)) will increase in a quasilinear (monotonic) manner with the HDL-C/ApoA-I ratio. By allowing \( TG/CE_{\text{core}} \) and \( F_{\text{ApoA-I}} \) to vary over the relevant physiological ranges, we generated a family of curves describing the theoretical relationship between \( d \) and HDL-C/ApoA-I (see online Supplemental Fig. S4). We further show in the online Supplemental Data (section 3) that for a heterogeneous system of HDL particles corresponding to 5 HDL subclasses detected by NMR spectroscopy (7), the \( d_{\text{mean,NMR}} \) will be 5% larger on average than the diameter for the homogenous case with the same HDL-C/ApoA-I ratio (see online Supplemental Data, Fig. S5). For this reason the theoretical curves derived for the homogeneous system should provide a reasonable prediction of the dependence of \( d_{\text{mean,NMR}} \) on HDL-C/ApoA-I ratio. Lastly, in the online Supplemental Data (section 4), we extend the concept of the HDL-C/ApoA-I ratio to the HDL-C/(ApoA-I + ApoA-II) ratio, previously used by Brinton and colleagues (20) as a surrogate for HDL size.

**Experimental Relationship Between HDL Size and HDL-C/ApoA-I Ratio**

For the WHS population (n = 26 772), the mean (SD) values of the measured variables were \( d_{\text{mean,NMR}} \) 9.02 (0.47) nm; HDL-C, 53.9 (15.0) mg/dL; ApoA-I, 150.9 (25.5) mg/dL; and HDL-C/ApoA-I ratio, 0.354 (0.063). The Pearson product–moment correlation matrix (Table 1A) shows that all of these variables are highly correlated with each other. The partial correlation matrix (Table 1), which corrects for the intercorrelations among the variables, indicates that the strongest direct correlates of \( d_{\text{mean,NMR}} \) are the HDL-C/ApoA-I ratio and ApoA-I concentration, whereas the partial correlation coefficient between \( d_{\text{mean,NMR}} \) and HDL-C is not significantly different from zero.

<p>| Table 1. Pearson product-moment correlation matrix and partial correlation matrix (r values).a |
|----------------------------------------|--------|--------|</p>
<table>
<thead>
<tr>
<th>HDL-C</th>
<th>ApoA-I</th>
<th>HDL-C/ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d_{\text{mean,NMR}} )</td>
<td>0.738</td>
<td>0.577</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.779</td>
<td>0.801</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.269</td>
<td></td>
</tr>
<tr>
<td>Partial correlation matrix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( d_{\text{mean,NMR}} )</td>
<td>−0.00015</td>
<td>0.130</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.971</td>
<td>0.972</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>−0.950</td>
<td></td>
</tr>
</tbody>
</table>

*a All r values and partial correlations are highly significant (\( P < 2.2 \times 10^{-16} \)) except for the partial correlation between \( d_{\text{mean,NMR}} \) and HDL-C (\( P = 0.981 \)).
given in Table 2. As \( r_{\text{mean,NMR}} \) increases over the observed range (8.0–10.8 nm), the median (and mean) HDL-C/ApoA-I ratio increases nearly 2-fold from 0.267 to 0.523; the corresponding CV values of the HDL-C/ApoA-I ratios vary between 9.6% and 16%.

Fig. 1 provides a graphical representation of the experimental relationship between the HDL diameter (\( r_{\text{mean,NMR}} \)) and HDL-C/ApoA-I ratio in the 26,772 WHS study participants using a combination scatter plot/heat map. Individual data points in the scatter plot fall on parallel horizontal lines, corresponding to \( r_{\text{mean,NMR}} \) values of 8.0, 8.1, 8.2, . . . 10.8 nm. Because many data points overlap, a heat map was used to indicate the density of data points in small rectangular regions of the scatter plot. The “hottest” region, i.e., greatest density of points, corresponds to the pink/red “cigar shape” within the larger “cloud” of data points and exhibits a positive slope. The median HDL-C/ApoA-I ratios and IQRs for data points with the same value of \( r_{\text{mean,NMR}} \) (Table 2), indicated by the diamonds and horizontal line segments, fall within the cigar-shaped region of the heat map and extend into the “cooler” region, where \( r_{\text{mean,NMR}} \) exceeds 10.1 nm. The least-squares regression line of the HDL-C/ApoA-I ratios vs \( r_{\text{mean,NMR}} \), also shown in Fig. 1, is discussed later.

### Table 2. Descriptive statistics and quartile analysis of HDL-C/ApoA-I ratios corresponding to each \( r_{\text{mean,NMR}} \) in the WHS population.

<table>
<thead>
<tr>
<th>( r_{\text{mean,NMR}} ) (nm)</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>CV (%)</th>
<th>Quartile 1 (25%)</th>
<th>Quartile 2 (50%)</th>
<th>Quartile 3 (75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>10</td>
<td>0.267</td>
<td>0.033</td>
<td>0.010</td>
<td>12.3</td>
<td>0.242</td>
<td>0.267</td>
<td>0.286</td>
</tr>
<tr>
<td>8.1</td>
<td>178</td>
<td>0.291</td>
<td>0.047</td>
<td>0.004</td>
<td>16.1</td>
<td>0.271</td>
<td>0.287</td>
<td>0.303</td>
</tr>
<tr>
<td>8.2</td>
<td>582</td>
<td>0.296</td>
<td>0.036</td>
<td>0.001</td>
<td>12.1</td>
<td>0.277</td>
<td>0.297</td>
<td>0.313</td>
</tr>
<tr>
<td>8.3</td>
<td>1085</td>
<td>0.303</td>
<td>0.041</td>
<td>0.001</td>
<td>13.4</td>
<td>0.282</td>
<td>0.302</td>
<td>0.321</td>
</tr>
<tr>
<td>8.4</td>
<td>1441</td>
<td>0.305</td>
<td>0.040</td>
<td>0.001</td>
<td>13.2</td>
<td>0.284</td>
<td>0.304</td>
<td>0.326</td>
</tr>
<tr>
<td>8.5</td>
<td>1620</td>
<td>0.314</td>
<td>0.041</td>
<td>0.001</td>
<td>13.2</td>
<td>0.291</td>
<td>0.313</td>
<td>0.334</td>
</tr>
<tr>
<td>8.6</td>
<td>1793</td>
<td>0.319</td>
<td>0.043</td>
<td>0.001</td>
<td>13.6</td>
<td>0.294</td>
<td>0.319</td>
<td>0.341</td>
</tr>
<tr>
<td>8.7</td>
<td>1884</td>
<td>0.326</td>
<td>0.046</td>
<td>0.001</td>
<td>13.9</td>
<td>0.302</td>
<td>0.327</td>
<td>0.350</td>
</tr>
<tr>
<td>8.8</td>
<td>1929</td>
<td>0.334</td>
<td>0.048</td>
<td>0.001</td>
<td>14.5</td>
<td>0.309</td>
<td>0.333</td>
<td>0.357</td>
</tr>
<tr>
<td>8.9</td>
<td>2023</td>
<td>0.343</td>
<td>0.050</td>
<td>0.001</td>
<td>14.6</td>
<td>0.318</td>
<td>0.343</td>
<td>0.368</td>
</tr>
<tr>
<td>9.0</td>
<td>1953</td>
<td>0.351</td>
<td>0.051</td>
<td>0.001</td>
<td>14.5</td>
<td>0.326</td>
<td>0.351</td>
<td>0.375</td>
</tr>
<tr>
<td>9.1</td>
<td>1991</td>
<td>0.358</td>
<td>0.050</td>
<td>0.001</td>
<td>14.0</td>
<td>0.332</td>
<td>0.358</td>
<td>0.383</td>
</tr>
<tr>
<td>9.2</td>
<td>1840</td>
<td>0.367</td>
<td>0.051</td>
<td>0.001</td>
<td>13.8</td>
<td>0.342</td>
<td>0.367</td>
<td>0.392</td>
</tr>
<tr>
<td>9.3</td>
<td>1723</td>
<td>0.376</td>
<td>0.053</td>
<td>0.001</td>
<td>14.1</td>
<td>0.350</td>
<td>0.376</td>
<td>0.401</td>
</tr>
<tr>
<td>9.4</td>
<td>1587</td>
<td>0.383</td>
<td>0.051</td>
<td>0.001</td>
<td>13.3</td>
<td>0.357</td>
<td>0.382</td>
<td>0.409</td>
</tr>
<tr>
<td>9.5</td>
<td>1318</td>
<td>0.394</td>
<td>0.053</td>
<td>0.001</td>
<td>13.5</td>
<td>0.368</td>
<td>0.394</td>
<td>0.417</td>
</tr>
<tr>
<td>9.6</td>
<td>1096</td>
<td>0.401</td>
<td>0.057</td>
<td>0.002</td>
<td>14.1</td>
<td>0.375</td>
<td>0.402</td>
<td>0.427</td>
</tr>
<tr>
<td>9.7</td>
<td>884</td>
<td>0.409</td>
<td>0.057</td>
<td>0.002</td>
<td>14.0</td>
<td>0.382</td>
<td>0.410</td>
<td>0.438</td>
</tr>
<tr>
<td>9.8</td>
<td>656</td>
<td>0.428</td>
<td>0.059</td>
<td>0.002</td>
<td>13.8</td>
<td>0.399</td>
<td>0.426</td>
<td>0.452</td>
</tr>
<tr>
<td>9.9</td>
<td>482</td>
<td>0.432</td>
<td>0.068</td>
<td>0.003</td>
<td>15.6</td>
<td>0.403</td>
<td>0.433</td>
<td>0.460</td>
</tr>
<tr>
<td>10.0</td>
<td>305</td>
<td>0.440</td>
<td>0.065</td>
<td>0.004</td>
<td>14.7</td>
<td>0.412</td>
<td>0.440</td>
<td>0.472</td>
</tr>
<tr>
<td>10.1</td>
<td>181</td>
<td>0.445</td>
<td>0.068</td>
<td>0.005</td>
<td>15.2</td>
<td>0.412</td>
<td>0.442</td>
<td>0.473</td>
</tr>
<tr>
<td>10.2</td>
<td>88</td>
<td>0.461</td>
<td>0.054</td>
<td>0.006</td>
<td>11.7</td>
<td>0.441</td>
<td>0.462</td>
<td>0.499</td>
</tr>
<tr>
<td>10.3</td>
<td>68</td>
<td>0.465</td>
<td>0.064</td>
<td>0.008</td>
<td>13.9</td>
<td>0.430</td>
<td>0.457</td>
<td>0.501</td>
</tr>
<tr>
<td>10.4</td>
<td>18</td>
<td>0.494</td>
<td>0.050</td>
<td>0.012</td>
<td>10.1</td>
<td>0.470</td>
<td>0.486</td>
<td>0.509</td>
</tr>
<tr>
<td>10.5</td>
<td>18</td>
<td>0.496</td>
<td>0.048</td>
<td>0.011</td>
<td>9.6</td>
<td>0.474</td>
<td>0.491</td>
<td>0.528</td>
</tr>
<tr>
<td>10.6</td>
<td>11</td>
<td>0.528</td>
<td>0.079</td>
<td>0.024</td>
<td>15.0</td>
<td>0.464</td>
<td>0.539</td>
<td>0.584</td>
</tr>
<tr>
<td>10.7</td>
<td>6</td>
<td>0.515</td>
<td>0.063</td>
<td>0.026</td>
<td>12.2</td>
<td>0.511</td>
<td>0.514</td>
<td>0.554</td>
</tr>
<tr>
<td>10.8</td>
<td>2</td>
<td>0.523</td>
<td>0.053</td>
<td>0.038</td>
<td>10.2</td>
<td>0.504</td>
<td>0.523</td>
<td>0.542</td>
</tr>
</tbody>
</table>
line Supplemental Fig. S4) onto the scatter plot/heat map shown in Fig. 1. For the literature-based ranges of the parameters TG/CEcore (0–0.4) and F_ApoA-I (50%–90%) used in the model calculations, the theoretical curves are in reasonable quantitative agreement with the WHS data, yielding comparable sizes and bracketing the cigar-shaped region of the heat map. The 2 curves defined by the parameter sets TG/CEcore = 0, F_ApoA-I = 70% and TG/CEcore = 0.133, and F_ApoA-I = 60% lie closest to the center of the cigar-shaped region and have a similar orientation. Theoretical curves with higher values of TG/CEcore (>0.2) or F_ApoA-I (>70%) are shifted to the left of the cigar-shaped region, whereas curves with a lower value of F_ApoA-I (50%) are shifted to the right. It should be appreciated that the theoretical curves shown in Fig. 2 were derived independently of the WHS data, with parameter values that were taken from the literature, rather than estimated by curve fitting (see online Supplemental Table S1).

**USE OF REGRESSION EQUATIONS TO ESTIMATE HDL SIZE FROM THE HDL-C/ApoA-I RATIO IN STATES OF CETP DEFICIENCY AND PHARMACOLOGICAL INHIBITION**

In contrast to the theoretical curves, the least-squares regression line shown in Fig. 1 was empirically derived by fitting the WHS data. It passes closely to the median HDL-C/ApoA-I values within the cigar-shaped region, deviating slightly to the left at HDL sizes above
10.3 nm, where the data are sparse. The intercept and slope of the regression line are given in Eq. 1:

\[ d_{\text{WHS}} = 4.66 \text{ nm} + 12.31(\text{HDL-C/Apo-I}), \]

(1)

where \( d_{\text{WHS}} \) is measured by NMR and expressed in nanometers (\( n = 26772; r = 0.608 \)).

We assessed the validity of Eq. 1 for estimating HDL size using published data from studies of individuals with homozygous and heterozygous CETP deficiency (16) and dyslipidemic individuals treated with the CETP inhibitors torcetrapib (17), dalcetrapib (18), and anacetrapib (19). Table 3 gives the mean values of HDL-C and ApoA-I in the respective study participant groups, the calculated HDL-C/ApoA-I ratio, the estimated HDL size from Eq. 1 (\( d_{\text{WHS}} \)), and the measured mean HDL size (\( d_{\text{meas}} \)).

For individuals with homozygous and heterozygous CETP deficiency (16), the calculated HDL-C/ApoA-I ratios were 0.827 and 0.541, respectively, and the corresponding \( d_{\text{WHS}} \) values were 14.8 and 11.3 nm, in excellent agreement with the mean (SD) \( d_{\text{meas}} \) values obtained by gel permeation chromatography, i.e., 14.5 (1.0) and 11.8 (0.6) nm. For the controls the HDL-C/ApoA-I ratio was 0.355 and \( d_{\text{WHS}} \) was 9.0 nm, somewhat smaller than the measured value, 10.5 (0.1) nm (16).

For dyslipidemic individuals treated with the CETP inhibitors torcetrapib (17) and dalcetrapib (18), the calculated HDL-C/ApoA-I ratios were consistent between studies and showed the expected dose–response for dalcetrapib (Table 3). Moreover, the predicted \( d_{\text{WHS}} \) values were in very close agreement with the \( d_{\text{meas}} \) values obtained by NMR. For the more potent CETP inhibitor anacetrapib, the calculated HDL-C/ApoA-I ratio was 0.504 after treatment vs 0.284 at baseline, and the corresponding \( d_{\text{WHS}} \) values were 10.9 and 8.2 nm. Although HDL size was not measured in this long-term safety study (19), a recent short-term study (20) revealed changes in the HDL size distribution that were qualitatively consistent with our prediction.

### ESTIMATION OF HDL PARTICLE CONCENTRATION USING THE UPDATED SHEN MODEL

On the basis of the NMR method (7, 12), the mean (SD) value of the measured HDL-P in the WHS population was determined to be 35.4 (6.3) \( \mu \text{mol/L} \). As outlined in the online Supplemental Data (section 5), the updated Shen model also provides an estimate of HDL-P in each individual from the HDL size (derived from Eq. 1) and the measured ApoA-I concentration. With this approach the estimated HDL-P in the WHS population averaged 15.6 (2.8) \( \mu \text{mol/L} \). Although the
estimated and measured HDL-P concentrations differ significantly ($P < 2.2 \times 10^{-16}$), they correlate closely on an individual basis, as shown in Fig. 3, and are described by the following regression equation:

$$\text{HDL-P}_{\text{meas}} = 4.77 \mu \text{mol/L} + 0.307(\text{HDL-P}_{\text{meas}}),$$

where HDL-P\text{meas} is expressed in micromoles per liter ($r = 0.676$).

We explored the disparity between the 2 measures by using the measured and estimated HDL-P values in each study participant to compute the apparent number of ApoA-I molecules per HDL particle from the ApoA-I/HDL-P ratio. With the measured HDL-P the apparent number of ApoA-I molecules per HDL particle averaged 1.5 (0.2), and with the estimated HDL-P it averaged 3.5 (0.4) ($P < 2.2 \times 10^{-16}$). As discussed later, the latter value is more consistent with the literature on HDL structure and composition. Further graphical analysis of these findings is presented in the online Supplemental Data (section 5).

### Discussion

**BIOPHYSICAL BASIS OF THE RELATIONSHIP BETWEEN HDL SIZE AND HDL-C/ApoA-I RATIO**

The quasilinear relationship between HDL size and the HDL-C/ApoA-I ratio predicted in the updated Shen model and observed in the WHS data (Figs. 1 and 2) is related to the well-known inverse relationship between the size and density of spherical lipoproteins (21). The latter derives from the fact that the lower-density lipid components (CE and TG) are located within the spherical core of the particle, whereas the higher-density apolipoproteins are located on the particle surface. On the basis of such considerations, Brinton et al. (22) used the HDL-C/(ApoA-I + ApoA-II) ratio as a surrogate for HDL size (see the derivation in the online Supplemental Data, section 4). Similarly, Miller (23, 24) used the HDL-C/ApoA-I ratio and noted its connection to the distribution of HDL2 and HDL3 particles, and Fournier reported a strong inverse relationship between TG concentrations and the HDL-C/ApoA-I ratio (25). Most recently, Kimak et al. (26) reported a lower HDL-C/ApoA-I ratio in post–renal transplant patients that was indicative of smaller particles.

The updated Shen model predicts that the apolipoprotein content of an HDL particle will be approximately proportional to the radius of its lipid core rather than its surface area (see online Supplemental Fig. S2E). This important prediction results from the curvature of the surface monolayer and the assumption that the apolipoproteins cover the unesterified cholesterol molecules and other hydrophobic areas exposed between the polar head groups of the phospholipid molecules (11). Although the predictions of the updated Shen model are admittedly based on an oversimplification of the complex interactions between apolipoproteins, un-
esterified cholesterol, and phospholipids, the underlying assumptions of this model are strongly supported by the close correspondence of these predictions with the number of ApoA-I molecules per particle in HDL subclasses reported by Kontush and Chapman (27) (see online Supplemental Fig. S3) and with Duverger et al.’s analysis of ApoA-I–containing HDL particles (28) (see online Supplemental Fig. S2). The model of spherical HDL structure and ApoA-I conformational state recently developed by Davidson and colleagues (29) and the molecular dynamics simulation of spherical HDL by Vuorela et al. (30) further refine these concepts.

POTENTIAL RELEVANCE OF THE UPDATED SHEN MODEL TO HDL REMODELING

HDL remodeling processes, such as particle fusion, lipid transfer, lipolysis, and esterification (8, 9), alter the size and composition of HDL particles by adding or removing molecules from the lipid core and surface monolayer of the particles. The relationship between particle size and composition given in the updated Shen model may explain some key experimental findings in HDL remodeling, e.g., the in vitro observation that phospholipid transfer protein–induced fusion of small HDL particles into large HDL particles generates lipid-poor ApoA-I molecules in the medium (8). Assuming that the small particles have a diameter of 8 nm and contain 3 ApoA-I molecules, the updated Shen model predicts that fusion of 2 small particles will create a large particle of approximately 9 nm containing about 4 ApoA-I molecules (see online Supplemental Fig. S3). To be compatible with the predicted surface composition, the fusion particle must release 2 ApoA-I molecules and small amounts of phospholipid and cholesterol. Such behavior has been postulated to occur in vivo as a pathway for pre–β1 formation and is thought to be important in the process of reverse cholesterol transport (1–3, 8). Similar considerations may apply to other remodeling mechanisms (31).

POTENTIAL USE OF THE HDL-C/APOA-I RATIO FOR ESTIMATING HDL SIZE

We have shown in the case of CETP-deficient populations and individuals treated with CETP inhibitors that the HDL-C/ApoA-I ratio, in conjunction with Eq. 1, provides an alternative approach for determining mean HDL size. The merits of this approach include the relative ease and availability of measuring HDL-C and ApoA-I and the straightforward calculation. The downside relates to measurement error in these variables, and the fact that the relationship between HDL size and the HDL-C/ApoA-I ratio also depends on other compositional variables, e.g., $F_{\text{ApoA-I}}$ and $\text{TG/CE}_{\text{core}}$ (Fig. 2). On the basis of the measurement CVs of the standardized HDL-C and ApoA-I assays used in the present study, e.g., $\leq 3\%$, the variation in HDL size predicted from Eq. 1 is estimated to be $\pm 0.1–0.2$ nm. From the cigar-shaped region of the heat map, the variation in HDL size corresponding to a given HDL-C/ApoA-I ratio is approximately $\pm 0.4$ nm, presumably because of the variation in $F_{\text{ApoA-I}}$ and $\text{TG/CE}_{\text{core}}$. Use of Brinton’s ratio (20), HDL-C/(ApoA-I + ApoA-II), would be expected to decrease some of this variability (see the online Supplemental Data, section 4).

POTENTIAL LIMITATIONS OF THE PRESENT STUDY

A potential limitation of this study was its focus on mean HDL size, rather than on HDL subclasses. In principle the updated Shen model can represent the relationship between size and composition of each HDL subclass, as we have done to simulate the effects of polydispersity on the relationship between $d_{\text{mean,NMR}}$ and HDL-C/ApoA-I (see the online Supplemental Data, section 3). Although these effects are expected to be small, examinations of the relationship between HDL size and HDL-C/ApoA-I ratio can be performed on fractionated HDL subclasses directly. Moreover, the relevance of mean HDL size as a biomarker of HDL metabolism, pathophysiology, and/or treatment response remains to be investigated in future experimen-
tal and theoretical studies. From the experimental perspective, a limitation of the NMR method is that it provides only an indirect measure of HDL size, calibrated by gradient gel electrophoresis (5, 7, 12, 32). Exploration of the updated Shen model using more direct methods for measuring HDL size would be valuable (5, 20, 33). Lastly, it should be noted that data on TG/CE core, F_ApoA-I, and ApoA-II were not available in the WHS study (13) to investigate the influence of these variables on HDL size.

A NEW APPROACH FOR ESTIMATING HDL PARTICLE CONCENTRATION
We have shown how one may use the HDL size (inferred from the HDL-C/ApoA-I ratio) and ApoA-I concentration to estimate HDL-P. The values of HDL-P obtained in this manner correlate with the NMR measurements of total HDL-P but are approximately 50%–60% lower. Using the HDL-P values obtained by both approaches, we have calculated the apparent number of ApoA-I molecules per HDL particle and have found that our new approach provides a result that is more concordant with literature values (27) for the number of ApoA-I molecules contained in HDL particles of different sizes (see online Supplemental Fig. S6). Further studies are needed to confirm this finding and reassess the predictive value of the estimated HDL-P values to cardiovascular risk, as recently reported for HDL-P values obtained by NMR in the MESA (Multi-Ethnic Study of Atherosclerosis) study (34).

RELATIONSHIP BETWEEN THE HDL-C/ApoA-I RATIO AND HDL SIZE TO CVD RISK
Miller et al. (23, 24) previously reported that the HDL-C/ApoA-I ratio was lower in patients with coronary heart disease compared to controls, suggesting that smaller mean HDL size may be associated with greater CVD risk. In a previous analysis of the WHS study (13), lower HDL size (measured by NMR) was also associated with greater CVD risk. In contrast, van der Steeg et al. (35) suggested that after controlling for ApoA-I and Apo-B concentrations, CVD risk in the European Prospective Investigation of Cancer (EPIC) study may actually increase with larger HDL size. In a later analysis of the EPIC study, Arsenault et al. (36) found no adverse effect of large HDL size after controlling for standard CVD risk factors (including diabetes) and HDL-C concentrations. In recent analyses of the WHS study, mean HDL size was found to be a stronger independent predictor of incident hypertension and diabetes than HDL-C (37, 38). Further research is needed to clarify the precise relationship between HDL size and CVD risk. In the absence of HDL size measurements, the HDL-C/ApoA-I ratio may be a useful surrogate biomarker for HDL size or used to estimate HDL size by Eq. 1.

References


33. Assaf BS, Schaefer EJ. High-density lipoprotein subclass populations in pathologic conditions. Am J Cardiol 2003;91:12E–17E.


