Increased Prevalence of Smaller and Denser LDL Particles in Asian Indians

Krishnaji R. Kulkarni, Jerome H. Markovitz, Navin C. Nanda, Jere P. Segrest

Abstract—There is increasing evidence to believe that Asian Indians are at an increased risk of coronary heart disease (CHD), which cannot be attributed to the common risk factors. Individuals with small, dense LDL phenotype are also known to be at increased risk of CHD. Our objective was to examine whether the prevalence of smaller and denser LDL particles is increased in Asian Indians. Thirty-nine Asian Indians (22 men and 17 women), aged 25 to 45 years, were matched with 39 whites for age and gender. Cholesterol profiles of lipoprotein classes and LDL subclasses were measured using the Vertical Auto Profile–II (VAP-II) and LDL-VAP-II methods, respectively. Six LDL subclasses (LDL1 to LDL6) have been identified using the LDL-VAP-II, with LDL1 and LDL6, respectively, being the most and least buoyant subclasses. The prevalence of small, dense LDL type (subjects with major LDL subclass 5 or 6) was significantly higher in Asian Indians compared with white subjects (44% versus 21%; \(P<0.05\)). The relative position of the major LDL density peak (LDL-Rf) on 0 to 1 scale in LDL-VAP-II density gradient was also significantly decreased in Asian Indians (0.462 ± 0.076 versus 0.505 ± 0.086; \(P<0.02\)), suggesting an increased LDL density. Furthermore, this increased prevalence of small, dense LDL type appears to be due to the increased triglycerides (TG) (\(r\) for LDL-Rf versus TG = 0.681, \(P<0.001\)), with fasting insulin being one of the important determinants of TG (\(r\) for TG versus fasting insulin = 0.572, \(P<0.001\)). In addition, fasting insulin was significantly increased in Asian Indians with small LDL type compared with other Asian Indians, suggesting a significant role of insulin resistance in increasing the prevalence of small, dense LDL type. We conclude that the increased prevalence of small, dense LDL observed in Asian Indians might contribute to their increased CHD risk. (Arterioscler Thromb Vasc Biol. 1999;19:2749-2755.)

Key Words: Asian Indians ■ small, dense LDL ■ insulin resistance ■ CHD risk

Sev eral epidemiological studies have shown that Asian Indians living outside India have an increased risk of coronary heart disease (CHD).1–5 CHD in this ethnic group is several-fold greater than many other ethnic groups known to have an increased prevalence of CHD.6 Although mortality due to CHD in many western countries generally declined in the last 2 decades, it has increased in immigrant Asians. Increased CHD incidence in immigrant Asian Indians in several countries such as UK,7 Malaysia,8 Trinidad,9 South Africa,10 and the United States11 have been reported.

The prevalence of classical risk factors such as total cholesterol (TC), LDL cholesterol (LDL-C), hypertension, and smoking are no more than in the western population.1 It appears, therefore, that the elevated CHD in Asian Indians may require an explanation by the uncommon risk factors. In this regard, we have recently reported increased platelet activation in Asian Indians, which might be a potential CHD risk factor.12 Some studies have also suggested that the increased CHD risk in Asian Indians may be due to a genetic predisposition and only accentuated by the westernized lifestyle.13 Thus, as a result of several studies, it is now believed that elevated lipoprotein(a) [Lp(a)], combined with a relatively elevated LDL caused by the western lifestyle in immigrants, is the primary cause of accelerated CHD in this population.6 Lp(a), whose plasma concentration is genetically determined, has proven to be a strong risk factor.14 Enas has proposed that the “lipid tetrad” consisting of Lp(a) concentration in the presence of high LDL cholesterol [augmenting pathological effects of Lp(a)], high triglycerides (TG), and low HDL cholesterol (HDL-C) best explains this increased propensity in Asian Indians.15 However, insulin resistance accompanied by a compensatory hyperinsulinemia as ascertained by increased fasting insulin and impaired glucose tolerance has been a more common finding in Asian Indians.1 Subjects with insulin resistance are characterized by an interrelated cluster of metabolic abnormalities that include higher fasting plasma TG, lower HDL-C, an enhanced degree of postprandial lipemia, increased levels of plasminogen activator inhibitor-1, hyperuricemia, and hypertension, all of which increase the risk of CHD.16 Recent findings also suggest that subjects with the predominance of small, dense LDL have higher TG and lower HDL than other types of LDL.17 These findings suggest that increased insulin resistance may be accompanied by increased levels of small, dense LDL particles, thus possibly increasing CHD in Asian Indians.18
HDL-C and HDL2-C concentrations, similar to the levels seen in subjects with insulin resistance and or hyperinsulinemia. Therefore, it appears that there is a close association of small, dense LDL with the insulin resistance. There is now an increasing evidence that suggests that small, dense LDL is a strong risk factor for CHD. Subjects with this phenotype are indeed at up to a 3-fold increased risk of myocardial infarction. Although this risk is usually attributed to the close association of small, dense LDL with low HDL-C and high TG, evidence is emerging regarding an independent role for small, dense LDL in atherosclerosis.

In the current report, we present evidence for increased prevalence of these potentially atherogenic LDL particles in Asian Indians.

Methods
Subjects
Thirty-nine Asian Indians, aged 25 to 45 years, and 39 whites residing in Birmingham, AL, matched for age (±3 years) and gender, were recruited by local advertisements and word of mouth. Each group consisted of 22 men and 17 women. Subjects were asked whether they are diabetic and those who reported diabetes were excluded from the study. All subjects were currently nonsmokers and were not taking lipid- or glucose-lowering or antihypertensive medications. However, 4 subjects were taking hormones or drugs, 3 white women were taking birth-control pills (1 woman with estrogen only and the other 2 with estrogen/progesterone combinations), and one Asian Indian woman was taking thyroid replacement medication. Data analysis performed by excluding these 4 subjects yielded similar results as compared with the analysis performed by including them, and hence, results obtained from all subjects are reported here. Of the 39 Asian Indians, 15 were completely vegetarians, whereas others consumed variable numbers of vegetarian-only meals in a week. All Asian Indians except one were first generation Asian Indians with an average stay of 9.6±6.3 years in the United States. Exclusion of the second generation Asian Indian from the data analysis did not change the results significantly, and hence, the data from this subject were also included for the final analysis. Informed consent was obtained from all subjects, and the study was approved by the Institutional Review Board of the University of Alabama at Birmingham.

Measurements
Subjects were tested after an overnight fast of at least 10 hours. After height, weight, and blood pressure (systolic blood pressure [SBP] and diastolic blood pressure [DBP]) were recorded, a venous blood sample was drawn into an ethylenediamine tetraacetic acid-containing (1 mg/mL) tube for the measurement of lipoproteins, LDL subclasses, and other risk factors, including fasting insulin and TG.

Lipoproteins
A complete lipoprotein cholesterol profile, which includes the plasma total cholesterol, HDL-C, LDL-C, LDL-H, LDL(a), LDL-R-C [LDL-real cholesterol], the LDL cholesterol without the HDL-C and Lp[(a)-cholesterol], IDL-C, and VLDL-C for each subject, was determined using the Vertical Auto Profile-II (VAP-II) methodology, developed in our laboratory. VAP-II is a single test direct measurement method, with no estimations involved. It is based on a combination of a rapid (45 minutes) single vertical spin ultracentrifugation and a novel continuous flow enzymatic cholesterol analyzer. VAP-II is highly sensitive (requiring only ≤40 μL of plasma) and reproducible. Furthermore, it separates the commonly measured LDL-National Cholesterol Education Program [LDL(NCEP)] into LDL-R, Lp[(a)- and IDL. Thus both the LDL-C (as commonly reported by the clinical laboratories) and its individual components (LDL-R-C, Lp[(a)-C, and IDL-C) are measured by the VAP-II method, making it amenable to compare with other commonly used LDL-C measurement methods.

LDL Subclass Measurement
Analysis of LDL subclass cholesterol profile was performed using the LDL-VAP-II method. LDL-VAP-II is a modification of VAP-II procedure designed to separate LDL subclasses. Six LDL subclasses (LDL1 to LDL6) have been previously identified using this method. The LDL-VAP-II computer deconvolution software developed in our laboratory also provides cholesterol concentrations of the 6 subclasses, while assigning the LDL subclass with the highest cholesterol concentration as the major LDL subclass. We have previously compared LDL subclass classification by the LDL-VAP-II procedure with the classification by 2% to 16% nondenaturing polyacrylamide gradient gel electrophoresis. LDL1 and LDL2 correspond well with LDL pattern A, LDL3 and LDL4 correspond with LDL pattern B or I (intermediate LDL pattern), and LDL5 and LDL6 correspond with LDL pattern B. Thus subjects with LDL1 or LDL2, LDL3 or LDL4, and LDL5 or LDL6 as the major subclass are, respectively, classified as having large, buoyant; intermediate density; and small, dense LDL types.

A continuous variable called LDL-Rf, which is a measure of the relative position of the major LDL peak (and hence its density) on a relative scale of 0 to 1 (with 0 and 1, respectively, corresponding to the origin of HDL peak and the LDL peak maximum in a LDL-VAP-II density gradient), was also calculated. In a study using 32 plasma samples, we found a correlation coefficient of 0.86 between LDL-Rf obtained by the LDL-VAP-II method and the major LDL peak particle diameter obtained by nondenaturing 2% to 16% polyacrylamide gradient gel electrophoresis. LDL-VAP-II is also highly sensitive requiring only 70 μL of plasma sample. Furthermore, it is also highly reproducible, with within-rotor coefficient of variation of cholesterol concentration of major LDL subclass ranging from 1.9% to 8.3% and between-rotor coefficient of variation of 5.2%.

Other Measurements
TG were measured using enzymatic assay, and fasting insulin was measured using immunoassay.

Statistical Analysis
All statistical calculations were performed using Jandel Scientific SigmaStat statistical software 2.0. Mean values of all variables are reported as mean (standard deviation). Z-test was used to test the significance of differences in prevalence of small, dense LDL type between groups. Because many variables used in this study are known to be non-normally distributed, normality test (Kolmogorov-Smirnov) was performed on all variable values. The data set that did not meet the normal distribution criteria (P<0.05) was transformed either to log10 or reciprocal values to achieve normality. Two-way ANOVA with ethnicity and gender as influencing factors was used to compare mean values of lipoproteins and other risk factors in subjects grouped by ethnicity and gender. As suggested by the software, Tukey’s all pairwise multiple comparison test was used to determine the groups that are different. Spearman’s rank order correlation method was used to assess the strengths of association between dependent and independent variables. Both multiple linear regression analysis and forward stepwise linear regression analysis methods were used to predict the variables that independently and significantly contributed to a dependent variable under consideration. Results with P<0.05 were considered statistically significant.

Results
Lipoproteins and Other Risk Factors in Asian Indians
Mean values of cholesterol concentrations of plasma, HDL, Lp[(a)- and LDL(NCEP) and of some other risk factors in this group of Asian Indian and white subjects have been reported by us previously. As both ethnicity and gender may affect levels of lipoproteins and their subclasses, as well as several other risk factors, we compared the mean values of these risk factors obtained by classifying all subjects into 4 groups based on ethnicity and gender (ie, Asian Indian men, Asian
Indian women, white men, and white women). Two-way ANOVA, with ethnicity and gender as 2 influencing factors, was used for this purpose. In addition, the interaction term between ethnicity and gender was also assessed for all variables. The summary of the results is shown in Table 1. Both ethnicity and gender independently and significantly influenced HDL-C, LDL2-C, HDL3-C, and SBP. However, there was no significant interaction between them. Asian Indians, both men and women, had significantly lower mean values of these variables than the corresponding values for white subjects. Only ethnicity significantly influenced VLDL-C and TG, with higher values in Asian Indians.

## Prevalence of Small, Dense LDL

Each subject was classified into 1 of 6 groups based on his/her major LDL subclass (LDL subclass 1 through 6) as determined from the LDL-VAP-II. Significantly higher number of Asian Indians had either LDL subclass 5 or 6 as the major subclass, which represent the small, dense LDL type, compared with the white subjects (17 Asian Indians versus 8 whites; \textit{P}<0.05). However, although statistically nonsignificant, more white subjects had either LDL subclass 1 or 2, which represent the large, buoyant LDL type, compared with Asian Indians (10 whites versus 5 Asian Indians; \textit{P}=0.25). Seventeen Asian Indians and 21 whites had either LDL subclass 3 or 4, which represent the intermediate density LDL type (\textit{P}=0.224). Forty eight percent of Asian Indian men and 41% of Asian Indian women had small, dense LDL type. The prevalence rates of small, dense LDL type for white men and women in our study (30% and 11%, respectively) were similar to the respective rates observed in the white population in the United States (men, 30% to 35%; women, 5% to 10%).28

### Association of LDL Density with Lipoproteins and Other Risk Factors

The results of the association of LDL-Rf, which is an inverse correlate of LDL density, with lipoproteins and other risk factors in Asian Indians and whites obtained by Spearman’s rank order correlation method are summarized in Table 2. HDL-C, HDL3-C, and HDL2-C were positively and significantly correlated with LDL-Rf in both groups, whereas TC, LDL-R-C, IDL-C, LDL(NCEP)-C, VLDL-C, and TG correlated negatively and significantly with LDL-Rf in both groups. However, all correlation coefficients for the above

### TABLE 1. Comparison of Mean (Standard Deviation) Values of Lipoproteins and Other Risk Factors Between Asian Indians and Whites Classified Based on Ethnicity and Gender Using 2-Way ANOVA

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Asian Indians</th>
<th>Whites</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>4.44 (0.83)</td>
<td>4.68 (0.80)</td>
<td>ns</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.90 (0.21)</td>
<td>1.09 (0.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp(a)-C</td>
<td>0.14 (0.10)</td>
<td>0.18 (0.10)</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-R-C</td>
<td>2.50 (0.67)</td>
<td>2.54 (0.60)</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.34 (0.15)</td>
<td>0.37 (0.12)</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-Rf**</td>
<td>0.453 (0.07)</td>
<td>0.478 (0.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>35.7 (6.8)</td>
<td>34.5 (5.8)</td>
<td>ns</td>
</tr>
<tr>
<td>SBP</td>
<td>115.6 (6.7)</td>
<td>119.9 (9.0)</td>
<td>0.047</td>
</tr>
<tr>
<td>DBP</td>
<td>77.8 (9.0)</td>
<td>75.6 (8.1)</td>
<td>ns</td>
</tr>
<tr>
<td>BMI</td>
<td>23.6 (3.9)</td>
<td>24.1 (2.6)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Cholesterol and triglyceride values are reported as mmol/L; to convert to mg/dL multiply cholesterol and TG values by 38.69 and 88.57, respectively. The insulin values were obtained from fasting plasma and are reported as \(\mu\)g/mL; to convert to pmol/L divide by 0.139. The mean values for age, SBP and DBP, and BMI are reported, respectively, in years, mm Hg and kg/m\(^2\).

*LDL-C indicates LDL(NCEP)-C; **LDL-Rf, the relative position of the major LDL peak on 0 to 1 scale (with 0 and 1, respectively, corresponding to the origin of HDL peak and the VLDL peak maximum) in LDL-VAP-II density gradient (the lower the LDL-Rf the higher the density of the major LDL peak); ns, nonsignificant (\textit{P}>0.05). Female =1, male =0 and Asian Indian =1, White =0 values were used for gender and ethnicity in ANOVA calculations.

The values for IDL-C, VLDL-C, TG, HDL2-C, insulin, age, SBP, and DBP were log10 transformed, and values for BMI were transformed to reciprocal values for the purpose of calculations. However, they were back transformed to their normal values to report in this table.
Variables, except for TG and VLDL-C, were somewhat higher with stronger statistical significance levels for the white group than for the Asian Indian group. Fasting insulin was negatively and significantly correlated ($P<0.001$) with LDL-Rf only in Asian Indians. Among other measurements, gender was positively (females having higher LDL-Rf) and SBP was negatively associated with LDL-Rf only in white subjects.

**Association of Fasting Insulin with Lipoproteins and Other Risk Factors**

The strengths of the association (correlation coefficients) of fasting insulin with lipoproteins and other risk factors obtained from Spearman’s rank order correlation method are shown in Table 3. Insulin was positively correlated with TC, LDL-R-C, LDL(NCEP)-C, VLDL-C, TG, and BMI in Asian Indians. As mentioned previously insulin was also negatively correlated with LDL-Rf in Asian Indians. On the other hand, insulin was correlated (positively) only with LDL-R-C and BMI in white subjects.

**Multiple Linear Regression Analysis**

To determine the variables that independently predicted LDL-Rf and TG, multiple linear regression analysis was used. The results of the analysis are shown in Table 4. To assess whether ethnicity is an independent predictor of LDL-Rf and TG, subjects from both groups were combined and ethnicity, gender, Lp(a)-C, LDL-R-C, IDL-C, TG, HDL3-C, HDL2-C, fasting insulin, age, SBP, DBP, and BMI were used as independent variables. As mentioned in the Methods section, transformed values were used when the distribution of the variable was non-normal.

When LDL-Rf was entered as a dependent variable in the combined group, TG, HDL2-C, and LDL-R-C remained as significant and independent predictors of LDL-Rf, whereas ethnicity and fasting insulin did not. However, when TG was entered as a dependent variable, ethnicity, along with fasting insulin, HDL2-C, and VLDL-C, remained as significant and independent predictors of TG, with higher TG concentrations associated with Asian Indians. Thus, although ethnicity and fasting insulin were not independent predictors of LDL-Rf, they were independent and strong predictors of TG ($P=0.003$ for ethnicity and $P=0.012$ for fasting insulin), which in turn was an independent predictor of LDL-Rf. To determine the independent predictors of TG within Asian Indians and whites, the above analysis was performed separately in Asian Indians and whites. Insulin, VLDL-C, and HDL2-C remained as independent predictors in Asian Indians, whereas only VLDL-C independently predicted TG in whites.

Forward stepwise linear regression analysis was also performed on the above data to verify our results. The results obtained were similar to the results obtained from multiple linear regression analysis (data not shown).

**LDL Subclasses and Fasting Insulin Levels**

When both Asian Indians and white subjects were classified based on their major LDL subclasses into large and buoyant LDL type, intermediate density LDL type, and small and dense LDL type, fasting insulin was significantly increased only in Asian Indian group with small, dense LDL type ($P<0.001$). The fasting insulin levels ($\mu$U/mL) in these groups were as follows: Asian Indians—large, buoyant LDL type, $5.54 \pm 1.84$; intermediate density LDL type, $5.55 \pm 2.12$; small, dense LDL type, $14.36 \pm 1.33$. Whites—large, buoyant LDL type, $12.21 \pm 12.65$; intermediate density LDL type, $6.6 \pm 2.05$; and small, dense LDL type, $9.94 \pm 7.58$.

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**TABLE 2. Spearman Rank Order Correlation Coefficients Between LDL-Rf* and Lipoproteins and Other Risk Factors in Asian Indian and White Subjects**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Asian Indians</th>
<th>$P$</th>
<th>Whites</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>$-0.437$</td>
<td>0.005</td>
<td>$-0.45$</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL-C</td>
<td>$0.453$</td>
<td>0.004</td>
<td>$0.678$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Lp(a)-C</td>
<td>$0.183$</td>
<td>ns</td>
<td>$0.131$</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-R-C</td>
<td>$-0.419$</td>
<td>0.007</td>
<td>$-0.557$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IDL-C</td>
<td>$-0.429$</td>
<td>0.006</td>
<td>$-0.546$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>LDL(NCEP)-C</td>
<td>$-0.433$</td>
<td>0.006</td>
<td>$-0.566$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>$-0.586$</td>
<td>$&lt;0.001$</td>
<td>$-0.60$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>TG</td>
<td>$-0.681$</td>
<td>$&lt;0.001$</td>
<td>$-0.574$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Insulin**</td>
<td>$-0.565$</td>
<td>$&lt;0.001$</td>
<td>$-0.092$</td>
<td>ns</td>
</tr>
<tr>
<td>HDL3-C</td>
<td>$0.375$</td>
<td>0.017</td>
<td>$0.518$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>HDL2-C</td>
<td>$0.512$</td>
<td>$&lt;0.001$</td>
<td>$0.683$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Age (y)</td>
<td>$-0.16$</td>
<td>ns</td>
<td>$-0.045$</td>
<td>ns</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>$-0.284$</td>
<td>ns</td>
<td>$-0.505$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>$-0.271$</td>
<td>ns</td>
<td>$-0.06$</td>
<td>ns</td>
</tr>
<tr>
<td>Gender</td>
<td>$0.052$</td>
<td>ns</td>
<td>$0.347$</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>$-0.206$</td>
<td>ns</td>
<td>$-0.061$</td>
<td>ns</td>
</tr>
</tbody>
</table>

*LDL-Rf indicates the relative position of the major LDL peak on 0 to 1 scale (with 0 and 1, respectively, corresponding to the origin of HDL peak and the VLDL peak maximum) in LDL-VAP-II density gradient (the lower the LDL-Rf the higher the density of the major LDL peak). **Insulin values were obtained from fasting plasma; ns indicates nonsignificant ($P>0.05$). Female = 1 and male = 0 values were used for calculations involving gender.

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**TABLE 3. Spearman Rank Order Correlation Coefficients Between Fasting Insulin and Lipoproteins and Other Risk Factors in Asian Indian and White Subjects**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Asian Indians</th>
<th>$P$</th>
<th>Whites</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>$0.379$</td>
<td>0.019</td>
<td>$0.285$</td>
<td>ns</td>
</tr>
<tr>
<td>HDL-C</td>
<td>$-0.224$</td>
<td>ns</td>
<td>$-0.087$</td>
<td>ns</td>
</tr>
<tr>
<td>Lp(a)-C</td>
<td>$-0.104$</td>
<td>ns</td>
<td>$-0.197$</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-R-C</td>
<td>$0.364$</td>
<td>0.025</td>
<td>$0.353$</td>
<td>0.03</td>
</tr>
<tr>
<td>IDL-C</td>
<td>$0.186$</td>
<td>ns</td>
<td>$0.118$</td>
<td>ns</td>
</tr>
<tr>
<td>LDL(NCEP)-C</td>
<td>$0.374$</td>
<td>0.02</td>
<td>$0.293$</td>
<td>ns</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>$0.348$</td>
<td>0.033</td>
<td>0.109</td>
<td>ns</td>
</tr>
<tr>
<td>TG</td>
<td>$0.572$</td>
<td>0.001</td>
<td>$0.233$</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-Rf</td>
<td>$-0.565$</td>
<td>$&lt;0.001$</td>
<td>$-0.092$</td>
<td>ns</td>
</tr>
<tr>
<td>HDL3-C</td>
<td>$-0.222$</td>
<td>ns</td>
<td>$-0.044$</td>
<td>ns</td>
</tr>
<tr>
<td>HDL2-C</td>
<td>$-0.245$</td>
<td>ns</td>
<td>$-0.106$</td>
<td>ns</td>
</tr>
<tr>
<td>Age (years)</td>
<td>$0.251$</td>
<td>ns</td>
<td>$0.314$</td>
<td>0.055 (ns)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>$0.032$</td>
<td>ns</td>
<td>$0.161$</td>
<td>ns</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>$0.149$</td>
<td>ns</td>
<td>$0.063$</td>
<td>ns</td>
</tr>
<tr>
<td>Gender</td>
<td>$-0.044$</td>
<td>ns</td>
<td>$-0.103$</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>$0.324$</td>
<td>0.05</td>
<td>$0.39$</td>
<td>0.016</td>
</tr>
</tbody>
</table>

ns indicates nonsignificant ($P>0.05$); female = 1 and male = 0 values were used in the calculations involving gender.
TABLE 4. Results of Multiple Linear Regression Analysis With LDL-Rf and Triglycerides as Dependent Variables

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Dependent Variable</th>
<th>Independent Variables</th>
<th>Variables Remaining in Model</th>
<th>P</th>
<th>Standardized Regression Coefficient</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>LDL-Rf</td>
<td>Ethnicity, Gender, Lp(a)-C, LDL-R-C, IDL-C, TG*, HDL3-C, HDL2-C*, Insulin*, Age*, SBP*,</td>
<td>LDL2-C</td>
<td>&lt;0.001</td>
<td>0.430</td>
<td>0.621</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INSulin†, BMI†</td>
<td>LDL-R-C</td>
<td>0.038</td>
<td>−0.194</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TG</td>
<td>0.04</td>
<td>−0.259</td>
<td></td>
</tr>
<tr>
<td>Asian Indians</td>
<td>TG</td>
<td>Ethnicity, Gender, Lp(a)-C, LDL-R-C, IDL-C, LDL-Rf, HDL3-C, HDL2-C*, VLDL-C*, Insulin*,</td>
<td>VLDL-C</td>
<td>&lt;0.001</td>
<td>0.846</td>
<td>0.874</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age*, SBP, DBP, BMI†</td>
<td>HDL2-C</td>
<td>0.003</td>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HDL2-C</td>
<td>0.01</td>
<td>−0.205</td>
<td></td>
</tr>
<tr>
<td>Whites</td>
<td>TG</td>
<td>Gender, Lp(a)-C, LDL-R-C, IDL-C, LDL-Rf, HDL3-C, HDL2-C†, VLDL-C*, Insulin*, Age*, SBP*,</td>
<td>VLDL-C</td>
<td>&lt;0.001</td>
<td>0.814</td>
<td>0.842</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age, SBP, DBP, BMI</td>
<td>HDL2-C</td>
<td>0.003</td>
<td>0.351</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Insulin†, BMI†</td>
<td>0.006</td>
<td>0.278</td>
<td></td>
</tr>
</tbody>
</table>

LDL-Rf indicates the relative position of the major LDL peak on 0 to 1 scale (with 0 and 1, respectively, corresponding to the origin of HDL peak and the VLDL peak maximum) in LDL-VAP-II gradient (the lower the LDL-Rf the higher the density of the major LDL peak). *Log10 transformed values; †reciprocal transformed values; R² adjusted for number of variables. Asian Indian = 1, whites = 0 values for ethnicity and female = 1, male = 0 values for gender were used for calculations.

Discussion

Our results suggest that the prevalence of small, dense LDL type is significantly increased in Asian Indians, who are generally characterized by the atherogenic lipoprotein profile (ie, lower HDL-C and their subfractions and higher TG), compared with the age- and gender-matched white subjects. This is further supported by a significantly lower LDL-Rf (and hence a higher LDL density) in Asian Indians compared with the white subjects. The increase in the prevalence of small, dense LDL type was particularly notable in Asian Indian women compared with the white women.

Few studies have examined the prevalence of small, dense LDL in Asian Indians. Abate et al reported an increased LDL size (mean±SD; 267.7±11.4 A° versus 260.1±12.8 A°) and an increased frequency of LDL pattern A (85% versus 59%) and a lower frequency of LDL pattern B (15% versus 41%) (P=0.0005) in Asian Indians (n=93) compared with whites (n=59). The differences in the results between the 2 studies may be explained by the differences in the study design and objectives. The primary objective of Abate et al’s study was to determine whether the prevalence of small, dense LDL in Asian Indians is caused by a genetical predisposition. Thus they matched their Asian Indian subjects with controls not only for age, gender, and BMI but also for the lipid and lipoprotein concentrations, including TC, TG, HDL-C, LDL-C, and VLDL-C. Furthermore, selection of Asian Indian subjects in their study was specific and confined to only physicians, and in addition, women were not included. In contrast, our objective was to determine the prevalence of small, dense LDL in a cross-sectional population of Asian Indians that included both men and women. The authors conclude that metabolic factors may be overwhelming genetical factors resulting in lower HDL-C and higher TG, generally observed in other studies involving Asian Indians. Lower HDL-C and higher TG are known to be closely associated with small, dense LDL, and thus the higher prevalence of small, dense LDL observed in our study agrees with the lower HDL-C and higher TG seen in our Asian Indian group.

In another study reported recently that involved 52 British Indian Asian men (Punjabi Sikhs) and 52 British whites, small, dense LDL was measured in a subset consisting of 8 myocardial infarction (MI) survivors and 9 controls of British Indian Asian men and 9 MI survivors and 8 controls of British whites. Small, dense LDL concentration was increased in British Indian Asian control subjects compared with the British white controls (44% versus 22% of the total LDL concentration) in this subset. Surprisingly, small, dense LDL was significantly lower in British Indian Asian MI survivors compared with their control subjects.

As found in many other studies, which involved different populations, small, dense LDL (ie, LDL with lower LDL-Rf) was also correlated with several lipoprotein and other risk factors in both groups of our study, particularly a positive correlation between LDL-Rf and HDL and a negative correlation between LDL-Rf and TG. In addition, LDL(NCEP)-C correlated negatively and significantly with LDL-Rf in both groups. Although many studies have found no significant correlation between LDL(NCEP)-C and LDL size or density, our results are consistent with significant correlation found in several other studies. 27,31–34

TG, LDL-R-C, and HDL2-C, but not ethnicity and fasting insulin, independently predicted LDL-Rf in multiple regres-
sion analysis (Table 4). As TG was an important independent predictor of LDL-Rf and because of its known significant role in small, dense LDL formation, TG in turn was considered as a dependent variable separately in Asian Indians and whites in multiple linear regression analysis (Table 4). Fasting insulin, and thus insulin resistance, was one of the important and significant predictors of TG only in Asian Indians. Increased fasting insulin levels have been good indicators of insulin resistance.35 Furthermore, fasting insulin was significantly increased only in Asian Indians with small, dense LDL type compared with other Asian Indians (P<0.001). Thus, although fasting insulin was not an independent predictor of LDL-Rf in multiple linear regression analysis, the above observations suggest an indirect but a relatively more important role for fasting insulin, and thus insulin resistance, in increasing prevalence of small, dense LDL type in Asian Indians. Thus, insulin resistance, which is considered as a common feature among CHD risk factors in Asian Indians,2 appears to be present only in Asian Indians with small, dense LDL type. However, insulin resistance only partly explains the increased prevalence of small, dense LDL type, because some Asian Indians with small, dense LDL type had normal fasting insulin levels.

In agreement with most other studies, fasting insulin was not significantly correlated with LDL(NCEP)-C [the most commonly reported LDL cholesterol, which is the sum of cholesterol concentrations of LDL-R-C, Lp(a)-C, and IDL-C] in our white subjects; however, it was significantly correlated with LDL-R-C. Although the reasons are not clear, the significant correlation between fasting insulin and LDL cholesterol is not an uncommon finding. In both univariate and multivariate analyses, the Coronary Artery Disease Risk in Young Adults (CARDIA) study, which involved both black and white young adults (18 to 23 years of age), reported a strong correlation between fasting insulin and LDL cholesterol.36 In another study, although involving only young blacks (28 to 33 years of age), Falkner et al also found a significant correlation between fasting insulin and LDL cholesterol.37 Fasting insulin was also found to correlate significantly with LDL-C in 323 non-diabetic first-degree relatives of insulin dependent diabetics.38 In addition, Knight et al have observed a significant correlation between fasting insulin and LDL cholesterol.39 In small, dense LDL type and hence increased prevalence of atherogenic lipoprotein profile. Thus it is this increased atherogenicity in Asian Indian women that may account for the frequently reported elevated risk of CHD in Asian Indians when considered as a single group. The smaller sample size employed combined with the fact that our study is not a population based study in a strict sense somewhat limits extrapolation of our results to Asian Indian and white populations at large. Thus further studies involving large sample sizes of both populations are required to confirm our findings. Nevertheless, our results show a clear trend toward the increased prevalence of small, dense LDL in Asian Indians, particularly in women, which might be contributing toward the increased risk of CHD in this population.

Acknowledgments
This study was supported by grants from the National Institutes of Health (HL 34343, HL 51806, and 1K08HL02975-01). We are grateful to Dr. William Bradley for his thoughtful suggestions during the preparation of this manuscript.

References


Increased Prevalence of Smaller and Denser LDL Particles in Asian Indians
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doi: 10.1161/01.ATV.19.11.2749
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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