LDL Subclass Phenotypes and the Insulin Resistance Syndrome in Women

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Background. Low-density lipoprotein (LDL) subclass phenotype B, characterized by predominance of small, dense LDL particles, is associated with elevated plasma triglycerides and apolipoprotein B and with lower high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I. Because these abnormalities resemble the dyslipidemia of insulin resistance, we examined associations of LDL subclass phenotype with plasma insulin levels and with other aspects of the insulin resistance syndrome.

Methods and Results. LDL subclass phenotypes were determined by gradient gel electrophoresis in 682 female twins aged 30 to 91 years who participated in the second examination of the Kaiser Permanente Women Twins Study. Prevalence of phenotype B and the intermediate phenotype (I) increased strongly with age, obesity, and non–insulin-dependent diabetes. In multivariate analysis of nondiabetic women, phenotype B or I was independently associated with each aspect of the insulin resistance syndrome, including higher plasma triglycerides, waist-hip ratio, fasting and postload insulin levels, and systolic blood pressure and lower HDL cholesterol levels after adjustment for age and body mass index. The prevalence of phenotype B or I rose progressively from 5.6% in women with no manifestations of the insulin resistance syndrome to 100% in women with four syndrome components. In 25 nondiabetic, monozygotic twin pairs discordant for subclass phenotype, the twins with phenotype B (or I) had significantly higher levels of body mass index, waist-hip ratio, and systolic blood pressure than their twins with phenotype A. Thus, nongenetic variation in these risk factors is important in explaining their associations with LDL subclass phenotype.

Conclusions. Small, dense LDL is an integral feature of the insulin resistance syndrome. Nongenetic (ie, behavioral or environmental) factors are important for the expression of the phenotype and for its association with other heart disease risk factors. (Circulation 1993;88:381-387)

Key Words: • insulin resistance • hypertension • human genetics • twins • low-density lipoprotein

There is substantial variation among individuals in the size and density of low-density lipoprotein (LDL) particles (density, 1.019 to 1.063).1,2 A predominance of small, dense LDL has been associated with increased risk of coronary heart disease3-6 and with non–insulin-dependent diabetes mellitus (NIDDM).4,7 Most individuals can be classified on the basis of the particle diameter of their predominant LDL subclass, using nondenaturing gradient gel electrophoresis, into one of two LDL subclass patterns.5 LDL subclass pattern A is characterized by a predominance of larger, more buoyant LDL; LDL subclass pattern B is characterized by a predominance of smaller, denser LDL.

Two family studies, one in normolipidemic families8 and one in families with familial combined hyperlipidemia,9 suggest that subclass pattern B represents the phenotypic expression of a single genetic locus with a dominant or additive mode of inheritance and complete penetrance in premenopausal women and men below age 20 years. Recently, evidence for linkage between LDL subclass phenotype B and the LDL receptor locus on chromosome 19 has been presented.10

The presence of small, dense LDL or phenotype B is associated with a lipid profile that includes higher levels of plasma triglycerides, very low-density lipoprotein (VLDL) mass, and apolipoprotein B and lower levels of high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I.9,11,12 For this reason, the LDL subclass phenotype has been designated the atherogenic lipoprotein phenotype (ALP).10,12 The syndrome of insulin resistance,13 or syndrome X,14 features a very similar dyslipidemia and a cluster of other coronary heart disease risk factors including obesity, central body fat distribution, hypertension, and glucose intolerance. This and the association of small, dense LDL with NIDDM suggest that LDL particle diameter and LDL subclass phenotype B may be related to hyperinsulinemia or insulin resistance. Moreover, the relations of phenotype B with other component risk factors of the insulin resistance syndrome are of interest and potentially of clinical importance.
In the present report, we examine cross-sectional associations of LDL subclass phenotypes with fasting and post–glucose load insulin levels and with other coronary heart disease risk factors that have been related to insulin resistance in a large sample of adult female twins. The availability of identical twin pairs who were discordant for LDL subclass phenotype allows us to determine whether nongenetic variation in factors such as obesity is related to its expression.

**Methods**

During 1989 to 1990, 704 women (comprising 206 monozygous [MZ] and 146 dizygous [DZ] twin pairs) participated in the second examination of Kaiser Permanente Women Twins Study. These women represented 81.1% of the original cohort of 868 women assembled 12 years earlier for a study of heart disease risk factors in women.13 Median age at the second examination was 51 years (range, 30 to 91 years). All except 36 pairs (approximately 10% of the sample) were white. Zygosity was previously determined by examination of 20 polymorphic loci such that the probability of misclassification as MZ twins was <.001.14 Five twin pairs are excluded from all analyses because one twin was pregnant. Four additional pairs are excluded because insufficient plasma was available for lipid analyses in at least one of the twins.

Height (in meters) and weight (in kilograms) were obtained with subjects dressed in lightweight clothes and their shoes removed. Body mass index was calculated as weight/height squared. Waist and hip circumferences were obtained with subjects standing. Waist circumference was measured at the natural indentation of the waist, or, if this could not be readily identified, at a point midway between the iliac crest and the lowermost extension of the rib cage in the midaxillary line. Hip circumference was measured at the maximal protrusion of the buttocks. Heart rate and blood pressure were measured after the patient had been seated for 5 minutes. A standard mercury sphygmomanometer was used for blood pressure measurements. Two blood pressure recordings (right arm) were obtained 1 minute apart and averaged to obtain the values used in these analyses.

Blood for lipid measurement was collected into EDTA-containing tubes after an overnight fast. Plasma was separated within 2 hours and stored under refrigeration for a maximum of 72 hours before processing. Nondenaturing gradient gel electrophoresis was performed on the LDL fraction using 2% to 16% polyacrylamide gradient gels (Pharmacia), as previously described.2 Peak particle diameter of the major LDL subclass was determined using a calibration curve constructed from high molecular weight standards run on the same gel. Electrophoretic patterns were then examined independently by three examiners (M.A.A., R.M.K., and the laboratory technician) to assign LDL subclass phenotype (A or B). Disagreements were resolved by negotiation among the three examiners. For 11% of subjects, an intermediate phenotype (hereafter designated phenotype I) was observed. For subjects with phenotype I, either the peak particle diameter was close to the 25.5-nm cutoff with little or no skewing of the curve, or two distinct major peaks were observed.

Plasma glucose and insulin levels were measured in the fasting state and again 2 hours after a 75-g oral glucose load (Glutol, Paddock Laboratories, Minneapolis, Minn).

Glucose values were measured by the glucose oxidase method. Insulin concentrations were measured by radioimmunoassay at Smith Kline Laboratories (Van Nuys, Calif) using commercial kits (RSL kit for 57% of the women in the present sample and Pharmacia for 43%). Coefficients of variation for the assays ranged from 4.8% to 6.5% for a range of standards. The correlation coefficient between the two assays was 0.97. However, the RSL kit tended to overestimate insulin level compared with the Pharmacia kit (Dr B. Scales, Smith Kline Laboratories, personal communication). All analyses involving insulin values are therefore adjusted for the assay used by inclusion of a dichotomous variable in the model.

Subjects who reported a physician diagnosis of diabetes and current use of insulin or oral hypoglycemic medication (n=21) were classified as diabetic. Remaining subjects were classified as having diabetes (n=28), impaired glucose tolerance (n=85), or normal glucose tolerance (n=530) on the basis of fasting and 2-hour glucose values using World Health Organization criteria.18 Four subjects (from three pairs) had missing values for both fasting and postload glucose and could not be classified. These three pairs were excluded from all analyses. Eighteen additional subjects failed either to take or to retain the glucose solution. Of these 18, five had fasting glucose levels between 100 and 140 mg/dL and were classified as probably diabetic and excluded along with diabetics from most analyses. The remaining 13 subjects, whose fasting glucose was <100 mg/dL, were included and classified as having normal glucose tolerance.

**Statistical Analyses**

Subjects with LDL subclass phenotype B or I were compared with those with phenotype A for several continuous heart disease risk factors, adjusting for age, body mass index, and the insulin assay in ANCOVA models. Because twins share many measured and unmeasured influences on most traits, error terms in regression models tend to be correlated between twins.17 This lack of independence can lead to underestimation of the standard errors of regression coefficients using standard regression methods. We therefore adjusted for intrapair correlation of errors, using generalized least squares models that allowed for separate error terms for MZ and DZ twin pairs.17

To examine multiple correlates of LDL subclass simultaneously, subclass phenotype was dichotomized (A versus B) and treated as the dependent variable. Generalized estimating equations for binary outcomes,18 which are essentially logistic regression methods that account for the within-twin pair correlation in outcome (LDL subclass phenotype) resulting from shared genetic and environmental factors, were used in these analyses. Analyses were conducted twice, once using the broad definition of phenotype B, in which subjects with the intermediate phenotype (I) are grouped with those having phenotype B, and again using the narrow definition, in which those with phenotype I are grouped with those having phenotype A.8,12
Bar graphs show prevalence of low-density lipoprotein subclass phenotypes B and I by decade of age (a) and diabetes status (b) in 682 women twins and by tertile of body mass index (BMI) (c) and tertile of waist-hip ratio (d) in the 628 nondiabetic women. IGT, impaired glucose tolerance.

The peak particle diameter of the major LDL subclass was also examined as a continuous dependent variable to determine whether associations noted with LDL subclass phenotype were also seen with particle diameter.

Both generalized least squares methods and the generalized estimating equations require complete data for both twins of each pair. Thus, if either twin was missing data for any variable in the models, that pair was excluded from all analyses. The only variables missing in more than three pairs were postload glucose and insulin levels, missing for at least one twin in 12 pairs. Exclusion of a total of 15 pairs left 564 women in whom multivariate models were examined.

There were 25 nondiabetic MZ twin pairs who were discordant for phenotype B using the broad definition (phenotype I’s treated as phenotype B’s). To study associations of LDL subclass phenotype and risk factors independent of genetic variation, we examined mean intrapair risk factor differences for these 25 pairs. Since MZ twins are genetically identical, all intrapair differences for both phenotype and risk factor levels must be due to nongenetic factors. If associations of phenotype with other variables persist in these analyses, these associations are also due to nongenetic sources of variation in the variables (ie, behavioral, environmental, or random effects). Intrapair differences for each risk factor were obtained by subtracting its value in the twin with phenotype B from that of the sister with phenotype A.

Associations of risk factors with peak LDL particle diameter were also evaluated using matched multiple linear regression models that included all 176 nondiabetic MZ twin pairs. In these analyses, the intrapair difference in peak particle diameter was regressed on intrapair differences in risk factors.

Results

The prevalence of phenotype B and the intermediate phenotype I were 10.4% and 10.9%, respectively, in the entire sample. The prevalence of both phenotypes B and I increased with age through the seventh decade (Figure, graph a) (P < .0001 for linear association). As previously reported,8,9 much of the association with age was apparently due to a strong association of menopausal status with phenotypic expression. The combined prevalence of phenotypes B and I was only 9.3% in premenopausal women compared with 31.2% for postmenopausal women. A very strong association of phenotype with diabetes status was also observed (Figure, graph b), with an increased prevalence of both phenotypes B and I in women with impaired glucose tolerance and a further increase in the 54 women classified as having NIDDM. The strong association with diabetes persisted after adjustment for age (P < .0001). Because of the very high prevalence of phenotypes I and B among diabetic women and the associations of each manifestation of insulin resistance with NIDDM, the 54 women with definite or probable NIDDM are excluded from all further analyses.

Among nondiabetic women, LDL subclass phenotype was strongly related to obesity as measured by body mass
index (Figure, graph c, P<.0001). An even stronger association was seen with waist-hip ratio (Figure, graph d) as judged by the $x^2$ (df=4) value of 82.8 (compared with 30.0 for body mass index). After adjustment by ANCOVA for age and body mass index, mean triglyceride levels were more than twice as high in women with phenotype B as in those with phenotype A (Table 1). Women with phenotype I had intermediate mean triglyceride levels. HDL cholesterol levels were significantly lower in twins with either B or I phenotype. Consistent with previous reports,9-12 the association of LDL cholesterol with subclass phenotype was weaker, although the mean value for women with phenotype I was significantly higher than for those with phenotype A. LDL cholesterol levels did not differ between those with phenotypes A and B.

Waist-hip ratio differed strongly by subclass phenotype after adjustment for body mass index and age. Systolic blood pressure level was significantly higher in those with either phenotype B or I, whereas diastolic blood pressure was significantly higher only in those with phenotype I. The strong associations with systolic blood pressure level were not reduced after further adjustment for self-reported current use of diuretics (n=88) or $\beta$-blockers (n=32) or after excluding users of either medication from the analyses. Fasting and 2-hour postload insulin levels as well as postload glucose concentration were highest in persons with phenotype B and intermediate in those with phenotype I. Fasting glucose levels did not differ by phenotype.

In multivariate models using the broad definition of LDL subclass phenotype as the outcome variable, we examined whether associations of waist-hip ratio and systolic blood pressure with phenotype were independent of the associations of phenotype with HDL and triglyceride levels (Table 2). Neither increasing age nor body mass index were predictive of subclass B once waist-hip ratio was included in the model (model 1). With HDL cholesterol and triglyceride levels added to the model, associations of waist-hip ratio and systolic blood pressure persisted, although the magnitude of the regression coefficient for waist-hip ratio was diminished (model 2). In a third model, fasting insulin and glucose were included to assess whether insulinemia or glucose

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**TABLE 1. Adjusted Mean Level of Cardiovascular Disease Risk Factors in 594 Nondiabetic Women by LDL Subclass Phenotype**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>LDL subclass phenotype</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n=496)</td>
<td>I (n=55)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>83.06</td>
<td>120.22</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>66.49</td>
<td>57.55</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>117.49</td>
<td>127.50</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.77</td>
<td>0.81</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>115.96</td>
<td>122.18</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>67.13</td>
<td>71.92</td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>70.41</td>
<td>71.92</td>
</tr>
<tr>
<td>Fasting insulin ((\mu)U/mL)</td>
<td>7.53</td>
<td>9.52</td>
</tr>
<tr>
<td>2-Hour postload insulin ((\mu)U/mL)</td>
<td>36.40</td>
<td>51.57</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>87.88</td>
<td>88.34</td>
</tr>
<tr>
<td>2-Hour postload glucose (mg/dL)</td>
<td>96.93</td>
<td>102.32</td>
</tr>
</tbody>
</table>

Means are calculated from generalized least squares models, adjusted for age, body mass index, and insulin assay. Three twin pairs were excluded from analyses of fasting glucose and insulin, and 11 pairs were excluded from analyses of postload glucose and insulin because of missing values. LDL, low-density lipoprotein; HDL, high-density lipoprotein; bpm, beats per minute.

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**TABLE 2. Multivariate Predictors of LDL Subclass Phenotype B in 564 Nondiabetic Women Twins**

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>$P$</th>
<th>Regression coefficient</th>
<th>$P$</th>
<th>Regression coefficient</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.001</td>
<td>.98</td>
<td>-0.018</td>
<td>.23</td>
<td>-0.016</td>
<td>.32</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-0.001</td>
<td>.98</td>
<td>-0.058</td>
<td>.06</td>
<td>-0.075</td>
<td>.04</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>10.301</td>
<td>&lt;.0001</td>
<td>4.778</td>
<td>.03</td>
<td>5.240</td>
<td>.01</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>0.021</td>
<td>.02</td>
<td>0.028</td>
<td>.002</td>
<td>0.027</td>
<td>.004</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0.022</td>
<td>&lt;.0001</td>
<td>0.023</td>
<td>&lt;.0001</td>
<td>0.023</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>-0.051</td>
<td>.0006</td>
<td>-0.048</td>
<td>.001</td>
<td>-0.048</td>
<td>.001</td>
</tr>
<tr>
<td>Fasting insulin ((\mu)U/mL)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.050</td>
<td>.03</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>...</td>
<td>...</td>
<td>-0.039</td>
<td>.04</td>
<td>-0.039</td>
<td>.04</td>
</tr>
</tbody>
</table>

For these analyses, the broad definition of phenotype B is used in which subjects with the intermediate phenotype I are grouped with those having phenotype B. Sample size is reduced because 15 pairs with missing data for fasting or postload glucose or insulin values were excluded. Regression coefficients from generalized estimating equations9 are the logarithm of the adjusted odds ratio for having phenotype B. Thus, a positive coefficient indicates the increase in risk for a one-unit increase in the independent variable. LDL, low density lipoprotein; HDL, high-density lipoprotein.
TABLE 3. Prevalence of LDL Subclass Phenotype B in Relation to the Number of Other Manifestations of the Insulin Resistance Syndrome in 564 Nondiabetic Women Twins

<table>
<thead>
<tr>
<th>No. of other manifestations of the insulin resistance syndrome</th>
<th>No. of women</th>
<th>No. (and %) with LDL subclass phenotype B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>349</td>
<td>20 (5.7%)</td>
</tr>
<tr>
<td>1</td>
<td>125</td>
<td>32 (25.6%)</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>21 (33.3%)</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>14 (63.6%)</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

For these analyses, the broad definition of phenotype B is used in which subjects with the intermediate phenotype I are grouped with those having phenotype B. "Other manifestations" refers to any combination of the specified number of manifestations of the insulin resistance syndrome. Manifestations include plasma triglycerides >250 mg/dL; plasma high-density lipoprotein cholesterol <45 mg%; fasting plasma insulin >140.0 μU/mL (75th percentile of the sample distribution); systolic blood pressure >160 mm Hg or current pharmaceutical treatment for hypertension; and waist-hip ratio >0.824 (75th percentile of the sample distribution). LDL, low-density lipoprotein.

level might explain associations of LDL subclass with blood pressure and waist-hip ratio. The addition of insulin and glucose levels had essentially no effect on the strength of associations with other risk factors in the model. Fasting insulin level itself remained independently predictive of phenotype in this model that included the lipid levels. Interestingly, the direction of the associations of body mass index and glucose level with LDL subclass phenotype in this last model became inverse and of borderline statistical significance.

Postload insulin, when substituted for the fasting value, was also positively related, but the coefficient estimate did not meet the criterion of statistical significance (P=.25). Using the narrow definition of phenotype B changed findings only slightly. However, the precision of estimates decreased somewhat because of the reduction in the number of subjects classified as phenotype B.

To verify findings of the multivariate model, we grouped nondiabetic women by the number of manifestations of the insulin resistance syndrome they demonstrated. For purposes of this categorization, plasma triglycerides, HDL cholesterol, fasting insulin, systolic blood pressure, and waist-hip ratio were dichotomized, with values more extreme than the following cutoffs considered consistent with the insulin resistance syndrome: triglycerides >250 mg/dL, HDL cholesterol <45 mg/dL, fasting insulin above the 75th percentile of the sample distribution (or >14.0 μU/mL), systolic blood pressure >160 mm Hg or current pharmaceutical treatment of hypertension, and waist-hip ratio above the 75th percentile of the sample distribution (or >0.824). Consistent with the model, the prevalence of phenotype B (broad definition) rose progressively (Table 3) as the number of manifestations of insulin resistance increased from zero to four. No participant demonstrated all five components.

With the exception of systolic blood pressure, similar findings were noted in a model that substituted LDL peak particle diameter, a continuous variable, for LDL subclass phenotype. Using multiple linear regression and including all variables from model 3 of Table 2, significant inverse associations with particle diameter were observed for triglyceride level, waist-hip ratio, and fasting insulin level; a strong positive association was seen for HDL cholesterol level. Systolic blood pressure was not a significant independent predictor of particle diameter in this model (β=-0.01, P=.24). When 78 pairs in which one or both twins were taking antihyperensive medications were removed, the coefficient estimate was somewhat larger (β=-0.02) although still not statistically significant (P=.15).

To determine whether these associations persisted after removing all genetic variation, mean intrapair differences were examined for each variable in the 25 nondiabetic MZ twin pairs discordant for LDL subclass phenotype B, using the broad definition (Table 4). Each manifestation of the insulin resistance syndrome was more apparent in the twin with phenotype B. Significantly higher mean plasma triglycerides and lower mean HDL cholesterol levels were found in the women with phenotype B than in their genetically identical sisters. Twins with pattern B were significantly more obese and had the higher waist-hip ratio and systolic blood pressure. The difference in postload insulin levels was quantitatively quite large, although it was of only borderline significance in this very small sample.

Similarly, in matched multiple linear regression analyses using all 176 nondiabetic MZ twin pairs, components of the insulin resistance syndrome remained as strongly related to LDL peak particle diameter as in the

TABLE 4. Means and Intrapair Differences in Risk Factor Levels in 25 Nondiabetic Monozygotic Twin Pairs Discordant for LDL Subclass Phenotype

<table>
<thead>
<tr>
<th>Twin with subclass phenotype B*</th>
<th>Twin with subclass phenotype A</th>
<th>Mean difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>182.7</td>
<td>111.9</td>
<td>70.88</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>49.0</td>
<td>55.9</td>
<td>-6.92</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.7</td>
<td>26.1</td>
<td>2.50</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.88</td>
<td>0.81</td>
<td>0.07</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>128.5</td>
<td>120.6</td>
<td>7.92</td>
</tr>
<tr>
<td>Fasting insulin (μU/dL)</td>
<td>19.1</td>
<td>13.3</td>
<td>6.99</td>
</tr>
<tr>
<td>Postload insulin (μU/dL) †</td>
<td>76.2</td>
<td>57.8</td>
<td>18.38</td>
</tr>
</tbody>
</table>

LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*Includes 15 twins with phenotype I and 10 twins with phenotype B.
†n=24 pairs for this analysis only because of missing postload insulin value for one twin.
unmatched regression analysis. That is, nongenetic differences in waist-hip ratio ($P<.0001$), triglycerides ($P<.0001$), and fasting insulin ($P=.0002$) were independently and inversely related to intrapair differences in peak particle diameter. In the same model, differences in HDL cholesterol were positively associated with particle diameter ($P=.002$). As in the unmatched model, systolic blood pressure was unrelated to LDL peak particle diameter.

**Discussion**

In this large sample of predominantly white women, LDL subclass phenotype was associated with each component of the insulin resistance syndrome, including central obesity, dyslipidemia, hypertension, glucose intolerance, and hyperinsulinemia. Moreover, each component was found to add independently to the likelihood of having phenotype B. Persons with the full insulin resistance syndrome had the highest probability of having phenotype B. With the exception of the blood pressure association, these relations were also seen with LDL particle diameter, a continuous measure of LDL heterogeneity.

Associations of LDL density and peak particle diameter with insulin resistance have been noted previously in a small clinical sample. Hyperinsulinemia and/or insulin resistance is associated with a lipoprotein profile similar to that accompanying LDL subclass phenotype B. Hepatic production of VLDL (and plasma triglyceride levels) are increased in insulin resistance, either as a direct influence of hyperinsulinemia on triglyceride synthesis or possibly because peripheral resistance to insulin's antilipolytic effect increases plasma free fatty acid substrate for VLDL synthesis. Hyperinsulinemia is also believed to increase catabolism of HDL cholesterol, leading to lower plasma levels. In our data, elevation of plasma triglyceride levels was by far the strongest lipid association with phenotype B. The $F$ test from the ANCOVA model (Table 1) for triglycerides was 225 compared with 32.5 for HDL cholesterol.

Persons with phenotype B were more obese than those with phenotype A, but adjustment for obesity, as measured by body mass index, did not substantially diminish any of the cross-sectional associations with other risk factors. Body mass index was no longer predictive of subclass phenotype ($P=.56$) once waist-hip ratio was included in the regression models, emphasizing the predominant importance of central obesity. A strong association of waist-hip ratio with LDL particle diameter has been reported previously in a clinical study in men.

To our knowledge, a relation of LDL subclass phenotype or particle diameter with blood pressure level has not been previously reported. This association was also quite strong and was not diminished by adjustment for either lipid or insulin levels. A specific association of $\beta$-blocker medication with small, dense LDL has been noted, but adjustment for use of $\beta$-blockers in this study did not diminish the blood pressure/LDL subclass association. Many previous reports have noted the co-occurrence of hypertension with low HDL cholesterol and elevated triglyceride levels. The label “familial dyslipidemic hypertension” has been proposed to describe this association. The present data suggest that LDL subclass phenotype B is an integral part of dyslipidemic hypertension.

We could not demonstrate a significant, independent, linear association of blood pressure level and LDL particle diameter. This discrepancy may result, at least in part, from the combined measurement errors for blood pressure level and LDL particle diameter. The increase in the magnitude of the coefficient with removal of persons using antihypertensive medications supports this explanation. An alternative explanation would be the presence of a nonlinear or threshold relationship between blood pressure and LDL particle diameter.

The very strong association of LDL subclass phenotype B with impaired glucose tolerance and NIDDM in this study is consistent with the report of Barakat et al showing that morbidly obese patients with either disorder have smaller, denser LDL particles than either normoglycemic obese individuals or lean control subjects. Thus, further clarification of the genetics of LDL subclass phenotypes may provide insights into the genetics of NIDDM as well.

Genetic influence on LDL subclass phenotypes or on LDL particle size has been demonstrated in this twin sample, in a large study of male twins, and in family studies. However, in each study, genetic factors appeared to explain no more than 50% of the variation in LDL particle size. Moreover, substantial discordance was noted for subclass phenotype B among identical twin pairs in this study. Twenty-five of the 40 non diabetic MZ pairs in which at least one twin had phenotype B were discordant for the phenotype.

Previous reports have shown potentially beneficial effects of weight reduction and increased physical activity in increasing LDL particle diameter. Within the 25 discordant MZ twin pairs, associations of LDL subclass phenotype and other coronary heart disease risk factors (lipoprotein levels, waist-hip ratio, and blood pressure) persisted in the absence of genetic variation, indicating that these associations are due at least in part to modifiable nongenetic influences. The strong association of LDL subclass phenotype with menopausal status is further evidence that nongenetic (possibly hormonal) factors may influence expression of the phenotype.

In each population studied to date, a small portion of individuals appear to have an electrophoretic pattern that is intermediate between subclass patterns A and B. The present analyses reveal that this intermediate phenotype also tends to be intermediate between phenotypes A and B in its associations with coronary heart disease risk factors (Table 1). Moreover, the associations with age, obesity, and central obesity show that phenotype B tends to become more prevalent (and phenotype I relatively less prevalent) at higher levels of these variables. This suggests that phenotype I could represent a partially penetrant phenotype B, with penetrance modulated by environmental factors in genetically susceptible individuals. Longitudinal observation of persons with phenotype I would be useful in examining this possibility.

Taken as a whole, the available information suggests that LDL subclass phenotype B is an integral part of the cluster of risk factors that has been termed the insulin-resistance syndrome or syndrome X. However, none
of the associations appeared to be accounted for by the level of insulinemia. Neither the previously reported associations of subclass phenotype with triglyceride and HDL cholesterol levels nor the strong independent associations with central obesity and blood pressure level were diminished by adjusting for insulin levels. Perhaps some aspect of insulin resistance not directly measured by plasma insulin levels may explain the associations. However, it is also possible that hyperinsulinemia is primarily a marker for this syndrome in which the primary abnormality could be a defect in lipid metabolism.31

Acknowledgments

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