Comparison of the Associations of Apolipoprotein B and Non–High-Density Lipoprotein Cholesterol With Other Cardiovascular Risk Factors in Patients With the Metabolic Syndrome in the Insulin Resistance Atherosclerosis Study

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Background—The metabolic syndrome (MetS) as defined by the National Cholesterol Education Panel definition has been proposed as an indicator of cardiovascular disease risk. Both apolipoprotein (apo)B and non–HDL cholesterol (NHDLC) have been proposed as an additional indicator to identify patients at higher risk in MetS patients.

Methods and Results—We studied 1522 individuals in the Insulin Resistance Atherosclerosis Study (IRAS) who were 40 to 69 years of age and from 3 ethnic groups. Their anthropometric measures, lipids, apoB, C-reactive protein, fibrinogen, plasminogen activator inhibitor-1, fasting and post–glucose load glucose, and insulin concentrations were measured, and insulin sensitivity was determined by intravenous glucose tolerance test. Differences in risk parameters in individuals with hyper-apoB/normo-NHDLC, and normo-apoB/hyper-NHDLC were compared in all IRAS subjects and again in those with MetS. In both cases, despite anticipated lower LDL cholesterol, the hyper-apoB/normo-NHDLC group had elevated risk indicated by greater waist circumference (both \( P < 0.05 \)) and fasting insulin (\( P < 0.01 \)) and lower insulin sensitivity (\( P < 0.001 \)). They also had higher C-reactive protein (\( P < 0.05 \)). Moreover, the Spearman correlation of apoB was significantly stronger (\( P < 0.05 \)) in the direction of greater associated risk than that of NHDLC with body mass index, waist circumference, systolic blood pressure, 2-hour glucose, fasting glucose, fasting insulin, 2-hour insulin, insulin sensitivity, C-reactive protein, fibrinogen, and plasminogen activator inhibitor-1.

Conclusions—In conclusion, apoB is more closely associated with central adiposity, insulin resistance, thrombosis, and inflammation than NHDLC. Our data suggest that apoB is a better candidate risk parameter than NHDLC for identifying a subgroup of individuals with or without MetS with elevated cardiovascular risk. (Circulation. 2004;110:2687-2693.)

Key Words: epidemiology ■ glucose ■ prevention ■ risk factors

An expanding body of evidence points to the superiority of apolipoprotein B (apoB) over LDL cholesterol (LDL-C) as a marker of the risk of vascular disease. For example, several large, prospective epidemiological studies have shown that apoB predicts coronary events better than LDL-C (for review, see Reference 1). In addition, clinical trial evidence indicates that apoB is a better marker than LDL-C of the residual risk of vascular events in patients on statin therapy.\(^1\) Part of the explanation for such findings may stem from our recent observation\(^2\) that apoB is more strongly associated than LDL-C with a spectrum of other risk factors for vascular disease. These included not only factors in the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATPIII) guidelines for lipid treatment but also important “novel” risk factor candidates, including abdominal obesity, insulin resistance, inflammation, and prothrombogenic markers.

ATPIII proposed a definition of the metabolic syndrome (MetS) to identify a group of individuals who would not otherwise be recognized to be at higher risk of cardiovascular disease\(^3\). A series of recent studies confirmed that the MetS as defined by ATPIII does indeed predict elevated risk of coronary heart disease (CHD), albeit to varying degrees, independently of LDL-C.\(^4–8\) Individuals with the MetS also have an elevated risk for type 2 diabetes.\(^4,9\) The practical difficulty, however, is the high incidence of MetS. For example, a recent US survey found that the prevalence of MetS was \( \approx 25\% \) in white Americans\(^10\) equating to \( \approx 47 \) million individuals on the basis of 2000 census data. Intensive medical intervention in 25% of the adult population would
represent a substantial challenge for any healthcare system. Nor is it clear that all individuals with the MetS are at the same risk. On the contrary, it seems almost certain that there is a gradient of risk within this group. Therefore, an urgent need exists to identify subgroups of patients with MetS who are at substantially elevated risk and require intensive medical intervention to reduce their risk.

Both apoB and non–HDL cholesterol (NHDLC) have been proposed as an additional indicator to identify such patients. NHDLC is thought to better “encapsulate” total risk from the atherogenic lipoproteins in patients with the MetS in whom LDL-C is characteristically normal and triglycerides concentrations are modestly elevated (>150 to 200 mg/dL). In agreement with this suggestion, a follow-up of the Lipid Research Clinic cohort showed a stronger correlation with coronary mortality for NHDLC than for LDL-C.11 NHDLC is the sum of the cholesterol in all the apoB-containing lipoproteins, and the high correlation between NHDLC and apoB that has been documented in a number of studies led to the proposal that NHDLC is an adequate surrogate for apoB. For clinical practice, however, that is not the case. The high correlation notwithstanding, there is substantial discordance between them.12

It is not certain, therefore, which is the better marker of risk to use in patients with MetS. We therefore compared the association of elevated apoB and NHDLC with the broad range of risk markers predictive of vascular disease. Having previously demonstrated the superior association of apoB over LDL-C to such risk factors in a diverse population, we hypothesized in the present study that apoB would also be better than NHDLC. If correct, then our data would provide more evidence to consider elevated apoB as a robust mechanism to categorize patients with or without MetS at higher risk for vascular disease.

Methods

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter, epidemiological study exploring relationships between insulin resistance and cardiovascular risk factors across different ethnic groups and various states of glucose tolerance. Having previously demonstrated the superior association of apoB over LDL-C to such risk factors in a diverse population, we hypothesized in the present study that apoB would also be better than NHDLC. If correct, then our data would provide more evidence to consider elevated apoB as a robust mechanism to categorize patients with or without MetS at higher risk for vascular disease.

Chosen Cutoffs for Phenotype Groupings

Cut points for apoB and NHDLC were based on the distribution of lipids and apolipoproteins in the North American population. The NHDLC cut point was derived from a VLDL cholesterol value of 30 mg/dL, added onto the optimal LDL-C cut point (<100 mg/dL). The chosen apoB cutoff of 120 mg/dL approximates the 75th percentile in prior studies.15–17

Statistical Analysis

Formal statistical comparisons were made between mutually exclusive groups defined by hyper-NHDLC, normo-NHDLC, hyper-apoB, and normo-apoB categories for all subjects and repeated in the subgroup fulfilling the NCEP MetS criteria. Analysis of covariance (Tables 1 and 2) without and with ethnicity, gender, glucose tolerance status interaction terms, and Spearman correlations with various adjustments (Figures 1 and 2) were calculated with SAS, version 9.0. Framingham score was calculated with the NCEP ATP III score sheet.3 Correlations were compared using the T1 method recommended by Steiger.18

Results

Direct Comparison of Hyper-NHDLC/Normo-ApoB Versus Normo-NHDLC/Hyper-ApoB in All IRAS Subjects

Comparing the hyper-NHDLC/normo-apoB group (Table 1, group B) with the normo-NHDLC/hyper-apoB group (group C) shows that NHDLC is significantly higher in hyper-NHDLC and apoB is higher in hyper-apoB by definition. The normo-NHDLC/hyper-apoB individuals have significantly higher body mass index, waist circumference, systolic blood pressure (SBP), fasting insulin, and CRP and lower insulin sensitivity index (SI) than the hyper–LDL-C/normo-apoB group (all P<0.05). Moreover, both fibrinogen and PAI-1 were also higher, although not significantly so. We also compared the percentages in the 2 groups with each of CRP, fibrinogen, and PAI-1 in the top quartile. In this case, proportionately more of the normo-NHDL/hyper-apoB group had high CRP (38.3% versus 25.9%; P=0.025), PAI-1 (34.9% versus 25.0%; P=0.067), and fibrinogen (37.6% versus 25.7%; P=0.030). As expected, LDL-C and TG concentrations were lower (both P<0.05) in the normo-NHDL/hyper-apoB group, despite lower insulin sensitivity in this group.

Direct Comparison of Other Groups in All IRAS Subjects

Individuals with combined hyper-apoB and hyper-NHDL (group A) appear to have results somewhat similar to those of subjects with normo-NHDL/hyper-apoB (group C). TG and LDL concentrations were higher in group A (as expected); otherwise, only SI was significantly different but paradoxically also elevated. It is also notable how much more different group C (normo-NHDL/hyper-apoB) is from group D (normo-NHDL/normo-apoB) than group B (hyper-NHDL/normo-apoB) is. In this case, common carotid intima-media thickness (IMT) was also significantly higher (P=0.0034) if the data in group C are compared with those in group D.

Direct Comparison of Hyper-NHDL/Normo-ApoB Versus Normo-NHDL/Hyper-ApoB in IRAS Subjects Fulfilling the NCEP MetS Criteria

Data in Table 2 are structured as per Table 1. In this case, waist circumference, fasting insulin, SI, and CRP were all significantly different in the direction of elevated risk in the
Correlations of ApoB and NHDLC With Insulin Sensitivity and Other Metabolic Parameters in the Entire Cohort

In a continuous analysis adjusted for age, gender, and ethnicity (Figure 1A), apoB correlated significantly \( (P<0.05) \) inversely with HDL cholesterol, LDL size, and insulin sensitivity and positively with body mass index, waist circumference, TG, diastolic blood pressure (DBP), SBP, 2-hour and fasting glucose and insulin levels, CRP, fibrinogen, PAI-1, and both common and internal carotid artery intima-media thicknesses (IMTs). NHDLC also correlated with all of the above parameters but less strongly in most cases. Indeed, when we formally tested whether the 2 correlations were different, apoB was stronger in its associations with body mass index, waist circumference, SBP, 2-hour and fasting glucose; fasting insulin levels, insulin sensitivity, CRP, fibrinogen, and PAI-1 compared with NHDLC. In contrast, NHDLC was not a significantly better correlate than apoB with any measured parameter.

Because our 2 criterion variables (NHDLC and apoB) are correlated with each other (Spearman \( r=0.74, P<0.0001 \)), it is possible that the correlations shown in Figure 1A for either variable reflect the association with the other. Thus, we adjusted the correlations with each criterion variable for the other, as shown in Figure 1B. All the apoB correlations except that with DBP remained significant and in the same, increasing-risk direction. Conversely, adjusting the NHDLC correlations for apoB substantially attenuated the significance of each correlation so that only the association with TG and DBP remained significant and in the direction associated with elevated risk. Otherwise, the association of increasing NHDLC with other parameters was reversed, some (waist circumference, 2-hour glucose, fasting insulin, and SI) now associated in the direction of significantly lower risk.
TABLE 2. Demographically Adjusted Mean±SE Among IRAS Subjects With MetS

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>P, Group A vs B</td>
<td>P, Group A vs C</td>
<td>P, Group B vs C</td>
<td>P, Group C vs D</td>
</tr>
<tr>
<td>n*</td>
<td>148–160</td>
<td>68–78</td>
<td>76–92</td>
<td>303–323</td>
</tr>
<tr>
<td>Age (unadjusted), y</td>
<td>56.7±0.64</td>
<td>0.32</td>
<td>57.8±0.92</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.8±0.45</td>
<td>0.71</td>
<td>31.5±0.65</td>
<td>0.11</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>99.6±0.93</td>
<td>0.40</td>
<td>98.2±1.35</td>
<td>0.013</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.46±0.01</td>
<td>&lt;0.001†</td>
<td>1.06±0.02</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>TG, mmol/L‡</td>
<td>2.55±0.09</td>
<td>&lt;0.001†</td>
<td>2.27±0.12</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>4.56±0.06</td>
<td>0.001†</td>
<td>4.23±0.08</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>0.98±0.02</td>
<td>0.65</td>
<td>0.47</td>
<td>0.31</td>
</tr>
<tr>
<td>NHDLC, mmol/L</td>
<td>12.4±0.11</td>
<td>&lt;0.001†</td>
<td>11.58±0.16</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>LDL size, μm</td>
<td>255.0±0.75</td>
<td>0.30</td>
<td>256.4±1.08</td>
<td>0.19</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>80.3±0.77</td>
<td>0.13</td>
<td>81.1±1.11</td>
<td>0.075</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>14.00±0.48</td>
<td>0.23</td>
<td>13.00±0.69</td>
<td>0.19</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L‡</td>
<td>8.44±0.25</td>
<td>0.24</td>
<td>7.93±0.35</td>
<td>0.27</td>
</tr>
<tr>
<td>2-h Insulin, pmol/L‡</td>
<td>118.0±5.27</td>
<td>0.010</td>
<td>108.5±7.07</td>
<td>0.002</td>
</tr>
<tr>
<td>SI, 10⁻⁴ · mm·μU·mL‡</td>
<td>504.0±32.50</td>
<td>0.77</td>
<td>520.5±49.14</td>
<td>0.26</td>
</tr>
<tr>
<td>CRP, mg/L‡</td>
<td>3.51±0.27</td>
<td>0.27</td>
<td>3.04±0.34</td>
<td>0.044</td>
</tr>
<tr>
<td>Fibrinogen, μmol/L</td>
<td>8.70±0.14</td>
<td>0.49</td>
<td>8.53±0.20</td>
<td>0.16</td>
</tr>
<tr>
<td>PAI-1, ng/mL‡</td>
<td>30.0±1.49</td>
<td>0.16</td>
<td>26.7±1.91</td>
<td>0.44</td>
</tr>
<tr>
<td>Common IMT, mm‡</td>
<td>0.828±0.015</td>
<td>0.22</td>
<td>0.796±0.022</td>
<td>0.055</td>
</tr>
<tr>
<td>Internal IMT, mm‡</td>
<td>0.882±0.024</td>
<td>0.026</td>
<td>0.793±0.032</td>
<td>0.26</td>
</tr>
</tbody>
</table>

BMI indicates body mass index.

*Nonmissing values vary in the range indicated.
†Probability value not bolded because difference is due to category definition.
‡Log-transformed for analysis and back-transformed for presentation.

Similarly, it is possible, on the basis of the evidence examined thus far, that the stronger association of apoB with the conventional risk factors included in the Framingham score may render it less independent than NHDLC in its associations with the less conventional risk factors. To address that possibility, we generated an additional set of correlation coefficients (Figure 1C) adjusted for the Framingham score itself. The pattern of this last set of correlations is similar to that of the sets presented above, with apoB showing associations of greater magnitude and thus greater statistical significance in the direction of higher risk and NHDLC showing either nonsignificant associations or significantly weaker associations in the direction of higher risk.

Correlations of ApoB and NHDLC With Insulin Sensitivity and Other Metabolic Parameters in the Subgroup With MetS

We noted that the patterns are similar to those in the total population in Figure 2A and even more strikingly in favor of apoB in Figure 2B and 2C. Indeed, when we adjusted the risk factor correlations with each criterion variable for the other (Figure 2B), the correlations of many risk factors with apoB were not attenuated compared with results in Figure 2A but in many cases were strengthened with further adjustment for NHDLC. Indeed, several apoB correlations attained greater significance in the same, increasing-risk direction after adjustment for NHDLC. Conversely, adjusting the NHDLC correlations for apoB substantially attenuated and in many cases reversed its association with many risk factors in the direction associated with lower risk. Similarly, with adjustment for Framingham score (Figure 2C), many more of the adjusted NHDLC correlations are in the direction of lower associated risk in the subgroup of MetS compared with the similar analysis in the entire cohort (Figure 1C).

**Discussion**

NHDLC has been proposed to be an acceptable surrogate of apoB in routine clinical practice on the basis of the fact they are highly correlated. However, they do not measure the same thing: NHDLC is the mass of cholesterol in the apoB-containing lipoproteins, whereas apoB is the number of atherogenic particles. The atherogenic risk resulting from lipoproteins does not relate primarily to their cholesterol content but rather to their size and number. In this regard, it is clearly important to examine whether apoB is superior to NHDLC in its prediction of the risk of vascular disease.

But does this difference relate only to lipoproteins as such? Our previous study comparing LDL-C and apoB showed that this might relate, at least in part, to closer associations of apoB than LDL-C to a series of other risk factors. Therefore,
our aim in the present study was to test whether apoB or NHDLC was more closely related to a series of other established and novel risk factors. The risk profile of the hyper-apoB group was more extensive than the hyper-NHDLC group, notwithstanding that plasma TGs were higher in the latter than in the former. Several cross-sectional studies\textsuperscript{19–23} and 2 prospective studies\textsuperscript{24,25} have demonstrated that risk is higher in hypertriglyceridemic patients with elevated apoB (the hyper-apoB group) than in hypertriglyceridemic patients with normal apoB (the hyper-NHDLC group). Thus, risk does not relate directly to TG levels but to the number of small dense LDL particles. Our data clearly demonstrate that apoB is more closely associated than NHDLC with central adiposity, insulin resistance, thrombosis, and inflammation. This was true in all IRAS subjects, in the subgroups with MetS, and in those who did not have MetS.
MetS. Furthermore, it appeared that relative to subjects with normo-NHDL/C/normo-apoB, individuals with isolated hyper-apoB had many more significant abnormalities than those with isolated hyper-NHDL/C, including significantly greater common carotid IMT. Moreover, once individuals had elevated apoB, risk factor abnormalities changed little with the addition of elevated NHDL/C. In other words, it appears that once apoB concentrations are elevated, then CHD risk can be considered high and the further transition from normo-NHDL/C to hyper-NHDL/C yields negligible additional risk.

The findings are consistent across glucose tolerance categories and ethnic groups. Moreover, our correlation analyses indicate that apoB has the potential to add more to the Framingham score than NHDL/C in the entire cohort and in the subgroup with MetS. Such findings are of significant clinical interest because they add explanatory power as to why apoB is superior not only to LDL-C but also to NHDL/C in its prediction of vascular risk.

A recent report from the Quebec Cardiovascular Study indicated that although apoB and NHDL/C were significantly correlated (r = 0.87), the actual concordance between these 2 parameters was only moderate. In fact, >25% of patients had apoB and NHDL/C values that did not fall in the same quintile of population distribution. The conclusion for the Quebec study was that apoB and various indexes of cholesterol are unlikely to be identical surrogates of CHD risk in a significant population.

In our examination of the linkages of apoB and NHDL/C with a wide range of risk pathways, we have now substantially strengthened this proposal. Perhaps the most striking data here are presented in Figure 1B, which could be considered a head-to-head comparison of apoB and NHDL/C in their associations with other risk markers. When we adjusted the correlations of apoB with each criterion variable for NHDL/C, all correlations except DBP remained significant and in the same, increasing-risk direction. Conversely, adjusting the NHDL/C correlations for apoB substantially attenuated the significance of each correlation; indeed, several were now associated in the direction of significantly lower risk. When we repeated these correlations in the subgroup with MetS (Figure 2B), the results were even more strikingly in favor of apoB. These observations clearly indicate the potential for apoB to give significantly greater insight into the basis for increased CHD risk than NHDL/C.

Our findings here lend strong mechanistic support to prior data comparing the predictive ability for vascular risk of apoB and NHDL/C or related ratios. For example, apoB has been shown to be superior to NHDL/C in its association with increased IMT in patients with familial combined hyperlipidemia. More impressively, the ratio of apoB to apoA-1 ratio in the AMORIS study was superior to the ratio of NHDL/C to HDL/C in predicting fatal myocardial infarction. It is important to point out that the measurement of apoB is standardized and automated, that it can be performed on nonfasting samples, and that it has been accepted as an alternative to LDL-C by all of the most recent Canadian guideline groups. It is also relevant that whereas apoB levels predict on-treatment CHD events in statin trials, other traditional lipid parameters such as LDL-C do not.

In conclusion, our data suggest that apoB is more closely associated than NHDL/C with central adiposity, insulin resistance, thrombosis, and inflammation and adds more to Framingham Risk Score than NHDL/C. Accordingly, we suggest that apoB is a better candidate risk parameter than NHDL/C for identifying a subgroup of individuals with or without MetS with elevated cardiovascular risk.

References
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