Comparison of the Associations of Apolipoprotein B and Low-Density Lipoprotein Cholesterol With Other Cardiovascular Risk Factors in the Insulin Resistance Atherosclerosis Study (IRAS)

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Background—Risk factors for vascular disease include obesity, dyslipidemia, hypertension, dysglycemia, insulin resistance, inflammation, thrombosis, and subclinical atherosclerosis. This study compares the associations of apolipoprotein B (apoB) and LDL cholesterol (LDLC) with a wide array of measures of these risk factors.

Methods and Results—In 1522 individuals in the Insulin Resistance Atherosclerosis Study, anthropometric measures and measures of lipids, apoB, C-reactive protein, fibrinogen, plasminogen activator inhibitor-1 (PAI-1), fasting and postglucose load glucose and insulin concentrations, and carotid artery intima-media thickness (IMT) were taken and insulin sensitivity was determined by frequently sampled intravenous glucose tolerance test. There were significant differences in measures of abdominal obesity, dyslipidemia, hyperinsulinemia, and thrombosis between subjects with elevated apoB but normal LDLC versus those with elevated LDLC but normal apoB. In each statistically significant comparison, the elevated-apoB group had higher associated risk than the elevated-LDLC group. Moreover, apoB is highly significantly (P<0.0001) correlated with each measure in the direction of higher risk, whereas LDLC was significantly correlated (P<0.05) only with blood pressure, triglyceride, fibrinogen, and C-reactive protein. After further adjustment for LDLC, apoB correlations remained significant, whereas several LDLC correlations adjusted for apoB became significant in the direction of lower risk.

Conclusions—Elevated apoB is more strongly associated than LDLC with other risk factors, including measures in the National Cholesterol Education Program guidelines for lipid treatment and other more recently established risk factors. This may provide new insight into why apoB is a better predictor of vascular risk than LDLC. (Circulation. 2003;108:2312-2316.)

Key Words: apolipoproteins • lipoproteins • risk factors • atherosclerosis

The risk factors for vascular disease frequently cluster. Thus, multiple risk factors may be present within the same individual, although to varying degrees, and the precocious and progressive vascular disease that results is a result of each of these factors interacting additively or perhaps even synergistically. A broad range of risk factors for vascular disease has been identified. These include dyslipidemia, dysglycemia, hypertension, abdominal obesity, inflammation, and a prothrombotic state.

The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recommended initiation of therapy and therapeutic goals within 3 different coronary heart disease (CHD) risk categories based on LDL cholesterol (LDLC) levels.1 However, LDL particles, which contain most of the cholesterol in plasma, may differ in composition.2 Small dense LDL species are more atherogenic than larger LDL particles but carry less LDLC. LDLC therefore is not always equivalent to LDL particle number.2 By contrast, each VLDL and LDL particle contains 1 molecule of apoB; therefore, plasma apoB is equivalent to the total atherogenic particle number, >90% of which are LDL particles.2 A series of large, prospective epidemiological studies has recently shown that apoB is superior to LDLC as a predictor of the risk of vascular disease (for review, see Sniderman et al3).

Our hypothesis was that apoB would provide a better approach than LDLC to characterizing the specific dyslipidemia that was most intimately linked to the broad range of risk factors predictive of vascular disease, including directly measured insulin resistance and subclinical inflammation.

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Such an approach, in turn, might provide a clue as to how they may all be linked in terms of pathogenesis.

Methods

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter, population-based epidemiological study exploring relationships between insulin resistance and cardiovascular risk factors across different ethnic groups and various states of glucose tolerance. Glucose, insulin, triglyceride (TG), and cholesterol levels were measured by standard measures.1 LDL cholesterol was calculated as total cholesterol minus HDL cholesterol and 20% of TG level, all in mg/dL. Plasma total apoB concentrations were assayed at Medstar Laboratory with an immunoprecipitation technique (SPQ kit from Instar, Inc). The coefficients of variation for LDL cholesterol and apoB were both 8%. Festa et al.2 described the methods used to measure C-reactive protein (CRP), fibrinogen, and plasminogen activator inhibitor-1 (PAI-1). Wagenknecht et al.4 described the overall design of IRAS and the methods used to obtain all the other measures in this analysis. The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent. A total of 1624 individuals participated in IRAS, including 719 men and 905 women from 3 ethnic groups (612 non-Hispanic whites, 548 Hispanics, and 464 blacks), 40 to 69 years old, with representation across glucose tolerance status (712 normal, 353 with impaired glucose tolerance, and 539 with diabetes). Of the 1624 subjects 102 were missing apoB or LDL cholesterol, leaving 1522 with the data required for this analysis.

Classifications of Subjects

Subjects were divided into groups based on NCEP ATP III guidelines5 for identifying patients to initiate therapeutic lifestyle changes (Table). Those subjects above the LDL cholesterol cut point in each CHD risk category are defined as hyper-LDL cholesterol. The remaining subjects, not recommended for treatment, are categorized as normo-LDL. We selected the apoB cut point in each risk category so that the prevalence of hyper-apoB was approximately equal to the prevalence of hyper-LDL cholesterol. Because exact equality was not possible because of ties in apoB values, the cutoff point providing the nearest prevalence greater than that for LDL cholesterol was chosen for the lowest risk category. For the other 2 risk categories, we selected cut points providing slightly fewer hyper-apoB subjects than hyper-LDL cholesterol subjects.

Subjects who self-reported having had a myocardial infarction, coronary artery angioplasty, coronary artery bypass surgery, or any other heart or vessel surgery; who were diagnosed as having diabetes on the basis of the 1999 WHO criteria applied to standard fasting and 2-hour glucose assays; or who had a Framingham Point Score >10-year risk ≥20% were categorized into the highest CHD risk category: CHD or CHD equivalent risk. Subjects in this risk category were defined as hyper-LDL cholesterol if their LDL cholesterol was ≥160 mg/dL. Anyone in this risk category with apoB ≥82 mg/dL was deemed to be hyper-apoB.

Of the remaining subjects, those with ≥2 of the following 5 major risk factors: smoking cigarettes as of the baseline examination; blood pressure ≥140/90 or on antihypertensive medication; HDL cholesterol <40 mg/dL; self-reported as having a male first-degree relative who had a heart attack before age 55 or female first-degree relative before age 65 years; and male ≥45 years old or female ≥65 years old were classified into the middle risk category: ≥2 risk factors (10-year risk ≥20%). In this category, subjects with LDL cholesterol ≥130 were classified as hyper-LDL cholesterol and apoB ≥99 mg/dL as hyper-apoB.

All subjects classified in neither of the ATP III risk categories described above were classified in the lowest risk category: 0 to 1 risk factor. Individuals with LDL cholesterol ≥160 mg/dL and apoB ≥112 mg/dL were classified as hyper-LDL cholesterol and hyper-apoB, respectively.

The numbers in each of the 12 combinations of these categories are shown in the Table.

Statistical Analysis

Formal statistical comparisons were made between mutually exclusive groups defined as hyper-LDL cholesterol versus normo-LDL cholesterol, hyper-apoB versus normo-apoB, and hyper-LDL cholesterol versus normo-LDL cholesterol hyper-apoB. In addition, we made this last set of comparisons stratified separately by gender, by ethnic group (white, black, and Hispanic), by glucose tolerance status (diabetic, impaired glucose tolerance, and normal glucose tolerance), and by the 3 ATP III risk categories described above.

ANCOVA (Table) and Spearman correlations (Figure) were calculated by use of SAS version 9.0. Correlations were compared by the T2 method recommended by Steiger.6 Although we have declared comparisons with a value of P<0.05 to be statistically significant, we present the actual probability values in the Table and identify each comparison in the Figure, in which the probability value is greater than the cut point for Bonferroni adjustment (0.005), for any readers who choose to adjust for multiple testing.

Results

Classifications of Subjects

Of the 942 subjects meeting the NCEP criteria for treatment, 801 (85%) were also defined in our approach as having elevated apoB (Table). Of the 580 subjects not meeting the NCEP criteria, 147 (25%) met the criteria we used to identify elevated apoB for comparison with the 141 subjects with elevated LDL cholesterol but normal apoB. The statistically significant agreement between these 2 classification schemes (κ (95% CI) = 0.60 (0.56 to 0.64)) is not surprising in light of the Spearman correlation between apoB and LDL cholesterol (r = 0.61, P < 0.0001). However, combining the 147 normo-LDL cholesterol/apoB with the 141 hyper-LDL cholesterol/normo-apoB, we note that 288 (19%) of the IRAS population would have a different treatment recommendation if apoB were used to guide treatment rather than LDL cholesterol.

Comparison of Hyper-LDL cholesterol Versus Normo-LDL cholesterol and Hyper-ApoB Versus Normo-ApoB

All but 3 of the differences between hyper-LDL cholesterol and normo-LDL cholesterol (Table, columns A and B combined versus columns C and D combined) and between hyper-apoB and normo-apoB (columns A and C combined versus B and D combined) were highly significant (P<0.0001). Although not as highly significant, the other 3 comparisons—diastolic blood pressure (DBP) in both the LDL cholesterol and apoB and insulin sensitivity in LDL cholesterol—were all significant (P<0.03). As expected, HDL cholesterol, LDL size, and insulin sensitivity had lower means for both hyper categories, whereas all other measures had higher means in the hyper than in the normo groups.

Direct Comparison of Hyper-LDL cholesterol Normo-ApoB versus Normo-LDL cholesterol Hyper-ApoB

The probability values in the middle column of the Table are from comparing the hyper-LDL cholesterol/normo-apoB group (column B) with the normo-LDL cholesterol/hyper-apoB group (column C). By definition, LDL cholesterol is significantly higher in hyper-LDL cholesterol and apoB is higher in hyper-apoB. The normo-LDL cholesterol/hyper-apoB individuals have significantly lower mean age, HDL cholesterol, and LDL particle size and higher body mass index, waist circumference, TG, fasting insulin, 2-hour insulin, and PAI-1 values than the hyper-LDL cholesterol/normo-apoB group (all P<0.05). In summary, each of the 8 significantly different risk factors except age revealed higher associated risk for the group with elevated apoB but normal LDL cholesterol than for the group with elevated LDL cholesterol but normal apoB.
When the same phenotypes were compared within each gender, ethnic group (white, black, and Hispanic), glucose tolerance status (normal, impaired, and diabetic), and ATP III risk category (detailed results available on request), the overall pattern was the same as in the entire data set. Each measure showing a significant difference reflected higher risk for normo-LDLC hyper-apoB than for the hyper-LDLC normo-apoB.

Other Metabolic Parameters

In continuous analysis adjusted for age, gender, and ethnicity (Figure, panel A), apo B correlated significantly \( (P<0.05) \) inversely with HDL cholesterol, LDL size, and insulin sensitivity.
A. Adjusted for demographics

B. Adjusted for demographics. LDL C also adjusted for apoB. ApoB also adjusted for LDL C

C. Adjusted for Framingham Point Score

Spearman correlations with LDL C (gray bars) and with apoB (black bars) in IRAS baseline examination. Correlations in A are adjusted for demographics, ie, age, gender, and ethnicity. In B, LDL C associations are further adjusted for apoB, and apoB associations are adjusted for LDL C. Correlations in C are adjusted for Framingham Point Score.

It appears from the correlations shown in the Figure, panel B, in the direction of lower risk. 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 LDL C in its associations with the less conventional risk factors. To address that possibility, we generated an additional set of correlation coefficients (Figure, panel C) adjusted for the Framingham score itself. The pattern of this last set of correlations is similar to the sets presented above, with apoB showing associations of greater magnitude, and thus greater statistical significance, in the direction of higher risk and LDL C showing either nonsignificant associations or associations in the direction of lower risk.

Discussion

The major finding of this study is that apoB was linked to a wider array of different potent risk factors for vascular disease than LDL C. Although there is considerable overlap between the NCEP classification of elevated LDL C and the classification we developed to identify elevated apoB for comparison, almost 10% of the subjects included in this analysis met the definition for elevated LDL C but not for apoB, and another 10% had normal LDL C but elevated apoB. These are the subjects of our concern. Hyper-apoB subjects (with normal LDL C) demonstrated stronger associations with abdominal obesity, other dyslipidemias, hyperinsulinemia, and thrombosis than hyper-LDLC subjects with normal apoB. We believe that these findings are robust because of the large number of individuals studied and because the entire range of glucose tolerance and a wide range in insulin sensitivity were included. The inclusion of different ethnic groups is also an advantage. The fact that the principal findings held in all groups suggests that our results have wide applicability.

Previous work has also shown correlations between TG and apoB with hyperglycemia and insulin resistance, as reviewed previously. However, this is the first study that directly compares the relation of each not only to dysglycemia and insulin resistance but also to a broad range of prothrombotic and inflammatory vascular risk factors. It is also the first to directly compare LDL C and apoB with this broad panel of risk factors with many more significant associations to apoB than to LDL C. It appears from the correlations shown in the Figure, panel B, in which the correlations of each criterion variable were adjusted for the other, that at a specific level of apoB, subjects with higher levels of LDL C have the same or lower associated risk than subjects with lower levels of LDL C. The same phenomenon occurs when the correlations are adjusted for the Framingham...
risk score (Figure, panel C). Thus, these results support the hypothesis that apoB might provide a better approach than LDLC to characterizing the specific dyslipidemia most intimately linked to the broad range of cardiovascular risk factors.

Understanding which risk factors cluster and why they do so is important if we wish to explain why some individuals but not others are so susceptible to premature and extensive vascular disease. Documenting these associations is also critical if we are to understand the pathophysiology that underlies the risk factors. A series of previous studies have pointed to important and interesting linkages among risk factors that were originally thought to be independent. For example, Lemieux et al.1 showed that abdominal obesity is associated with a greater degree of hyperinsulinemia and higher TG and apoB levels than gynoid obesity. Moreover, recent prospective studies have shown that inflammatory markers predict the risk of type 2 diabetes.5,8 Ridker et al.6 reported that CRP predicted the risk of coronary events independently and more powerfully than did LDL-C. The present data show that CRP is more strongly associated with apoB than with LDL-C. It would be of interest to examine predictability of CRP in models containing apo B. A wide range of proatherogenic metabolic abnormalities may all be traced back to adipose tissue dysfunction, as suggested by Yudkin et al.10 and extended by Sniderman et al.2,11 Mohamed-Ali et al.12 Heinrich et al.13 Mutch et al.14 and others.

One virtue of such models is that they explain how an apparently disconnected set of risk factors may be present to variable degrees in a large number of individuals. Also, it reconnects the present complex pathophysiology of vascular disease to the simple foundational epidemiological observations, which established that coronary disease is common only in societies with excess energy intake and high-fatty-acid diets.15 Thus, simple changes in our environment and behavior may result in complex adaptations that, individually and collectively, are responsible for common disease.

At the present time, apoB is in limited use in clinical practice, whereas LDL-C is widely used to assess the risk of vascular disease and the response to therapy. These findings further support the need for a reevaluation in that regard. They accord with evidence from several recent prospective epidemiological studies (for review, see Reference 3) that have shown that apoB is superior to LDL-C as an index of the risk of vascular disease. They also accord with evidence from clinical trials that apoB is a better marker of residual risk of vascular events in patients on statin therapy than is LDL-C despite the fact that these clinical trials were not specifically designed to test the efficacy of managing apoB. The evidence presented in this article suggests that treatment will be more effective in reducing absolute risk among subjects with normal LDL-C but elevated apoB than among subjects with elevated LDL-C but normal apoB. It should also be noted that the apoB thresholds for this analysis were selected to make the comparisons with LDL-C categories as fair as possible. We advocate setting apoB cut points for therapy recommendations on the basis of prospective risk analyses rather than on cross-sectional analyses such as those presented in this article.

In conclusion, these results establish that the combination of elevated apoB and conventional risk factors other than LDL-C generates greater associated risk than the combination of elevated LDL-C with the same other conventional risk factors. These observations underscore that a particularly atherogenic profile is present in patients with elevated apoB, which may explain why apoB is superior to LDL-C as a marker of the risk of vascular disease.

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