Nonfasting Apolipoprotein B and Triglyceride Levels as a Useful Predictor of Coronary Heart Disease Risk in Middle-Aged UK Men

Philippa J. Talmud,* Emma Hawe,* George J. Miller, Steve E. Humphries

Objective—The Apolipoprotein-related Mortality Risk (AMORIS) study concluded that the apolipoprotein (apo)B/apoA-I ratio was the best predictor of coronary heart disease (CHD) risk. We have compared the pairwise combinations of total cholesterol, triglycerides (TGs), apoB, high density lipoprotein (HDL) cholesterol, low density lipoprotein cholesterol, and apoA-I on CHD risk prediction in middle-aged men.

Methods and Results—Healthy middle-aged men (n=2508), free of CHD at baseline, were examined prospectively. Over 6 years of follow-up, there were 163 CHD events (including acute myocardial infarction, coronary artery surgery, and ECG evidence of silent myocardial infarction). The relative risk (RR) of CHD associated with cholesterol, TGs, apoB, apoA-I, apoB/apoA-I, low density lipoprotein cholesterol, and HDL cholesterol were examined by survival analysis. The apoB/apoA-I ratio was associated with the strongest effect on the RR (3.58, 95% CI 2.08 to 6.19). In multivariate analysis, apoA-I had no significant effect on risk. Examining RR by quartiles, apoB and HDL in combination (RR 8.38, 95% CI 3.21 to 21.92) were better predictors of CHD risk than apoB and TGs (RR 4.05, 95% CI 1.57 to 6.23). However, apoB and TGs in combination added risk information over and above lifestyle factors, whereas apoB and HDL cholesterol did not.

Conclusions—The combined evaluation of apoB with TGs provides useful diagnostic criteria for CHD risk. (Arterioscler Thromb Vasc Biol. 2002;22:1918-1923.)

Key Words: follow-up studies ■ apolipoprotein B ■ triglycerides ■ HDL cholesterol ■ apolipoprotein A-I

Raised cholesterol,1 raised triglycerides (TGs),2 low levels of HDL cholesterol (HDL-C),3 and small dense LDLs4 are all considered to be independent predictors of coronary heart disease (CHD) risk. However, there is mounting evidence indicating that measures of plasma apoB and apoA-I, over and above lipid measures, may be better predictors of CHD risk in adults5,6 and in children.7 Walldius et al.8 recently published results from the Apolipoprotein-related Mortality Risk (AMORIS) study of 175 553 men and women; these results clearly demonstrate the power of apoB, apoA-I, and the apoB/apoA-I ratio in predicting CHD risk, suggesting that the ratio of apoB/apoA-I helps define cholesterol transport and reverse cholesterol transport to and from the peripheral tissue. Furthermore, in that study, the value of apoB as a risk predictor was evident even at LDL cholesterol (LDL-C) levels below the median, showing the strength of apoB compared with LDL-C in evaluating CHD risk. Making use of the newly derived formulas for HDL-C and LDL-C estimates by Walldius et al.,8 we have examined the combination of TGs and cholesterol, TGs and apoB, HDL-C and apoB, apoB and apoA-I, and HDL-C with LDL-C in predicting CHD risk in a large prospective study of healthy middle-aged men in the United Kingdom.

Methods

Study Sample
Healthy men (n=3052), aged 50 to 61 years and registered with 9 general medical practices, were recruited for prospective surveillance. The study had full ethical approval, with subjects giving informed consent. Methods have been described in detail elsewhere.9,10 Briefly, all men were free of a history of unstable angina, myocardial infarction (MI), ECG evidence of a silent infarction, coronary surgery, aspirin or anticoagulant therapy, other cerebrovascular disease, malignancy, or any condition precluding informed consent. A standard 12-lead ECG was recorded and coded according to Minnesota criteria.11 Height (in meters) was measured on a stadiometer, and weight (in kilograms) was determined by a balance scale for the calculation of body mass index (BMI, in kilograms per square meter). During the first 5 years of follow-up, survivors were recalled annually for an interview, and blood samples were taken for quantitative trait measures. The ECG was repeated at the sixth examination. CHD events taken as end points were fatal (sudden or

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not) and nonfatal MI, based on World Health Organization criteria,12 plus coronary artery surgery and silent MI on the follow-up ECG. Clinical information for each event was assembled by inquiries through the participating practices, hospitals attended, and, for fatal events, coroners’ offices. This information was collated and submitted to an independent assessor who assigned qualifying events to the appropriate category.

**Plasma Measures**

Each participant attended the study in a nonfasting state and was instructed as follows: “If you have a morning appointment avoid a cooked breakfast and more than one cup of tea or coffee. Use skimmed or semi-skimmed milk. If you have a later appointment, you may have a light tea with one cup of tea and coffee beforehand, but have your main meal after examination.”

In addition, men were requested to refrain from smoking or vigorous exercise from midnight beforehand. Those that did not adhere to these requirements were given a further appointment. The time of day of venipuncture was noted. A 5-mL sample of venous blood was taken by Vacutainer technique (Becton Dickinson). Serum was stored briefly at −40°C and then at −80°C, pending analysis. Cholesterol and TG concentrations were determined by automated enzyme procedures with reagents from Sigma Chemical Co and Wako Chemicals, respectively. Serum apoA-I (coefficient of variation 3.1%) and apoB (coefficient of variation 6.5%) concentrations were measured by immunoturbidometry with reagents from Incstar. HDL-C measures were made on year-6 samples,13 but because risk was assessed from the start of the study, HDL-C was calculated by using the formula of Walldius et al,8 taking baseline measurements into account.

**Statistical Methods**

Only individuals with TGs, cholesterol, apoB, and apoA-I measured at baseline were considered throughout the analysis. TGs, apoB, LDL-C, and HDL-C were logarithmically transformed before inclusion in any analysis, as a result of the nonnormality of their distributions. Logarithmically transformed data are presented together with geometric means and approximate SDs. HDL-C and LDL-C were calculated according to the formula of Walldius et al.8 Differences in baseline characteristics by coronary event status were considered via 1-way ANOVA, with appropriately transformed dependent variables as required. A categorical variable with 7 levels, corresponding to different times of the day, was created from the venipuncture time. Differences in TG levels by venipuncture time were corrected for time of venipuncture, they remained unchanged. Considering the TG levels across the day, the differences were <0.4 mmol/L. As expected, there was no evidence to suggest that CHD risk differed by venipuncture time, with hazards ratios (HRs) close to 1 for comparison of time slots during the day; thus, it did not confound any relationship between TGs and risk.

The univariate risk ratios (adjusted for age and clinical practice and for quartiles of apoB and TGs, cholesterol and apoA-I, calculated LDL-C and HDL-C, and the ratio of apoB/apoA-I) are presented in Figure 1. For all traits, risk was significantly different for the top quarter compared with the lowest quarter, and the risk ratios for the highest quarter of TGs, cholesterol, LDL-C, and apoB were higher than for apoB/apoA-I. For HDL-C and apoA-I, these were 0.84 (95% CI 0.77 to 0.91), 0.74 (95% CI 0.67 to 0.82), and 0.72 (95% CI 0.64 to 0.81), respectively. By comparison, the relative risk for the ratio of apoB/apoA-I was 3.58 (95% CI 2.08 to 6.19); thus, this ratio appeared to be the best risk predictor.

By use of multivariate analysis, the effect on CHD risk, as assessed by the impact on the HR of a 1-SD increase in these lipid and apolipoprotein traits, when considered in the same model, is presented in Table 2. In the pairwise models, when TGs were paired with cholesterol, both had significant effects on risk. Replacing cholesterol with apoB in combination with TGs showed similar risk estimates, although with greater

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**TABLE 1. The Mean Baseline Characteristics (SD) of the Men in NPHSII Who Had a CHD Event or Not and in Whom Apolipoprotein and Lipid Measures Were Available**

<table>
<thead>
<tr>
<th></th>
<th>No CHD Event</th>
<th>CHD Event</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>56.17 (3.52)</td>
<td>56.83 (3.72)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>26.21 (3.45)</td>
<td>26.99 (3.55)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Current smoking</strong></td>
<td>651 (27.76%)</td>
<td>61 (37.42%)</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure,† mm Hg</strong></td>
<td>83.77 (11.26)</td>
<td>86.57 (12.18)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Systolic blood pressure,† mm Hg</strong></td>
<td>136.69 (18.58)</td>
<td>142.65 (20.38)</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Cholesterol, mmol/L</strong></td>
<td>5.71 (1.02)</td>
<td>6.06 (1.04)</td>
<td>&lt;0.0000</td>
</tr>
<tr>
<td><strong>Triglycerides, † mmol/L</strong></td>
<td>1.80 (0.95)</td>
<td>2.13 (1.17)</td>
<td>&lt;0.0000</td>
</tr>
<tr>
<td><strong>ApoB, † g/L</strong></td>
<td>0.85 (0.24)</td>
<td>0.92 (0.24)</td>
<td>0.0004</td>
</tr>
<tr>
<td><strong>ApoA-I, g/L</strong></td>
<td>1.64 (0.32)</td>
<td>1.58 (0.27)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>LDL-C, † mmol/L</strong></td>
<td>3.07 (1.00)</td>
<td>3.41 (0.96)</td>
<td>&lt;0.0000</td>
</tr>
<tr>
<td><strong>HDL-C, † mmol/L</strong></td>
<td>1.71 (0.61)</td>
<td>1.53 (0.56)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

*Percentage of current smokers.*
†Means given are the anti-log of the log-transformed mean, and standard deviations are approximated.
‡Calculated according to Walldius et al.8
statistical significance for apoB. However, when cholesterol was added to the model together with TGs and apoB, the relative risk associated with cholesterol levels was no longer statistically significant. The risk prediction of the pairwise combination of apoB and HDL-C was almost identical to that of apoB and TGs. To compare these, the HR for 1-SD decrease in HDL-C (as opposed to the increase presented in Table 2) was 1.25 (95% CI 1.06 to 1.47) compared with 1.42 (95% CI 1.20 to 1.70) for apoB. When apoB and apoA-I were considered in the model together, only apoB remained statistically significant, whereas apoA-I had a nonsignificant effect on risk. TGs and the ratio of apoB/apoA-I provided no additional information over and above that of the model with TGs and apoB alone. HDL-C combined with LDL-C was a strong predictor, but not as strong as apoB and TGs or apoB and HDL-C.

The pairwise relative risk ratios for CHD by quartiles of TGs and cholesterol, TGs and apoB, and HDL-C and apoB are presented in Figure 2a through 2c. Because apoA-I, when combined in a model with apoB, did not have a significant effect on the relative risk of CHD, the pairwise relationship of apoB and apoA-I was not examined. Men in the top quartile of TGs and cholesterol had a relative risk of 3.12 (95% CI 1.93 to 8.51) compared with risk in the lowest quartile of each. For TGs and apoB, the relative risk in the top quarter was 4.05 (95% CI 1.57 to 6.23). Effects with apoB and HDL-C were more striking. Considering the top quarter of apoB and the bottom quarter of HDL, the relative risk ratio was 8.38 (95% CI 3.21 to 21.92). Tests for the interaction between apoB and TGs and apoB and HDL-C were null (ie, the effects were not significantly different from additive). We next considered whether the combination of apoB and TGs or of apoB and HDL-C significantly added to a risk model that included the lifestyle factors, age, BMI, systolic blood pressure, smoking, and alcohol consumption. Although the difference in the models that included lifestyle factors only or lifestyle factors plus apoB and TGs was highly statistically significant (P<0.00005), comparing the basic lifestyle risk model with one including apoB and HDL was not statically significant (P=0.13). Thus, apoB and HDL-C did not add significantly to the risk predicted by lifestyle parameters only.

**Discussion**

The AMORIS study, with >175 000 participants, convincingly demonstrated that apoB and apoA-I and the ratio of apoB/apoA-I are important predictors of CHD risk, providing information over and above that of total and LDL-C and of TGs and HDL-C. Furthermore, Walldius et al\(^8\) validated their
formulas for HDL-C and LDL-C by comparing them with values for standard measurements of HDL-C and LDL-C, which were calculated by use of the Friedewald equation. The relative effectiveness of the risk prediction of pairwise combinations of lipids, apoA-I, and apoB is an important issue because the ultimate aim is to identify the most effectual minimum number of predictors of CHD risk that can be used to maximal effect in the clinical setting.

Many prospective studies have examined the relative predictive values of different CHD risk factors. The Quebec Cardiovascular Study, a prospective study with a size similar to that of the second Northwick Park Heart Study (NPHSII), examined which parameters best define CHD risk and reported that the ratio of total cholesterol to HDL-C is superior to that of LDL-C to HDL-C. A subset of men with normal TG levels but low HDL-C levels were at increased risk, thus suggesting the importance of HDL-C measures. Finally, when apoB and apoA-I levels were taken into account, the results strongly supported the importance of apoB as a risk factor for CHD, whereas apoA-I showed borderline risk ratios and, after adjustment for other plasma lipid and lipoproteins, was eliminated from the association. However, the combined effect of apoB and TGs on risk was not examined. The Atherosclerosis Risk in Communities (ARIC) study (a multiethnic study of males and females) examined whether apoB and apoA-I added significant information over and above LDL-C, TGs, and HDL-C. LDL-C proved to be the best risk predictor, whereas apoB, although having a strong effect in univariate analysis, did not have a significant effect on risk in multivariate analysis.

<table>
<thead>
<tr>
<th>Model</th>
<th>Hazard Ratio (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride*</td>
<td>1.23 (1.05, 1.45)</td>
<td>0.009</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.25 (1.08, 1.47)</td>
<td>0.004</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>1.23 (1.05, 1.44)</td>
<td>0.009</td>
</tr>
<tr>
<td>ApoB*</td>
<td>1.42 (1.19, 1.70)</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglyceride*</td>
<td>1.22 (1.03, 1.44)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.05 (0.86, 1.30)</td>
<td>0.61</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.37 (1.08, 1.73)</td>
<td>0.009</td>
</tr>
<tr>
<td>ApoB*</td>
<td>1.47 (1.23, 1.75)</td>
<td>0.000</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.86 (0.73, 1.02)</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>1.30 (1.11, 1.01)</td>
<td>0.001</td>
</tr>
<tr>
<td>ApoB*/ApoA-I</td>
<td>1.24 (1.07, 1.43)</td>
<td>0.004</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.29 (1.02, 1.50)</td>
<td>0.001</td>
</tr>
<tr>
<td>ApoB*/ApoA-I</td>
<td>1.22 (1.04, 1.42)</td>
<td>0.01</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.42 (1.19–1.70)</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.78 (0.68–0.94)</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.79 (0.67, 0.93)</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.31 (1.12, 1.52)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Log-transformed.

Figure 2. Stratification of proportion of CHD events by quartiles of apoB and TGs (a), TGs and cholesterol (b), and apoB and HDL-C (c) adjusted for age and clinical practice. For apoB and TGs and for TGs and cholesterol, the lowest quartiles were set at 1. For apoB and HDL-C, the lowest quartile for apoB and highest quartile for HDL-C were used as references and were set at 1.
Lovastatin Primary Prevention Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), baseline apoB, LDL-C, and HDL-C and on-treatment apoB and apoB/apoA-I ratio were the best predictors of risk.

In the present analysis, we examined the pairwise effect of cholesterol, TGs, LDL-C, HDL-C, apoB, and apoA-I on CHD risk in a series of parallel analyses in an all-male all-white study. Results from the univariate analysis show that the risk ratios associated with each of these variables and the apoB/apoA-I ratio are very similar to those reported in the AMORIS study; these results give strong credence to our subsequent comparisons. CHD risk was highest in men in the top quartile of the apoB/apoA-I ratio, with an almost 4-fold increase in risk compared with men in the lowest quartile. However, in multivariate analyses, the strongest risk predictions came from the combination of apoB with TGs or apoB and HDL and apoB and LDL-C. When cholesterol, TGs, and apoB were considered together, cholesterol no longer remained significant (as reported in the AMORIS study). The comparison of the relative risks associated with quartiles of total cholesterol and TGs, apoB and TGs, and apoB and HDL-C suggested that HDL and apoB were the best predictors of risk, inasmuch as men in the top quartile of apoB and the lowest quartile of HDL had HRs twice the level of those for men in the top quartile of apoB and TGs: 8.38 (95% CI 3.21 to 21.92) and 4.02 (95% CI 1.57 to 6.23), respectively. To assess whether these effects contributed to risks over and above those of lifestyle risk factors, a comparison was made of a model that included, in addition, both apoB and TGs or apoB and HDL-C. The efficacy of these measures is supported by results from the Physicians Health Study (PHS), in which 85% of the patents had not fasted and in which TGs were found to be a strong predictor of CHD risk even after correcting for LDL size. Similarly, a proportion of the AMORIS participants had not fasted. Furthermore, any variability caused by the differences in degree in fasting must be random, and the effect of such random variation in TG levels would be to add “noise” to the risk estimate and is unlikely to confound it. This suggests that the data in the present study may, if anything, be underestimating the risk association. Thus, the prospective NPHSII supports the results from the nested case-control PHS study, ie, that nonfasting TG levels are a strong risk predictor and are thus useful for risk assessment. Furthermore, our results confirm the effectiveness of the formulas for HDL-C and LDL-C estimates used in the AMORIS study for CHD risk prediction.

The mechanism by which raised apoB and TG levels in combination increase the risk of CHD is as yet uncertain. There is only 1 apoB molecule for each VLDL and LDL particle, and because VLDL particles are cleared much faster than are LDLs, apoB levels in essence reflect LDL number, and high apoB levels reflect a relative reduction in cholesterol mass. The consequence is a relatively small, dense LDL particle derived from VLDL2 overproduction, with a reduced cholesterol/apoB ratio. In a recent meta-analysis, Austin reported a 60% increase in the risk of CHD for every 10-Å decrease in LDL size. Adjustment for TGs and HDL-C reduced this to 30% increased risk, but the odds ratio remained statistically significant, demonstrating that small LDL is an independent risk factor.

Whatever the genetic or metabolic cause, the high CHD risk associated with the combined phenotype of elevated TGs and apoB opens up the possibility of using these simple diagnostic criteria for further studies. Confirmation of these results in additional large prospective studies is necessary. It will also be of interest to use family and association studies in these subjects to determine the heritability of this phenotype and to identify genetic factors that are involved in raised apoB and raised TG levels, inasmuch as these may be useful for identifying subjects at inherited risk of developing this phenotype.

Acknowledgments

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References


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