Apolipoprotein Concentrations During Treatment and Recurrent Coronary Artery Disease Events


Abstract—The effect of untreated total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) as cardiovascular risk factors in both primary and secondary prevention has been extensively investigated. The predictive value of on-treatment lipid and apolipoprotein levels on subsequent cardiovascular events is as yet uncertain. Eight hundred forty-eight patients (675 men and 173 women) with angiographically proven coronary artery disease (CAD) who received effective statin therapy (≥30% decrease of baseline TC) were studied. We analyzed the predictive value of on-treatment levels of TC, LDL-C, triglycerides (TG), apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) on subsequent myocardial infarction (MI) and all cause mortality. On-treatment LDL-C levels were 2.55±0.55 mmol/L and 2.58±0.62 mmol/L for men and women respectively. Age-adjusted Cox regression analysis showed that only on-treatment apoA-I was predictive for future CAD events in both men and women, whereas on-treatment HDL-C was exclusively predictive in women. On-treatment apoB levels were predictive for recurrent CAD events in the total population but not after separate analysis for men and women. On-treatment levels of TC, LDL-C, and TG did not predict subsequent events. Multivariate analysis showed that on-treatment apoA-I and apoB were the only significant predictors for future cardiovascular events. On-treatment levels of TC, LDL-C, and TG were no longer associated with increased risk of recurrent cardiovascular events in CAD patients treated to target levels, which justifies the current guidelines. However, on-treatment levels of apoB and in particular apoA-I (and HDL-C in women) were significantly predictive for MI and all-cause mortality and may therefore be more suitable for cardiovascular risk assessment in this population. (Arterioscler Thromb Vasc Biol. 2000;20:2408-2413.)

Key Words: lipids ■ apolipoproteins ■ risk factors ■ coronary disease

Major randomized lipid intervention trials have convincingly demonstrated the clinical benefit of lipid lowering therapy in both primary and secondary prevention.1–5 Therefore, elevated levels of total cholesterol (TC), and more specifically elevated levels of low density lipoprotein cholesterol (LDL-C), have become highly modifiable risk factors. Besides TC and LDL-C, elevated triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) levels are other important lipid factors associated with an increased risk on coronary heart disease.6,7 Furthermore, apolipoprotein B (apoB) and A-I (apoA-I), the major apolipoproteins of LDL-C and HDL-C particles, have been suggested as better markers than LDL-C and HDL-C in the assessment of risk on coronary artery disease (CAD).8–10

M ajor randomized lipid intervention trials have convincingly demonstrated the clinical benefit of lipid lowering therapy in both primary and secondary prevention.1–5 Therefore, elevated levels of total cholesterol (TC), and more specifically elevated levels of low density lipoprotein cholesterol (LDL-C), have become highly modifiable risk factors. Besides TC and LDL-C, elevated triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) levels are other important lipid factors associated with an increased risk on coronary heart disease.6,7 Furthermore, apolipoprotein B (apoB) and A-I (apoA-I), the major apolipoproteins of LDL-C and HDL-C particles, have been suggested as better markers than LDL-C and HDL-C in the assessment of risk on coronary artery disease (CAD).8–10

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Because of their safety and efficacy, statin therapy plays a major role in the management of patients who are at risk for CAD events. An increasing number of patients with hyperlipidemia will therefore achieve target lipid levels as recommended by international guidelines. The National Cholesterol Education Program (NCEP) guidelines recommend target levels of LDL-C of <2.6 mmol/L (100 mg/dL) in patients with established CAD.11 However, the risk on recurrent coronary events in patients with LDL-C levels treated to these targets has never been established. In addition, the predictive value of lipid and apolipoprotein levels during lipid lowering therapy on subsequent cardiovascular events has not sufficiently been investigated.

Accordingly, the aim of this study was to investigate the effect of both lipids and apolipoproteins A-I and B on myocardial infarction (MI) and all-cause mortality in men and women with documented CAD who were adequately treated with lipid lowering therapy.

Methods

Patient Population and Follow-Up

The study population derived from a population of 3095 consecutive patients who underwent a first diagnostic coronary angiogram between July 1981 and January 1998 at the Oosterschelde Hospital.
TABLE 1. Clinical Baseline Characteristics in Men and Women

<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>Men (n=675)</th>
<th>Women (n=173)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.2±9.5</td>
<td>66.9±9.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Angiographic results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-vessel disease</td>
<td>286 (40.9%)</td>
<td>77 (44.5%)</td>
<td></td>
</tr>
<tr>
<td>2-vessel disease</td>
<td>193 (28.9%)</td>
<td>51 (29.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>3-vessel disease</td>
<td>175 (25.9%)</td>
<td>36 (20.8%)</td>
<td></td>
</tr>
<tr>
<td>Left main disease</td>
<td>31 (4.5%)</td>
<td>9 (5.2%)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.8±3.8</td>
<td>26.5±3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoking</td>
<td>131 (20.1%)</td>
<td>12 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>History of MI</td>
<td>327 (49.2%)</td>
<td>67 (39.4%)</td>
<td>0.025</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>176 (26.1%)</td>
<td>80 (46.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>77 (11.9%)</td>
<td>35 (20.7%)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

BI indicates body mass index; MI, myocardial infarction.

in Goes, the Netherlands. Patients who were referred for coronary angiography for reasons other than suspicion of CAD (eg, valvular pathology, cardiomyopathy) were excluded from analysis (n=365). We next excluded patients without hemodynamically significant coronary artery disease (defined as ≥60% luminal narrowing, n=682). We then excluded 1200 patients who did not show a ≥30% reduction of TC by lipid lowering therapy (statin therapy either alone or in combination with cholestyramine or gemfibrozil) or in whom no complete lipid profile was known. As a result 848 patients (675 men and 173 women) could be included for final analysis.

To acquire mortality data, the computerized hospital system was screened. Subsequently, all family practitioners in the region were asked to verify whether patients who underwent coronary angiography and belonged to their practice were currently alive.

Using this information, we sent a questionnaire containing questions about clinical and lifestyle characteristics to all patients who were known to be alive. Letters were submitted to population registers and family members to obtain information about patients who could not be located or who did not return their questionnaires. Through these procedures, 3084 (99.7%) of all patients could be traced. The questionnaires were returned by 2285 (93.0%) of the 2457 eligible patients. Medical records were consulted to obtain clinical and lifestyle characteristics of the remaining patients. Myocardial infarction was enzymatically diagnosed. The follow-up period of each patient was calculated from the date of the first complete lipid profile that showed a ≥30% decrease of baseline TC level until death, non-fatal myocardial infarction, or until September 1, 1999.

Coronary Angiography
Coronary angiography was performed according to the standard Judkins technique. An obstruction in 1 of the 3 major epicardial coronary arteries of ≥60% on visual examination was considered hemodynamically significant.5,12 The coronary angiograms were evaluated visually and independently by 2 experienced cardiologists. In case of disagreement, a third observer was consulted to reach consensus.

Lipids and Apolipoproteins
All plasma lipid and apolipoprotein concentrations were determined from overnight fasting blood samples. TC and TG concentrations were measured enzymatically (Vitros analyzer, Johnson & Johnson). HDL-C fractions were prepared by precipitation from plasma of the apoB containing lipoproteins with the use of dextran sulfate and MgCl². Plasma LDL-C was calculated with the Friedewald formula (TC−[HDL-C]−[0.45×TG]).13 ApoA-I and apoB were measured by immunonephelometry on a Beckman array protein system: Beckman reagents, calibrators, and standards were used. All patients in this study were on statin therapy (simvastatin, atorvastatin, fluvastatin, or pravastatin). For each patient, a baseline TC level without statin therapy was used as reference value. Additionally, baseline LDL-C level was known of 290 men and 81 women, LDL-C level of 270 men and 76 women, TG of 297 men and 80 women, and apoA-I/apoB level of 188 men and 54 women. There were no important differences between baseline total cholesterol levels in patients with or without complete baseline lipid profile. The first complete lipid profile (TC, HDL-C, LDL-C, TG, apoA-I, and apoB) that demonstrated a ≥30% decrease of baseline TC level was used for analysis. Previous studies have shown that a ≥30% lowering of TC can be considered effective lipid treatment.4,5

Statistical Analysis
Changes in lipids and apolipoproteins were described as average percent decrease from baseline values. Continuous and categorical variables were analyzed by Student’s t test and χ² analysis respectively. A Cox proportional-hazards regression model was used to estimate the effect of various age-adjusted risk factors as independent predictors of MI and all-cause mortality. A multivariate Cox proportional-hazards regression analysis was performed to measure the combined effect of the variables on MI and all-cause mortality. All lipid and apolipoprotein variables were analyzed as continuous variables unless stated otherwise. Because of interdependency of several lipid factors, LDL-C and HDL-C could not enter the multivariate model. Therefore a ridge regression analysis was performed for these variables. Values were expressed as hazard ratios (HR) with 95% confidence intervals (CI). Cut-off levels for high versus low HDL, apoB, and apoA-I were based to the values that divide the subjects into 2 equal groups. A P value <0.05 was considered statistically significant.

Results
Baseline Characteristics and Follow-Up
Baseline characteristics of the study population for men and women are shown in Table 1. Age ranged from 33 to 84 years for men and 30 to 87 years for women. Women were older than men (66.9±9.9 years versus 64.2±9.5 years, P<0.001, respectively). Diabetes was more prevalent in women than in...
men (20.7% versus 11.9%, \( P = 0.005 \), respectively). Fewer women than men were current smokers (7.1% versus 20.1%, \( P < 0.001 \), respectively). Results of coronary angiography were similar for men and women in terms of extent of coronary artery disease. Mean follow-up of the 675 men and 173 women was 2.95 years (0–6.61) and 3.03 years (0.1 to 6.55), respectively. The 1-, 2-, and 3-year event-free survival rates were 93.8%, 91.0%, and 88.6% for men and 92.9%, 90.1%, and 87.6% for women.

### Serum Lipids

Baseline lipids and apolipoprotein levels before lipid lowering therapy for men and women showed that both sexes had significantly higher baseline TC and higher HDL-C levels than men (TC: 7.46 ± 6.00 mmol/L, \( P < 0.0001 \)), higher HDL-C (1.1 ± 0.65 mmol/L, \( P < 0.0001 \)), ApoA-I (1.1 ± 0.55 mmol/L, \( P < 0.0001 \)), ApoB (1.3 ± 0.65 mmol/L, \( P < 0.0001 \)), apoA-I (1.11 ± 0.55 mmol/L, \( P < 0.0001 \)), ApoB (1.3 ± 0.65 mmol/L, \( P < 0.0001 \)), LDL-C (1.1 ± 0.55 mmol/L, \( P < 0.0001 \)), and HDL-C (1.1 ± 0.55 mmol/L, \( P < 0.0001 \)). The 1-, 2-, and 3-year event-free survival rates were 93.8%, 91.0%, and 88.6% for men and 92.9%, 90.1%, and 87.6% for women.

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Population (n=848)</th>
<th>Men (n=675)</th>
<th>Women (n=173)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.98 (0.71–1.34)</td>
<td>1.09 (0.76–1.58)</td>
<td>0.74 (0.38–1.43)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.37 (0.17–0.80)</td>
<td>0.60 (0.25–1.46)</td>
<td>0.08 (0.01–0.45)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.16 (0.80–1.67)</td>
<td>1.04 (0.68–1.60)</td>
<td>1.59 (0.82–3.10)</td>
</tr>
<tr>
<td>TG</td>
<td>1.08 (0.84–1.40)</td>
<td>1.24 (0.95–1.60)</td>
<td>0.56 (0.25–1.23)</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.20 (0.08–0.49)</td>
<td>0.29 (0.10–0.86)</td>
<td>0.06 (0.01–0.35)</td>
</tr>
<tr>
<td>ApoB</td>
<td>3.21 (1.10–9.35)</td>
<td>3.41 (0.99–12.00)</td>
<td>3.16 (0.38–26.52)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.00 (0.55–1.81)</td>
<td>0.86 (0.41–1.82)</td>
<td>1.67 (0.60–4.69)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.43 (0.82–2.51)</td>
<td>1.38 (0.77–2.48)</td>
<td>1.73 (0.21–14.10)</td>
</tr>
</tbody>
</table>

#### Figure 1

Probability of recurrent cardiovascular events (myocardial infarction or all-cause mortality) adjusted for age, from time of ≥30% decrease of baseline TC level before statin therapy in patients with low HDL-C (<1.1 g/L) (black line) versus higher HDL-C (≥1.1 g/L) (gray line).
the only significant predictors for subsequent future events. LDL-C could not be included in the model because of collinearity with TC. The effect of HDL-C could not be estimated due to collinearity between apoA-I and TC. When using a ridge regression approach, the HR of HDL-C was 0.99, CI 0.37 to 2.62 ($P_{\text{NS}}$).

**Discussion**

Our study revealed that only on-treatment levels of apoB and especially apoA-I were significantly predictive for MI and all-cause mortality in CAD patients receiving effective statin therapy, whereas on-treatment HDL-C level was a significant risk factor exclusively for women. On-treatment levels of TC, LDL-C, and TG were no longer associated with increased risk of recurrent cardiovascular events in this population.

**LDL Metabolism and Recurrent Cardiovascular Events**

Clinical intervention trials have shown that lowering LDL-C plasma levels with statins reduces the relative risk on major coronary events by \(\approx 30\%\) dependent on baseline risk. The National Cholesterol Education Program (NCEP) guidelines recommend target levels of LDL-C of <2.6 mmol/L (100 mg/dL) in patients with established CAD. However, the risk on recurrent coronary events in patients with LDL-C levels treated to these targets have never been established. In our population, patients were on aggressive lipid lowering therapy, resulting in average levels of LDL-C of 2.55 mmol/L for men and 2.58 mmol/L for women. We found that neither TC nor LDL-C levels during statin therapy were predictive for all-cause mortality and non-fatal myocardial infarction. This finding has several implications. First, the value of lipid lowering therapy is once more confirmed, because we showed that effective treatment of elevated LDL-C levels eliminates LDL-C as risk factor for recurrent cardiovascular events. Second, our results imply that it is unlikely that further lowering of LDL-C has any effect when treated to NCEP standards. This is consistent with several previous studies that showed that the magnitude of beneficial clinical effects is diminished or even eliminated at lower LDL-C and TC levels. Results from the Multiple Risk Factor Intervention Trial (MRFIT) and the Scandinavian Simvastatin Survival Study (4S) support a curvilinear model showing a continuous but progressive decrease of benefit on CHD risk with increased reduction of TC levels. The Cholesterol and Recurrent Events (CARE) study demonstrated that no further decline in CHD risk was to be expected with LDL-C levels below 3.2 mmol/L (125 mg/dL). Accordingly, our findings along with these the current results provide evidence for justification of the current guidelines.

Nevertheless, as Supero previously emphasized, CAD risk management cannot be simplified to LDL-C reduction. We found that apoB was a better predictor for recurrent coronary events than LDL-C. This finding is biologically plausible. Atherogenic lipoproteins including LDL-C parti-

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**TABLE 4. Relationship Between On-Treatment Lipid/Apolipoprotein Levels and Clinical Variables on Subsequent Myocardial Infarction/All-Cause Mortality by Multivariate Cox Regression Analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio (95% C.I.)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.73 (0.38–1.38)</td>
<td>0.330</td>
</tr>
<tr>
<td>TG</td>
<td>0.95 (0.70–1.29)</td>
<td>0.754</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.29 (0.09–0.97)</td>
<td>0.044</td>
</tr>
<tr>
<td>ApoB</td>
<td>7.94 (1.09–57.72)</td>
<td>0.041</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.90 (0.52–1.55)</td>
<td>0.700</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.94 (0.51–1.72)</td>
<td>0.848</td>
</tr>
<tr>
<td>Age</td>
<td>1.06 (1.03–1.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Angiographic results</td>
<td></td>
<td>0.404</td>
</tr>
<tr>
<td>1 vessel disease</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2 vessel disease</td>
<td>1.31 (0.75–2.31)</td>
<td></td>
</tr>
<tr>
<td>3 vessel disease</td>
<td>1.57 (0.90–2.73)</td>
<td></td>
</tr>
<tr>
<td>Left main disease</td>
<td>1.73 (0.69–4.32)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>1.34 (0.76–2.37)</td>
<td>0.318</td>
</tr>
</tbody>
</table>

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**Figure 2.** Probability of recurrent cardiovascular events (myocardial infarction or all cause mortality) adjusted for age, from time of \(\approx 30\%\) decrease of baseline TC level before statin therapy in patients with apoB (<0.89 g/L) (black line) versus higher apoB (>0.89 g/L) (gray line).

**Figure 3.** Probability of recurrent cardiovascular events (myocardial infarction or all cause mortality) adjusted for age, from time of \(\approx 30\%\) decrease of baseline TC level before statin therapy in patients with low apoA-I (<1.3 g/L) (black line) versus higher apoA-I (>1.3 g/L) (gray line).
cles and remnants of triglyceride-rich particles each contain 1 molecule of apoB. Consequently, apoB gives an accurate estimation of the total number of atherogenic particles. The composition of LDL-C particles, each containing 1 molecule of apoB, is heterogeneous because of the variable content of cholesterol. Smaller, denser LDL-C are more atherogenic than larger ones. Therefore apoB is superior compared with LDL-C in determining CAD risk. A great number of studies have confirmed that apoB is a better marker for atherogenicity and CAD than LDL-C and TC both in men and women. Recently Goto et al showed in data from the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) that on-treatment apoB was the only significant predictor for subsequent cardiovascular events for primary prevention. This study is as yet the only large study that evaluated the predictive value of on-treatment lipid and apolipoprotein levels. As our study provides information on secondary prevention our data are complementary to their results on primary prevention. In agreement with their findings, we showed that, in patients with effectively treated lipid levels, measurement of apoB seems more appropriate than LDL-C and TC plasma levels as predictor of recurrent cardiovascular events.

HDL Metabolism and Recurrent Cardiovascular Events

Low levels of plasma HDL-C are directly associated with a significantly increased risk of CAD. Compared with the knowledge of the LDL-C metabolism, our insight of HDL-C metabolism including reverse cholesterol transport, is incomplete. Judged by the number of recently published studies dedicated to HDL-C, it appears that lipid research has ‘discovered’ HDL-C, due to the remarkable progress in unraveling the HDL-C metabolism. Major breakthroughs include the discovery of the HDL-C receptor, the scavenger receptor B-1 (SR-B1), which is expressed at high levels at the main sites of selective uptake of HDL-C. In addition, along with epidemiologic evidence, recent major clinical intervention trials have shown the importance of HDL-C on CAD, in particular in patients without elevated LDL-C levels. Miller et al demonstrated the significance of low HDL-C as risk factor on the incidence of recurrent cardiovascular events in a follow-up study of patients with angiographically proven CAD and desirable TC levels. The AFCAPS/TexCAPS study showed that, in a population without a history of CAD and with normal TC and LDL-C levels, a below-average HDL-C level (0.94 mmol/L for men and 1.03 mmol/L for women) forms an important risk factor for a first major coronary event. The recently published Veterans Affairs Cooperative Studies Program High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) proved that a modest increase in HDL-C levels in CAD patients with normal LDL-C levels (≥3.6 mmol/L) resulted in a significant reduction of risk on major cardiovascular events. In our study, we found that only in women, on-treatment plasma HDL-C was inversely correlated with the risk for recurrent events. This is consistent with some other studies that found that the impact of HDL-C on coronary risk was greater in women than in men.

ApoA-I, the major apolipoprotein of HDL-C, showed a predictive value for CHD events similar to HDL-C in women and was the only significant predictor in men. Similar to our findings, Kwiterovich et al found that the level of apoA-I, but not the level of HDL-C, was an indicator of future CAD for men. ApoA-I has previously been reported as a better indicator of CHD than HDL-C. Biologically, this finding can be explained because not all HDL-C particles are equally protective. Two predominant classes of HDL-C can be recognized; HDL-C particles that only contain apoA-I (lipoprotein A-I) and particles that contain both apoA-I and apoA-II (lipoprotein A-I:A-II). In general, lipoprotein A-I is considered to be more protective against CHD than lipoprotein A-I:A-II. Therefore, apoA-I levels may be a better marker for functional reverse cholesterol transport than HDL-C.

Considerations of the Study

Recently it has been stated that clinical investigators should rely on all-cause death as an objective, unbiased end-point, rather than rely on cardiovascular mortality alone. Therefore, we chose to assess total mortality because of the bias existing in the use of cardiovascular mortality, especially in a population with known CAD. In our institution, all patients had complete lipid profiles measured after 1993 and we do not expect to create a bias in outcome by excluding patients with incomplete profiles. We analyzed patients with 1 or more significant coronary artery stenosis and, therefore, our results only apply to this group and cannot be generalized. Furthermore, we cannot answer the question whether patients with <30% reduction in total cholesterol should be given a higher dose of statins as we did not assess these patients. In our opinion, the current guidelines are appropriate as far as it concerns LDL-C target levels as we showed that on-treatment LDL-C levels do not have prognostic relevance anymore. To establish the prognostic value of on-treatment apolipoproteins, large prospective trials are necessary. Therefore, we do not think that the current guidelines should be changed directly as a result of our study.

Conclusion

On-treatment levels of TC, LDL-C, and TG were no longer associated with increased risk of recurrent cardiovascular events in CAD patients treated to target levels, which justifies the current guidelines. However, on-treatment levels of apoB and in particular apoA-I (and HDL-C in women) were significantly predictive for MI and all-cause mortality and may therefore be more suitable for cardiovascular risk assessment in this population.

References


Apolipoprotein Concentrations During Treatment and Recurrent Coronary Artery Disease Events
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