Apolipoprotein B and Coronary Artery Disease in Women
A Cross-sectional Study in Women Undergoing Their First Coronary Angiography


Abstract—The association between plasma apolipoprotein (apo) B concentrations and angiographically determined coronary artery disease (CAD) was investigated in women in a cross-sectional study. Stenosis of >60% in 1 or more coronary arteries was classified as CAD+. CAD− was defined as a maximum stenosis of 10% in any coronary artery. Fasting plasma concentrations of apoB, apoA-I, cholesterol (chol), low density lipoprotein cholesterol (LDL-chol), high density lipoprotein cholesterol (HDL-chol), and triglycerides (TGs) were determined. Information on nonlipid risk factors was obtained from questionnaires. CAD+ women (n=160) were older than CAD− women (n=129), 64.0±7.8 vs 57.8±11.1 years, respectively. CAD+ compared with CAD− women had higher frequencies of diabetes (14.7% vs 5.8%, P<0.05), hypertension (53% vs 37%, P=0.018), and ever-smoking (48% vs 35%, P<0.001). CAD+ women had higher plasma concentrations of apoB (1.48±0.32 vs 1.25±0.34 g/L, P<0.001), chol (7.01±1.19 vs 6.38±1.22 mmol/L, P=0.001), LDL-chol (4.74±1.09 vs 4.13±1.13 mmol/L, P<0.001), and TGs (1.98±0.84 vs 1.71±0.93 mmol/L, P=0.007) and lower levels of HDL-chol (1.28±0.28 vs 1.37±1.38 mmol/L, P=0.028). After correction for nonlipid risk factors, apoB, chol, LDL-chol, HDL-chol, and TG were independently related to CAD. In the lowest quartiles of chol, LDL-chol, and TG, CAD+ women had higher apoB concentrations than CAD− women. In contrast, chol, LDL-chol, TG, or HDL-chol levels were not different in any quartile of apoB. ApoB showed the most significant relation with the number of stenotic vessels, and apoB was associated with CAD in the normolipidemic subgroup. In conclusion, apoB was superior to chol, LDL-chol, HDL-chol, TG, and apoA-I in discriminating between CAD+ and CAD−. (Arterioscler Thromb Vasc Biol. 1998;18:1101-1107.)

Key Words: apoB ■ coronary artery disease ■ coronary angiography ■ women

The atherogenic lipoprotein particles LDL, VLDL remnants, or IDL, and chylomicron remnants each contain 1 molecule of apoB as the structural protein. The plasma apoB concentration reflects the number of atherogenic lipoproteins, and studies in men have demonstrated that apoB can be a valuable predictor for CAD.1,2 Data on apoB as a risk indicator for CAD in women are limited.1 Although CAD is the major cause of death in women, the absolute numbers of clinical manifestations of CAD are low.3-4 Thus, long-term studies in large numbers of women are required to evaluate the risk factors on CAD. Studies with surrogate end points, such as coronary angiograms, are useful.

ApoB has been associated with angiographically determined CAD in a small group of Asian women1 and in women with premature (<60 years) CAD.5 ApoB was also related to CAD in a larger angiography study that included women on lipid-lowering medication and women for whom nonfasting lipid measurements were available.9 Fasting plasma samples are essential for comparison between different lipid parameters because TG concentrations increase10-12 and HDL-chol concentrations decrease in the postprandial state in both men10 and women.11,13 In the current angiography study in a large group of women, only fasting plasma samples were used for determination of lipids and apolipoproteins. Angiographies were performed in a community-referral hospital. No age or lipid selection criteria were used in an attempt to obtain a representative sample of women from the general population referred for angiography. We investigated whether apoB was an independent risk factor for CAD and whether apoB was superior to chol, LDL-chol, HDL-chol, TG, or apoA-I in discriminating between CAD+ and CAD− women. Frequency distributions of age, lipids, and apolipoproteins in CAD+ and CAD− women were also obtained.

Methods

Design

This cross-sectional study was performed in a community-referral cardiology clinic in the southwestern part of the Netherlands, the Oosterschelde Hospital Goes. The study protocol was approved by...
Selected Abbreviations and Acronyms

- CAD+, CAD− = with, without coronary artery disease
- chol = cholesterol
- HRT = hormone replacement therapy
- TG = triglyceride

the Ethics Committee of the hospital, and all subjects gave their informed consent before participation in the study.

Study Population

The study population consisted of women who were undergoing their first coronary angiography between January 1992 and January 1997. The indications for angiography were suspicion of CAD or preoperative screening for CAD in subjects with valvular disease. Women undergoing elective angiographies were included to avoid the influence of stress situations, such as a myocardial infarction, on plasma lipids. Women using lipid-lowering medication were excluded from analysis. Plasma concentrations of chol, TGs, HDL-chol, LDL-chol, apoA-I, and apoB were determined after an overnight fast during the week preceding the angiography.

Angiography

Coronary angiographies were performed according to the standard Judkins technique.14 Women were classified as CAD+ if 1 or more coronary arteries had a stenosis >60% on visual examination. The other women were classified as CAD−.

Lipids and Apolipoproteins

Chol and TG concentrations were measured enzymatically (Vitros analyzer, Johnson & Johnson). HDL-chol fractions were prepared by precipitation from plasma of the apoB-containing lipoproteins with the use of dextran sulfate and MgCl2.15 Plasma LDL-chol was calculated by using the Friedewald formula (total chol−[HDL-chol]−[0.45×TG]). ApoA-I and apoB were measured by immunonephelometry on a Beckman array protein system. Beckman reagents, calibrators, and standards were used. From June 1995 onward, the reference values for apoB were changed as a result of international standardization according to IFCC/WHO standards (SP3–07). The assigned values according to IFCC/WHO standards for apoB after June 1995 were identical to those before June 1995 multiplied by 0.82. In the current study the old values were converted to new values. ApoA-I reference values, based on SP1–01, did not change.

Clinical and Lifestyle Characteristics

Questionnaires were sent to the participants to retrospectively obtain self-reported information about clinical and lifestyle characteristics during the year preceding coronary angiography. Height and weight were recorded. Women were classified as never-smokers, past smokers, or current smokers. Diabetes was diagnosed as non insulin-dependent if the age of onset was >30 years and the first treatment was with diet modification or oral hypoglycemic medication. Diabetes was diagnosed as insulin dependent if the age of onset was <20 years and the first treatment was with insulin. The diagnosis of diabetes with an onset between 20 and 30 years of age was obtained from the patient’s records. Women were diagnosed as hypertensive if a physician had told them they had hypertension or if they were using antihypertensive medication. Family history was considered positive if at least 1 of the parents, siblings, or children had manifestations of cardiovascular disease before the age of 60 years. Ages at menarche and, if appropriate, at menopause (surgical or natural) were recorded. Finally, the premenopausal use of oral contraceptives and the postmenopausal use of HRT in any form were recorded.

Statistics

Logistic regression was used to analyze the influence of continuous and dichotomous variables on the presence of CAD (the dependent variable). ANCOVA with age as a covariate was used to analyze the effect of lipid and apolipoprotein values on 1-, 2-, or 3-vessel disease. Student’s t tests for independent samples were used to analyze differences in apoB concentrations between CAD− and CAD+ groups for each quartile of the other lipid or apolipoprotein variable. Because the distribution of TG values was skewed, all TG calculations were performed after logarithmic transformation. Values are expressed as mean±SD. Two-tailed P values <0.05 were considered significant. The SPSS (SPSS) software program was used.

Results

Subjects

In the 5-year interval between 1992 and 1997, 289 women met the inclusion criteria. By January 1997, 10 women had died and 3 women were considered ineligible to fill out the questionnaire (according to their general practitioners) because of dementia, hypochondriasis, and analphabetism, respectively. Seven women had changed their places of residence without leaving their new addresses. Two hundred sixty-nine questionnaires were mailed, and 235 (87%) were returned and adequately filled out. Therefore, information on nonlipid risk factors was available for 235 of the 289 women (81%). The women who did not receive or return questionnaires (n=57) were older than the responders (64.33±9.68 versus 61.12±9.95 years, P=0.028), but the plasma concentrations of chol, TGs, LDL-chol, HDL-chol, apoA-I, and apoB and the distribution of CAD− and CAD+ were similar in both groups (Student’s t tests for independent samples). Therefore, we assumed that the questionnaire group was representative of the entire study population so far as lipids, apolipoproteins, and CAD were concerned.

Angiography

The CAD− group consisted of 129 women. The indications for angiography in the CAD− group were suspicion of CAD (81%) or preoperative screening for CAD in valvular disease (19%). All CAD− women only had vascular wall irregularities and stenosis <10%. The CAD+ group consisted of 160 women, and the indications for angiography in the CAD+ group were suspicion of CAD (98%) or preoperative screening for CAD in valvular disease (2%). The CAD+ group was further divided into 1-vessel (n=78), 2-vessel (n=41), and 3-vessel (n=32) disease groups. Nine women had a stenosis in the left main coronary artery.

Clinical and Lifestyle Characteristics

CAD+ women were significantly older than CAD− women. Non–insulin-dependent diabetes mellitus was present in 24 women and insulin-dependent diabetes mellitus in 1 CAD+ woman. Both forms of diabetes were pooled for analysis (Table 1). Diabetes, smoking, and hypertension were significantly associated with CAD+ after correction for age. The body mass index was not different between CAD− (25.94±3.3 kg/m2) and CAD+ (26.24±4.04 kg/m2) women. Premature CAD, diagnosed as disease onset at <60 years of age, was present in 26.3% of CAD+ women. In the normolipidemic subgroup, defined as chol <6.5 mmol/L and TG <2.3 mmol/L, CAD was associated with age (P=0.008), but after correction for age, smoking was the only nonlipid risk factor that was significantly associated with CAD (P=0.03).
Hormonal Status
The majority of the women (88.5%) were postmenopausal. The age at menopause was 49.17 ± 4.3 years for women with a natural menopause (n = 168) and 41.65 ± 7.3 years for women with surgical menopause (n = 23) (Table 1). After correction for age, postmenopausal status (natural or surgical) was no longer a predictor of CAD. Ever-use of HRT was associated with reduced CAD (P = 0.012), but after correction for age, this was no longer significant (P = 0.07). Only 12.4% of the postmenopausal women had ever used HRT in any form, and the mean duration of HRT was 3.2 ± 4.0 years (range, 0.2 to 16). Twenty percent of the postmenopausal women had undergone hysterectomy.

Lipids and Apolipoproteins
Plasma chol concentrations in 5-year age categories of the entire study group were in the same range as chol concentrations in Dutch women from the general population (Tables 2, 3, and 4 and Figure 1).15,16 Fasting plasma concentrations of chol, LDL-chol, TGs, and apoB were significantly higher and HDL-chol was significantly lower in CAD+ women than in CAD− women (Table 2), and this association remained so after correction for age. The extent of CAD, expressed as the number of stenotic coronary arteries, was associated with chol, LDL-chol, apoB, and age. After correction for age, apoB remained the most significant parameter (Table 3). In a normolipidemic subgroup (chol ≤6.5 mmol/L and TGs ≤2.3 mmol/L), apoB and LDL-chol were the only parameters associated with CAD after correction for age (Table 4).

Each lipid parameter was an independent risk factor for CAD (logistic regression). After correction for confounders in clinical and lifestyle characteristics, the odds ratios for CAD were 11.1 for apoB (g/L), 1.5 for chol (mmol/L), 1.66 for LDL-chol (mmol/L), 0.68 for HDL-chol (mmol/L), and 5.1 for log TGs (log scale). Owing to differences in the units of measure, odds ratios cannot be directly compared. Therefore, lipid and apolipoprotein parameters were divided into quartiles. In any quartile of apoB, no significant differences between CAD− and CAD+ women were observed for chol, LDL-chol, HDL-chol, or TG. In contrast, apoB was signifi-
TABLE 3. Fasting Lipids and Lipoproteins in Women With 1-, 2-, and 3-Vessel Disease on Coronary Angiograms

<table>
<thead>
<tr>
<th></th>
<th>1 Vessel</th>
<th>2 Vessel</th>
<th>3 Vessel</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chol, mmol/L</td>
<td>6.74±1.08 (n=78)</td>
<td>7.36±1.39 (n=41)</td>
<td>7.27±1.08 (n=32)</td>
<td>0.034</td>
</tr>
<tr>
<td>TGs, mmol/L</td>
<td>1.89±0.79 (n=71)</td>
<td>2.28±0.90 (n=35)</td>
<td>1.95±0.85 (n=30)</td>
<td>NS (0.07)</td>
</tr>
<tr>
<td>HDL-chol, mmol/L</td>
<td>1.33±0.29 (n=71)</td>
<td>1.17±0.25 (n=36)</td>
<td>1.27±0.29 (n=30)</td>
<td>0.017</td>
</tr>
<tr>
<td>LDL-chol, mmol/L</td>
<td>4.44±0.99 (n=71)</td>
<td>5.05±1.21 (n=36)</td>
<td>5.09±1.04 (n=30)</td>
<td>0.014</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.51±0.26 (n=66)</td>
<td>1.42±0.24 (n=29)</td>
<td>1.50±0.21 (n=28)</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.39±0.29 (n=66)</td>
<td>1.55±0.33 (n=29)</td>
<td>1.64±0.34 (n=28)</td>
<td>0.009</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.9±8.4 (n=78)</td>
<td>64.2±8.2 (n=41)</td>
<td>68.9±6.2 (n=32)</td>
<td>0.074</td>
</tr>
</tbody>
</table>

*General factorial ANOVA with linear contrast and age as a covariate.

Discussion

In the current study we report the association between plasma apoB concentrations and CAD in women from the general population who were referred for angiography. Chol, LDL-chol, HDL-chol, TGs, and apoB were independent risk factors for CAD in women after correction for nonlipid risk factors. ApoA-I was not related to CAD. ApoB was superior to the “traditional lipids” chol, LDL-chol, HDL-chol, and TG in predicting the presence or absence of CAD. In the lowest quartiles of chol, LDL-chol, and TG, CAD+ women still had higher apoB concentrations than CAD− women. In the highest HDL-chol quartile, apoB concentrations were higher in CAD+ compared with CAD− women, but this association only approached (P=0.07) significance. In contrast, chol, LDL-chol, TGs, or HDL-chol gave no additional information for any apoB quartile. ApoB was also associated with the extent of CAD, expressed as the number of vessels involved, which has recently been reported to predict cardiovascular mortality.17

The role of apoB as an important risk factor is biologically plausible,18,19 since plasma apoB concentrations reflect the number of atherogenic lipoprotein particles.20 The atherogenic lipoproteins are LDL, containing predominantly chol, VLDL remnants (IDL), and chylomicron remnants, which contain both chol and TGs. LDL and remnant particles each contain 1 molecule of apoB as the structural protein, whereas the amount of chol and TGs per particle varies, and with it, the atherogenicity of the particle. Large, TG-rich VLDL particles are not considered atherogenic,21 whereas smaller remnants of TG-rich lipoprotein particles are atherogenic.22 Small, dense LDL particles are more atherogenic than are LDL particles of normal composition.23 Increased small, dense LDL concentrations are reflected by a more pronounced elevation of LDL apoB than of LDL-chol, and this trend is often accompanied by elevated plasma TG concentrations. Chol is a constituent of both atherogenic, apoB-containing lipoproteins and antiatherogenic, apoA-containing lipoproteins. This heterogeneity of lipoprotein particle composition can explain the superiority of apoB over chol and TG as a CAD risk factor.

TABLE 4. Fasting Lipids and Apolipoproteins in Normolipidemic* CAD− and CAD+ Women as Assessed by Angiography

<table>
<thead>
<tr>
<th></th>
<th>CAD−</th>
<th>CAD+</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chol, mmol/L</td>
<td>5.63±0.69 (n=75)</td>
<td>5.87±0.54 (n=52)</td>
<td>0.041</td>
</tr>
<tr>
<td>TGs, mmol/L</td>
<td>1.37±0.42 (n=66)</td>
<td>1.43±0.47 (n=50)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-chol, mmol/L</td>
<td>1.35±0.33 (n=64)</td>
<td>1.35±0.31 (n=50)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-chol, mmol/L</td>
<td>3.58±0.78 (n=64)</td>
<td>3.89±0.59 (n=50)</td>
<td>0.020</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.48±0.33 (n=58)</td>
<td>1.49±0.28 (n=49)</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.08±0.28 (n=58)</td>
<td>1.21±0.20 (n=49)</td>
<td>0.008</td>
</tr>
<tr>
<td>Age, y</td>
<td>57.2±12.06 (n=75)</td>
<td>62.6±8.87 (n=52)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

*Plasma chol =6.5 mmol/L and TGs ≤2.3 mmol/L.
†By logistic regression.
Prospective population-based studies have designated HDL-chol as the most powerful lipid risk factor in women, but in these studies apoB was not determined. Cross-sectional analysis of the Framingham Offspring population showed that apoB concentrations were related to CAD in women. Data on apoB as a risk factor for CAD in the general population will also become available from a prospective, population-based observational study, including a large number of US women.

Although apoB was the best discriminant between CAD− and CAD+ in the current study, there was still considerable overlap in apoB between CAD− and CAD+. Other studies have shown that the predictive power of apoB can be increased by separation of the plasma into different apoB-containing lipoprotein fractions. The fasting remnant, or IDL fraction, was superior to the LDL fraction in predicting the presence or severity of CAD in women with premature CAD. Postprandial chylomicron remnant concentrations were higher in CAD+ than in CAD− women while LDL apoB was similar. IDL-chol was also related to the progression of CAD in a combined group of men and women (n=63) with premature CAD, and IDL mass was related to the progression of carotid artery intima-media thickness in a study in men and women (n=24) combined. Early data from Framingham showed that in women, and in particular in postmenopausal women, remnant chol was related to CAD. Thus, in women, parameters for remnant particles are potentially better CAD risk indicators than is plasma apoB.

A limitation of the current study is the selection of women for angiography. Although only women who were undergoing their first angiography were included and women on lipid-lowering medication were excluded, the women who were suspected of having CAD could have changed their lifestyle. The CAD− women, who were referred for angiography because of suspected CAD, were likely to have more risk factors compared with CAD+ women from the general population. These considerations would more likely underestimate than overestimate the strength of apoB as a risk factor. Still, the findings from the current study can be applied to women who are referred for angiography but cannot be extrapolated directly to the general population.

Currently, guidelines for CAD prevention are focussed on LDL-chol, although in women, LDL-chol is not a very powerful CAD risk factor. For secondary prevention, the target level for LDL-chol is ≤2.6 mmol/L, and lipid-lowering therapy should be initiated if LDL-chol is ≥3.4 mmol/L. In the current study, 99% of CAD+ women had an LDL-chol concentration >2.6 mmol/L. Ninety percent of CAD+ women had an LDL-chol concentration ≥3.4 mmol/L, and therefore, had an indication for lipid-lowering interventions. A generally accepted apoB target level for prevention of cardiovascular disease may be defined in the future.

**Hormonal Status**

CAD is a disease of postmenopausal women. In the current study 95% of the CAD+ women were postmenopausal. Estrogen deficiency has been reported to be an independent risk factor for CAD assessed by angiography. In the current study, menopause was no longer a CAD risk factor after correction for age. This was probably due to the relatively small number of premenopausal women. Therefore, another measure of exposure to endogenous estrogens was used: the age at menopause. Recently it has been reported that the age at menopause is inversely associated with cardiovascular mortality. This association was not found in the Nurses’ Health Study, in which the population was rather young. In the current study the age at menopause was not associated with CAD after correction for age. These findings suggest...
that age is a more important factor than endogenous estrogens. Another explanation could be that the current study group is different from the general population. In addition, endogenous estrogens are potentially related to CAD through mechanisms that are not detectable on angiography, such as vascular wall reactivity.\textsuperscript{39–42} This would imply that angiography studies are not an appropriate tool to evaluate the effect of estrogens. Nevertheless, the effect of HRT on angiographically defined CAD has repeatedly been demonstrated\textsuperscript{43–46} and even linked to survival.\textsuperscript{17} In the current study, few women ever used HRT, and the mean duration of HRT was short.

### Nonlipid Risk Factors
Smoking emerged as a stronger risk factor than hypertension and diabetes. This also applied to the normolipidemic subgroup of women. Cessation of smoking has been reported to reduce CAD risk by 50% to 80% in observational studies.\textsuperscript{47} In conclusion, in this community-based angiography study in women, plasma concentrations of apoB, reflecting the number of atherogenic lipoproteins, was the best lipoprotein or lipid discriminant between CAD$^-$ and CAD+. The majority of CAD$^+$ women had an LDL > 2.5 mmol/L, the target level for secondary prevention. The majority of the CAD$^+$ women were also postmenopausal, and HRT has been associated with protection against CAD.\textsuperscript{48} It remains to be determined which intervention, lipid lowering per se, HRT (also known to reduce apoB\textsuperscript{49–51}), or a combination of both will be the best strategy.

### Acknowledgment
The authors wish to thank Dr Ir. J.A.J. Faber for statistical advise and E. Bouwens for secretarial help.

### Appendix
The parameters in the current study were also analyzed in a forward logistic regression model. The forward logistic regression model designated apoB as the most powerful discriminative factor between CAD$^+$ and CAD$^-$, as shown in Table 5. The interrelation between the different lipid and apolipoprotein variables and differences in the units of measure, ie, a logarithmic scale for TGs, millimoles per liter for chol, and grams per liter for apolipoproteins, make interpretation of this analysis less straightforward. A second-best parameter that is very close to the first and almost equal in discriminating potential as the first can be completely lost in a forward logistic model. We therefore chose not to report the data from the logistic regression analysis in our article but instead divided the lipid and apolipoprotein parameters into quartiles and analyzed differences in the other lipid parameters for any given quartile. Nevertheless, both ways of analysis show consistent results and depict apoB as the strongest factor associated with CAD.

### References


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