Apolipoprotein A-II Is Inversely Associated With Risk of Future Coronary Artery Disease

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Background—Although the vasculoprotective effects of apolipoprotein A-I (apoA-I), the major protein associated with high-density lipoprotein, have been universally accepted, apoA-II has been suggested to have poor antiatherogenic or even proatherogenic properties. To study this suggestion more closely, we evaluated how serum levels of apoA-II and apoA-I relate to the risk of future coronary artery disease (CAD) in a large, prospective study.

Methods and Results—We performed a nested case-control study in the prospective EPIC-Norfolk (European Prospective Investigation into Cancer and Nutrition–Norfolk) cohort. Case subjects (n=912) were apparently healthy men and women aged 45 to 79 years who developed fatal or nonfatal CAD during a mean follow-up of 6 years. Control subjects (n=1635) were matched by age, gender, and enrollment time. Conditional logistic regression was used to quantify the relationship between serum apoA-II levels and risk of CAD. Serum apoA-II concentration was significantly lower in case subjects (34.5±6.3 mg/dL) than in control subjects (35.2±5.8 mg/dL) and was inversely associated with risk of CAD, such that patients in the upper quartile (>38.1 mg/dL) had an odds ratio of 0.59 (95% confidence interval 0.46 to 0.76) versus those in the lowest quartile (<31.1 mg/dL; P for linearity <0.0001). After adjustment for fasting time, alcohol use, and cardiovascular risk factors (systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, smoking, diabetes mellitus, and C-reactive protein), the corresponding risk estimate was 0.48 (95% confidence interval 0.34 to 0.67, P for linearity <0.0001). Surprisingly, additional adjustment for serum apoA-I levels did not affect risk prediction of apoA-II for future CAD (odds ratio 0.49, 95% confidence interval 0.34 to 0.68, P for linearity <0.0001). Also, after adjustment for high-density lipoprotein particle number and size, apoA-II was still associated with the risk of future CAD (odds ratio 0.62, 95% confidence interval 0.43 to 0.90, P for linearity 0.02).

Conclusions—ApoA-II is associated with a decreased risk of future CAD in apparently healthy people. These findings imply that apoA-II itself exerts effects on specific antiatherogenic pathways. On the basis of these findings, discussion of the potential proatherogenic effects of apoA-II can cease. (Circulation. 2007;116:2029-2035.)

Key Words: apolipoproteins ♦ coronary disease ♦ risk factors ♦ cholesterol ♦ atherosclerosis

A polipoprotein A-I (apoA-I), the major structural apolipoprotein of HDL, has been widely acknowledged to have potent antiatherogenic properties. This is less clear for apolipoprotein A-II (apoA-II), the second most abundant structural apolipoprotein associated with HDL.

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In the past few years, attempts have been made to dissect the pathophysiological relevance of HDL particles that contain only apoA-I (LpA-I) versus particles that contain both apoA-I and apoA-II (LpA-I:A-II). LpA-I particles have been reported to have antiatherogenic capacity, demonstrated in both experimental and observational studies. O’Brien and colleagues have even reported that LpA-I had a larger cardioprotective capacity than LpA-I:LpA-II particles. However, in a post hoc analysis from the Framingham Offspring Study and the Veterans-Affairs HDL Intervention Trial, no relationship between cardiovascular events and LpA-I or LpA-I:A-II particles was obvious.

Although the latter studies show that measuring these HDL subclasses has no additional value for predicting cardiovascular risk, it remains to be demonstrated whether apoA-II is associated with cardiovascular event rate. Currently, relatively small case-control studies have demonstrated an inverse relation between...
cardiovascular disease and plasma apoA-II levels. Likewise, low serum apoA-II levels have been put forward as a powerful predictor for cardiovascular risk in individuals with diabetes mellitus. However, other studies suggested that elevated apoA-II levels may in fact be proatherogenic. One study in middle-aged men revealed no relation between cardiovascular disease and plasma apoA-II levels. In fact, polymorphisms of the apoA-II gene associated with decreased apoA-II levels were not related to CAD. In view of these contradictory results, we performed a nested case-control study in the large, prospective EPIC (European Prospective Investigation into Cancer and Nutrition)–Norfolk study cohort to evaluate the relationship between both serum apoA-I and serum apoA-II levels and risk of future CAD.

Study Design
Between 1993 and 1997, the EPIC-Norfolk cohort study was performed. This is a prospective population study of 25,663 male and female inhabitants of Norfolk, United Kingdom, between the ages of 45 and 79 years who completed a baseline questionnaire survey and attended a clinic visit. EPIC-Norfolk is part of the 9-country collaborative EPIC study designed to investigate dietary and other determinants of cancer. Additional data were obtained to enable assessment of determinants of other diseases. The study cohort was similar to UK population samples with regard to many characteristics, including anthropometry, blood pressure, and lipids, but with a lower proportion of smokers. Participants were recruited by mail from age-gender registers of general practices. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire, and additional data collection was performed by trained nurses at a clinic visit as described previously. All individuals have been flagged for mortality at the UK Office of National Statistics, with vital status ascertained for the entire cohort. Death certificates for all decedents were coded by trained nosologists according to the International Classification of Diseases (ICD) 9th revision. Death was considered due to CAD if the underlying cause was coded as ICD 410 to 414. In addition, participants admitted to a hospital were identified by their unique National Health Service number by data linkage with ENCORE (East Norfolk Health Authority database), which identifies all hospital contacts throughout England and Wales for Norfolk residents. Participants were identified as having CAD during follow-up if they had a hospital admission and/or died with CAD listed as an underlying cause. We report results with follow-up up to January 2003, an average of 6 years. The Norwich District Health Authority Ethics Committee approved the study, and all participants gave signed informed consent.

Participants
For the present nested case-control study, we identified 912 apparently healthy individuals who developed fatal or nonfatal CAD during follow-up. Apparently healthy individuals were defined as study participants who did not report a history of heart attack or stroke at the baseline clinic visit. Control subjects were apparently healthy study participants who remained free of CAD during follow-up. Two control subjects were matched to each case subject by gender, age (within 5 years), and date of visit (within 3 months).

Biochemical Analyses
Nonfasting blood samples were taken by vein puncture into serum tubes. Blood samples were processed for assay at the Department of Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands, and stored at −80°C before analysis. Serum levels of total cholesterol, HDL cholesterol, and triglycerides were measured on fresh samples with the RA 1000 autoanalyzer (Bayer Diagnostics, Basingstoke, United Kingdom). LDL cholesterol levels were calculated with the Friedewald formula to closely approach current clinical procedures. Serum levels of apoA-I and apoB were measured by rate immunonephelometry (Behring Nephelometer BNII, Marburg, Germany) with calibration traceable to the International Federation of Clinical Chemistry primary standards. The interassay coefficients of variation of the apoA-I and apoB measurements were 5% and 3%, respectively. Serum concentrations of apoA-II were measured with a commercially available immunoturbidimetric assay (Wako Pure Chemicals Industries, Ltd, Osaka, Japan) on a Cobas-Mira autoanalyzer (Roche, Basel, Switzerland). The intra-assay and interassay variations for this assay are 2.5% and 3.1%, respectively.

HDL particle number and HDL size were measured with an automated nuclear magnetic resonance spectroscopic assay as described previously. C-reactive protein (CRP) levels were measured as described previously. Samples were analyzed in random order to avoid systematic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

Statistical Analysis
Baseline characteristics were compared between case and control subjects with a mixed-effect model for continuous variables or conditional logistic regression for categorical variables. Because triglycerides and CRP levels had a skewed distribution, values were log-transformed before statistical analysis. Our primary objective was to evaluate the relationships between serum apoA-II levels, cardiovascular risk factors, and the risk of CAD; therefore, apoA-II levels were categorized into (gender-specific) quartiles based on the distribution that was identified in the control subjects. Mean levels of cardiovascular risk factors were calculated per apoA-II quartile. Conditional logistic regression analysis was used to calculate ORs and corresponding 95% CIs with gender-specific apoA-II quartiles as an estimate of the relative risk of CAD. Serum apoA-II concentrations were analyzed as categorical variables after division into quartiles, with the lowest quartile used as the reference category. ORs were calculated after we took into account the matching for age and gender, and ORs were adjusted for systolic blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, body mass index (BMI), smoking, alcohol use, diabetes mellitus, CRP, and fasting time (the time between the last meal and the moment that blood was drawn). ORs were also calculated after additional adjustment for apoA-I and HDL particle number and size. C-statistic analysis (receiver operating characteristic analysis) was performed to assess the effect of apoA-I and apoA-II on risk prediction. For Pearson correlation, we used continuous versions to measure correlation between apoA-II levels and cardiovascular risk factors. Statistical analyses were performed with SPSS software (version 12.0.1). A probability value <0.05 was considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Serum samples from 912 case subjects and 1635 matched control subjects were available. From these individuals, 689 case subjects were matched to 2 control subjects each, whereas 223 case subjects could only be matched to 1 control. Matching ensured that age and gender were comparable between case subjects and control subjects. Despite accurate matching, age was only matched within 5 years; therefore, we also adjusted for age in a separate analysis. However, the addition of age did not change the results. As expected, individuals who developed CAD during follow-up were more likely than control subjects to smoke and to have diabetes mellitus (Table 1). The use of alcohol was comparable between case subjects and control subjects. Likewise, in both men and women, levels of total cholesterol, LDL cholesterol,
Triglycerides, apoB, systolic and diastolic blood pressure, BMI, and CRP were significantly higher in case subjects than in control subjects. In contrast, HDL cholesterol and apoA-I levels were lower in case subjects than in control subjects (34.5 ± 3.5 vs. 36.3 ± 2.5 mg/dL, P < 0.0001). Even additional adjustment for apoA-I did not affect the relationship between apoA-II and risk of future CAD (OR 0.49, 95% CI 0.34 to 0.68, P for linearity < 0.0001). Even additional adjustment for apoA-I did not affect the relationship between apoA-II and risk of future CAD (OR 0.49, 95% CI 0.34 to 0.68, P for linearity < 0.0001). Even additional adjustment for apoA-II weakened the risk estimate of apoA-I with an OR of 0.69 (95% CI 0.43 to 1.09, P for linearity = 0.06, highest versus lowest quartile). As expected, further adjustment for HDL particles and size abolished the apoA-I risk estimate (P for linearity = 0.72). Comparable to apoA-I, apoA-II was a potent, inverse predictor of cardiovascular risk, even after adjustment for traditional risk factors.

C-statistics revealed that apoA-I and apoA-II alone and combined did not improve risk prediction above that of the Framingham risk score. In brief, we compared the following models: (1) a model with the Framingham risk score alone (area under the curve 0.599; 95% CI 0.575 to 0.622); (2) a model with Framingham risk score and apoA-I (area under the curve 0.600; 95% CI 0.576 to 0.623); (3) a model with the Framingham risk score and apoA-II (area under the curve 0.599; 95% CI 0.575 to 0.622); and (4) a model with the Framingham risk score and both apoA-I and apoA-II (area under the curve 0.600; 95% CI 0.576 to 0.623). We performed receiver operating characteristic analysis with the models that included total cholesterol, HDL cholesterol, diabetes mellitus, smoking, systolic blood pressure, and either apoA-I or apoA-II or both combined.

Table 4 shows the Pearson correlation between apoA-I and apoA-II and HDL cholesterol, HDL size, and HDL particle number. HDL cholesterol correlated with apoA-II (r = 0.44, P < 0.0001) but not as much as with apoA-I (0.80, P < 0.0001). Although apoA-I correlated well with HDL size (r = 0.59, P < 0.0001), a much weaker correlation was found between HDL size and apoA-II (r = 0.11, P < 0.0001). No major differences existed in correlations between men and women (see online-only Data Supplement for gender-specific data).

**Discussion**

In this large, prospective, case-control study among apparently healthy men and women, we observed a strong inverse relationship between serum apoA-II levels and risk of future CAD. Individuals in the highest apoA-II quartile had an unadjusted OR of 0.59 (95% CI 0.46 to 0.76) compared with those in the lowest apoA-II quartile (P for linearity < 0.0001). With increasing apoA-II quartiles, the OR for future CAD, adjusted for systolic blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, BMI, smoking, alcohol use, diabetes mellitus, CRP, and fasting time, decreased in a linear pattern (OR 0.48, 95% CI 0.34 to 0.67, P for linearity < 0.0001).
CAD events. After adjustment for cardiovascular risk factors including HDL cholesterol and apoA-I, increased apoA-II levels still were associated with a lower risk for future CAD events. In contrast, adjustment for apoA-II significantly weakened the association between apoA-I and risk of future CAD events. These data indicate that apoA-II is associated with cardiovascular protection, even after adjustment for apoA-I. Given these findings, discussions on the potential proatherogenic effects of apoA-II can cease.

**ApoA-I, ApoA-II, and Risk of CAD**

In the present study, we found that serum apoA-II levels were lower among case subjects than control subjects. These data are in accordance with previous case-control studies that reported lower plasma apoA-II levels among 246 and 283 patients, respectively, with CAD than among control subjects.21,22 We also found an inverse relation between apoA-II and CAD risk, even after adjustment for apoA-I and HDL particle numbers and size. The latter finding contrasts with previous studies in which no protective effect for apoA-II against atherosclerosis was found in apoA-I–deficient heterozygotes.23 In fact, most animal and human studies have associated apoA-II levels with increased receptiveness toward atherosclerosis,21–23 increased fatty acid levels,24,25 increased body fat,25,26 decreased paraoxonase-1 activity, and decreased insulin sensitivity.25,27 Several explanations can account for this discrepancy. First, the present findings are derived from a large, prospective, case-control cohort, whereas results from other observational studies have been hampered because of small sample size or the retrospective design of these studies.9,19 In addition to this, the only human study with apoA-II–deficient heterozygotes comprised just 2 participants.28 Second, apoA-II accounts for ≈20% of HDL protein, and mean serum apoA-II concentrations are generally 4 times lower than mean apoA-I levels in normolipidemic subjects. This increases the prospect of spurious results. A study with a large sample size similar to the present study would reduce this possibility. Finally, most of the animal studies have been performed in either knockout or transgenic mice, which have been shown to poorly reflect HDL metabolism in humans, whereas the present data are exclusively human.

To illustrate the (patho)physiological relevance of apoA-II in relation to CAD risk, we assessed its discrimination beyond that of standard cardiovascular disease risk factors. ApoA-I and apoA-II, alone or combined, did not improve risk prediction beyond that of the Framingham risk score. Obviously, we do not propose to put forth apoA-II as a risk predictor in clinical practice. Most importantly, the present findings clearly do not support previous controversies with regard to the potential proatherogenic effects of apoA-II.

**ApoA-II and HDL**

Interestingly, the protective effect of apoA-II persisted after adjustment for HDL cholesterol, particle numbers, and size.
The latter finding may have different implications. First, the inverse relation between apoA-II and cardiovascular disease may be a consequence of the fact that apoA-II is predominantly present on large HDL particles. In this scenario, apoA-II could merely serve as a marker for a large HDL subfraction, which has been suggested to be associated with beneficial cardiovascular outcome.29 However, this is less likely, because we found no correlation between apoA-II and larger HDL and a very weak one between apoA-II and HDL size in the present study. Likewise, others have demonstrated a relatively larger proportion of LpA-I to be present in larger HDL (HDL), whereas LpA-I:A-II was predominantly present on smaller HDL (HDL),3,30 Second, apoA-II may exert direct vasculoprotective effects. In fact, apoA-II has been shown to increase lecithin:cholesterol acyltransferase activity, promote cholesterol efflux,31,32 and reduce cholesteryl ester hydroperoxides,33 whereas HDL from human apoA-II transgenic mice failed to protect LDL from oxidative modification.34 It has also been shown that overexpression of human apoA-II per se results in a proatherogenic phenotype.21,22,35 Conversely, other studies have demonstrated apoA-II to be protective against atherosclerosis.36,37 Although the protective effect of apoA-II also persists after correction for HDL, <50% of HDL particles contain LpA-I:A-II. More interestingly, the relation between HDL particle number and apoA-II was even stronger than for apoA-I. The potential explanation for this phenomenon pertains to the fact that apoA-II is only present in LpA-I:A-II HDL, which contains 2 molecules of apoA-II per particle. Thus, the concentration of apoA-II provides a “robust” measure of the number of LpA-I:A-II particles. In contrast, apoA-I is present in both LpA-I and LpA-I:A-II, which contain anywhere from 2 to 4 apoA-I molecules. As a consequence, apoA-I concentration is less well correlated with either LpA-I or LpA-I:A-II particles.

The fact that the protective capacity of apoA-II is comparable to that of apoA-I in spite of its lower abundance lends further support to a potent antiatherogenic effect of apoA-II. In support of this, apoA-II has been shown to share similar structural domains with apoA-I, such as the amphipathic α-helices that have been associated with antiatherogenic functions.38

### Table 3. ORs for Future CAD Events by Gender-Specific ApoA-II Quartiles

<table>
<thead>
<tr>
<th>ApoA-II Quartile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men and women, n</td>
<td>635</td>
<td>643</td>
<td>636</td>
<td>633</td>
<td>...</td>
</tr>
<tr>
<td>Quartile range, mg/dL</td>
<td>&lt;31.1</td>
<td>31.1–34.3</td>
<td>34.3–38.1</td>
<td>&gt;38.1</td>
<td>...</td>
</tr>
<tr>
<td>OR unadjusted</td>
<td>1.00</td>
<td>0.72 (0.57–0.91)</td>
<td>0.72 (0.57–0.91)</td>
<td>0.59 (0.46–0.76)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OR adjusted*</td>
<td>1.00</td>
<td>0.71 (0.53–0.95)</td>
<td>0.66 (0.49–0.89)</td>
<td>0.48 (0.34–0.67)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OR adjusted†</td>
<td>1.00</td>
<td>0.72 (0.54–0.95)</td>
<td>0.67 (0.49–0.90)</td>
<td>0.49 (0.34–0.68)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OR adjusted‡</td>
<td>1.00</td>
<td>0.78 (0.58–1.05)</td>
<td>0.78 (0.57–1.08)</td>
<td>0.62 (0.43–0.90)</td>
<td>0.02</td>
</tr>
<tr>
<td>Men, n</td>
<td>397</td>
<td>395</td>
<td>396</td>
<td>399</td>
<td>...</td>
</tr>
<tr>
<td>Quartile range, mg/dL</td>
<td>&lt;30.4</td>
<td>30.4–33.6</td>
<td>33.6–36.8</td>
<td>&gt;36.8</td>
<td>...</td>
</tr>
<tr>
<td>OR unadjusted</td>
<td>1.00</td>
<td>0.83 (0.61–1.13)</td>
<td>0.66 (0.48–0.89)</td>
<td>0.57 (0.41–0.79)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OR adjusted*</td>
<td>1.00</td>
<td>0.79 (0.52–1.20)</td>
<td>0.50 (0.32–0.78)</td>
<td>0.35 (0.21–0.58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OR adjusted†</td>
<td>1.00</td>
<td>0.79 (0.52–1.20)</td>
<td>0.50 (0.32–0.78)</td>
<td>0.36 (0.22–0.59)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OR adjusted‡</td>
<td>1.00</td>
<td>0.93 (0.60–1.44)</td>
<td>0.63 (0.39–1.01)</td>
<td>0.78 (0.66–0.94)</td>
<td>0.009</td>
</tr>
<tr>
<td>Women, n</td>
<td>240</td>
<td>240</td>
<td>240</td>
<td>240</td>
<td>...</td>
</tr>
<tr>
<td>Quartile range, mg/dL</td>
<td>&lt;32.6</td>
<td>32.6–35.9</td>
<td>35.9–39.5</td>
<td>&gt;39.5</td>
<td>...</td>
</tr>
<tr>
<td>OR unadjusted</td>
<td>1.00</td>
<td>0.81 (0.55–1.18)</td>
<td>0.69 (0.46–1.02)</td>
<td>0.81 (0.54–1.19)</td>
<td>0.21</td>
</tr>
<tr>
<td>OR adjusted*</td>
<td>1.00</td>
<td>0.69 (0.42–1.10)</td>
<td>0.64 (0.38–1.06)</td>
<td>0.71 (0.42–1.20)</td>
<td>0.22</td>
</tr>
<tr>
<td>OR adjusted†</td>
<td>1.00</td>
<td>0.68 (0.42–1.09)</td>
<td>0.61 (0.37–1.02)</td>
<td>0.70 (0.41–1.21)</td>
<td>0.20</td>
</tr>
<tr>
<td>OR adjusted‡</td>
<td>1.00</td>
<td>0.72 (0.44–1.17)</td>
<td>0.68 (0.40–1.17)</td>
<td>0.83 (0.46–1.50)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

ORs for the risk of future CAD events for both genders and males and females separately.

*Adjustment for traditional risk factors (systolic blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, BMI, smoking, diabetes mellitus, CRP), alcohol use, and fasting time.
†Adjustment for above-mentioned variables plus apoA-I.
‡Adjustment for above-mentioned variables plus HDL particle numbers and size.
P linear trend with 1 degree of freedom.

### Table 4. Correlation Between ApoA-I/A-II and HDL Cholesterol, HDL Size, and HDL Particle Numbers

<table>
<thead>
<tr>
<th></th>
<th>ApoA-I</th>
<th>ApoA-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.59</td>
<td>0.44</td>
</tr>
<tr>
<td>HDL size, nm</td>
<td>0.59</td>
<td>0.11</td>
</tr>
<tr>
<td>No. of HDL particles, nmol/L</td>
<td>0.55</td>
<td>0.44</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ApoA-II, mg/dL</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

R indicates 2-tailed Pearson’s correlation with the corresponding P value.
One contradictory finding of interest was the positive correlation between apoA-II and HDL cholesterol and triglycerides. This observation is odd, because HDL cholesterol and triglycerides are inversely related. One explanation might be that apoA-II inhibits lipoprotein lipase and hepatic lipase activities. In fact, some studies have demonstrated these effects of apoA-II. Consequently, the inhibition of lipoprotein lipase and hepatic lipase activities results in less hydrolysis of triglyceride-rich lipoproteins, which leads to increased triglycerides.

**Study Limitations**

When interpreting the results of the present study, one must be aware of its potential limitations. First, case ascertainment is an issue in the design of every prospective study, including this one. However, a validation study indicated that case ascertainment in the present study was at least equivalent to that of other large, prospective cohort studies. Second, the findings of the present study obviously apply mainly to whites, because it was performed in the United Kingdom. Therefore, extrapolation of these results to nonwhite populations must be done with caution. Another issue pertains to the fact that in prospective case-control studies, it is sometimes difficult to establish causality because of observational study design. Previously, apoA-II levels have been positively related with free fatty acids, VLDL, and triglycerides, although a clear-cut relation has not yet been demonstrated. The most important results have been produced mainly through analysis of genetically modified mice, although a few human studies have been able to confirm this relation. However, in the present study, we did not measure free fatty acid levels, because a large variation exists in these levels, especially when samples are obtained in the nonfasting state. Of note, we did measure triglycerides and VLDL concentrations, which revealed a weak but significant association with apoA-II.

**Conclusions**

In the present study, we found a potent inverse relation between apoA-II and risk of future CAD in apparently healthy subjects. The fact that the protective effect of apoA-II persisted even after adjustment for apoA-I, HDL cholesterol, and HDL particle number and size implies that apoA-II itself exerts effects on specific antiatherogenic pathways. Because there has been continued uncertainty about the relationship between apoA-II and CAD, the present findings clearly do not support previous controversies with regard to potential proatherogenic effects of apoA-II.

**Acknowledgments**

We thank the participants, general practitioners, and staff in EPIC-Norfolk. We gratefully acknowledge LipoScience, Inc for performing the nuclear magnetic resonance measurements.

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**Disclosures**

None.

**References**


CLINICAL PERSPECTIVE
The antiatherogenic effects of apolipoprotein A-I (apoA-I), the major structural apolipoprotein of high-density lipoprotein (HDL), have been widely acknowledged. These effects are less clear for apolipoprotein A-II (apoA-II), the second-most-abundant apolipoprotein of HDL, which has been suggested to have poor antiatherogenic or even proatherogenic properties. To study this suggestion more closely, we evaluated the relation between serum levels of apoA-II and the risk of future coronary artery disease in the large prospective EPIC (European Prospective Investigation into Cancer and Nutrition)–Norfolk cohort. We observed a strong inverse relationship between apoA-II levels and risk of future coronary artery disease in apparently healthy men and women. Even after adjustment for cardiovascular risk factors, including apoA-I, HDL cholesterol, HDL particle numbers, and HDL size, increased apoA-II levels were still associated with a lower risk for future coronary artery disease events, an association that is indicative of the direct and potent antiatherogenic potential of apoA-II. These findings provide strong arguments against earlier concepts of adverse proatherogenic effects of apoA-II. Whereas an additional value of apoA-II beyond that of the Framingham risk score was not observed with C-statistic analysis, further studies addressing the value of combined apoA-I and apoA-II as an index of antiatherogenic apolipoproteins appear mandatory.
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