Low Apolipoprotein A-IV Plasma Concentrations in Men With Coronary Artery Disease

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OBJECTIVES The objective of this study was to evaluate the relation between apolipoprotein A-IV (apoA-IV) plasma concentrations and coronary artery disease (CAD).

BACKGROUND Experimental in vitro and in vivo studies favor apoA-IV to be protective against the development of atherosclerosis. Mice that overexpress either human or mouse apoA-IV demonstrated a significant reduction of aortic atherosclerotic lesions compared with control mice. Data on apoA-IV plasma concentrations and CAD in humans are lacking.

METHODS We determined in two independent case-control studies of a Caucasian and an Asian Indian population whether apoA-IV plasma concentrations are related to the presence of angiographically assessed CAD.

RESULTS Plasma apoA-IV levels were significantly lower in 114 male Caucasian subjects with angiographically defined CAD when compared with 114 age-adjusted male controls (10.2 ± 3.8 mg/dL vs. 15.1 ± 4.0 mg/dL, p < 0.001). Logistic regression analysis indicated that the association between apoA-IV levels and CAD was independent of the high-density lipoprotein cholesterol and triglyceride concentrations. The inverse relationship between plasma levels of apoA-IV and the presence of CAD was confirmed in an independent sample of 68 male Asian Indians with angiographically documented CAD and 68 age-matched controls.

CONCLUSIONS The results of this cross-sectional study demonstrate for the first time an association between low apoA-IV concentrations and CAD in humans and suggest that apoA-IV may play an antiatherogenic role in humans. (J Am Coll Cardiol 2000;36:751–7) © 2000 by the American College of Cardiology

Human apolipoprotein A-IV (apoA-IV) is a 46 kDa glycoprotein (1) that is almost exclusively produced in intestinal enterocytes and secreted into the lymph as a structural protein of chylomicrons (2). The mean plasma level of apoA-IV is about 15 mg/dL. The distribution of apoA-IV among plasma lipoproteins varies depending on the plasma fractionation method. In the fasting state the majority of apoA-IV circulates in plasma as a lipid-poor, small high-density lipoprotein (HDL)-like particle that does not contain apolipoprotein A-I (apoA-I) (3,4). The remaining moiety of apoA-IV is associated with apolipoprotein A-I (apoA-I) containing HDL particles of larger size, and its levels vary over a wide range in the literature (3,5,6).

The physiological function of apoA-IV is not clear. It was postulated to be involved in fat absorption (7), but recently this hypothesis has been challenged by findings in transgenic mice in which the gene has been inactivated (8,9). Both strains of genetically modified mice absorb lipids normally and are phenotypically indistinguishable from wild type mice.

Intravenous apoA-IV infusion to rats resulted in a reduction of food intake, therefore suggesting it may function as a satiety factor (10). However, A-IV knockout mice failed to reveal any abnormalities in their feeding behavior (9).

Numerous in vitro studies suggest that apoA-IV participates in several steps of the reverse cholesterol transport pathway, which removes cholesterol from peripheral cells and transports it to the liver or steroidogenic organs where cholesterol can be metabolized to bile acids and hormones, respectively. Apolipoprotein A-IV binds to peripheral cells, promotes cholesterol efflux and enhances the formation of small HDL particles (11,12) by activating lecithin: cholesterol acyltransferase (13,14). In addition, apoA-IV may participate in the binding and uptake of HDL by hepatocytes (15). Moreover, apoA-IV modulates the activation of lipoprotein lipase (16) and the cholesteryl ester transfer protein (CETP)-mediated transfer of cholesteryl esters from HDL to low-density lipoprotein (LDL) in tissue culture studies (17). Taken together, these in vitro functions suggest that apoA-IV may represent an antiatherogenic factor.

In vivo studies in animals support this antiatherogenic role for apoA-IV. Fat-fed mice that overexpress either human (18) or mouse apoA-IV (19) demonstrated a significant reduction of aortic atherosclerotic lesions compared with control mice. Atherosclerosis was even inhibited by overexpression of human apoA-IV in apoE-deficient mice.
which are hyperlipidemic and develop severe atherosclerosis even on chow diets (18).

In this study we aimed to investigate whether the findings in transgenic mice have a clinical correlative in humans. We, therefore, measured apoA-IV plasma concentrations in two different ethnic populations of patients with angiographically verified coronary artery disease (CAD) and compared them with those in controls of the same population.

METHODS

Caucasian study population. A total of 114 consecutive male subjects who underwent coronary angiography and had coronary stenosis of $\geq 50\%$ of the luminal diameter in at least one coronary artery were included in the study. These patients were recruited from the geographical area of Tyrol, Austria. The mean age was 60 $\pm$ 10 years, and 43 had single-vessel disease; 33 had double-vessel disease, and 38 had a stenotic lesion in three or more vessels. Sixty-two of the 114 patients had suffered a coronary event (myocardial infarction, aortocoronary bypass of percutaneous transluminal coronary angioplasty) at least six weeks and up to 24 years before the coronary angiography. Patients were compared with 114 male controls with a mean age of 53 $\pm$ 8 years who were recruited in 1997 during a follow-up project of the Münster Heart Study (PROCAM) from one of the PROCAM study centers in Germany (20). Apolipoprotein A-IV levels in these controls were identical with the levels obtained in a previous group of blood donors (21) recruited from the same geographical area as the patients of this study and similar to controls from Northern, Middle and Southern Europe in the European Atherosclerosis Research Study (EARS) (22). Subjects with renal impairment (serum creatinine $>1.5$ mg/dL or a macroalbuminuria) (6,21) were excluded from the analysis. Patients and controls gave informed consent to the investigation.

Indian study population. We also analyzed the apoA-IV plasma concentrations in 68 male patients from India with entry criteria as mentioned above and 68 age-matched male controls from the same geographical area as patients. The mean age was 52 $\pm$ 8 years, and 22 of them showed a single-vessel disease; 16 had double-vessel disease, and 30 had stenosis of three or more arteries.

Samples and laboratory procedures. Venous blood was obtained in tubes containing ethylenediamine tetraacetate (EDTA) one to three days before coronary angiography after an overnight fasting period. The plasma was isolated and frozen at $-70^\circ$C before analysis (on average 6 to 12 weeks after withdrawal) (23). Storing samples under those conditions is without any consequence on the measured values within a storage period of six months (23).

Plasma apoA-IV concentrations were determined using an enzyme-linked immunosorbent assay that employs affinity-purified rabbit antihuman apoA-IV polyclonal antiserum as the capture antibody and the same antibody coupled with horseradish peroxidase as detection antibody (23,24). Plasma with known content of apoA-IV (standardized with purified apoA-IV after phenylalanin quantification by high pressure liquid chromatography) served as calibration standard. Intraassay and interassay coefficients of variation of this assay are 4.5% and 6.6%, respectively (23). Samples from the patients and controls were analyzed in duplicate within one series in a blinded fashion and after a similar time of sample storage at $-70^\circ$C.

Total cholesterol, HDL cholesterol and triglycerides were measured using kits from Boehringer Mannheim (Mannheim, Germany). Measurements were made on microtitre plates as previously described (23). Low-density lipoprotein cholesterol was calculated with the Friedewald formula.

Statistical methods. Because controls were on average seven years younger than patients in the Caucasian study population, we adjusted total, HDL and LDL cholesterol as well as triglycerides using linear regression analysis. No correlation was observed between apoA-IV and age neither in patients ($r = 0.09, p = 0.30$) nor in controls ($r = 0.06, p = 0.54$). Therefore, unadjusted apoA-IV concentrations were used for the analysis. Continuous variables were compared between patients and controls by unpaired $t$ test or by the Mann-Whitney $U$ test (triglycerides). Power calculation revealed a power of 99% at a significance level of $p < 0.05$ to detect a difference in apoA-IV plasma concentration of 2.4 mg/dL between patients and controls in the Caucasian study population and of 2.8 mg/dL in the Asian Indian population.

Categorial variables were compared in the Caucasian study group using Pearson’s chi-square test. Correlation coefficients between apoA-IV and total HDL and LDL cholesterol were calculated by the method of Pearson. Because of their non-Gaussian frequency distribution, correlations of triglycerides were calculated according to Spearman. Logistic regression analysis was performed to evaluate whether apoA-IV concentrations are associated with CAD independently of HDL cholesterol and triglycerides. Odds ratios (OR) as an approximation of the true relative risk of having CAD were calculated from the regression coefficients while adjustment was made for other variables.

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) for Windows 9.0 (SPSS Inc., Chicago, Illinois). A $p$ value $<0.05$ was used as level of significance for hypothesis testing.
RESULTS

Table 1 shows the clinical characteristics as well as the differences of cardiovascular risk factors between Caucasian patients with CAD and their controls. The subjects with CAD had markedly lower apoA-IV concentrations than controls (10.2 ± 3.8 mg/dL vs. 15.1 ± 4.0 mg/dL, p < 0.001). The frequency distribution of apoA-IV concentrations in patients and controls is shown in panel A of Figure 1. The distribution was shifted to lower concentrations in the Caucasian subjects with CAD, so a markedly higher percentage of the subjects with CAD had low plasma apoA-IV concentrations. Concentrations of plasma HDL cholesterol were significantly lower and triglycerides higher in the patients than they were in controls. Plasma levels of total and LDL cholesterol were lower in the CAD group, probably because a large percentage of the patients (64%) were on lipid-lowering medication.

Correlation of apoA-IV with other variables. We examined the relationship between the plasma apoA-IV concentrations and the other variables that differed significantly in frequency between patients and controls. Subjects receiving antihypertensive drugs had similar plasma apoA-IV concentrations to those without these medications in both patients (10.2 ± 3.7 mg/dL vs. 9.9 ± 4.2 mg/dL) and controls (15.4 ± 4.2 mg/dL vs. 15.0 ± 4.0 mg/dL). Patients who received lipid-lowering drugs had similar apoA-IV concentrations compared to those without (10.1 ± 3.9 mg/dL vs. 10.3 ± 3.6 mg/dL). Furthermore, apoA-IV concentrations in patients who had already suffered a major coronary event previously did not differ from those without an event (10.0 ± 3.7 mg/dL vs. 10.3 ± 3.9 mg/dL).

Plasma apoA-IV concentrations correlated slightly with HDL cholesterol in both the patient and control groups (Fig. 2), but no significant relationship was seen between apoA-IV levels and total cholesterol and LDL cholesterol or triglyceride concentrations.

Association of apoA-IV, HDL cholesterol and triglycerides with CAD. Figure 3 presents the odds of being a patient as an approximation of the relative risk in case of low or high plasma concentrations of apoA-IV, HDL cholesterol or triglycerides. The median levels of these variables

<table>
<thead>
<tr>
<th>Table 1. Comparison of Caucasian Patients With CAD and Controls</th>
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<tbody>
<tr>
<td><strong>Controls</strong></td>
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<tr>
<td>Age (yrs)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
</tr>
<tr>
<td>Current smokers (%)</td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
</tr>
<tr>
<td>Lipid-lowering drugs (%)</td>
</tr>
</tbody>
</table>

Results of coronary angiography (n %)

| Single-vessel disease | 43 (38) | – |
| Double-vessel disease | 33 (29) | – |
| Three or more vessel disease | 38 (33) | – |
| ApoA-IV (mg/dL) | 15.1 ± 4.0 (14.3–15.8) | 10.2 ± 3.8 (9.5–10.9) | 0.001 |
| Total cholesterol (mg/dL*) | 217 ± 40 (209–224) | 194 ± 46 (186–203) | 0.001 |
| HDL cholesterol (mg/dL*) | 40.7 ± 8.8 (39.1–42.4) | 34.0 ± 11.3 (31.9–36.1) | 0.001 |
| LDL cholesterol (mg/dL*) | 144 ± 36 (137–150) | 120 ± 39 (113–127) | 0.001 |
| Triglycerides (mg/dL*) | 162 ± 105 (143–182) | 201 ± 132 (176–225) | 0.005 |

Median 135 164

Values are presented as mean ± SD (95% confidence interval), unless otherwise stated. CAD = coronary artery disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

*Adjusted for age.

Figure 1. Distribution of apolipoprotein A-IV (apoA-IV) plasma concentrations in patients with coronary artery disease and controls. Panel A shows the results of 114 Caucasian male patients and 114 male controls. Panel B describes the apoA-IV distribution in 68 male Asian Indian patients and 68 age-matched male controls.
from the control group were used as categorization cutpoints. Having low compared with high plasma HDL cholesterol concentrations increased the probability of being a patient about 2.3 and 2.5 times in the group with high and low apoA-IV plasma concentrations, respectively. On the other hand, having low apoA-IV concentrations increased the odds about six-fold in both groups with high and low HDL cholesterol concentrations (Fig. 3, panel A). Very similar ORs were observed for apoA-IV and triglyceride concentrations (Fig. 3, panel B). This clearly shows that the association of apoA-IV with CAD is independent from HDL cholesterol or triglycerides but additive to their association with CAD.

**Logistic regression analysis.** We further examined the association of apoA-IV with CAD using a logistic regression analysis (Table 2). All three variables, apoA-IV, HDL cholesterol and triglycerides, significantly predicted CAD in univariate analysis. High-density lipoprotein cholesterol and triglycerides, however, predicted CAD with a much lower probability than apoA-IV concentrations. Multivariate analysis showed that the association of apoA-IV with CAD was independent of HDL cholesterol and triglycerides for two reasons: first, no large differences in the ORs of apoA-IV were seen between univariate analysis and multivariate analysis including either HDL cholesterol (model 1) or triglycerides (model 2). Each 2 mg/dL decrease of apoA-IV increased the odds for being a patient between 79% and 93% depending on the model. The inclusion of HDL cholesterol or triglycerides in the model did not increase the frequency of subjects of about 75% correctly classified as patient or control. Second, the introduction of an interaction term between apoA-IV and HDL cholesterol or triglycerides did not contribute to the model. Repeating the analysis excluding patients who already had a history of CAD or those taking lipid-lowering drugs revealed a similar
OR of 1.88 (1.42 to 2.48), which was close to the results obtained in the whole patient group as presented in Table 2.

**Asian Indian study population.** Finally, we confirmed the association of apoA-IV with CAD in an independent ethnic population. We compared apoA-IV levels of 68 male Asian Indian patients with CAD to those of age-matched male controls from the same geographical area. Patients showed significantly lower apoA-IV concentrations compared with controls (7.6 ± 3.5 mg/dL vs. 10.4 ± 4.1 mg/dL, p < 0.001) with a markedly different distribution of apoA-IV concentrations (Fig. 1, panel B).

**Discussion**

**Novel findings.** The physiological role of apoA-IV in lipoprotein metabolism is still not clear. Both in vitro and in vivo studies suggest that apoA-IV is antiatherogenic due to its involvement in reverse cholesterol transport. In this study we provide compelling evidence of a relationship between low apoA-IV concentrations and CAD in two different ethnic populations. Patients with CAD had 30% lower apoA-IV concentrations when compared with their respective controls. This difference in apoA-IV levels between patients and controls was even seen in the Asian Indian study population, which is characterized by markedly lower concentrations of apoA-IV when compared with Caucasians. These relative differences between the ethnic groups is most probably explained by the different diet, which is known to influence apoA-IV concentrations (25).

**Previous studies.** Our data are in contrast with two previous studies, which are different in study design, and the populations investigated. First, the EARS described similar apoA-IV concentrations in children whose fathers had suffered a myocardial infarction before age 55 years and controls (22). This might be explained by a recent investigation in 119 nuclear families using a variance component model, which favored an environmental over a genetic model (26). Even if genetic factors determine apoA-IV concentrations partially, it might take major efforts to detect significant differences of apoA-IV concentrations in children of fathers with myocardial infarction and age-matched controls without a family history of coronary heart disease. An association of apoA-IV with myocardial infarction in fathers is expected to be diluted in offsprings due to the genetic contribution from the mothers’ side. The other study investigated patients with noninsulin-dependent diabetes mellitus and described significantly higher apoA-IV concentrations in patients compared with those without macrovascular complications (CAD, cerebral vascular disease and peripheral arteriopathy) (27). One explanation for this discrepancy to our data is the increased prevalence of microalbuminuria and the concomitant renal impairment in diabetic patients with macrovascular complications. Unfortunately, the authors did not provide detailed data on renal function in their patients (27). A slight impairment of renal function with creatinine concentrations >1.5 mg/dL with or without microalbuminuria is already associated with a dramatic increase in apoA-IV concentrations (Kronenberg et al., unpublished observation 2000). Because diabetic patients with macrovascular complications are more likely to have renal disease than diabetic patients without vascular disease, high levels of apoA-IV in diabetic patients with macrovascular complications may simply reflect their impaired renal function. For this reason we excluded patients and controls with renal impairment from our analysis.

Studies that investigated the relationship between genetic polymorphisms of apoA-IV and CAD found no associations (22,28–30). This is not surprising since these polymorphisms do not impact significantly on lipoprotein metabolism or apoA-IV concentrations (22,28,29,31–33). The correlation of apoA-IV plasma levels with HDL cholesterol

### Table 2. Logistic Regression Analysis Investigating the Predictive Value of ApoA-IV, HDL Cholesterol and Triglyceride Concentrations for CAD

<table>
<thead>
<tr>
<th>Variable (Increment)</th>
<th>Coefficient</th>
<th>SEM</th>
<th>Chi-square</th>
<th>OR (95% CI)</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Univariate analysis</td>
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<tr>
<td>ApoA-IV (–2 mg/dL)</td>
<td>0.628</td>
<td>0.088</td>
<td>50.6</td>
<td>1.89 (1.59–2.22)</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (–5 mg/ dL)</td>
<td>0.346</td>
<td>0.076</td>
<td>20.6</td>
<td>1.41 (1.22–1.64)</td>
<td>0.0001</td>
</tr>
<tr>
<td>In-transformed triglycerides*</td>
<td>0.727</td>
<td>0.258</td>
<td>7.9</td>
<td>2.07 (1.25–3.43)</td>
<td>0.0049</td>
</tr>
<tr>
<td>Multivariate analysis: model 1</td>
<td></td>
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<tr>
<td>ApoA-IV (–2 mg/dL)</td>
<td>0.587</td>
<td>0.091</td>
<td>42.0</td>
<td>1.79 (1.52–2.13)</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (–5 mg/ dL)</td>
<td>0.194</td>
<td>0.081</td>
<td>5.7</td>
<td>1.22 (1.03–1.43)</td>
<td>0.0171</td>
</tr>
<tr>
<td>Interaction term†</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.86</td>
</tr>
<tr>
<td>Multivariate analysis: model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA-IV (–2 mg/dL)</td>
<td>0.651</td>
<td>0.092</td>
<td>50.2</td>
<td>1.93 (1.61–2.27)</td>
<td>0.0001</td>
</tr>
<tr>
<td>In-transformed triglycerides*</td>
<td>0.920</td>
<td>0.301</td>
<td>9.0</td>
<td>2.46 (1.37–4.44)</td>
<td>0.0027</td>
</tr>
<tr>
<td>Interaction term†</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.87</td>
</tr>
</tbody>
</table>

*aNo linear increment can be provided because triglycerides were logarithmically transformed before inclusion into the model; interaction term of the two variables included in the model.

ApoA-IV = apolipoprotein A-IV; CAD = coronary artery disease; CI = confidence interval; HDL = high-density lipoprotein; OR = odds ratio.
observed in this study (Fig. 2) was also found in the EARS study (22). The correlation was slightly stronger in CAD patients \( (r = 0.28) \) than it was in controls \( (r = 0.22) \). Since only about 8\% \( (r^2 = 0.08) \) and 5\% \( (r^2 = 0.05) \), respectively, of the variance was explained by the other variable, apoA-IV and HDL cholesterol were independently associated with CAD in the logistic regression analysis (Table 2). This may reflect the observation from in vitro experiments that apoA-IV forms distinct lipid-poor and apoA-I-free particles, which are very effective mediators of cholesterol efflux (3,4) and are not assessed by the measurement of HDL cholesterol.

### Possible pathophysiological mechanisms
One explanation of the inverse association of apoA-IV with CAD is its involvement in several steps of the reverse cholesterol transport. Low apoA-IV concentrations might result in a decreased efflux of cholesterol from peripheral cells, decreased esterification of free cholesterol as well as a diminished CETP-mediated transfer of cholesterylesters from HDL to LDL as shown in cell culture studies (3,4,11–17,34). A promotion of lipid-induced atherosclerotic changes might be the consequence. Support for this hypothesis comes from experiments in mice overexpressing apoA-IV. High-density lipoprotein-sized lipoproteins of these animals promoted cholesterol efflux from cholesterol-loaded human monocytes more efficiently than HDL from control animals. Furthermore, plasma from these apoA-IV overexpressing animals exhibited a higher endogenous cholesterol esterification rate (19). Another potentially antiatherogenic effect of apoA-IV is its endogenous antioxidative quality described recently; apoA-IV significantly inhibited the copper-mediated oxidation of lymph and LDL and the macrophage-mediated oxidation of fasting lymph, and it increased the time of conjugated diene-formation (35).

### Study limitations
A high percentage of the Caucasian patients had already suffered a cardiovascular event at the time of evaluation, and, therefore, 62\% were already under antilipemic treatment. This treatment, however, has probably not caused the lower apoA-IV concentrations in patients since treated and untreated patients showed similar concentrations, and the logistic regression analysis excluding treated patients revealed similar results. Moreover, a similar relative concentration difference in apoA-IV was observed in the Indian study population whose CAD patients were not under antilipemic treatment. Because of the influence of antilipemic treatment on other lipoproteins, an extensive multivariate modeling in the statistical analysis including total and LDL cholesterol as well as triglyceride concentrations is not possible. We, therefore, confined our calculation to the question of whether apoA-IV concentrations predict CAD independently of HDL cholesterol and triglyceride concentrations.

Since apoA-IV plasma concentrations are significantly lower in women than they are in men and are influenced by hormonal status and use of oral contraceptives (22), we confined our study to men. This allowed a better controlling for confounders but necessitates investigating the observed association in a future study in women.

We had no reliable possibility to control for physical activity and diet, two possible confounders of apoA-IV concentrations (22,25). Moderate and hard exercise levels in men tended to increase apoA-IV levels slightly in the EARS study although the highest apoA-IV levels were measured in subjects with a low level of activity (22). Since a lower activity level is more often expected in patients with CAD and since this association is only weak in men, we do not expect a considerable influence on our results. Diet, on the other hand, is expected to have a stronger influence on apoA-IV levels. Weinberg et al. (25) described apoA-IV to be positively correlated with the percent of total daily caloric intake ingested by fat. However, this might have diminished rather than increased the difference in apoA-IV levels between patients and controls of our study because patients with CAD are expected to have a higher fat intake than control subjects (36). Nevertheless, we found similar results when we excluded all patients from the analysis who had already suffered a major coronary event and who might, therefore, have changed their diet.

### Conclusions
Low apoA-IV levels are associated with CAD, and this association is independent of triglycerides and HDL cholesterol concentrations. This cross-sectional study cannot answer the question whether low apoA-IV concentrations are a cause or a consequence of CAD. A causal relationship is, however, likely in view of the inhibitory effect of transgenic apoA-IV expression on atherosclerosis in mice, and the in vitro findings pointing to the role of apoA-IV in the antiatherogenic reverse cholesterol transport. Presently, apoA-IV concentrations can at least serve as a valuable diagnostic marker for CAD.

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