Circulating Retinol-Binding Protein 4 and Subclinical Cardiovascular Disease in the Elderly

ERIK INGELSSON, MD, PHD1
LARS LIND, MD, PHD2

OBJECTIVE — We evaluated associations of serum retinol-binding protein 4 (RBP4) with subclinical cardiovascular disease (CVD).

RESEARCH DESIGN AND METHODS — Subclinical CVD was measured with echocardiography, carotid artery ultrasound, brachial artery ultrasound, and invasive forearm endothelial vasoreactivity in 1,008 70-year-old participants (50% women) of the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study.

RESULTS — In analyses adjusted for multiple CVD risk factors, we observed inverse associations of RBP4 with carotid artery intima-media (β = −0.39, 95% CI −0.55 to −0.22) and plaque (β = −0.33, 95% CI −0.60 to −0.05) echogenicity (gray scale median).

CONCLUSIONS — Circulating RBP4 concentrations were inversely associated with intima-media and plaque echogenicity in carotid arteries. These findings imply that RBP4 could be involved in the development of atherosclerosis.

RESEARCH DESIGN AND METHODS — The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study has been described elsewhere (4) (http://www.medsci.uu.se/ pivus/pivus.htm). Briefly, 1,016 free-living 70-year-old individuals from Uppsala County attended the baseline examination. The present study sample consisted of 1,008 participants with valid RBP4 measurements. The study was approved by the Uppsala University Ethics Committee, and participants gave written informed consent.

RBP4 was assayed using a commercially available ELISA kit (Phoenix Europe, Karlsruhe, Germany). The average intra- and interassay CVs were 4.6 and 9.8%. A comprehensive two-dimensional and Doppler echocardiography was performed; measurements included left atrial diameter, interventricular septal thickness (IVS), left ventricular posterior wall thickness (PW), ejection fraction, and left ventricular diameter in end diastole (LVEDD). Left ventricular wall thickness was calculated as IVS + PW, and left ventricular mass as 0.8 [1.04(IVS + LVEDD + PW) − (LVEDD)] + 0.6 g/cm (5). The carotid artery was assessed by external B-mode ultrasonography. Intima-media thickness (IMT) was evaluated in the far wall in the common carotid artery, 1–2 cm proximal to the bulb. No overt plaques were included in the IMT measurement. The images were digitized, and gray scale median (GSM) in IMT (GSM-IM) and plaques were performed using semiautomated methods. Common carotid artery distensibility was calculated as the percentage change in the diameter divided by the central pulse pressure obtained by pulse-wave analysis. Endothelium-dependent vasodilation was evaluated with brachial artery B-mode ultrasound (flow-mediated vasodilation) and invasive forearm technique with intrabrachial infusion of acetylcholine (endothelium-dependent vasodilation). Endothelium-independent vasodilation was evaluated with invasive forearm technique with infusion of sodium nitroprusside.

Age- and sex-adjusted and multivariable-adjusted (adjusted for age, sex, BMI, systolic blood pressure, antihypertensive medication, log plasma glucose, antidiabetes medication, total cholesterol, HDL cholesterol, creatinine, current/former smoking, and physical activity) linear regressions were used to relate RBP4 concentrations to subclinical CVD. In confirmatory analyses, we used multiple imputation methods to impute missing data. Multiple testing corrections were performed by calculation of empirical P values using bootstrap methods. Statistical software package Stata 10.1 (Stata, College Station, TX) was used.

RESULTS — Characterization of subclinical CVD in our sample and associations of RBP4 with subclinical CVD are...
shown in Table 1. In multivariable-adjusted analyses, RBP4 was inversely associated with IM-GSM and plaque GSM (corrected for multiple testing, $P < 0.0001$ and 0.056). Creatinine was found to be a strong negative confounder of both associations of RBP4 with IM-GSM and plaque GSM. 

CONCLUSIONS — Several lines of evidence support a potential role for RBP4 in pathways linking adiposity with atherosclerosis. Serum RBP4 levels are increased and correlate with subclinical inflammation in childhood obesity (2), and RBP4 mRNA expression in adipose tissue is associated with inflammatory markers (3). RBP4 concentrations are associated with pro-atherogenic VLDL cholesterol and triglycerides in patients with type 2 diabetes or coronary artery disease (6). Also, RBP4 was associated with incident coronary artery disease in a recent nested case-control study (7).

We report that RBP4 was inversely associated with intima-media and plaque GSM in the carotid arteries of individuals with normal kidney function. GSM is a measure of echogenicity of the vessel wall; a lower value corresponds to a darker ultrasound image, i.e., a higher fat content (8). IM-GSM and plaque GSM are highly correlated (8), and plaque echogenicity is an independent risk factor for ischemic cerebrovascular disease (9). Our finding that higher RBP4 is associated with a higher fat content in the vessel wall and in atherosclerotic plaques might reflect the known lipid-modulating activities of retinoids and retinol-binding proteins, such as expression of several genes involved in triglyceride metabolism, including regulators of ApoC-III production, hepatic and intestinal triglyceride production and secretion, and $eta$-oxidation (10).

There were some limitations of our
study. First, because our study sample consisted of elderly men and women of European ethnicity, the generalizability to other age-groups and ethnicities is unknown. Second, even though we corrected for multiple testing, our results could represent false-positive findings and should be considered hypothesis generating. Third, because our study was cross-sectional, we cannot assess causality or longitudinal tracking of subclinical CVD. Fourth, even though RBP4 remained significantly associated with GSM in multivariable models adjusting for potential confounders, we cannot rule out the possibility of some residual confounding or confounding by unmeasured factors.

In our community-based sample, circulating RBP4 concentrations were associated with intima-media and plaque echogenicity in carotid arteries in individuals with normal kidney function. These findings imply that RBP4 could be involved in the development of atherosclerosis. We did not find an effect-modifying role of type 2 diabetes in the associations. Further studies are needed to validate and evaluate clinical implications of our findings.

Acknowledgments—This work was supported by the Swedish Research Council (grant no. 2007-2135); the Swedish Heart-Lung Foundation (grant no. 20070212); the Linnéus Foundation for Medical Research; the Erik, Karin, och Gösta Selander Foundation; the Fredrik and Ingrid Thuring Foundation, the Ake Wiberg Foundation, and the Swedish Society for Medical Research.

No potential conflicts of interest relevant to this article were reported.

Parts of this study were presented in abstract form at the American Heart Association Scientific Sessions, New Orleans, Louisiana, 8–12 November 2008.

The authors thank Barbro Simu for excellent technical assistance.

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