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

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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.

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Retinol-binding protein 4 (RBP4) is elevated in individuals with insulin resistance and type 2 diabetes (1). Recent observations that serum RBP4 correlates with subclinical inflammation early in obesity development (2) and that RBP4 mRNA expression in adipose tissue is related to inflammation rather than insulin resistance (3) have prompted the hypothesis that RBP4 may elicit subclinical inflammation leading to cardiovascular disease (CVD). We hypothesized that RBP4 would be associated with atherosclerosis, left ventricular geometry and function, and/or endothelial function—intermediate phenotypes on pathways from insulin resistance and subclinical inflammation to clinical CVD—and that these associations would be modified by type 2 diabetes.

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)^3 − (LVEDD^3) + 0.6 g] (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

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Table 1—*Associations of serum RBP4 with measures of subclinical CVD (N = 1,008)**

		Age- and sex-adjusted			Multivariable-adjusted	
	n	Mean ± SD	β (95% CI)	Nominal *P*	β (95% CI)	Nominal *P*
Echocardiographic measures						
LV mass (g)	916	181 ± 60	0.28 (−0.13 to 0.69)	0.19	−0.12 (−0.47 to 0.23)	0.50
LV wall thickness (mm)	916	21 ± 4	0.03 (0.01–0.06)	0.016	0.00 (−0.03 to 0.02)	0.76
LV end-diastolic diameter (mm)	918	47 ± 6	0.00 (−0.04 to 0.03)	0.80	−0.01 (−0.05 to 0.03)	0.62
Ejection fraction (%)	830	67 ± 8	0.01 (−0.06 to 0.07)	0.85	0.01 (−0.05 to 0.08)	0.70
LV isovolumic relaxation time (ms)	888	121 ± 21	0.10 (−0.05 to 0.25)	0.20	−0.05 (−0.20 to 0.10)	0.50
Left atrial diameter (mm)	946	39 ± 7	0.00 (−0.05 to 0.05)	0.98	−0.02 (−0.07 to 0.02)	0.30
log E/A ratio	932	0.97 ± 0.28	0.00 (0.00–0.00)	0.85	0.00 (0.00–0.00)	0.94
Carotid ultrasound measures						
IMT (mm)	947	0.89 ± 0.16	0.00 (0.00–0.00)	0.34	0.00 (0.00–0.00)	0.12
IM-GSM	983	79 ± 24	−0.21 (−0.37 to −0.05)	0.011	−0.39 (−0.55 to −0.22)	<0.0001
Number of carotid arteries with plaques†	936		0.00 (0.00–0.01)	0.59	0.00 (0.00–0.01)	0.94
0		324 ± 35				
1		340 ± 36				
2		272 ± 29				
Plaque GSM‡	589	74 ± 32	−0.17 (−0.43 to 0.08)	0.19	−0.33 (−0.60 to −0.05)	0.020
Measures of arterial compliance						
logCCA distensibility (%)	872	−2.5 ± 0.5	0.00 (−0.01 to 0.00)	0.30	0.00 (0.00–0.00)	0.26
Endothelial function measures						
FMD (%)	982	4.8 ± 3.6	0.01 (−0.01 to 0.04)	0.28	0.01 (−0.02 to 0.04)	0.49
logEDV (%)	859	524 ± 311	0.00 (−0.01 to 0.00)	0.19	0.00 (0.00–0.00)	0.79
logEIDV (%)	876	369 ± 213	0.00 (0.00–0.00)	0.62	0.00 (0.00–0.00)	0.85
RBP4 (μg/ml)	1,008	31.1 ± 9.1	NA	NA	NA	NA

*Data from models with measures of subclinical disease as dependent variables and RBP4 with age and sex (in age- and sex-adjusted analyses) or age, sex, BMI, systolic blood pressure, antihypertensive medication, log plasma glucose, antidiabetes medication, total cholesterol, HDL cholesterol, creatinine, current or former smoking, and physical activity (in multivariable-adjusted analyses) as independent variables. β-coefficients are for a 1-unit increase of RBP4 and represent a change of subclinical measures in natural units (or log natural units for E/A ratio, common carotid artery distensibility, endothelium-dependent vasodilation, and endothelium-independent vasodilation). †Data represent *n* (%) of individuals with 0, 1, or 2 carotid arteries with plaques and the β-coefficient per additional artery with a plaque. ‡Lowest GSM in a plaque at either side, except for when the Grey-Weal scale at either side was 4, in which case the highest plaque GSM was recorded. CCA, common carotid artery; EDV, endothelium-dependent vasodilation; EIDV, endothelium-independent vasodilation; FMD, flow-mediated dilation; LV, left ventricular; NA, not applicable.

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 of these associations and to be an effect modifier in the relation of RBP4 to IM-GSM (β −0.44, $P < 0.0001$, and β −0.08, $P = 0.65$, in individuals with creatinine below or above the 75th percentile; P for interaction = 0.004).

The mean concentrations of RBP4 did not differ between men and women (31.2 vs. 30.9 μg/ml, $P = 0.50$). There were no sex differences regarding associations of RBP4 with IM-GSM, and there was no evidence that type 2 diabetes ($n = 118$, 12%) acted as an effect modifier of the relation of RBP4 with IM-GSM or plaque GSM.

In secondary analyses including total energy intake by 7-day registration and use of hormone replacement therapy ($n = 229$) in the multivariable models, the results were unchanged. In confirmatory analyses using multiple imputation methods, we obtained essentially the same results. In a post hoc power analysis, we had 82% or higher statistical power to detect an increment to the model R^2 of 0.0125 (at $\alpha = 0.05$) for all subclinical CVD measures.

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 β-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.

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