Retinol binding protein 4 and incident diabetes – the Atherosclerosis Risk in Communities Study (ARIC Study)

Proteína carreadora de retinol 4 e diabetes incidente – Atherosclerosis Risk in Communities Study (ARIC Study)

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Abstract

Background: Retinol binding protein 4 (RBP4) has been described as a link between impaired glucose uptake in adipocytes and systemic insulin sensitivity. **Objective:** To determine whether RBP4 fasting levels predict the development of type 2 diabetes. **Methods:** Using a case-cohort design, we followed 543 middle-aged individuals who developed diabetes and 537 who did not over ~9 years within the population-based Atherosclerosis Risk in Communities Study. Weighted Cox proportional hazards analyses permitted statistical inference of the RBP4 – incident diabetes associations to the entire cohort. **Results:** Women in the highest tertile of RBP4 presented greater risk of developing diabetes (HR = 1.74; 95%CI 1.03 – 2.94) in analyses adjusted for age, ethnicity, study center, parental history of diabetes, hypertension, glomerular filtration rate, body mass index, waist-hip ratio, nonesterified fatty acids, adiponectin, leptin, triglycerides and HDL-C. When additionally adjusted for fasting insulin, this association's significance became borderline (HR = 1.68; 95%CI 1.00 – 2.82). No association between RBP4 levels and incident diabetes was found in men. **Conclusion:** These findings suggest that RBP4 levels may be directly involved in the pathogenesis of type 2 diabetes in women.

Keywords: Diabetes. Retinol-Binding Proteins. RBP4 protein, human. Epidemiologic Studies.

Resumo

Introdução: A proteína carreadora de retinol 4 (RBP4) tem sido descrita como elo entre uma menor captura de glicose pelos adipócitos e sensibilidade sistêmica à insulina. **Objetivo:** Determinar se os níveis de RBP4 em jejum predizem diabetes tipo 2. **Método:** Em um delineamento de caso-coorte, foram acompanhados 543 indivíduos de meia-idade que desenvolveram diabetes e 537 que não desenvolveram diabetes ao longo de 9 anos no estudo *Atherosclerosis Risk in Communities Study* (ARIC). Foi realizada análise ponderada de riscos proporcionais de Cox para inferência estatística da associação entre os níveis de RBP4 e diabetes incidente na coorte. **Resultados:** Mulheres com níveis de RBP4 no terceiro tercil apresentaram maior risco de desenvolver diabetes (HR = 1,74; 95% CI 1,03 – 2,94) em análises ajustadas para idade, etnia, centro, história familiar de diabetes, hipertensão, taxa de filtração glomerular, índice de massa corporal, razão cintura-quadril, níveis de ácidos graxos não esterificados, adiponectina, leptina, triglicerídeos e HDL-C. Quando adicionalmente ajustado para os níveis de insulina de jejum, a significância dessa associação se tornou limítrofe (HR = 1,68; 95% CI 1,00 – 2,82). Nenhuma associação foi observada entre RBP4 e diabetes incidente em homens. **Conclusão:** Esses achados sugerem que os níveis de RBP4 possam estar diretamente envolvidos na patogênese do diabetes tipo 2 em mulheres.

Palavras-chave: Diabetes. Proteína carreadora de retinol. RBP4. Estudo epidemiológico.

Introduction

Type 2 diabetes is a leading cause of morbidity and mortality[1]. It results in a considerably reduced life expectancy, diminished quality of life, and enormous health costs[2]. Improving our understanding of the molecular biology underlying type 2 diabetes will hopefully lead to more efficient prevention and more efficacious treatment.

Retinol binding protein 4 (RBP4) is the principal transport protein for retinol (vitamin A) in the circulation. However, there is no compelling evidence that dietary vitamin A regulates circulating levels of RBP4. Some studies have shown that high fasting levels of RBP4 are associated with insulin resistance and diabetes[3,4], although the molecular mechanisms underlying this effect are not clearly understood[5]. This association was first demonstrated in mice, where increases in RBP4 through genetic manipulation or injection caused insulin resistance[3]. In humans, although findings are not entirely consistent[6,7], serum levels of RBP4 have been shown to be elevated with obesity and type 2 diabetes, and changes in insulin sensitivity in response to exercise training were associated with changes in RBP4 levels[4].

To clarify the predictive role of RBP4 on diabetes pathogenesis, ethnically diverse prospective studies, well-suited to minimize bias and control for confounding are needed. In the present study, we tested the hypothesis that higher fasting plasma RBP4 concentrations predict the development of type 2 diabetes in a long-term cohort of middle-aged adults.

Methods

Between 1987 and 1989, the Atherosclerosis Risk in Communities (ARIC) study recruited a population-based cohort of 15,792 men and women aged 45–64 years from four US communities. The ARIC was designed to investigate the etiology and natural history of atherosclerosis, the etiology of clinical atherosclerotic diseases,

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and variation in cardiovascular risk factors, medical care and disease by ethnic group, gender, location, and date. For the present analyses, we used a case-cohort design, as previously described in the investigation of the role of several inflammation biomarkers in the development of diabetes in the ARIC Study[8-10]. Prior to sampling, we excluded 2,018 participants with prevalent diabetes, 95 members of minority ethnic groups with small numbers, 853 individuals who did not return to any follow-up visits, 26 with no valid diabetes determination at follow-ups, 7 with restrictions on stored plasma use, 12 with missing baseline anthropometric measurements, 2,506 participants in previous ARIC case-control and case-cohort studies involving cardiovascular disease for whom stored plasma was either previously exhausted or held in reserve, 45 participants with incomplete fasting (< 8 h), 490 not having values for all covariates, and 31 for whom RBP4 was not measured. From the 9729 eligible individuals, we selected ethnicity-stratified (50% African-American, 50% white) random samples of both incident diabetes (cases) and members of the full eligible ARIC cohort, resulting in 1095 individuals. We additionally excluded 15 individuals having missing data for inflammation markers, non-esterified fatty acid or alcohol intake. Our final sample was composed by 543 diabetes cases and 537 non-cases. A few of the incident cases of diabetes overlapped with the cohort random sample (full cohort-representative random sample = 630). Incident diabetes was defined on the basis of a reported physician diagnosis, use of antidiabetic medications, or a fasting glucose value $\geq$ 7.0 mmol/l at 3 follow-up visits, each occurring approximately 3 years after the previous one. The date of diabetes incidence was estimated by linear interpolation using glucose values at the ascertaining visit and the prior one, as previously described[8]. Cohort-representative controls were followed for a median of 9 years, and incident cases were followed until the onset of diabetes (median = 3 years).

Human-subject research review committees at the involved institutions approved the study, and all participants gave written informed consent.

RBP4 was measured in duplicate using an ELISA technique (R&D Systems). These samples, stored for approximately 20 years at −70°C, were thawed and maintained at 4°C until measured, no longer than 24 h later. Analysis of replicate pair samples drawn at baseline from a subset of 39 subjects yielded a reliability coefficient of 0.93 and a coefficient of variation of 8.9% for RBP4. Laboratory measurements and reliability coefficients, as well as methods for measurement of other covariates, are described in previous reports[8-10]. Low-grade systemic inflammation is estimated by a score ranging from 0 to 6, attributing one point for a value greater than the median of the cohort sample for each of the measured inflammation markers (IL-6, CRP, orosomucoid, and sialic acid, and white cell count and fibrinogen)[8].

Glomerular filtration rate (GFR) was estimated using the CKD-EPI serum creatinine equation, considering gender, ethnicity and age[11].

We used weighted Spearman correlations to describe crude associations between RBP4 and other variables, and weighted ANCOVA to compute adjusted RBP-4 means in diabetes cases and non-cases. We performed weighted Cox proportional hazards regression to estimate the relation between plasma RBP4 and time of onset of diabetes, with weights defined as the inverse of the ethnicity-specific sampling fractions to enable estimation and inference relevant to the entire eligible cohort. In proportional hazard analyses, leptin gender-specific quartiles were modeled as a continuous variable[10]. Adiponectin, body mass index, waist-hip ratio and other continuous variables were centered on their means in order to avoid multicollinearity. Centering was performed subtracting from each observed value the mean of the variable in question and then dividing the difference by the standard deviation of the variable's distribution: (observed − mean) / standard deviation. To test heterogeneity in the RBP4 – incident diabetes associations across categories of

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covariates, we characterized RBP4 in tertiles (according to the cohort random sample distribution) and tested the interactions in the final model.

Linear trend was tested by fitting tertiles of RBP4 as a continuous variable in the regression models. Statistical analyses were performed using the SAS (SAS Institute Inc., Cary, NC) and SUDAAN (Research Triangle Institute, Raleigh, NC) statistical software packages, reflecting the case-cohort sampling design. The proportional hazards assumption was examined through plots of Martingale and Schoenfeld residuals[12]. Collinearity across independent variables was investigated with linear regression models: variance inflation factors were < 2.5.

Results

Among incident diabetes cases, median age (interquartile ranges) was 53 (49–58) years, there were 76 (13%) African-American men, 201 (35%) African-American women, 153 (26%) white men and 151 (26%) white women. Among non-cases, median age was 52 (48–57) years, and the corresponding numbers were 75 (14%), 164 (30%), 117 (21%) and 189 (35%), respectively. Other characteristics of cases and cohort representative non-cases have previously been reported[9]. RBP4 distribution, expressed by mean values and standard deviations, in diabetes cases and non-cases was 29.2 ± 8.2 g/mL and 31.3 ± 8.9 g/mL, respectively. Spearman correlations assessed in the cohort random sample (n=630) showed that RBP4 levels were most highly correlated with triglycerides (r = 0.37, p < 0.01), kidney function markers (serum creatinine, r = 0.34, p < 0.01; glomerular filtration rate, r = − 0.21, p < 0.01), and central obesity (waist-hip ratio, r = 0.26, p < 0.01); and to a lesser, yet still statistically significant, extent with fasting insulin (r = 0.08, p = 0.03), the adipokines adiponectin (r = − 0.15, p < 0.01) and leptin (r = − 0.13, p < 0.01), some inflammation markers (orosomucoid, sialic acid, and white blood count), other metabolic variables (non-esterified fatty

acids, HDL-cholesterol), blood pressure, glucose, habitual ethanol intake, and cigarette smoking. Important differences in association were seen across gender/ethnic groups, indicating potential for confounding (Table 1).

RBP4 mean values were somewhat higher in men than women (32.3 vs. 27.8 g/mL, p < 0.01), African-Americans than whites (30.9 vs. 29.1 g/mL, p = 0.03), and in those in the lowest (vs. the highest) quartile of glomerular filtration rate (32.1 vs. 28.6g/mL, p < 0.01), independently of studied confounders (Table 2).

Women who developed diabetes had higher RBP4 mean levels at baseline: 29.3 (95%CI 28.1–30.6) g/mL vs. 27.8 (95% CI 27.2–28.4) g/mL (p = 0.03), adjusted for age, ethnic group, parental history of diabetes, ethanol intake, smoking, hypertension, body mass index (BMI) and waist-hip ratio, an inflammation score[8], adiponectin, leptin, triglycerides, HDL-cholesterol, non-esterified fatty acids, insulin and glucose levels. This difference was not observed in men: 33.0 (95% CI: 31.7–34.4) g/mL vs. 32.5 (95%CI: 31.6–33.4) g/mL (p = 0.56).

Survival analyses comparing the highest (vs. lowest) RBP4 quartile in all individuals indicated no overall association with diabetes when adjusted for age, ethnic group, ethanol intake, smoking, BMI and waist-hip ratio (HR = 1.26, 95% CI 0.82–1.92, p = 0.29). The size of the association diminished further when additionally adjusted for other covariates (parental history of diabetes, hypertension, glomerular filtration rate, inflammation score[8], adiponectin, leptin, triglycerides, HDL-cholesterol, and non-esterified fatty acids): HR = 1.09, 95% CI 0.66–1.80 (p = 0.73). In analyses stratified by gender (Table 3), even though gender interaction was not statistically significant (p = 0.51), women presenting RBP4 levels at the highest tertile were at increased risk of diabetes, while no association between RBP4 and diabetes was observed in men. Table 3 also shows that, in women, the association between RBP4 and diabetes was significant independently of multiple

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Table 1 – Spearman correlations for crude associations of RBP4 levels with inflammation and metabolic variables at baseline in the cohort random sample.

Tabela 1 – Correlações de Spearman para associações brutas entre níveis de RBP4 com inflamação e outras variáveis metabólicas, na linha de base, na amostra aleatória da coorte.

	Overall	White Women (n=206)	African-American Women (n=208)	White Men (n=131)	African-American Men (n=85)
	r	R	r	r	r
Possible confounders					
Age (years)	0.20	0.34	0.19	-0.01	0.06
Usual ethanol intake (g/wk)	0.16	0.00	0.05	0.12	0.38
N cigarettes per day * N years of smoking	0.15	0.08	0.16	-0.04	0.09
Body mass index (kg/m^2)	0.05	0.10	0.05	-0.05	-0.13
Waist (cm)	0.14	0.14	0.09	0.05	-0.08
Waist-to-hip ratio	0.26	0.21	0.12	0.07	0.19
Creatinine (mg/dL)	0.34	0.20	0.37	0.23	0.07
GFR (mL/min per 1.73m^2)	-0.21	-0.26	-0.38	-0.21	-0.12
Inflammation markers and adipokines					
C-reactive protein (mg/mL)	-0.03	0.13	0.03	-0.26	-0.10
IL-6 (pg/mL)	-0.09	-0.01	-0.07	-0.27	-0.10
Fibrinogen (µmol/L)	-0.05	0.04	0.06	-0.12	-0.07
Orosomucoid (mg/dL)	0.13	0.13	0.25	0.09	0.28
Sialic acid (mg/dL)	0.15	0.21	0.11	0.18	-0.08
White blood count (10^9/L)	0.09	0.05	0.12	0.03	0.12
Complement component 3 (mg/dL)	0.08	0.20	0.17	0.01	-0.01
Adiponectin (ug/mL)	-0.15	-0.05	-0.04	-0.10	-0.20
Leptin (ng/mL)	-0.13	0.17	0.00	0.06	0.00
Other metabolic variables					
Non-esterified fatty acids (mEq/L)	0.10	0.24	0.05	0.12	0.25
Triglycerides (mmol/L)	0.37	0.34	0.32	0.34	0.25
HDL-cholesterol (mg/dL)	-0.08	0.09	-0.04	-0.04	0.11
Systolic blood pressure (mmHg)	0.13	0.18	0.00	0.06	0.17
Diastolic blood pressure (mmHg)	0.08	0.07	-0.02	0.00	0.29
Fasting insulin (µU/mL)	0.08	0.18	0.08	0.03	-0.07
HOMA-IR	0.09	0.19	0.10	0.04	-0.05
Fasting glucose (mg/dL)	0.12	0.11	0.13	0.04	0.09

Bolding indicate *p* value < 0.05. GFR – Glomerular filtration rate[13].
Negrito indica valor p < 0,05. GFR – Taxa de filtração glomerular[13]

diabetes risk factors (HR = 1.74, 95% CI 1.03–2.94, comparing the highest vs. lowest RBP4 tertile). Statistical significance became borderline when additionally adjusted for fasting insulin (HR = 1.68; 95% CI 1.00–2.82), a marker of insulin resistance presumably on the RBP4-diabetes causal pathway, and it was not nominally statistically significant when baseline fasting glucose was added to the model (HR = 1.24, 95% CI 0.70–2.19).

No heterogeneity in these associations was found comparing African-Americans

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Table 2 – RBP4 levels at baseline in the cohort random sample.
Tabela 2 *– Níveis de RBP4, na linha de base, na amostra aleatória da coorte.*

		Minimal adjustment		Full adjustment	
		Mean (95%CI), g/mL	p value	Mean (95%CI), g/mL	p value
Gender	Female	27.8 (27.0 – 28.7)	<0.01	27.8 (27.1 – 28.6)	<0.01
	Male	32.3 (31.2 – 33.3)		32.3 (31.2 – 33.5)	
Ethnicity	African-American	29.2 (28.1 – 30.3)	0.73	30.9 (29.5 – 32.2)	0.03
	White	29.5 (28.7 – 30.2)		29.1 (28.5 – 29.8)	
Age	45–54 years	26.7 (24.7 – 28.7)	<0.01	29.9 (28.1 – 31.7)	0.58
	55–64 years	28.7 (27.9 – 29.5)		29.6 (28.9 – 30.3)	
Ethanol (4th quartile)[a]	Yes	31.2 (29.7 – 32.8)	<0.01	30.6 (29.2 – 31.9)	0.06
	No	28.9 (28.1 – 29.6)		29.1 (28.5 – 29.7)	
GFR (1st quartile)[b]	Yes	31.9 (30.5 – 33.4)	<0.01	32.1 (30.9 – 33.3)	<0.01
	No	28.6 (27.9 – 29.3)		28.6 (28.0 – 29.2)	
Smoking	Current	29.7 (28.1 – 31.3)	<0.01	29.6 (28.2 – 31.0)	0.12
	Former	31.3 (30.0 – 32.5)		30.2 (29.2 – 31.2)	
	Never	28.1 (27.2 – 29.1)		28.9 (28.1 – 29.7)	
Inflammation score[8]	High	29.5 (28.5 – 30.6)	0.77	28.9 (28.1 – 29.7)	0.11
	Low	29.3 (28.5 – 30.1)		29.8 (29.1 – 30.4)	
Body mass index	Normal	29.3 (28.1 – 30.4)	0.86	30.1 (28.9 – 31.2)	0.30
	Overweight	29.4 (28.6 – 30.2)		29.0 (28.3 – 29.7)	
	Obese	29.8 (28.3 – 31.2)		29.3 (27.8 – 30.7)	
WHR[c]					
Men	Yes	33.1 (31.5 – 34.8)	0.26	33.2 (31.9 – 34.6)	0.15
	No	31.9 (30.6 – 33.3)		31.9 (30.7 – 33.0)	
Women	Yes	28.5 (27.3 – 29.7)	0.08	27.9 (26.9 – 28.9)	0.61
	No	27.0 (25.9 – 28.1)		27.5 (26.7 – 28.4)	
Fasting insulin[d]	< P25	28.3 (26.7 – 29.9)	0.30	28.5 (27.1 – 29.9)	0.06
	P25 – P75	29.7 (28.8 – 30.5)		30.0 (29.3 – 30.6)	
	> P75	29.7 (28.5 – 31.0)		28.8 (27.5 – 30.1)	

[a] Ethanol intake ≥ 52.8g per week; *[a] Ingestão de álcool ≥ 52,8g por semana.*
[b] Glomerular filtration rate < 61.8 mL/min per 1.73 m²; *[b] Taxa de filtração glomerular < 61,8 mL/min por 1,73 m².*
[c] Waist-to-hip ratio ≥ 0.95, for men, or ≥ 0.89, for women; *[c] Razão cintura-quadril ≥ 0,95 para homens ou ≥ 0,89 para mulheres.*
[d] Percentile 25 = 5 µU/mL, Percentile 75 = 13 µU/mL; *[d] Percentil 25 = 5 µU/mL, Percentil 75 = 13 µU/mL.*
Minimal adjustment: Adjusted for age, gender, and ethnic group/centre indicators;
Ajuste mínimo: Ajustado para idade, sexo e etnia/centro.
Full adjustment: Adjusted for age, gender, ethnic group/centre indicators, plus ethanol intake, ethanol intake², N cigarettes per day * N years of smoking, BMI, BMI², waist-to-hip ratio, glomerular filtration rate, inflammation score[8], adiponectin, leptin (gender-specific quartiles), ln-triglycerides, ln-triglycerides², HDL-cholesterol, non-esterified fatty acids, hypertension, family history of diabetes, ln-insulin, and glucose.
*Ajuste máximo: Ajustado idade, sexo e etnia/centro, consumo de etanol², número de cigarros por dia * número de anos de tabagismo, índice de massa corporal², razão cintura-quadril, taxa de filtração glomerular, escore de inflamação[8], adiponectina, leptina (quartis específicos por sexo), ln-triglicerídeos², HDL-c, ácidos graxos não esterificados, hipertensão, história familiar de diabetes, ln-insulina e glicose*

and whites, obese and non-obese individuals, current smokers and non-smokers, or in those with a higher vs. lower inflammation score[8], glomerular filtration rate, or ethanol intake [data not shown]. Women having impaired fasting glucose at baseline showed a considerably smaller RBP4-diabetes association (HR = 1.24, CI 95% 0.62–2.47) than those with baseline normoglycemia (HR = 2.38, CI 95% 0.83–6.77), though this

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Table 3 – Hazard ratios (95% CIs) of incident diabetes by tertiles of RBP4, stratified by gender.

Tabela 3 – Razão de riscos proporcionais de Cox (Hazard Ratios, 95% CI) para diabetes incidente de acordo com os níveis de RBP4, estratificado por sexo.

	Men			Women		
	2nd tertile	3rd tertile	Linear trend	2nd tertile	3rd tertile	Linear trend
	HR (95%CI)	HR (95%CI)	*p* value	HR (95%CI)	HR (95%CI)	*p* value
Model 1	0.96 (0.59–1.57)	1.07 (0.66–1.73)	0.77	1.48 (1.01–2.16)	1.92 (1.32–2.79)	<0.01
Model 2	1.02 (0.62–1.69)	1.12 (0.67–1.85)	0.67	1.49 (1.01–2.18)	1.94 (1.33–2.83)	<0.01
Model 3	0.89 (0.51–1.56)	1.00 (0.58–1.71)	0.93	1.26 (0.82–1.92)	1.70 (1.11–2.59)	0.01
Model 4	0.89 (0.51–1.56)	1.03 (0.60–1.77)	0.90	1.27 (0.83 – 1.94)	1.83 (1.19 – 2.82)	0.01
Model 5	0.98 (0.54–1.76)	0.94 (0.51–1.73)	0.83	1.27 (0.79– 2.03)	1.74 (1.03–2.94)	0.04
Model 6	1.01 (0.57–1.80)	0.93 (0.51–1.71)	0.83	1.28 (0.81–2.04)	1.68 (1.00–2.82)	0.05
Model 7	1.04 (0.56–1.92)	0.83 (0.44–1.57)	0.57	1.12 (0.67–1.85)	1.24 (0.70–2.19)	0.47

Reference: 1st tertile. / *Referência: 1° tercil.*
In men: 1st tertile = 12.8–28.1 g/mL, 2nd tertile = 28.2–35.1 g/mL, 3rd tertile = 35.2–59.2.1 g/mL. / *Em homens: 1° tercil = 12,8–28,1 g/mL, 2° tercil = 28,2-35,1 g/ml, 3° tercil = 35,2-59,2 g/mL*
In women: 1st tertile = 8.8–23.9 g/mL, 2nd tertile = 24.0–30.1 g/mL, 3rd tertile = 30.3–70.0 g/mL. / *Em mulheres: 1° tercil = 8,8-23,9 g/mL, 2° tercil = 24,0-30,1 g/mL, 3° tercil = 30,3-70,0 g/mL.*
Model 1: Adjusted for age, ethnic group/centre indicators. / *Modelo 1: ajustado para idade, sexo e etnia/centro.*
Model 2: Model 1 plus ethanol intake, ethanol intake², and N cigarettes per day * N years of smoking. / *Modelo 2: Modelo 1 + consumo de etanol², número de cigarros por dia * número de anos de tabagismo.*
Model 3: Model 2 plus BMI, BMI², and waist-to-hip ratio. / *Modelo 3: Modelo 2 + índice de massa corporal², razão cintura-quadril.*
Model 4: Model 3 plus glomerular filtration rate. / *Modelo 4: Modelo 3 + taxa de filtração glomerular.*
Model 5: Model 4 plus inflammation score⁸, adiponectin, leptin (gender-specific quartiles), ln-triglycerides², HDL-cholesterol, non-esterified fatty acids, hypertension, and family history of diabetes. / *Modelo 5: Modelo 4 + escore de inflamação⁸, adiponectina, leptina (quartis específicos por sexo), ln-triglicerídeos², HDL-c, ácidos graxos não esterificados, hipertensão, história familiar de diabetes.*
Model 6: Model 5 plus ln-insulin. / *Modelo 6: Modelo 5 + ln-insulina.*
Model 7: Model 6 plus glucose. / *Modelo 7: Modelo 6 + glicose.*

difference was not statistically significant (p = 0.47). Excluding 27 individuals identified as non-cases but having 2-h glycaemia ≥ 11.1 mmol/l during the oral glucose tolerance test performed at the last follow-up visit did not materially alter the results [data not shown].

Discussion

In this case cohort study of middle-aged African-Americans and whites, RBP4 was correlated with several metabolic variables and inflammation markers, most notably so with triglycerides, waist-hip ratio and creatinine. It was minimally correlated with fasting insulin. Values were higher in men than women. Though no association was found in the overall sample, higher RBP4 values predicted the development of diabetes in women in multivariable-adjusted analyses.

It has been suggested that adipocytes detect decreased glucose uptake in early insulin resistance and, in response, secrete adipokines such as RBP4 to restrict glucose uptake in skeletal muscle and increase glucose production in the liver, thereby increasing the blood glucose level, as a consequence of the "adipocytes' starvation state"[13,14]. RBP4 appears to reduce glucose uptake in the muscle tissue by interfering with the phosphorylation of the insulin receptor substrate-1 (IRS-1) and phosphoinositide-3-kinase (PI-3-kinase) activity, which are necessary components of the insulin signaling pathway, while, in the liver, RBP4 has been shown to increase the expression of the gluconeogenic enzyme phosphoenol-pyruvate carboxykinase (PEPCK), resulting in increased hepatic glucose output[3]. In addition to this, it has been also proposed that oversecretion of RBP4 may adversely affect B-cell function[15], although mechanisms for such an action remain to be described and other authors have failed in the attempt to confirm this hypothesis[16,17].

Several other studies have tried to elucidate the association between RBP4 and diabetes in humans, yielding conflicting results[4,6,7,18-20]. These inconsistencies likely result from differences in study design, sample size, population composition, and assay methods used. Analytical strategies are also important. An advantage of our study is the ability to adjust our results for many factors that may influence RBP4 levels (such as ethanol intake and kidney function) and factors associated with diabetes, previously suggested to be possible confounders in the RBP4-diabetes association[6].

In agreement with the present findings, other studies have demonstrated lower RBP4 levels in women compared to men[18,19], but, to our knowledge, we are the first group to demonstrate that gender may modify the ability of RBP4 to predict diabetes. Possible reasons exist to explain why the RBP4-diabetes association may be different between men and women, especially in middle age. First, metabolic differences between genders has been described to impact on insulin secretion, insulin action, and hepatic insulin extraction, and result in substantial differences in the regulation of glucose metabolism in men and women[21], although the underlying mechanisms are yet to be elucidated. Second, women seem to have different regulatory mechanisms of RBP4 levels. RBP4 was shown to be associated with gonadotropin concentrations in healthy women and to be higher postmenopausally[22].

Regarding the effect of covariates on the RBP4-diabetes association, the increased risk of diabetes in those women with higher RBP4 levels seen in the minimally adjusted models was weakened after adjustment for obesity indices. Despite this, higher RBP4 levels were still associated with diabetes in these adjusted analyses, indicating that the RBP4-diabetes association may be somewhat independent of the presence of obesity. On the other hand, we confirmed that the RBP4-diabetes association is not independent of fasting glucose levels, although this result may well reflect over-adjustment of this final model. It has been stated that the RBP4-diabetes association is an artifact of renal function [6]. However, the association in women was little altered by adding estimated glomerular filtration rate to the model. Some associations still need to be better understood, as the somewhat weak negative correlation found between IL-6 and RBP4 levels.

Our study has some limitations. Selection bias, either due to participants not returning for follow-up or not having a sample available for measurement, or to exclusion of those participants with cardiovascular disease, could conceivably have influenced our results. However, we have little *a priori* reason to believe that the associations here demonstrated should be stronger or weaker among them. Second, we only assessed fasting RBP4 levels, and thus we may not have fully captured the effect of RBP4 in the postprandial period. If so, our findings might underestimate the size of the associations reported. However, it has been recently demonstrated that no significant difference exists in RBP4 plasma concentrations between fasting levels and those 2h, 4h and 6 hours after a high-fat meal intake[23]. This finding suggests that not assessing postprandial RBP4 levels in the present study is probably not an important weakness. Third, errors in RBP4 measurements resulting from long periods of storage are unknown, and could be compromising our ability to measure a true association. Fourth, the fact that the RBP4-diabetes association appeared somewhat weaker in those with impaired fasting glycemia at baseline makes a reverse causality less likely, but, given the multiple stratifications performed and the modest size of the association that we described, we are unable to rule out that RBP4 levels may be linked to type 2 diabetes in a secondary and non-causal manner[24], Thus, our results must be considered as preliminary at best.

Conclusion

To our knowledge, this is the first long-term prospective study testing the

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hypothesis that higher plasma RBP4 concentrations predict incident diabetes. Our results suggest that higher RBP4 levels predict type 2 diabetes differently in men and women, and perhaps are important in the development of diabetes in the latter.

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