Association of Serum Retinol-Binding Protein 4 Levels and the Risk of Incident Type 2 Diabetes in Subjects With Prediabetes

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OBJECTIVE

To explore the association of serum retinol-binding protein 4 (RBP4) levels and risk for the development of type 2 diabetes in individuals with prediabetes.

RESEARCH DESIGN AND METHODS

A population-based prospective study was conducted among 1,011 Chinese participants with prediabetes (average age 55.6 ± 7.2 years). Incident type 2 diabetes was diagnosed according to the American Diabetes Association 2010 criteria. Serum RBP4 levels were measured using a commercially available ELISA. We analyzed the association of serum RBP4 levels with the risk of incident type 2 diabetes using the Cox proportional hazards model.

RESULTS

During a median follow-up period of 3.1 years, 153 participants developed incident type 2 diabetes. A U-shaped association was observed between serum RBP4 levels and the risk of incident type 2 diabetes, with the lowest risk in the RBP4 range of 31–55 μg/mL. Multivariate Cox regression model analysis showed that serum RBP4 levels <31 μg/mL and RBP4 levels >55 μg/mL were associated with an increased risk of incident type 2 diabetes. The adjusted hazard ratios (95% CI) were 2.01 (1.31–3.09) and 1.97 (1.32–2.93), respectively, after adjusting for age, sex, BMI, waist circumference, γ-glutamyltransferase, HOMA of insulin resistance index, fasting plasma glucose, 2-h plasma glucose, and glycated hemoglobin (HbA_{1c}) levels.

CONCLUSIONS

A U-shaped relationship exists between serum RBP4 levels and the risk of incident type 2 diabetes in subjects with prediabetes.

Retinol-binding protein 4 (RBP4) was initially identified as the primary vitamin A transport protein that facilitates the delivery of retinol from liver to peripheral tissues (1). Circulating RBP4 primarily comes from hepatocytes and, to a lesser extent, from adipocytes and other cell types (2). RBP4 has recently been recognized as an adipokine, and multiple epidemiological studies suggested that elevated serum RBP4 levels play a critical role in the development of metabolic diseases, including insulin resistance and type 2 diabetes (3–6). According to the results of animal studies, increasing serum RBP4 concentrations through transgenic overexpression or an injection of the purified recombinant RBP4 protein induces insulin resistance in wild-type mice (7). However, decreasing serum RBP4 levels with a fenretinide treatment or

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gene knockout significantly enhances insulin sensitivity and improves glucose tolerance in mice. In addition, serum RBP4 concentrations are increased in insulin-resistant humans with obesity and type 2 diabetes, even before overt diabetes develops. Cross-sectional studies reported increased serum RBP4 levels in individuals with type 2 diabetes that inversely correlated with insulin sensitivity (8,9). A prospective observational study reported an independent association between higher circulating RBP4 levels and incident diabetes (10). In addition to these endocrine functions of RBP4 in glucose disposal and insulin resistance, a few studies have identified a pathophysiological link between RBP4 and insulin secretion and β-cell dysfunction (11,12). Nevertheless, RBP4 is proposed to be an additional predictor of the risk of development of type 2 diabetes in the general population.

Prediabetes, an intermediate metabolic state of hyperglycemia that is higher than normal but lower than the clinical diabetes threshold, is a heterogeneous subclinical status of diabetes. Patients with this condition have a higher risk of developing diabetes, and the lifetime conversion rate to type 2 diabetes is as high as 74% (13). Circulating RBP4 levels are also increased in subjects with prediabetes (14); however, little is currently known about the link between serum RBP4 levels and incident type 2 diabetes in high-risk populations, with the exception of only one study based on a very small sample size (15). Therefore, the current study aimed to investigate the association between serum RBP4 levels and the risk of incident type 2 diabetes in a prospective cohort of Chinese adults with prediabetes.

RESEARCH DESIGN AND METHODS

Study Participants

Participants were recruited from a community-based cohort study in Guangzhou, China, which was designed as a single-center prospective observational study to evaluate chronic diseases in the Chinese population. During the recruitment period, local permanent residents (39–79 years of age) were invited to participate in a screening examination for diabetes. From June to December 2011, 3,620 residents were successfully recruited, among whom 1,256 participants were diagnosed with prediabetes, which is defined as a fasting blood glucose (FBG) level of 5.6–6.9 mmol/L (100–125 mg/dL), a 2-h blood glucose level of 7.8–11.0 mmol/L (140–199 mg/dL), or a glycated hemoglobin (HbA_{1c}) level of 5.7–6.4% (39–46 mmol/mol) according to the American Diabetes Association (ADA) guidelines from 2010 (16). In 2014, participants returned for a 3-year follow-up survey, and 1,097 participants completed the baseline and follow-up surveys (follow-up rate was 87.3%). Among these subjects, 61 participants were excluded due to the presence of a malignant tumor, hepatitis, liver or kidney failure, acute inflammation, and immune diseases, and 25 participants were excluded based on RBP4 outliers, which were defined as extreme value (lower or upper 1% of distribution) or repeated measurements coefficient of variation >15%, resulting in the inclusion of 1,011 participants with prediabetes in the final analysis (Supplementary Fig. 1).

The study protocol was approved by the Sun Yat-sen Memorial Hospital Ethics Committee and complied with the Declaration of Helsinki. All participants provided written informed consent before data collection.

Data Collection

At baseline, a questionnaire, physical examination, anthropometric measurements, and laboratory measurements were completed. Information about sociodemographic characteristics, medical histories, and lifestyle factors, including physical activity, smoking, and drinking habits, was collected by trained staff using a standard questionnaire. We used the short form of the International Physical Activity Questionnaire and added questions about the frequency and duration of moderate or vigorous activities and walking to estimate physical activity during leisure time (17). Current smokers or drinkers were defined as participants who had a regular smoking or drinking status in the past 6 months (18).

All participants underwent anthropometrical measurements conducted by well-trained examiners using standard protocols. Body weight, standing height, and waist circumference were measured when participants were dressed in light clothes without shoes. BMI was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was consecutively measured three times within a 5-min interval using an automated electronic device (OMRON Model HEM-752 FUZZY; Omron Company, Dalian, China), and the mean values were used for analysis. We measured the waist circumference at the umbilical level when the participant was in a standing position at the end of gentle expiration. According to the World Health Organization classifications, overweight was defined as a BMI ≥25 kg/m^2, and waist circumference reference values were 80 cm for women and 94 cm for men.

Clinical and Biochemical Measurements

Prior to the baseline survey, participants were asked to fast overnight for at least 10 h, and fasting venous blood samples were collected by experienced nurses. Blood samples were centrifuged at 2,500*g* for 15 min to obtain serum or plasma within 2 h after blood was collected and were then cooled and stored at −80°C. All participants received a standard 75-g oral glucose tolerance test (OGTT), and plasma glucose concentrations were determined at 0 and 2 h. FBG, triglyceride (TG), total cholesterol, HDL cholesterol (HDL-c), LDL cholesterol (LDL-c), creatinine (Cr), γ-glutamyltransferase (GGT), AST, and alanine aminotransferase (ALT) concentrations were measured using an autoanalyzer (CX-7 Biochemical Autoanalyzer; Beckman Coulter, Brea, CA). The 2-h postprandial blood glucose (2-h PBG) level was measured after participants had completed the 75-g OGTT. HbA_{1c} levels were determined using high-performance liquid chromatography (Bio-Rad, Hercules, CA). The estimated glomerular filtration rate (eGFR) was estimated using the equation of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), which is based on the serum creatinine concentration, age, sex, and ethnicity (19). Fasting insulin levels were measured with an enzyme immunosorbent assay kit (Mercodia, Uppsala, Sweden). The HOMA of insulin resistance (HOMA-IR) was used to evaluate insulin resistance, which was calculated using the following equation: HOMA-IR = insulin (μU/mL) × glucose (mmol/L)/22.5.

Measurement of Serum RBP4 Levels

Serum RBP4 levels were measured using an ELISA (AdipoGen, Seoul, Korea)

according to the manufacturer's instructions and compared with purified human RBP4 standards. The lowest limit of detection was 1 ng/mL. All samples were assayed in duplicate, and the mean intra- and interassay coefficients of variation were 2.64–9.22% and 3.48–10.27%, respectively.

Prospective Follow-up and End Point Definitions

In 2014, all participants were asked to return to complete a 75-g OGTT and questionnaires to diagnose new cases of diabetes. Questionnaires on the current health status included questions about medication, hospital admissions, and outpatient diagnosis of diabetes. The end point was defined as incident type 2 diabetes based on the results of the OGTT and a review of all available hospital records. Incident type 2 diabetes included newly diagnosed and undiagnosed diabetes cases. Newly diagnosed diabetes was defined as a positive response from the participant to the question, "Have you been told that you have diabetes by a doctor since 2011?" New undiagnosed diabetes was defined according to the ADA 2010 criteria: FBG $\geq$7.0 mmol/L ($\geq$126 mg/dL), 2-h PBG $\geq$11.1 mmol/L ($\geq$200 mg/dL), or HbA_{1c} $\geq$6.5% (48 mmol/mol). We did not record type of diabetes among incident cases, but given that all the participants in our study were at least 39 years old at enrollment, these participants were unlikely to have type 1 diabetes because the fasting glucose was only mildly elevated and insulin levels were high normal (Table 1). All potential events of prediabetes and type 2 diabetes were independently adjudicated by two study physicians. In cases of disagreement, a consensus was achieved after a consultation with an endocrinologist.

Statistical Analysis

Normally distributed continuous variables are reported as means $\pm$ SDs, and skewed variables are presented as medians (interquartile ranges). Categorical variables are presented as frequencies (%). The participants were divided into two groups according to the incident type 2 diabetes status. For comparisons between groups, we conducted an independent-samples Student *t* test for normally distributed variables and a Mann-Whitney *U* test for variables with highly skewed distributions. The χ^2 test was used to compare categorical variables. The correlation coefficients between serum RBP4 levels and baseline metabolic risk factors were calculated using the Spearman correlation analysis. Person-years of type 2 diabetes were estimated. Multivariate Cox regression models with a cubic natural spline analysis were used to determine the potential nonlinear association between RBP4 levels (continuous) and the risk of incident type 2 diabetes. Hazard ratios (HRs) and 95% CIs for the relationship between RBP4 categories and the incidence of type 2 diabetes were generated with the Cox proportional hazards model. Model 1 was adjusted for age, sex, smoking, drinking, and physical activity; model 2 was adjusted for the variables in model 1 plus BMI; model 3 was adjusted for the variables in model 2 plus waist circumference; model 4 was adjusted for the variables in model 3 plus eGFR, HDL, TG, systolic blood pressure (SBP), GGT, and HOMA-IR; and model 5 was adjusted for the variables in model 4 plus FBG, 2-h PBG, and HbA_{1c}. RBP4 categories depended on the inflection points obtained from the cubic natural spline regression analysis. The reference group was the group presenting the RBP4 concentration associated with the lowest risk of incident type 2 diabetes.

Several risk factors affect the association between RBP4 levels and incident type 2 diabetes, particularly BMI, waist circumference, eGFR, and TG levels. Therefore, we performed a subgroup analysis to assess whether the relationship between RBP4 levels and the risk of incident type 2 diabetes was robust in the presence of potential confounders. Subgroups were divided according to BMI $<$25 kg/m^2 vs. BMI $\geq$25 kg/m^2, waist circumference $<$80 cm for women and 94 cm for men vs. waist circumference $\geq$80 cm for women and 94 cm for men, eGFR $<$90 mL/min/1.73 m^2 vs. eGFR $\geq$90 mL/min/1.73 m^2, and TG $<$1.7 mmol/L vs. TG $\geq$1.7 mmol/L.

We did two sensitivity analyses. First, we repeated the analysis and restricted prediabetes to participants who met at least two of the three glycemic criteria for prediabetes established by the ADA in 2010. Second, we investigated the effect of excluding incident cases ascertained only on the basis of a single glycemic abnormality. *P* values of $<$0.05 were considered statistically significant in all of the analyses. All analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL) and R version 3.5.1.

RESULTS

Baseline Characteristics of Subjects With or Without Incident Type 2 Diabetes

Table 1 summarizes the baseline characteristics of 1,011 participants who were stratified according to the presence or absence of incident type 2 diabetes after follow-up. The mean age of the study participants was 55.6 $\pm$ 7.2 years. Notably, 29.2% of the participants were men and 23.7% had hypertension. Participants who experienced events (incident type 2 diabetes) had a higher prevalence of hypertension, higher BMI, waist circumference, TG levels, and Cr levels, and a lower eGFR. Higher RBP4 levels were observed in males than in females (48.79 μg/mL [36.15–63.86] vs. 44.61 μg/mL [33.20–57.05], $P < 0.05$), and no significant differences in baseline RBP4 levels were observed between participants with or without incident type 2 diabetes ($P > 0.05$).

Association of Serum RBP4 Levels With Clinical Characteristics

Supplementary Table 1 presents the associations between serum RBP4 levels and clinical characteristics obtained from the bivariate correlation analysis. In the univariate model, associations between RBP4 levels and FBG levels, 2-h PBG levels, HbA_{1c} levels, and BMI were not observed ($P > 0.05$), whereas serum RBP4 levels positively correlated with fasting insulin levels, HOMA-IR, waist circumference, Cr levels, TG levels, and LDL-c levels and inversely correlated with eGFR. The associations were still significant even after adjustment for sex, age, smoking, drinking, and physical activity ($P < 0.05$).

U-Shaped Association Between Serum RBP4 Levels and Incident Type 2 Diabetes

During a median follow-up period of 3.1 years (interquartile range 3.0–3.2, representing 3,086 person-years of follow-up), 153 subjects with prediabetes developed type 2 diabetes (cumulative incidence of type 2 diabetes 49.9/1,000 person-years [45.5–54.2]). A nonlinear relationship was observed between the

Table 1—Baseline characteristics of participants with or without incident type 2 diabetes

Variables	Total (n = 1,011)	Incident type 2 diabetes		P value
		Without (n = 858)	With (n = 153)	
Age (years)	55.59 ± 7.20	55.59 ± 7.22	56.27 ± 7.52	0.288
Male, n (%)	295 (29.2)	247 (28.8)	48 (31.4)	0.517
Current smoker, n (%)	179 (17.7)	147 (17.1)	32 (20.9)	0.259
Current drinker, n (%)	273 (27.0)	228 (26.6)	45 (29.4)	0.466
Physical activity, n (%)	363 (35.9)	309 (36.0)	54 (35.3)	0.864
Hypertension, n (%)	240 (23.7)	191 (22.3)	49 (32.0)	0.009
IFG, n (%)	442 (43.7)	351 (45.9)	91 (59.5)	<0.001
IGT, n (%)	441 (43.6)	339 (39.5)	102 (66.7)	<0.001
iHbA_{1c}, n (%)	693 (68.5)	569 (66.3)	124 (81.0)	<0.001
SBP (mmHg)	125.63 ± 15.88	124.83 ± 15.54	130.13 ± 17.06	<0.001
DBP (mmHg)	75.05 ± 9.48	74.80 ± 9.55	76.45 ± 9.01	0.048
BMI (kg/m^2)	23.33 ± 2.74	23.21 ± 2.73	23.99 ± 2.71	0.001
Waist circumference (cm)	81.70 ± 8.96	81.25 ± 8.88	84.18 ± 9.01	<0.001
Male	85.09 ± 8.84	84.12 ± 8.63	90.07 ± 8.32	<0.001
Female	80.30 ± 8.63	80.10 ± 8.73	81.48 ± 8.00	0.129
FBG (mmol/L)	5.50 ± 0.57	5.46 ± 0.56	5.69 ± 0.61	<0.001
2-h PBG (mmol/L)	7.53 ± 1.65	7.36 ± 1.58	8.49 ± 1.75	<0.001
HbA_{1c} (%)	5.87 ± 0.32	5.85 ± 0.32	6.01 ± 0.33	<0.001
HbA_{1c} (mmol/mol)	40.50 ± 3.50	40.30 ± 3.50	42.01 ± 3.60	<0.001
Insulin (mU/L)	7.20 (5.96–9.50)	7.20 (5.93–9.40)	7.40 (6.17–9.76)	0.216
HOMA-IR	1.76 (1.41–2.35)	1.73 (1.40–2.34)	1.92 (1.52–2.47)	0.013
HDL-c (mmol/L)	1.33 ± 0.36	1.35 ± 0.36	1.24 ± 0.35	0.001
LDL-c (mmol/L)	3.18 ± 0.94	3.19 ± 0.95	3.11 ± 0.94	0.365
Total cholesterol (mmol/L)	5.26 ± 1.25	5.29 ± 1.25	5.13 ± 1.20	0.154
TG (mmol/L)	1.53 ± 1.03	1.50 ± 0.96	1.70 ± 1.32	0.022
ALT (U/L)	13.08 ± 8.70	12.98 ± 8.6	13.69 ± 9.25	0.353
AST (U/L)	19.20 ± 6.29	19.16 ± 6.19	19.42 ± 6.88	0.634
GGT (mg/dL)	24.03 ± 17.32	23.52 ± 15.09	26.88 ± 26.51	0.028
Serum creatinine (μmol/L)	69.53 ± 13.70	69.20 ± 12.95	71.38 ± 17.25	0.070
eGFR (mL/min/1.73 m^2)	91.32 ± 12.76	91.78 ± 12.10	88.75 ± 15.77	0.007
RBP4 (μg/mL)	45.86 (33.86–59.23)	45.33 (34.34–58.03)	48.62 (29.71–69.25)	0.452

Data are mean ± SD or median (interquartile range) unless otherwise indicated. DBP, diastolic blood pressure; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; iHbA_{1c}, impaired HbA_{1c}. IFG defined as FBG 100–125 mg/dL (5.6–6.9 mmol/L). IGT defined as 2-h PBG 140–199 mg/dL (7.8–11.0 mmol/L). iHbA_{1c} defined as HbA_{1c} >5.7% (>39 mmol/mol).

ranges of serum RBP4 concentrations and the incidence of type 2 diabetes (Supplementary Table 2). The P value for the test of linearity hypotheses was <0.001, confirming the nonlinear relationship between RBP4 levels and risk of incident type 2 diabetes. Multivariate Cox regression models with cubic natural spline analyses revealed that the association of serum RBP4 levels with incident type 2 diabetes was U shaped on a continuous scale, as both high and low RBP4 levels resulted in elevated risks of incident type 2 diabetes (Fig. 1). A progressively higher risk of incident type 2 diabetes was observed when the serum RBP4 level exceeded 55 μg/mL. Notably, the risk of type 2 diabetes incidence also increased when the RBP4 level was <31 μg/mL. Below the inflection point of RBP4 at 31 μg/mL, the risk of incident type 2 diabetes decreased with increasing RBP4 levels (HR 2.09 [95% CI 1.18–3.69] at 20 μg/mL and 1.21 [1.01–1.44] at 30 μg/mL). Above the inflection point at 55 μg/mL, the risk of incident type 2 diabetes increased with increasing RBP4 levels (1.68 [1.06–2.65] at 60 μg/mL, 2.00 [1.26–3.17] at 70 μg/mL, and 2.22 [1.37–3.59] at 80 μg/mL) (Fig. 2).

We used Cox proportional hazards models with the middle range of RBP4 levels (31–55 μg/mL) as a reference to further assess the relationship between RBP4 levels and the risk of incident type 2 diabetes. As shown in Table 2, compared with the reference group, lower and higher serum RBP4 levels were significantly associated with a higher risk of incident type 2 diabetes (HR 2.01 [95% CI 1.31–3.09] and 1.97 [1.32–2.93], respectively) after adjustment for age, sex, smoking, drinking, physical activity, BMI, waist circumference, eGFR, HDL levels, TG levels, SBP, GGT, HOMA-IR, FBG, 2-h PBG, and HbA_{1c} levels (P = 0.001, model 5).

Subgroup Analyses

We divided participants into three groups based on RBP4 cutoff values from this study to elucidate the potential mechanisms and compared with the low RBP4 group; the high RBP4 group included a higher proportion of males, high levels of insulin, waist circumference, LDL-c, TG, GGT, and Cr, and lower

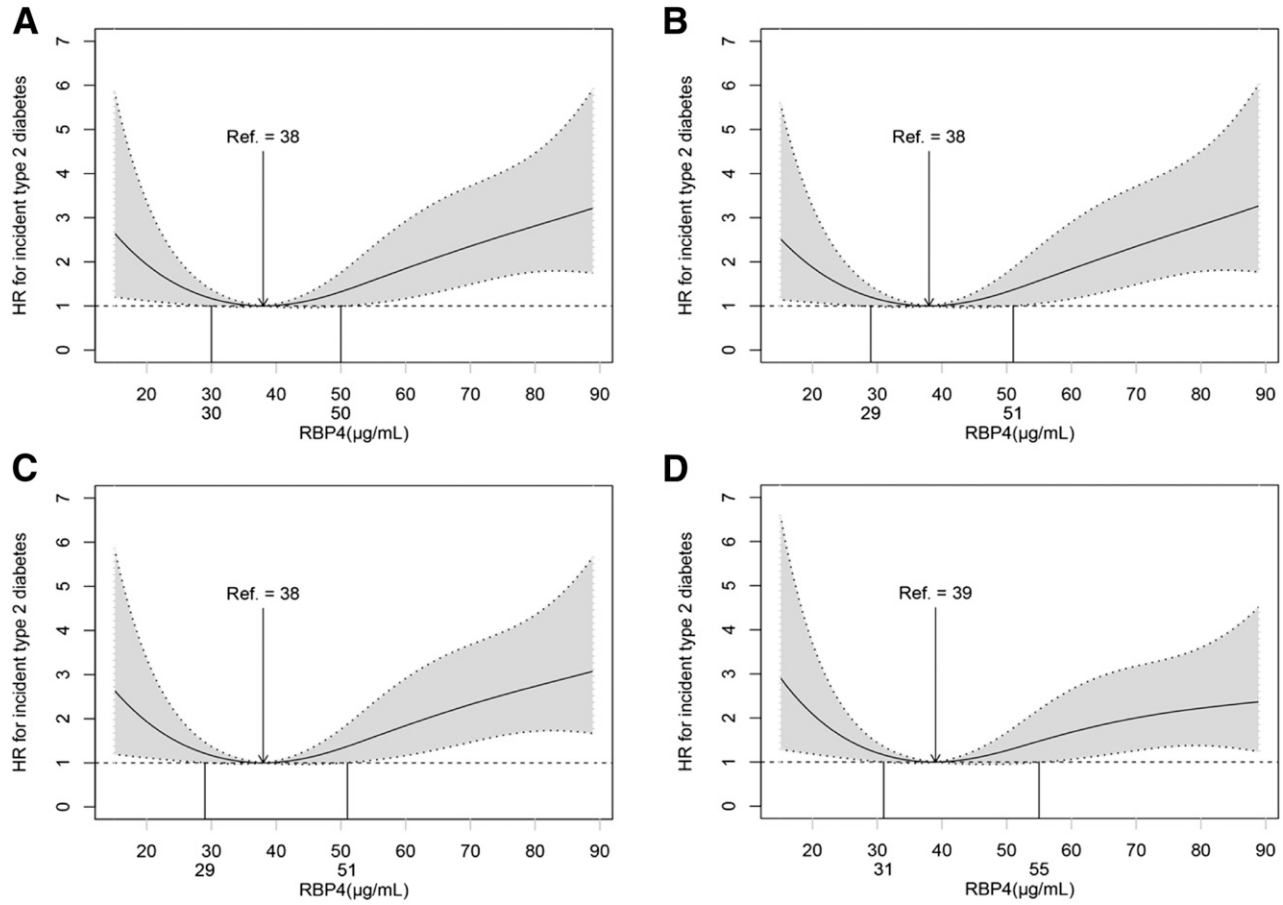

Figure 1—RBP4 levels on a continuous scale and risk of incident type 2 diabetes. HRs (solid line) and 95% CIs (dotted lines) were derived from multivariable Cox regression using cubic natural spline. Model 1 was adjusted for age, sex, smoking, drinking, and physical activity (*A*). Model 2 was adjusted for the variables in model 1 plus BMI (*B*). Model 3 was adjusted for the variables in model 2 plus waist circumference (*C*). Model 4 was adjusted for the variables in model 3 plus eGFR, HDL, TG, SBP, GGT, and HOMA-IR (*D*).

levels of HDL-c and eGFR (Supplementary Table 3). According to the results of the interaction analysis, RBP4 interacted with waist circumference, BMI, TG levels, and eGFR ($P < 0.05$). Therefore, we examined the association of RBP4 levels with incident type 2 diabetes in different subgroups of waist circumference, BMI, TG levels, eGFR, and sex. The U-shaped associations between RBP4 levels and the risk of incident type 2 diabetes remained consistent across all subgroups; both RBP4 levels <31 μg/mL and >55 μg/mL were associated with a higher risk of incident type 2 diabetes, with the exception of waist circumference subgroups (Supplementary Fig. 2). Compared with the reference group (RBP4 levels 31–55 μg/mL), the RBP4-diabetes relationship was not statistically significant in participants with a high waist circumference when RBP4 levels were <31 μg/mL, or in participants with a low waist circumference when RBP4 levels were >55 μg/mL. Furthermore, we compared the metabolic parameters between the low RBP4 group and the high RBP4 group among the participants who developed diabetes. A significant difference in metabolic parameters was not observed between the low and high RBP4 groups, except for a higher proportion of males in the high RBP4 group (Supplementary Table 4). We further analyzed the association between RBP4 levels and incident type 2 diabetes in males and females separately, and results showed that the U-shaped relationship still exists (Supplementary Fig. 3).

Sensitivity Analyses

In the sensitivity analysis, 553 participants who met at least two of the three glycemic criteria for prediabetes were included, including 124 participants who progressed to type 2 diabetes (Supplementary Fig. 4). We also performed another sensitivity analysis to assess the robustness of our findings only in confirmed cases (Supplementary Fig. 5). Results from the sensitivity analysis were consistent with the primary analyses.

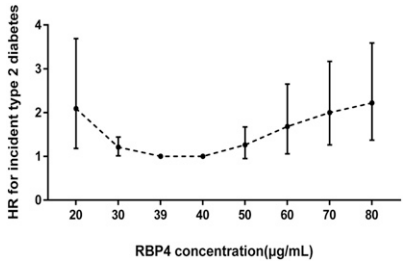

Figure 2—HRs (95% CI) for 3-year risk of incident type 2 diabetes at different levels of RBP4 (20–80 μg/mL) were analyzed by multivariable Cox regression models with cubic natural spline adjusting for age, sex, smoking, drinking, physical activity, BMI, waist circumference, eGFR, HDL, TG, SBP, GGT, and HOMA-IR (39 μg/mL as reference).

Table 2—Association of serum RBP4 with incident type 2 diabetes

	Total (n = 1,011)			
	RBP4 levels			
	<31 μg/mL (n = 191)	31–55 μg/mL (n = 507)	>55 μg/mL (n = 313)	P value
Incidence of type 2 diabetes				
n (%)	42 (22.0)	51 (10.1)	60 (19.2)	<0.001
Per 1,000 person-years (95% CI)	72.4 (61.1–83.7)	33.2 (28.0–38.5)	63.1 (54.4–71.9)	—
Model 1 HR (95% CI)	1.95 (1.29–2.93)	1.00	2.41 (1.65–3.52)	<0.001
Model 2 HR (95% CI)	1.90 (1.26–2.86)	1.00	2.41 (1.65–3.52)	<0.001
Model 3 HR (95% CI)	1.93 (1.28–2.91)	1.00	2.36 (1.61–3.44)	<0.001
Model 4 HR (95% CI)	1.97 (1.30–2.99)	1.00	2.03 (1.37–3.00)	<0.001
Model 5 HR (95% CI)	2.01 (1.31–3.09)	1.00	1.97 (1.32–2.93)	0.001

Incidence of type 2 diabetes is presented as the number of events (%) and the number of events per 1,000 person-years (95% CI). Model 1 was adjusted for age, sex, smoking, drinking, and physical activity. Model 2 was adjusted for the variables in model 1 plus BMI. Model 3 was adjusted for the variables in model 2 plus waist circumference. Model 4 was adjusted for the variables in model 3 plus eGFR, HDL, TG, SBP, GGT, and HOMA-IR. Model 5 was adjusted for the variables in model 4 plus FBG, 2-h PBG, and HbA_{1c}. Participants with a serum RBP4 level of 31–55 μg/mL served as the reference group.

CONCLUSIONS

In this prospective population-based cohort study, we found a U-shaped association between RBP4 and incident type 2 diabetes, with both higher and lower RBP4 levels being associated with a high risk for incident type 2 diabetes. To the best of our knowledge, this is the largest study to investigate the association between RBP4 and type 2 diabetes risk in subjects with prediabetes. Previous studies have suggested that RBP4 levels were associated with a higher risk of diabetes (15,20). Conversely, other investigations failed to identify a relationship between RBP4 and diabetes (21–23). Such an ambiguous correlation is mainly due to a limited sample size (15,21–23), cross-sectional study design (21–23), methodology and population characteristics (15,22), and especially an incomplete adjustment for potential confounders such as eGFR (15,22). By extensively adjusting for potential confounders and stratifying several potential risk factors that may have an effect on the RBP4–type 2 diabetes relationship, our large cohort study confirmed that RBP4 independently predicted incident type 2 diabetes in subjects with prediabetes.

We observed that compared with the middle range of serum RBP4 levels of 31–55 μg/mL, RBP4 levels <31 μg/mL and >55 μg/mL were associated with a 2.01-fold and 1.97-fold increase in the risk of incident type 2 diabetes, respectively. Our findings are consistent with the previous study, which reported that risk of type 2 diabetes starts to increase when RBP4 concentrations are >50 μg/mL (10). However, there were no previous studies that analyzed the relationship between lower RBP4 levels and the risk of diabetes. Our findings, on one hand, corroborated the link between high RBP4 levels with increased incident type 2 diabetes. On the other hand, we highlighted the notion that low RBP4 levels are also significantly associated with increased risk of incident type 2 diabetes. Thus, our data provide important prognostic information to both physicians and patients that serum RBP4 levels might be an additional predictor of the development of type 2 diabetes in subjects with prediabetes.

High circulating RBP4 has been reported to be associated with insulin resistance, which is a major contributor to type 2 diabetes (24). Previous studies reported that RBP4 contributed to the development of diabetes by inducing insulin resistance through various ways, including inducing the hepatic expression of phosphoenolpyruvate kinase, suppressing muscle insulin signaling, activating antigen-presenting cells, and stimulating an inflammatory state in adipose tissue (7,25–27). However, the RBP4-diabetes association in our study was independent of adiposity and HOMA-IR, indicating that RBP4 may increase the risk of type 2 diabetes through pathway(s) not largely overlapping with insulin resistance. In fact, both insulin resistance and β-cell dysfunction are responsible for pathogenesis of type 2 diabetes (28), and studies have reported that β-cell failure, rather than aggravated insulin resistance, might be the main reason for subjects with prediabetes to develop diabetes (29). What's more, previous studies suggested that RBP4 might be involved in the pathogenesis of β-cell dysfunction, which could be behind the association between higher RBP4 levels and type 2 diabetes (11,30). Nevertheless, to our knowledge, there was no available mechanistic evidence regarding how RBP4 facilitates the pathogenesis of β-cell dysfunction. More intensive investigations are needed in the future.

The most striking finding in the current study was the association of low RBP4 levels with increased risk of diabetes in participants with prediabetes. Yet, the underlying mechanisms of this association are still unknown. RBP4 is the only specific transporter protein for retinol in the circulation and is responsible for maintaining normal levels of retinol (31), an essential, fat-soluble nutrient for maintaining normal physiology (32). Our findings further raise the possibility that retinol might influence the action of RBP4 and the risk of type 2 diabetes. Studies have shown that RBP4 knockout mice have dramatically lower serum retinol levels, similar to the levels in the later stages of retinol deficiency in humans (33). In addition, retinol deficiency was also reported to cause hyperglycemia and loss of pancreatic β-cell mass, indicating an important role of maintaining normal levels of retinol in glucose metabolism (34). Thus, we hypothesized that the association between RBP4 and insulin resistance might be explained

by lower retinol to some extent. Nevertheless, the exact molecular mechanisms driving this phenomenon need to be examined in future studies.

The strengths of the current study include the large-scale, longitudinal follow-up of a high-risk population, the use of the nonlinear test rather than a simple linear analysis, and the extensive adjustment for key confounders, including waist circumference, TG levels, GGT, HOMA-IR, and eGFR, which has been shown to provide a reasonably accurate and unbiased estimate of the relationship between RBP4 levels and incident type 2 diabetes.

Several potential limitations of the current study should also be considered. First, the use of a single baseline RBP4 measurement to predict outcomes was a simplified and practical approach but was unable to assess the relationship between changes in RBP4 levels and the risk of incident type 2 diabetes. Second, all study participants were Chinese, and the conclusions may not be generalizable to other ethnicities. Further studies are needed in more diverse groups. Third, we did not distinguish incident diabetes in terms of type, but the incidence of type 1 diabetes is very low, the fasting glucose was only mildly elevated, and insulin levels were high normal among the participants; these participants are unlikely to develop type 1 diabetes. Fourth, our ascertainment of incident type 2 diabetes included subjects with undiagnosed diabetes. Although sensitivity analysis performed on confirmed cases yielded the same result as the original analysis, the RBP4-diabetes relationship should also be exclusively analyzed in confirmed cases in future studies.

In summary, our population-based longitudinal study identified a U-shaped association between RBP4 levels and the risk of incident type 2 diabetes in subjects with prediabetes. RBP4 levels >55 μg/mL or <31 μg/mL predicted higher risks of type 2 diabetes in individuals with prediabetes.

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Author Contributions. J.F. and S.Y. analyzed data and drafted the manuscript. J.F., S.Y., and D.L. performed the experiments. J.F., S.Y., D.L., Y.L., N.C., X.B., Q.K., J.S., L.Yo., X.L., F.L., F.H., and L.Ya. collected data. J.F., S.Y., D.L., Y.L., N.C., X.B., Q.K., J.S., L.Yo., X.L., F.L., F.H., L.Ya., C.C., and M.X. were involved in discussing and revising the manuscript and provided final approval of the submitted versions. J.F., C.C., and M.X. conceived and designed the study. L.Ya., C.C., and M.X. reviewed the manuscript. C.C. and M.X. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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