Published in final edited form as:
Diabetes Res Clin Pract. 2013 January ; 99(1): 48–53. doi:10.1016/j.diabres.2012.10.023.

Association of Retinol Binding Protein 4 with Risk of Gestational Diabetes

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Abstract

Aim—We investigated association of maternal retinol binding protein 4 (RBP4) with risk of gestational diabetes (GDM).

Methods—GDM cases (N=173) and controls (N=187) were selected from among participants of a cohort study of risk factors of pregnancy complications. Early pregnancy (16 weeks on average) serum RBP4 concentration was measured using an ELISA-based immunoassay. Logistic regression was used to estimate unadjusted and adjusted odds ratios (ORs/aORs) and 95% confidence intervals (95%CI).

Results—Mean serum RBP4 was significantly higher among GDM cases compared with controls (47.1 vs. 41.1 μg/ml, respectively; p-value<0.05). Participants in the highest quartile for serum RBP4 had a 1.89-fold higher risk of GDM compared with participants in the lowest quartile (95%CI: 1.05-3.43). However, this relationship did not reach statistical significance after adjustment for confounders (aOR: 1.54; 95%CI: 0.82-2.90). Women who were ≥35 years old and who had high RBP4 (≥38.3 μg/ml, the median) had a 2.31-fold higher risk of GDM compared with women who were < 35 years old and had low RBP4 (<38.3 μg/ml) (aOR: 2.31; 95%CI: 1.26-4.23; p-value for interaction=0.021).

Conclusion—Overall, there is modest evidence of a positive association of early pregnancy elevated RBP4 concentration with increased GDM risk, particularly among women with advanced age.

Keywords

Retinol binding protein 4; gestational diabetes; pregnancy; maternal age

Introduction

Gestational diabetes (GDM), a common complication of pregnancy occurring in 5-10% of all pregnancies [1,2,3,4], is characterized by glucose intolerance that first appears during

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pregnancy [5,6,7,8]. GDM is associated with adverse maternal (e.g., C-sections and increased risk of type 2 diabetes [T2DM]) and offspring (e.g., fetal hyperinsulinism, macrosomia, and birth injuries) outcomes [2,4,8,9]. Underlying pathophysiological disturbances commonly identified in GDM include reduced insulin secretion and abnormal insulin resistance [10,11,12,13,14,15].

Retinol binding protein 4 (RBP4) is a recently identified adipokine that has been linked to obesity and related co-morbidities including insulin resistance and T2DM [10,16]. Hepatocytes are the main sources of RBP4; and, RBP4 is known to be highly expressed in adipose tissues [5,16,17,18,19,20]. An increase in adipose tissue mass is associated with an increase in RBP4, IL-6 and TNF-α production [17]. Using an animal model, Yang et al [21] have shown that RBP4 may serve as an adipocyte derived signal that plays a role in the development of insulin resistance. Decreased adiponectin and increased RBP4 concentration have been associated with decreased fatty acid oxidation, increased hepatic gluconeogenesis, and insulin resistance [17].

Several investigators have reported associations of RBP4 concentrations with insulin resistance and T2DM [5,21,22,23]. RBP4 is encoded by the RBP4 gene, which maps to chromosome 10q23-q24, the genomic region that has been linked to an increased risk of T2DM [5,17]. Further, advanced maternal age has previously been shown to influence the association of RBP4 concentrations with insulin sensitivity, percent fat mass and lipid profiles [24]. Despite previous reports of associations of serum concentrations of RBP4 with insulin resistance and risk of T2DM, the role of RBP4 in GDM risk is not well established. In a cross-sectional study, Lewandowski et al documented elevated serum RBP4 concentrations at 28 weeks of gestation among women with GDM as compared with controls [25]. However, Teper et al, in a prospective cohort study, reported that neither circulating RBP4 nor the RBP4:retinol molar ratio was significantly different between GDM cases and controls [18]. Given the scarcity of research on this topic and inconsistency of findings, we investigated whether early pregnancy serum RBP4 concentrations is associated with risk of GDM later in pregnancy. We also examined whether this relationship is modified by maternal age.

Methods

Overview

Study participants for this nested case-control study were drawn from participants of the Omega study, a prospective cohort study designed to examine maternal dietary and life style risk factors of pregnancy complication such as preeclampsia and gestational diabetes [26,27,28,29]. The study population was comprised of women attending prenatal care clinics affiliated with Swedish Medical Center (SMC) in Seattle, WA and Tacoma General Hospital (TGH) in Tacoma, WA. Women were eligible if they began prenatal care before 20 weeks of gestation, were older than 18 years, were able to speak and read English, planned to carry the pregnancy to term, and planned to deliver at either of the two research hospitals.

Study population

All study participants underwent a screening test, a 50 gram of 1-hour oral glucose challenge test, at 24-28 weeks of gestation. For participants who failed this test (blood glucose ≥ 140 mg/dl), a follow-up test consisting of a 100g, 3-hour oral glucose tolerance test (OGTT) was administered. Based on the American Diabetes Association (ADA) recommendations, women were diagnosed with GDM if two or more of results of the OGTT exceeded the ADA criteria [30] as follows: fasting ≥95 mg/dl; 1-hour ≥ 180 mg/dl; 2-hour ≥ 155mg/dl; 3-hour ≥ 140mg/dl [30]. We included all 173 GDM cases and 187 randomly selected controls

in the current study. Study protocols were approved by the Institutional Review Boards of SMC and TGH. All participants provided written informed consent.

Data collection

Using standardized questionnaires administered by trained-interviewers at or near the time of enrollment (16 weeks on average), information was gathered on maternal characteristics including maternal age, pre-pregnancy weight, height, pre-pregnancy body mass index (BMI), medical and reproductive history, gestational age at glucola, maternal socio-demographic factors, and family history of medical illness in first degree family members. At the end of the pregnancy period, maternal and infant medical records were reviewed for information on the course and outcomes of pregnancy.

At or near the time of enrollment, blood samples were collected for biomarker measurement. Biological samples were either processed immediately or stored at −80°C until further processing. A commercial enzyme-linked immunoassay (Catalog number DRB400, Quantikine TM, R&D Systems, Minneapolis, MN, USA) was used to measure serum RBP4 concentrations according to the manufacturer's instructions. All assays were performed by one technician who was blinded to the case control status of study participants.

Statistical analysis

We examined general characteristics of the study population using mean (standard deviation) for continuous variables and numbers (%) for categorical variables. We compared maternal characteristics between GDM cases and controls using Student's T-test (for continuous variables) and chi square tests (for categorical variables). We used unadjusted and multivariable adjusted logistic regression models to compute unadjusted/adjusted odds ratios (ORs/aORs) and 95% confidence intervals (95% CI) comparing risk of GDM among the upper three quartiles for RBP4 concentrations with the referent (the lowest quartile). Besides *a priori* identified potential confounding variables (maternal age and race/ethnicity), variables that resulted in >10% difference in estimated ORs (comparing unadjusted and adjusted regression coefficients) were considered as confounders and were retained in final multivariable models. We explored possibility of a nonlinear relation between RBP4 concentrations and GDM odds, using generalized additive logistic regression modeling procedures (GAM) [31]. We also evaluated potential effect modification of the relationship between serum RBP4 concentrations and risk of GDM by maternal age using stratified models and interaction terms in multivariable logistic regression models. For these analyses, we defined advanced maternal age as age ≥35 years. We used the median RBP4 concentration (38.3ug/ml) as the cut-off to define high and low RBP4. Overweight was defined as BMI ≥ 25 kg/m^2. For the interaction analyses, we defined three variables for age (<35 years or ≥35years), RBP4 concentrations (< 38.3ug/ml or ≥ 38.3ug/ml), and their interaction. The P-value for the interaction term was the interaction p-value.

All analyses were conducted using SPSS version 14 and S-Plus (version 6.1, release 2, Insightful Inc. Seattle, WA). Statistical significance was defined as two sided $p<0.05$.

Results

GDM cases were older compared with controls (34.2 vs. 33.0 years, respectively) (Table 1). Controls were more likely to be non-Hispanic White compared with GDM cases (86% vs. 71%). Mean (standard deviation, SD) pre-pregnancy BMI among cases and controls was 26.6(6.5) kg/m^2 and 23.4(5.1) kg/m^2, respectively (p-value<0.05). GDM cases were more likely to report a positive history of hypertension, family history of diabetes and family history of hypertension compared with controls (all p-values<0.05).

Mean (SD) serum RBP4 concentrations were significantly higher among GDM cases 47.1 (30.0) μg/ml compared with controls 41.1 (21.3) μg/ml ($P<0.05$). Participants in the highest quartile for serum RBP4 had a 1.89-fold higher risk of GDM compared with participants in the lowest quartile (95% CI: 1.05-3.43) (Table 2). However, this relationship was greatly attenuated and became statistically insignificant after adjustment for confounders including maternal age, race/ethnicity, family history of diabetes, and pre-pregnancy overweight status (aOR: 1.54, 95%CI: 0.82-2.90). In analyses employing GAM procedures, higher odds of GDM risk was observed with increasing concentrations of RBP4 (Figure 1).

Among women who were ≥35 years of age, those in the highest quartile for RBP4 had a greater than 2-fold increase in risk of GDM compared with women in the lowest quartile (aOR: 2.39, 95%CI: 0.91-6.29) (Table 3). This relationship, however, was not observed among women < 35 years of age (trend p-value=0.94). We observed statistically significant interaction between higher RBP4 concentration and maternal age (p value for interaction = 0.021). Women who were ≥35 years of age and who had high RBP4 (≥38.3 μg/ml, the median) had a greater than 2-fold higher risk of GDM compared with women who were < 35 years of and who had low RBP4 (<38.3 μg/ml) (aOR: 2.31, 95%CI: 1.26-4.23) (Table 4).

Discussion

Overall, there is modest evidence to support association of serum RBP4 concentrations in early pregnancy with an increased risk of GDM. Maternal age may be a potential effect modifier of the association between serum RBP4 and GDM. The relationship between RBP4 and GDM was observed only among women ≥ 35 years of age.

Our findings are similar to some previous reports of associations of RBP4 with insulin resistance, obesity and diabetes in non-pregnant and pregnant populations [2,5]. An increase in RBP4 in serum was observed among T2DM patients in a cross-sectional study by Takebayashi et al [32]. They reported a significant elevation of RBP4 concentrations in diabetic patients compared with healthy controls (mean and SD, 23.8±8.8 vs. 21.1±4.3 μg/ml, respectively). T2DM shares a number of risk factors with GDM; and some investigators consider GDM as a precursor of T2DM [4,5,7,22]. RBP4, like other adipokines, may participate in GDM pathogenesis [8]. A correlation between RBP4 concentrations and insulin resistance has been demonstrated among obese or T2DM study participants [5,22,23]. Chan et al reported that serum concentrations of RBP4 measured in samples collected between 24-28 weeks of gestation were significantly higher among women with GDM pregnancies compared with serum RBP4 concentrations among women with pregnancies uncomplicated by GDM (mean and SD, 42.4 ± 13.8ng/ml vs. 32.0 ± 8.7ng/ml; p=0.007)[5].

Our findings are similar to those reported by Su et al. In a cross-sectional study, Su et al measured RBP4 concentrations among pregnant women with and without GDM (at median gestational age of 26 weeks), as well as non-pregnant women. They found significantly increased mean RBP4 concentrations in GDM cases (mean and SD, 41.64 ± 12.21 mg/l) compared to concentrations among non-GDM pregnant women (mean and SD, 34.50 ± 9.80 mg/L) and healthy non-pregnant women (mean and SD, 30.64 ± 9.46 mg/l) [23]. In contrast, Krzyzanowska et al found that women with GDM had lower RBP4 concentrations as compared to controls in a study that involved enzyme immunometric assay (EIA) (median 6.8; inter-quartile range, 3.9-14.3; vs. median 11.3; inter-quartile range 7.8-19.9 ug/ml, for GDM cases and controls respectively; p< 0.001) and western blot (median 25.1; inter-quartile range 21.7-29.6 vs. median 26.6, inter-quartile range 23.5-32.2 ug/ml, for GDM cases and controls respectively; p=0.026) based measurements [6]. However, they found that women with GDM had a higher RBP4 to retinol molar ratio than their control counterparts

[6]. In the study conducted by Teper et al, neither circulating RBP4 nor RBP4 to retinol molar ratio was significantly different between GDM cases and corresponding controls [18]. Inconsistent findings in previous reports can result from differences in study population characteristics, including age and socio-economic status indicators. For instance, the study population investigated by Teper et al comprised of a cohort of borderline obese women who may have different underlying physiological and patho-physiological characteristics to our study population.

Advanced maternal age is one of the risk factors for GDM [33]. For instance, investigators have reported that women ≥40 years of age have greater than 10-fold increased risk of GDM as compared with women 20-40 years of age [33]. RBP4 concentrations were also higher among older study participants compared with younger participants [24,34]. Among characteristics that have been related to aging are changes in body fat distribution and renal function [24]. Both of these are direct or indirect risk factors for GDM. Possible hypotheses for observed relationships in our study could be that age related alteration in body fat distribution and/or changes in renal function may account for differences in association of serum RBP4 concentrations with GDM risk among younger or older pregnant women.

Several strengths and limitations should be considered when interpreting results from our study. Unlike previous studies which collected samples between 24 and 28 weeks of gestation, we collected serum samples early in pregnancy, and thus were able to determine maternal early pregnancy RBP4 concentrations. Therefore, we can safely identify temporal relationship between the exposure and outcome. We controlled for several variables that could be potential confounders. Finally, we examined potential effect modification by maternal age. Some limitations of our study deserve mention. Although we adjusted for several potential confounders, we cannot exclude the possibility of residual confounders or confounding by unmeasured variables. Generalizability of our findings may be limited because our study participants were predominantly non-Hispanic White and residents of the Pacific Northwestern region of the USA.

Conclusion

There is modest evidence of a positive association of early pregnancy elevated RBP4 concentration with increased risk of GDM, particularly among women with advanced maternal age. Additional research is warranted to further explore these relationships.

Acknowledgments

The authors are very thankful to the participants of the Omega study and staff of Center for Perinatal Studies, Swedish Medical Center, Seattle, WA, USA for their technical support with this research. This research was supported by awards from the National Institutes of Health (R01HD-055566 and R01HD-32562). Dr. Enquobahrie was supported by a K01 award (K01HL-103174).

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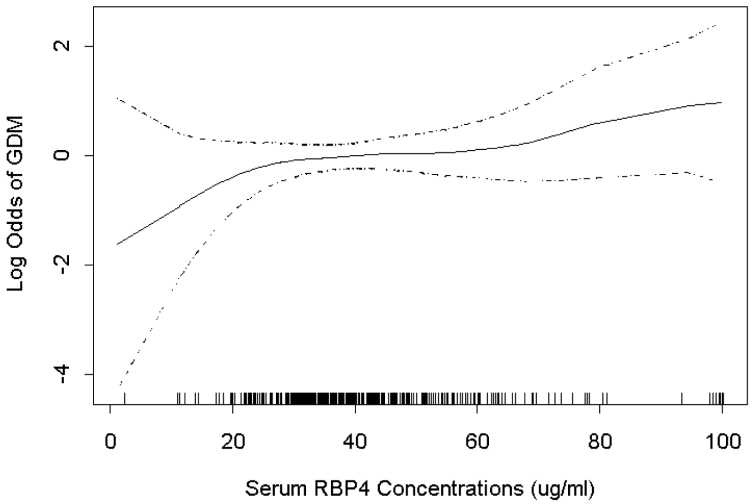

Figure 1.

Relation between maternal serum RBP4 concentrations (X-axis) and adjusted relative odds of gestational diabetes (GDM) (Y-axis). Estimates are depicted by the solid line while 95% CIs are depicted by the dotted lines. The vertical bars along the X-axis indicate distribution of study subjects. The estimates were adjusted for maternal age, race/ethnicity, family history of diabetes and pre-pregnancy overweight status.

Table 1

Selected clinical characteristics of the study population Seattle and Tacoma, Washington, USA 1996 - 2008

Characteristics	GDM Cases N (%)	Controls N (%)	P-value
Numbers	173 (48.1)	187 (51.9)	
Maternal Age (years) *	34.15±4.56	32.95±4.32	
<35	89 (51.4)	117 (62.6)	
≥35	84 (48.6)	70 (37.4)	
			0.011
Race, non-Hispanic White	122 (70.9)	161 (86.1)	<0.001
Education ≤ high school	8 (5.0)	5 (2.9%)	0.316
Unmarried	29 (16.8)	27 (14.4)	0.543
Multiparous	82 (47.4)	82 (43.9)	0.499
Pre-pregnancy BMI (kg/m^2) *	26.62±6.50	23.38±5.11	
<18.5	4 (2.3)	9 (4.8)	
18.8-24.9	84 (48.6)	130 (69.5)	
25.0-29.9	48 (27.7)	39 (20.9)	
≥30.0	37 (21.4)	9 (4.8)	
			<0.001
Gestational age at blood collection	15.52±2.82	15.65±2.94	0.67
Exercise during pregnancy	137 (79.2)	158 (84.5)	0.191
Worked during pregnancy	140 (81.9)	152 (84.0)	0.599
History of chronic hypertension	18 (10.4)	7 (3.7)	0.013
Family history of diabetes mellitus	53 (30.6)	28(15.0)	<0.001
Family history of hypertension	99 (57.2)	87 (46.5)	0.042
smoking during pregnancy	13 (7.5)	9 (4.8)	0.346
Serum Retinol Binding Protein 4 µg/ml *	47.13±30.02	41.14±21.29	0.029

* Mean (SD) otherwise N (%)

Table 2

Gestational diabetes mellitus (GDM) risk according to quartiles of maternal serum RBP4 concentrations in early pregnancy

Serum RBP4 (μg/ml)	GDM N= 173	Controls N= 187	Unadjusted OR (95%CI)	Adjusted * OR (95%CI)
	N (%)	N (%)		
Quartile 1 (<31.2)	31 (17.9)	46 (24.6)	Referent	Referent
Quartile 2 (31.2 - 38.2)	39 (22.5)	46 (24.6)	1.25 (0.67-2.34)	0.99 (0.50-1.93)
Quartile 3 (38.3 - 46.3)	43 (24.9)	48 (25.7)	1.32 (0.72-2.45)	1.30 (0.67-2.51)
Quartile 4 (≥ 46.4)	60 (34.7)	47 (25.1)	1.89 (1.04-3.43)	1.53 (0.81-2.89)
P -value for the trend			0.035	0.14

* OR and 95%CI adjusted for maternal age, race/ethnicity, family history of diabetes and pre-pregnancy overweight status

Table 3

Gestational diabetes mellitus (GDM) risk according to quartiles of early pregnancy maternal serum RBP4 concentrations stratified by advanced maternal age

Serum RBP4 (μg/ml)	GDM N= 173	Controls N= 187	Unadjusted OR (95%CI)	Adjusted OR (95%CI) *
	N (%)	N (%)		
Maternal Age ≥35 years				
Quartile 1 (<31.2)	11 (13.1)	16 (22.9)	Referent	Referent
Quartile 2 (31.2 - 38.2)	14 (16.7)	18 (25.7)	1.13 (0.40-3.19)	0.96 (0.32-2.89)
Quartile 3 (38.3 - 46.3)	22 (26.2)	16 (22.9)	2.00 (0.73-5.44)	1.92 (0.67-5.51)
Quartile 4 (≥ 46.4)	37 (44.0)	20 (28.6)	2.69 (1.05-6.89)	2.40 (0.89-6.45)
p-value for the trend			p=0.02	p=0.03
Maternal Age< 35 years				
Quartile 1 (<31.2)	20 (22.5)	30 (25.6)	Referent	Referent
Quartile 2 (31.2 - 38.2)	25 (28.1)	28 (23.9)	1.33 (0.61-2.92)	0.98 (0.41-2.35)
Quartile 3 (38.3 - 46.3)	21 (23.6)	32 (27.4)	0.98 (0.44-2.16)	1.03 (0.43-2.45)
Quartile 4 (≥ 46.4)	23 (25.8)	27 (23.1)	1.27 (0.57-2.82)	1.02 (0.42-2.45)
p-value for the trend			0.8	0.93

* OR and 95%CI adjusted for maternal race/ethnicity, family history of diabetes, and pre-pregnancy overweight status.

Number (%)

Table 4

Higher early pregnancy serum maternal RBP4 concentrations, advanced maternal age, and gestational diabetes mellitus (GDM) risk

Higher RBP4 and Age ≥35 years	GDM (N=173)	Controls (N= 187)	Unadjusted OR (95%CI)	Adjusted OR (95%CI) *
	N (%)	N (%)		
RBP4 < 38.3 and <35years	45 (26.0)	60 (32.1)	Referent	Referent
RBP4 ≥ 38.3 and <35years	44 (25.4)	57 (30.5)	1.02 (0.59-1.78)	1.08 (0.60-1.96)
RBP4 < 38.3 and ≥35years	25 (14.5)	34 (18.2)	0.98 (0.51-1.86)	1.01 (0.50-2.01)
RBP4 ≥ 38.3 and ≥35years	59 (34.1)	36 (19.3)	2.18 (1.24-3.85)	***2.31 (1.26-4.23)***
p-value for interaction			0.018	*0.021*

* OR and 95%CI adjusted for maternal race/ethnicity, family history of diabetes, and pre-pregnancy overweight status.