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Retinol-binding protein 4 is not associated with insulin resistance in pregnancy

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ABSTRACT

Retinol-binding protein 4 (RBP4) is an adipokine proposed to be specifically associated with insulin resistance (IR). We examined whether serum levels of RBP4 were associated with IR in pregnancy. One hundred seventy-two women with gestational diabetes mellitus (GDM) and 361 pregnant Thai women who did not have GDM but had a positive 50-g glucose challenge test result (plasma glucose level was ≥7.2 mmol/L after 1 hour) were enrolled. We measured fasting serum levels of RBP4 and assessed IR at a 100-g oral glucose tolerance test. We found a higher degree of IR in the GDM group compared with the non-GDM group, but serum RBP4 levels between the 2 groups were not different. Retinol-binding protein 4 levels were associated with serum triglyceride levels but were not associated with the degree of IR assessed by homeostasis model assessment or quantitative insulin sensitivity check index. Our results suggest that serum RBP4 levels in pregnancy are not associated with IR.

1. Introduction

Pregnancy is a physiologic state of progressive insulin resistance (IR). Failure to increase insulin secretion to compensate for enhanced IR in pregnancy plays a pathogenic role in gestational diabetes mellitus (GDM). Retinol-binding protein 4 (RBP4) is a recently discovered protein secreted by adipocytes [1]. Several studies have shown that RBP4 levels are associated with IR in subjects with diabetes mellitus and obesity [1,2], whereas other studies failed to find a strong association with IR [3-6]. Nevertheless, data in nonpregnant subjects suggest that serum levels of RBP4 correlate with IR more than other adipokines [2,7].

In women with GDM, not all studies have shown an increase in RBP4 levels [8-11]. In addition, a relationship between RBP4 levels and IR in pregnancy has not yet been demonstrated [9-12]. A small number of subjects and lack of validation of RBP4 enzyme-linked immunosorbent assay (ELISA) in most studies [13] might have compromised previous conclusions. We therefore measured RBP4 levels using validated ELISA in a large group of pregnant women to investigate this issue.

2. Subjects and methods

Pregnant women were consecutively enrolled using a 2-step approach of glucose testing for diagnosis of GDM [14]. Initial screening was performed by determining plasma glucose concentration 1 hour after a 50-g glucose challenge test (GCT). If the result was positive (glucose value was ≥7.2 mmol/L), a

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diagnostic 100-g oral glucose tolerance test (OGTT) was scheduled within 1 week. We used the Carpenter and Coustan criteria for diagnosis of *GDM*, which was defined as at least 2 abnormal values higher than the cutoff of 5.3, 10.0, 9.2, and 8.1 mmol/L at 0, 1, 2, and 3 hours, respectively. Subjects were screened for GDM at 24 to 28 weeks of gestation. For those who had a high risk for GDM [14], glucose testing was performed as soon as feasible. If the result of the initial glucose testing was negative, another testing was performed at 24 to 28 weeks of gestation.

All women who had positive GCT result and successfully underwent a 100-g OGTT were enrolled. We collected fasting blood samples at the time of an OGTT. Sera were kept at –80°C until assay. Among 549 subjects initially enrolled, 17 subjects were excluded because of missing samples or data (n = 14), twin pregnancy (n = 2), and pregestational diabetes mellitus (n = 1). Of the 532 subjects that remained in the study, 171 had GDM, whereas 361 women had positive 50-g GCT result but did not have GDM. Fasting blood samples from 22 women with negative GCT result were also collected. All were followed to delivery. Written informed consent was obtained from each participant. The study protocol was approved by our Ethics Committee. Procedures were performed in accordance with the principles expressed in the Declaration of Helsinki.

Serum RBP4 levels were measured using ELISA from R&D Systems (Minneapolis, MN). The sensitivity of the assay was 0.22 ng/mL; and the intra- and interassay coefficients of variations were 7.6% and 16.3%, respectively. Because certain commercially available ELISA assays gave unreliable values [13], we validated our assay using quantitative Western blotting. A strong correlation between RBP4 levels, determined by our ELISA and Western blot, was observed ($r = 0.96$, $P < .001$). Plasma glucose, insulin, and lipid levels were measured using standard methods. Assessment of IR by homeostasis model assessment (HOMA) 1-IR and quantitative insulin sensitivity check index (QUICKI), which is comparable to log (HOMA-IR), was calculated. For HOMA2-IR, a computer program (www.dtu.ox.th) was used.

Variables with normal distribution are expressed as means ± SD, and variables with a skewed distribution are expressed as median (interquartile range). For continuous variables, differences between the groups were compared using Student *t* test, Mann-Whitney test, or analysis of variance, where appropriate. χ^2 test or Fisher exact test was used for categorical variables. Differences in pairs of the means among groups were compared using the Bonferroni post hoc test. Correlations were examined using linear regression analysis. A stepwise linear regression analysis was performed to investigate independent predictors of plasma RBP4 levels. All *P* values were 2-sided and considered significant if less than .05. We used SPSS version 16 (Chicago, IL) to perform the statistical analyses.

3. Results

Table 1 shows clinical characteristics of study subjects. Women with GDM had significantly higher prepregnancy

Table 1 – Clinical characteristics of the study subjects

	Positive GCT result				Negative GCT result (n = 22)
	Total (n = 532)	GDM (n = 171)	Non-GDM (n = 361)	*P* values	
Age (y)	33 (29-36)	33 (29-37)	33 (28-36)	.05	32 (26-39)
Gravidity	2 (1-3)	2 (1-3)	2 (1-3)	.53	2 (1-4)
History of GDM	5 (0.9%)	4 (2.3%)	1 (0.3%)	.04	0
History of macrosomia [a]	9 (1.7%)	4 (2.3%)	5 (1.4%)	.28	0
Family history of DM	173 (33%)	72 (42%)	101 (28%)	.002	8 (36%)
Prepregnancy weight (kg)	54.0 (48.0-62.0)	55.5 (49.0-65.0)	53.0 (48.0-60.5)	.06	51.5 (45.8-58.5)
Prepregnancy BMI (kg/m^2)	22.3 (20.0-25.5)	23.1 (20.6-26.2)	21.9 (19.9-25.3)	.003	21.1 (18.9-24.1)
GA at the time of OGTT (wk)	29 (25-32)	29 (25-32)	29 (24-32)	.91	NA
GA at delivery (wk)	38 (38-39)	38 (37-39)	38 (38-39)	.08	39 (38-39) [c]
Weight gain in pregnancy (kg)	14.1 (11.2-17.6)	13.3 (10.4-16.2)	14.5 (11.6-18.0)	.004	15.8 (10.9-21.7) [c]
FPG (mmol/L)	4.22 (3.94-4.55)	4.50 (4.11-5.00)	4.17 (3.89-4.39)	<.001	NA
1-h PG (mmol/L)	9.00 (7.89-10.4)	10.9 (10.2-11.9)	8.33 (7.33-9.17)	<.001	NA
2-h PG (mmol/L)	8.00 (6.89-9.28)	9.89 (9.06-10.9)	7.33 (6.33-8.03)	<.001	NA
3-h PG (mmol/L)	6.39 (5.39-7.50)	8.00 (6.61-9.06)	6.00 (5.11-6.78)	<.001	NA
FPI (pmol/L)	68.1 (45.1-104)	85.7 (51.9-128)	63.3 (43.7-91.9)	<.001	NA
HOMA1-IR	1.75 (1.16-2.79)	2.47 (1.44-3.94)	1.63 (1.10-2.44)	<.001	NA
HOMA2-IR [b]	1.40 (1.00-2.00)	1.80 (1.10-2.60)	1.30 (0.90-1.85)	<.001	NA
QUICKI	0.35 ± 0.04	0.34 ± 0.04	0.36 ± 0.04	<.001	NA
RBP4 (μg/mL)	35.8 ± 10.7	36.0 ± 10.4	35.6 ± 10.9	.69	34.6 ± 6.7

Data are presented as median (interquartile range), mean ± SD, or number (percentage). Difference between the GDM group and the non-GDM group was compared using Student *t* test or Mann-Whitney test for continuous variables and Fisher exact test for categorical variables. GA indicates gestational age; FPG, fasting plasma glucose; PG, plasma glucose; FPI, fasting plasma insulin; NA, not available.

[a] *Macrosomia* was defined as birth weight >4000 g.

[b] Because the HOMA2-IR model does not accept low values of plasma glucose and insulin, data from 29 subjects who had fasting plasma glucose <3.0 mmol/L or fasting plasma insulin <18 pmol/L were excluded. n = 503 (total), 162 (GDM), and 341 (non-GDM).

[c] Data are available only in 18 subjects.

Table 2 – Stepwise linear regression of independent predictors of serum RBP4 levels

Variables	Unstandardized coefficients		P	95% Confidence intervals for B	
	B	SE		Lower bound	Upper bound
Triglyceride levels	0.036	0.005	<.001	0.026	0.045
Weight gain during pregnancy	0.271	0.085	.001	0.107	0.436

Dependent variable was serum RBP4 level. Independent variables included were gestational age at the time of OGTT, fasting levels of plasma triglyceride and high-density lipoprotein cholesterol, and the amount of weight gain during pregnancy. Criteria were as follows: probability of F to enter ≤0.05 and probability of F to remove ≥0.10.

body mass index (BMI) and higher fasting and postload plasma glucose values compared with those in the non-GDM group. Similarly, indices of IR (HOMA1-IR, HOMA2-IR, and QUICKI) indicated that women with GDM were more insulin resistant than those without GDM.

We next measured RBP4 levels and found that the levels were not significantly different between the GDM and non-GDM group (Table 1). Significant positive correlations were found between RBP4 levels and gestational age at the time of OGTT (r = 0.093, P = .032), fasting triglyceride levels (r = 0.305, P < .001), and the amount of weight gain in pregnancy (r = 0.154, P < .001). Significant negative correlation was also found between RBP4 levels and high-density lipoprotein cholesterol levels (r = –0.121, P = .005). However, correlations between RBP4 levels and fasting insulin levels or HOMA-IR were not statistically significant. Stepwise linear regression analysis showed that fasting triglyceride levels and weight gain in pregnancy were independent predictors of serum RBP4 levels (Table 2).

To further evaluate an association between RBP4 level and IR, we divided 361 non-GDM women into 2 groups: women with completely normal glucose results on an OGTT (false-positive GCT, n = 280) and those with only one abnormal value (n = 81). As shown in Fig. 1 (A-D), fasting plasma insulin and the degree of IR, assessed by HOMA1-IR, HOMA2-IR, and QUICKI, were lowest in women who had no abnormal value on OGTT. Women with GDM (≥2 abnormal values on OGTT) had the highest degree of IR, whereas those with one abnormal value had the intermediate degree (P values for linear trend among the 3 groups for fasting plasma insulin, HOMA1-IR, HOMA2-IR, and QUICKI were <.001 in all). In contrast, we found that serum levels of RBP4 were not significantly different among the 3 groups with varying degrees of IR (Fig. 1E). Furthermore, we obtained additional samples from a group of women who had negative GCT result (n = 22) and found that fasting serum RBP4 levels in this group (34.6 ± 6.7 μg/mL) were not significantly different from those in any group of women with positive GCT result (P = not significant). When serum RBP4 levels from 361 non-GDM women were divided into 3 tertiles, we found that plasma glucose levels, both fasting and after an OGTT; fasting plasma insulin levels; and indices of IR, both HOMA-IR and QUICKI, were not statistically different among the 3 groups. Collectively, our results indicated that RBP4 levels in pregnancy were not associated with IR.

4. Discussion

Retinol-binding protein 4 is an adipokine proposed to be involved in IR and glucose homeostasis [1]. The relationship between RBP4 and IR in humans, however, remains controversial. Some studies reported a close correlation between RBP4 levels and the degree of IR [2]. Others failed to do so [3-6]. These conflicting results may in part be due to differences in the study populations, the study design, and/or the assays used [13].

Pregnancy is a physiological state of enhanced IR. Varying degrees of IR in pregnant women result in metabolically distinct clinical phenotypes [15,16]. The highest degree of IR is found in women with GDM, whereas those with completely normal glucose values on glucose testing have the lowest degree of IR. Women who have one abnormal value on OGTT demonstrate an intermediate degree of IR or a mild degree of glucose intolerance [15].

In pregnant women, 3 studies using various ELISA kits showed that RBP4 levels were elevated in those with GDM [8-10]; but 1 study using a Western blot analysis demonstrated a decrease in GDM [11]. The small number of subjects in each study and the lack of validation of RBP4 ELISA might have compromised interpretation. Furthermore, it is still unclear whether RBP4 level in pregnancy is associated with enhanced IR [9-12]. Our results using a validated ELISA, in the largest cohort of pregnant women to date, showed that the RBP4 level in pregnancy was not associated with IR. Despite the clear differences in indices of IR in women who had 0, 1, or at least 2 abnormal values on OGTT, there were no differences in RBP4 levels. We concluded that RBP4 was not causally linked to IR in pregnancy.

The limitation of this study was that all women were Thai with a relatively low BMI. Whether the results are similar in other ethnic groups with a higher BMI requires further investigations. Although RBP4 levels have been associated with certain pregnancy outcomes [17], our data suggest that RBP4 is not a good surrogate marker of IR in pregnancy.

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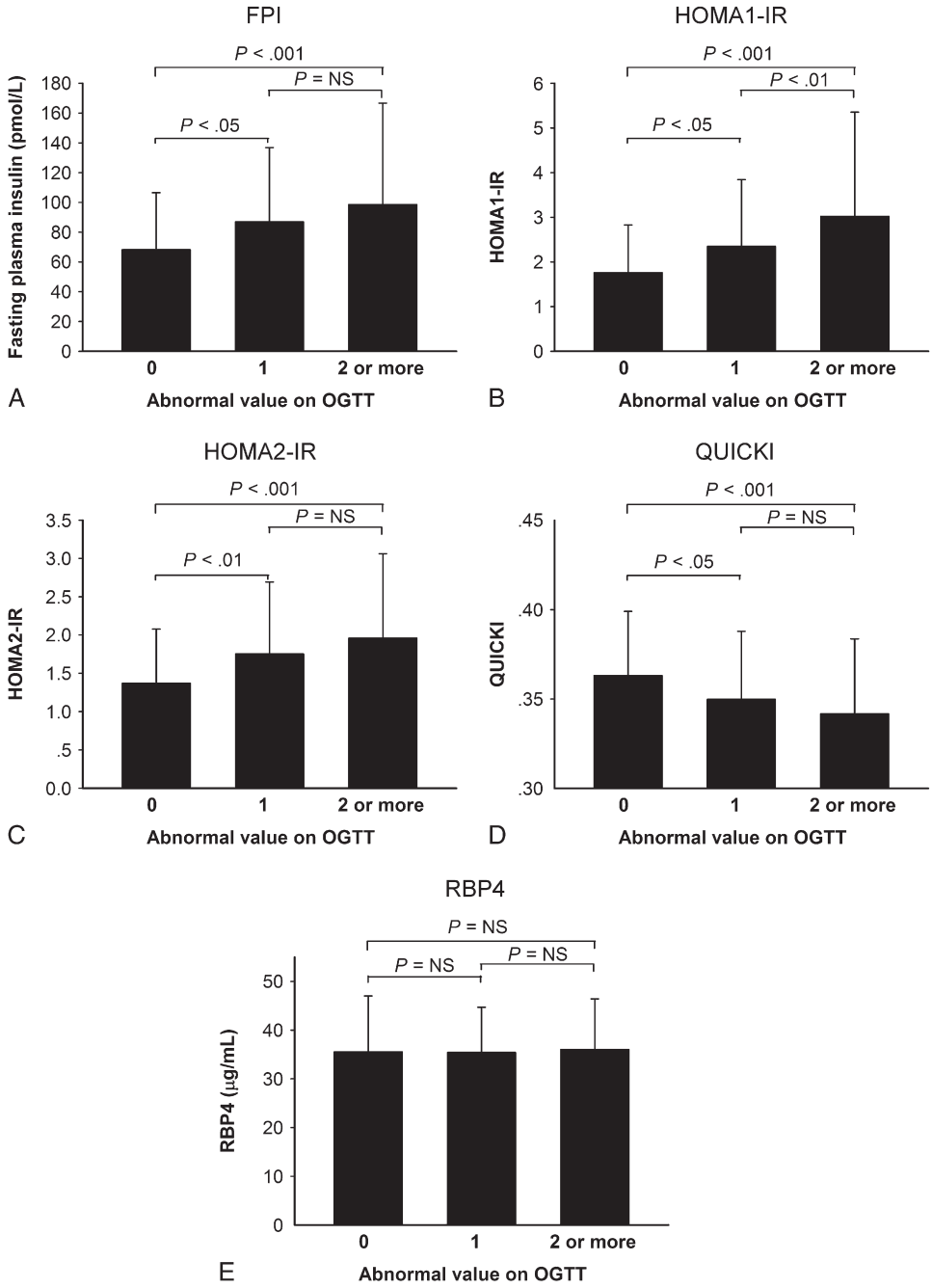

Fig. 1 – Fasting plasma insulin levels (A), HOMA1-IR (B), HOMA2-IR (C), QUICKI (D), and RBP4 levels (E) in pregnant women with 0, 1, or at least 2 abnormal glucose values on a 100-g OGTT. Values are expressed as mean and SD. Differences between the groups were compared using analysis of variance. The number of subjects was 280, 81, and 171, except for HOMA2-IR, where n = 264, 77, and 162, respectively. NS indicates nonsignificant; FPI, fasting plasma insulin. Conversion factor to SI unit for insulin: multiply by 6.945.

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Conflicts of Interest

The authors report no conflict of interests related to this topic area or this manuscript.

REFERENCES

[1] Yang Q, Graham TE, Mody N, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356-62.

[2] Graham TE, Yang Q, Bluher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354:2552-63.

[3] von Eynatten M, Lepper PM, Liu D, et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. Diabetologia 2007;50: 1930-7.
[4] Lewis JG, Shand BI, Elder PA, et al. Plasma retinol-binding protein is unlikely to be a useful marker of insulin resistance. Diabetes Res Clin Pract 2008;80:e13-5.
[5] Chavez AO, Coletta DK, Kamath S, et al. Retinol-binding protein 4 is associated with impaired glucose tolerance but not with whole body or hepatic insulin resistance in Mexican Americans. Am J Physiol Endocrinol Metab 2009;296:E758-64.
[6] Hutchison SK, Harrison C, Stepto N, et al. Retinol-binding protein 4 and insulin resistance in polycystic ovary syndrome. Diabetes Care 2008;31:1427-32.
[7] Choi SH, Kwak SH, Youn BS, et al. High plasma retinol binding protein–4 and low plasma adiponectin concentrations are associated with severity of glucose intolerance in women with previous gestational diabetes mellitus. J Clin Endocrinol Metab 2008;93:3142-8.
[8] Chan TF, Chen HS, Chen YC, et al. Increased serum retinol-binding protein 4 concentrations in women with gestational diabetes mellitus. Reprod Sci 2007;14:169-74.
[9] Kim SH, Choi HJ, Im JA. Retinol-binding protein 4 responses during an oral glucose tolerance testing in women with gestational diabetes mellitus. Clin Chim Acta 2008;391:123-5.
[10] Lewandowski KC, Stojanovic N, Bienkiewicz M, et al. Elevated concentrations of retinol-binding protein–4 (RBP-4) in gestational diabetes mellitus: negative correlation with soluble vascular cell adhesion molecule–1 (sVCAM-1). Gynecol Endocrinol 2008;24:300-5.
[11] Krzyzanowska K, Zemany L, Krugluger W, et al. Serum concentrations of retinol-binding protein 4 in women with and without gestational diabetes. Diabetologia 2008;51: 1115-22.
[12] Ueland T, Dalsoren T, Voldner N, et al. Retinol-binding protein–4 is not strongly associated with insulin sensitivity in normal pregnancies. Eur J Endocrinol 2008;159:49-54.
[13] Graham TE, Wason CJ, Bluher M, et al. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetologia 2007;50:814-23.
[14] Metzger BE, Coustan DR. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee. Diabetes Care 1998;21(Suppl 2):B161-7.
[15] Retnakaran R, Zinman B, Connelly PW, et al. Impaired glucose tolerance of pregnancy is a heterogeneous metabolic disorder as defined by the glycemic response to the oral glucose tolerance test. Diabetes Care 2006;29:57-62.
[16] Retnakaran R, Qi Y, Sermer M, et al. Isolated hyperglycemia at 1 hour on oral glucose tolerance test in pregnancy resembles gestational diabetes mellitus in predicting postpartum metabolic dysfunction. Diabetes Care 2008;31:1275-81.
[17] Vaisbuch E, Romero R, Mazaki-Tovi S, et al. Retinol binding protein 4—a novel association with early-onset preeclampsia. J Perinat Med 2010;38:129-39.