**Online only**

Supplemental Materials: the possible mechanism that RBP4 involved in GDM

Sup table I: Multivariable-adjusted Spearman correlation coefficients of RBP4 and other factors

Sup table II: Risk factors at admission prediction of GDM with AUROC

Sup Table III: Univariate and multivariate logistic regression of GDM according to RBP4 and obese status

Sup figure I: Distribution of RBP4 in different groups.

**The possible mechanism that RBP4 involved in GDM**

Mechanism that RBP4 involved in glucose homeostasis is not yet fully understood. In general, RBP4 plays a role in progression of insulin resistance through immunity [1] and inflammatory mechanisms [2] in adipose and vascular tissues. Some possible mechanisms underlying the phenomena illustrated by this study.

(1) It now seems that elevated serum RBP4 might be a mechanistic link by which downregulation of GLUT4 in adipocytes contributes to the development or worsening of systemic insulin resistance [3].

(2) Retinol-dependent mechanisms by which RBP4 may influence insulin action include, but are not limited to, increased production or altered tissue metabolism of retinoic acid isomers, the active forms of retinol that interact with retinoic acid receptors (RARs) and retinoic acid-X receptors (RXRs) to regulate gene transcription [4].

(3) Several gluconeogenic enzymes, including phosphoenolpyruvate kinase (Pepck), are regulated by dietary retinol deficiency and/or retinoic acid treatment [5]. A previous study indicated that hepatic Pepck is regulated, either directly or indirectly, by circulating RBP4[3]. RBP4 can act directly to induce Pepck expression, increase basal glucose production, and reduce insulin action to suppress glucose production in hepatocytes. In skeletal muscle, RBP4 reduces insulin sensitivity by inhibiting both insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activation, while increasing hepatic glucose production by increasing PEPCK expression [3].

(4) RBP4 might also cause insulin resistance through a retinol-independent mechanism. Evidence suggests that RBP4 binds with high affinity and high specificity to cell surface receptors [3].

(5) Inflammation may be the crucial way through which RBP4 exerts its function in the pathogenesis of insulin resistance [6]. Biological studies have shown that RBP4 induces the secretion of certain inflammatory risk markers, including TNF-a, IL-6, MCP-1, IFN-g, IL-1b, IL-2, IL-12, IL-10, and IL-8 in macrophages [7], as well as ICAM-1, VCAM-1, E-selectin, IL-6, and MCP-1 in endothelial cells [2]. RBP4’s role in inducing endothelial cell inflammation through NAD(P)H and NF-kB pathways occurs independently of STRA6 and retinol [7]. RBP4 binds to STRA6 and directly induces insulin resistance in adipocytes through c-Jun N-terminal kinases [8].

(6) RBP-4 induce endothelial inflammation by stimulating expression of proinflammatory molecules whose effects are mediated via the activation of NADPH oxidase and NF-κB[9]. Macrophage infiltration into adipose tissue is likely increased by the secretion of chemotactic molecules, such as MCP1, which is expressed by macrophages, as well as by adipocytes and other cells, especially in obese, insulin-resistant subjects [10]. As described previously, there was a significant positive relationship between adipose tissue CD68 and RBP4 expression, and also a significant relationship between adipose MCP1 and RBP4 ex pression. CD68 is a macrophage surface marker, and the expression of CD68 correlates with SI, and with the production of TNF\_α and IL-6 [11].

(7) Most of the studies reported a positive relation between RBP4 and oxidative stress markers and a negative relation between RBP4 and antioxidant glutathione [12]. Thus, RBP4 may have a role in oxidative stress. Cellular oxidative stress leads to the activation of vascular inflammation [13].

(8) A study suggested that RBP4 may be associated with vascular endothelial dysfunction as a diabetic complication [14]. RBP4 may affect endothelial function directly through inhibition of insulin- mediated pathways for nitric oxide (NO) production in endothelial cells because in the insulin-resistant state, NO dependent vasodilation is impaired by decreased NO production [15].

**References**

1. Moraes-Vieira PM, Yore MM, Dwyer PM, Syed I, Aryal P, Kahn BB. RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. Cell Metab 2014; 19: 512–526.

2. Farjo KM, Farjo RA, Halsey S, Moiseyev G, Ma J-x. Retinol-binding protein 4 induces inflammation in human endothelial cells by an NADPH oxidase-and nuclear factor kappa B-dependent and retinolindependent mechanism. Mol Cell Biol 2012; 32: 5103–5115.

3. Yang Q, Graham T E, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes[J]. Nature, 2005, 436(7049): 356-362.

4. Chambon P. A decade of molecular biology of retinoic acid receptors. The FASEB Journal, 1996, 10(9): 940-954.

5. Shin D J, Odom D P, Scribner K B, Ghoshal S, McGrane MM. Retinoid regulation of the phosphoenolpyruvate carboxykinase gene in liver. Molecular and cellular endocrinology, 2002, 195(1-2): 39-54.

6. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelialdependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002; 39: 257–26549.

7. Norseen J, Hosooka T, Hammarstedt A, Yore MM, Kant S, Aryal P, et al. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase-and Tolllike receptor 4-dependent and retinol-independent mechanism. Mol Cell Biol 2012; 32: 2010-2019.

8. Berry DC, Jin H, Majumdar A, Noy N. Signaling by vitamin A and retinol-binding protein regulates gene expression to inhibit insulin responses. Proc Natl Acad Sci USA 2011; 108: 4340–4345.

9. Agasthi P, Aloor S, Chenna A, Harris R. Association Between Serum Retinol Binding Protein-4 Level and Coronary Artery Disease: A Meta-Analysis [J]. Arteriosclerosis, Thrombosis, and Vascular Biology. 2015; 35: A344

10. Yao-Borengasser A, Varma V, Bodles A M, Rasouli N, Phanavanh B, Lee MJ, et al. Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone[J]. The Journal of Clinical Endocrinology & Metabolism, 2007, 92(7): 2590-2597.

11. Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, et al. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. Diabetes 2005; 54:2305–2313.

12. Codoñer-Franch P, Mora-Herranz A, Simó-Jordá R, Pérez-Rambla C, Boix-García L, Faus-Pérez A. Retinol-binding protein 4 levels are associated with measures of liver and renal function and oxidant/antioxidant status in obese children. J Pediatr 2013;163(2):593–595.

13. Frey RS, Ushio–Fukai M, Malik AB. NADPH oxidase-dependent signaling in endothelial cells: role in physiology and pathophysiology. Antioxid Redox Signal 2009; 11:791–810

14. Takebayashi K, Suetsugu M, Wakabayashi S, Aso Y, Inukai T. Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. The Journal of Clinical Endocrinology & Metabolism, 2007, 92(7): 2712-2719.

15. Steinberg H O, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance[J]. The Journal of clinical investigation, 1996, 97(11): 2601-2610.

Sup table I Multivariable-adjusted Spearman correlation coefficients of RBP4 and other factors

|  |  |  |
| --- | --- | --- |
|  | R[spearman] | P |
| Age | 0.098 | 0.012 |
| Gestational age | 0.056 | 0.109 |
| BMI | 0.154 | <0.001 |
| SBP | 0.043 | 0.189 |
| DBP | 0.038 | 0.235 |
| Total cholesterol | 0.033 | 0.276 |
| HDL cholesterol | -0.082 | 0.015 |
| Triglyceride | 0.179 | <0.001 |
| FPG | 0.173 | <0.001 |
| Insulin | 0.124 | <0.001 |
| HOMA-IR | 0.213 | <0.001 |
| HbA1c | 0.155 | <0.001 |
| CRP | 0.181 | <0.001 |
| IL-6 | 0.163 | <0.001 |
| Adiponectin | -0.127 | <0.001 |

SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; BMI, body mass index; CRP, C-reactive protein; FPG, Fasting plasma glucose; HDL, high-density lipoprotein; IL-6, Interleukin 6; RBP4: [Retinol binding protein 4](https://en.wikipedia.org/wiki/Retinol_binding_protein_4)

Sup table II Risk factors at admission prediction of GDM with AUROC

|  |  |  |  |
| --- | --- | --- | --- |
| Risk factors | AUC | 95%CI | P |
| Maternal age | 0.609 | 0.549-0.608 | <0.001 |
| Pre-gestational BMI | 0.634 | 0.573-0.695 | <0.001 |
| FPG | 0.590 | 0.529-0.650 | <0.001 |
| Insulin | 0.592 | 0.536-0.648 | <0.001 |
| HOMA-IR | 0.621 | 0.575-0.673 | <0.001 |
| HbA1c | 0.612 | 0.561-0.664 | <0.001 |
| CRP | 0.581 | 0.519-0.643 | <0.001 |
| IL-6 | 0.601 | 0.543-0.659 | <0.001 |
| Adiponectin | 0.591 | 0.537-0.648 | <0.001 |
| RBP4 | 0.737 | 0.690-0.784 | Reference |

HbA1c, glycosylated hemoglobin; BMI, body mass index; CRP, C-reactive protein; FPG, Fasting plasma glucose; HDL, high-density lipoprotein; IL-6, Interleukin 6; RBP4: [Retinol binding protein 4](https://en.wikipedia.org/wiki/Retinol_binding_protein_4); ROC, Receiver operating characteristic; AUC, area under the curve

**Sup Table III** Univariate and multivariate logistic regression of GDM according to RBP4 and obese status

|  |  |  |  |
| --- | --- | --- | --- |
| Groups (N) \* | GDM event | | |
| GDM/ALL, (%) | Crude OR(95%CI)ξ | Adjusted OR(95%CI)ǂξ |
| Not obese with low RBP4 | 12/375, (3.2) | Reference | Reference |
| Obese with low RBP4 | 6/40, (15.0) | 5.34(1.88-15.12) | 3.04(2.11-5.21) |
| Not obese with high RBP4 | 54/348, (15.5) | 5.56(2.92-10.15) | 3.12(2.20-5.03) |
| Obese with high RBP4 | 29/64, (45.3) | 25.06(11.76-54.73) | 9.83(4.76-16.13) |

\*Obesity was defined as BMI>30kg/m2, low RBP4 was defined as plasma levels <23.7 [ug/ml](https://onlineconversion.vbulletin.net/forum/main-forums/convert-and-calculate/5415-ug-ml), and high RBP4 was defined as plasma levels ≥23.7[ug/ml](https://onlineconversion.vbulletin.net/forum/main-forums/convert-and-calculate/5415-ug-ml). With women not obese with low RBP4 (<median) as reference

ǂ adjusted for maternal age, Pre-gestational BMI, gestational age at sampling, smoking, ethnicity, pre-existing hypertension and CVD gestational weeks at admission, conception status, family history of diabetes, physical activity, SBP, DBP, total cholesterol, triglyceride, HDL, FPG, CRP, Insulin, IL-6 and adiponectin.

ξP value for the trend <0.001

OR, odds ratio; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; CVD, cardiovascular disease; CRP, C-reactive protein; FPG, Fasting plasma glucose; HDL, high-density lipoprotein; IL-6, Interleukin 6; RBP4: [Retinol binding protein 4](https://en.wikipedia.org/wiki/Retinol_binding_protein_4); GDM, gestational diabetes mellitus; ROC, Receiver operating characteristic; AUC, area under the curve

**Sup figure I** Distribution of RBP4 in different groups. (A) plasma levels of RBP4 in obesity GDM patients and in GDM patients without obese. (B) plasma levels of RBP4 in older GDM patients and in other GDM patients (age <30 years). Horizontal lines represent medians and inter-quartile ranges (IQR). *P* values refer to Mann-Whitney *U* tests for differences between groups. RBP4=[Retinol binding protein 4](https://en.wikipedia.org/wiki/Retinol_binding_protein_4); GDM=gestational diabetes mellitus.

