ORIGINAL ARTICLE

Relation between Elevated Plasma Retinol Binding Protein-4 & Glucose Dysregulation in Type 2 Diabetes

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Abstract

A prospective study was done to find out the relation between plasma retinol binding protein-4 and glucose dysregulation in 41 type 2 diabetic patients aged 43±6 years (mean±SD). 51 healthy normal subjects aged 41±5 years were served as control. Anthropometric measurements and total body fat mass were determined in both the diabetic and control group. Blood glucose, lipid profile, fasting insulin level and plasma retinol binding protein-4 were estimated. Insulin secreting capacity and insulin sensitivity were also determined. Waist-hip ratio and total body fat mass between the groups did not show any statistical difference. Fasting total cholesterol and triglyceride level was significantly higher in diabetic group. HDL-cholesterol level did not show any significant difference. Fasting insulin in diabetic group was significantly higher. Insulin secretory capacity and insulin sensitivity were found significantly higher in diabetic group when compared to control. Mean retinol binding protein-4 was also significantly higher in diabetic group. Bivariate Pearson's correlation analyses were done for statistical purpose. In control group plasma retinol binding protein-4 showed significant positive correlation with serum triglyceride and total body mass fat, but did not show any association with other testing variables. In diabetic group retinol binding protein-4 did not show any association with other testing variables. Multiple linear regression analyses were performed with insulin secretory capacity and fasting glucose, retinol binding protein-4, BMI & serum triglyceride among control and diabetic group. Insulin secretory capacity showed significant negative association with fasting glucose in all four models. In regression analyses with insulin sensitivity and fasting glucose, BMI and serum triglyceride, insulin sensitivity showed positive association with fasting glucose and serum triglyceride. When linear regression analysis were performed with retinol binding protein-4 and fasting glucose, BMI, waist circumference and triglyceride, retinol binding protein showed no association with the variables. So, it can be concluded that retinol binding protein-4 may not associated with glucose intolerance in diabetic patients.

Key words : Plasma retinol binding protein-4, Glucose dysregulation.

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Introduction

Diabetes mellitus, a long considered disease of minor significance to world health is now taking its place as a main threats to human health in the 21st century.[1] Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and/or abnormal insulin secretion.[2] The precise pathogenesis and pathophysiological sequence resulting in insulin resistance are still not to be clearly understood. Recent studies on insulin resistant patients focused on the molecular dysregulations which are suggested to play an important role in the development of insulin resistance and T2DM.[3] Adipose tissue has traditionally been considered an energy storage organ, but over the last decade, a novel role of the adipose tissue as an endocrine organ has emerged.[4] Adipose tissue is currently known to secrete a large number of factors with diverse functions. These factors include free fatty acids (FFA) with well described physiological and pathophysiological effects on glucose homeostasis[5] and proteins, termed adipocytokines, that act as autocrine, paracrine or endocrine fashion to control various metabolic functions. Some of these adipocytokines have been implicated in the development of insulin resistance. However, precise roles of these adipocytokines have not been clearly understood. Recently characterized adipocytokine is retinol binding protein-4 (RBP4)[6] have also been

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implicated in the pathogenesis of type 2 diabetes. However, its role in the glucose dysregulation is still not to be clearly understood. Retinol binding protein (RBP4) which is a single-chain polypeptide glycoprotein.[7] RBP4 has found to be increased in insulin-resistant subjects. Graham et al reported increased serum RBP4 concentration in subjects with obesity or T2DM compared with lean subjects.[6] Insulin resistance was positively associated with serum RBP4 concentration and invoked to be causally related with T2DM. In fact, RBP4 is upregulated in the adipose tissue of several insulin-resistant mouse models.[6] Yang et al demonstrated that RBP4 can impair insulin sensitivity throughout the whole body by modulating glucose homeostasis.[8] However, questions still remain unresolved. Various authors have proposed the possible mechanism of insulin resistance and development of T2DM induced by RBP4. Hence, it is important to explore RBP4 in the pathogenesis of T2DM.[6]

Materials and Methods

41 T2DM patients were recruited purposively and 51 healthy subjects were served as control. Anthropometric measurements and total body fat mass were determined. Glucose and lipids were measured by standard biochemical methods. Insulin and RBP4 were estimated by using enzyme linked immunosorbent assay (ELISA). Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were determined by homeostasis model assessment (HOMA) using HOMA-Sigma software. Data were analyzed by appropriate univariate as well as multivariate tools.

Results

The study subjects in the 2 groups were age and BMI matched. Waist to hip ratio and total body fat mass among the groups did not show statistical difference between groups. Fasting triglyceride (TG) and total cholesterol (TC) were significantly higher in T2DM groups when compared to controls (p=<0.001 and 0.005 respectively). Mean HDL cholesterol (HDL-chol) did not show significant difference between groups. Fasting insulin in the T2DM groups was significantly higher when compared to controls (p=<0.001). HOMA%B was 110.0±44.6 and 78.9±17 in control and T2DM respectively; the value was significantlly higher in T2DM (p=0.019) compared to the control. HOMA%S was 68.1±17.6 and 56.9±17 which was significantly higher in the T2DM groups compared to controls (p<0.001). Fasting plasma RBP4 in control and T2DM groups was 30.4±6.0 and 37.2±6.8 respectively. Mean RBP4 value in T2DM was significantly higher when compared to control group (p<0.001). Bivariate Pearson's correlation analyses were then performed for plasma RBP4 with anthropometric and biochemical variables. In control group plasma RBP4 showed significant positive correlation with serum triglyceride (r=0.454, p=0.001) and subscapular-tricep skin fold ratio (r=0.305, p=0.031), but did not showed any association with other testing variables. Plasma RBP4 did not show any association with other testing variables in T2DM group. Multiple linear regression analyses were then performed with HOMA%B as dependant variable and fasting glucose, BMI and TG as independent variables, HOMA%B as dependable variable showed significant negative association with fasting glucose in all four models (Table IV). In regression analyses with HOMA%S as dependent variable and fasting glucose, BMI and TG as independent variables showed association of HOMA%S with fasting glucose and TG (Table III). Multiple linear regression analyses were then performed with RBP4 as dependant variable and fasting glucose, BMI, waist circumference and TG as independent variables. RBP4 showed no association with the variables (Table V).

Table I : Clinical characteristics of the study subjects

Parameters	Controls (n=51)	T2DM (n=41)	t/p value Control vs T2DM
Age(years)	41±5	43±6	1.22/0.225
BMI (kg/m^2)	24.4±4.1	24.9±3.9	0.83/0.527
WHR	0.91±.06	0.93±0.05	1.79/0.076
BFM (%)	28.2±6.2	28.0±6.5	0.12/0.901

Data were expressed as mean±SD. Unpaired students't' test was performed. p<0.05 was considered as level of significance.

n = number of subjects BMI=Body mass index
WHR=Waist hip ratio BFM= Body fat mass.

Table II : Biochemical characteristics of the study subjects

Parameters	Controls (n=51)	T2DM (n=43)	t/p value Control vs T2DM
Fasting Glucose (mmol/l)	5.1±0.41	6.3±0.20	4.35/<0.001
2h Glucose (mmol/l)	5.9±1.2	6.3±1.2	6.11/<0.001
Fasting insulin (μU/ml)	9.7±2.4	11.3±4.4	5.02/<0.001
RBP4 (μg/ml)	30.4± 6.07	32.2± 10.9	4.99/<0.001
HOMA% B	107± 25	76.5± 18.0*	2.93/0.004
HOMA %S	81.9± 18	72±23	6.85/<0.001

Data were expressed as mean±SD. Unpaired student's 't' test was performed. $p<0.05$ was considered as level of significance.
*Significantly different
2h glucose = 2 hours after 75 gm glucose load

RBP4 = Retinol binding protein 4

HOMA%B = β cell function assessed by homeostasis model assessment

HOMA%S= Insulin sensitivity assessed by homeostasis model assessment

Table III: Multiple stepwise regression analysis with HOMA%S as dependent variable and F glucose, RBP4, BMI and TG as independent variables among control and T2DM subjects

Independent Variables	Model 1		Model 2		Model 3		Model 4	
		p		*p*		*p*		*p*
F Glucose	-0.506	<0.001	-0.457	<0.001	-0.460	<0.001	-0.376	<0.001
RBP4			-0.228	0.015	-0.215	0.022	-0.144	0.119
BMI					-0.091	0.318	-0.093	0.281
TG							-0.287	0.003
Adjusted R^2	*0.248*		*0.290*		*0.290*		*0.352*	

F glucose= Fasting glucose
RBP4= Retinol binding protein 4
BMI= Body mass index TG=Triglyceride

Table IV : Multiple stepwise regression analysis with HOMA%B as dependent variable and F glucose, RBP4, BMI and TG as independent variables among control and T2DM subjects

Independent Variables	Model 1		Model 2		Model 3		Model 4	
		p		*p*		*p*		*p*
F Glucose	-0.697	<0.001	-0.710	<0.001	-0.711	<0.001	-0.714	<0.001
RBP4			0.061	0.441	0.064	0.424	0.062	0.459
BMI					-0.023	0.770	-0.023	0.772
TG							0.009	0.915
Adjusted R^2	*0.480*		*0.478*		*0.472*		*0.466*	

Table V : Multiple stepwise regression analysis of RBP4 as dependent variable with F glucose, BMI, WC and TG as independent variables among control and T2DM subjects

Independent Variables	Model 1		Model 2		Model 3		Model 4		Model 5	
		p		*p*		*p*		*p*		*p*
F Glucose	0.191	0.099	0.184	0.097	0.191	0.082	0.192	0.083	0.167	0.153
BMI					0.171	0.120	0.181	0.156	0.191	0.138
WC							-0.021	0.871	-0.035	0.789
TG									0.088	0.504
Adjusted R^2	*0.023*		*0.105*		*0.123*		*0.111*		*0.104*	

WC=Waist circumference

Discussion:

The major objective of the present study was to explore the association of plasma RBP4 with the basic pathophysiology of diabetes, namely insulin secretory defect and insulin resistance in diabetic subjects. Both healthy and T2DM controls were used to compare the results. The age and BMI of the two groups were matched (Table I) which excluded the possibility of the interference by two of the most important confounders of adipocytokines. Again, the association between plasma RBP4 and T2DM has previously been shown in only obese (BMI 31.6±4.5) subjects.[6] Moreover, Kloting et al[10] observed association of RBP4 with obese IGT (BMI 35.0±6.3). But, in present study an association has been demonstrated even in predominantly mild to moderate overweight T2DM (BMI 24.9±3.9) subjects. Although the adipocytokine was raised in the hyperglycemic states, there was no significant correlation of fasting blood glucose with RBP4 in univariate as well as multivariate analysis when adjusted with BMI, waist circumference and TG. The present findings indicate that blood glucose itself may not be a major determinant of circulating RBP4.

The association of the RBP4 with insulin seceretory capacity and insulin sensitivity were analysed with univariate as well as multivariate analyses RBP4 was not associated with insulin seceretory function as well as insulin sensitivity when adjusted with BMI and TG (Table IV and V) in T2DM subjects. Broch et al[11] found a negative correlation of B-cell seretory capacity with RBP4 in T2DM subjects.

Conclusions

- T2DM have both insulin resistance and secretory defects.
- RBP4 are not associated with glucose mild to moderately overweight T2DM subjects.

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