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Retinol Binding Protein 4 And Insulin Resistance In Egyptian Type 2 Diabetics

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Abstract: Retinol-binding protein-4 (RBP4), a novel adipokine secreted mainly by visceral adipose tissue, increased serum levels has been associated with obesity, type 2 diabetes and metabolic syndrome. The aim of our study is to detect the relation between various parameters of insulin resistance and serum RBP4 in type 2 Egyptian diabetic obese patients (T2DM). In a case control study 60 obese type 2 diabetic patients (40 females/20 males) with mean BMI (34.63±4.22 Kg/m^2) compared to 15 healthy controls subjects, age and sex matched. Laboratory and anthropometric measurements including: fasting blood glucose, HbA_1C, lipid profile, urea, creatinine, RBP4 serum levels, BMI, and waist/hip ratio. Our results demonstrated statistically significant elevation in serum RBP4 concentrations in T2DM subjects compared to control subjects (P<0.001). Significant correlations were observed between the serum RBP4 concentration and fasting blood glucose(r=0.237, P=0.005). In obese diabetic there was negative correlation between serum RBP4 concentration and insulin (r= -0.348, P = 0.845). No correlation was observed between HOMA-IR and serum RBP4 concentrations (r= 0.344, P = 0.007). No correlation between serum RBP4 concentration, BMI (r = 0.217, P=0.96), and waist/hip ratio (r=0.078, P=0.554). We concluded that elevated serum RBP4 may not play a role in the development of insulin resistance in obese type 2 diabetic Egyptian patients.

Key words: Retinol binding protein4, T2DM, insulin and BMI.

INTRODUCTION

Adipose tissue secretes several cytokines that affect, both positively and negatively insulin sensitivity (DeFronzo, 2004; George, 2007; Alberto, 2009).

Retinol-binding protein-4 (RBP4), a fat-derived adipokine, thought to be secreted mainly by adipose tissue and the liver (Yang, 2005). RBP4 has been shown to be associated with insulin resistance and decreased expression of GLUT4 (Broch, 2007; Haider, 2007; Eduardo, 2009). In skeletal muscle, RBP4 causes insulin resistance by impairing insulin signaling, and in the liver RBP4 increases gluconeogenesis (Eduardo, 2009). RBP4 is an exciting new biomarker for the determination of insulin resistance and type 2 diabetes (Gao, 2009).

RBP4 is the principal transport protein for retinol (vitamin A) (Yang, 2005), and is encoded by the *RBP4* gene, localized in the chromosome 10q23-q24 (Kovacs, 2007). A large number of subsequent studies confirmed an association between increase in the circulating RBP4 levels and various aspects of adiposity (Munkhtulga 2010), insulin resistance (An C, 2009; Suh J-B, 2010) diabetes mellitus (Klein, 2010), and metabolic syndrome (Gao S, 2009). However, there were also other studies that have been unable to establish these associations (Santoro, 2009; Sasaki, 2010). The reason for this discrepancy may be explained in part by the different methods that were used to measure the RBP4 and the different populations employed in these various studies.

Therapeutic interventions, like weight loss, exercise, and gastric bypass surgery, have been shown to reduce RBP4 levels in subjects with morbid obesity and T2DM (Haider, 2007; Graham, 2006; Balagopal, 2007; Broch, 2010). However, data concerning the effects of oral antidiabetic agents on plasma RBP4 levels are less clear. Treatment of impaired glucose tolerance (IGT) subjects with pioglitazone improved whole body insulin sensitivity, yet increased RBP4 gene expression in adipose tissue (Yao-Borengasser, 2007). Similarly, treatment with metformin, while leading to improvement in insulin sensitivity, was not associated with a significant decrease in plasma RBP4 in patients with polycystic ovarian syndrome (Hutchison, 2008).

The aim of our study is to detect the relation between insulin resistance and RBP4 in type 2 diabetic obese patients

Patients And Methods:

Sixty type 2 diabetic obese patients attending outpatient endocrinology clinic in Al Kasr Al Ani, Faculty of Medicine Cairo University. They were 40 females and 20 males; their mean age was 52.27± 7.22 years, and with mean BMI of 34.63±4.22 Kg/m^2.

Twenty healthy volunteers matched for age and sex as a control group with no family history of type 2 diabetes. All participants gave their approval to participate in the study and oral consent were obtained from each subject in the study. The study meets the ethical committee.

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All patients were subjected to: Complete history taking, detailed physical examination, fundus examination, BMI calculated by dividing the subject weight by the square height (BMI=Kg/m^2).

Waist/hip ratio (WHR) waist and hip circumferences were measured to nearest 0.1 cm, Waist measured at narrowest point between lowest ribs; hip measured at upper most lateral border of right iliac crest.

About 5 mls of fasting venous blood samples (12-14 hours of fasting) were taken from each subject participating in the study and divided into aliquots: The 1st aliquot about 1.5 mls of venous blood was added to tube containing EDTA for determination of glycosylated hemoglobin (HbA_1C) by cation exchange resin (Maquart, 1980). The 2nd aliquot (about 3.5 mls) of venous blood was left to clot and the serum was separated by centrifugation and fasting blood glucose was determined immediately by glucose oxidase method. The rest of the serum was stored at -20°C for determination of the followings: Serum urea, serum creatinine, serum total cholesterol, serum triglycerides, serum high density lipoprotein, serum low density lipoprotein, serum insulin and measurement of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and serum RBP4.

The determination of fasting blood glucose, lipid profile, and serum urea and serum creatinine was carried out by colorimetric methods on Hitachi auto analyzer (Hitachi 736, Hitachi, Japan). For determination of HDL-cholesterol, phosphotungestic acid and magnesium ions are used for precipitating all lipoproteins except HDL fraction that was present in the supernatant and measured by auto analyzer. LDL cholesterol was measured by Friedwald formula (Friedwald, 1972).

Fasting serum insulin was determined using radio immuno assay (Perez-Fontan, 2004). Insulin resistance was calculated as HOMA-IR using the following equation: HOMA-IR=fasting blood glucose (mmol/l) x fasting serum insulin (mU/l)/22.5 (Wallace, 2004).

Fasting serum RBP4 was determined using sandwich enzyme immunoassay technique (ELISA) (Murata, 2009) and the kit was supplied by Immunodiagnostic AG, Stubenwald-Allee 8a, D 64625 Bensheim, and Germany.

Statistical analysis: The data were analyzed using SPSS package version 10, and expressed as mean ± SD. Student t-test was used for comparing the mean of 2 groups. Spearman correlation coefficient was used for measuring the mutual correspondence between 2 groups. Chi square was used.

Results:

T2DM subjects were matched for age and gender to control (Table 1) higher serum triglyceride and lower serum high-density lipoprotein cholesterol concentrations compared with control subjects. In T2DM, hemoglobin A_1C (HbA_1C) was (6.47±2.14), indicating reasonably good glycemic control. There was 32 patients on sulfonylurea, 10 patients on metformin, 7 patients on combination of both, 16 patients on insulin, 12 patients on insulin with metformin. No patients were on thiazolidinedione.

Table 1: Clinical characteristics of patients in the study (Mean± SD).

	T2DM(n=60)	control(n=15)	P value
Gender(M/F)	20/40	5/10	0.002
Age(years)	52.27±7.22	45.53±7.77	<0.001*
BMI (Kg/m^2)	34.63±4.22	22.13±1.85	<0.001*
FBG(mmol/l)	34.2±0.4	23.8±0.5	<0.001*
Insulin(mU/I)	18.04±2.69	10.09±1.07	<0.001*
HOMA-IR	9.10±2.14	2.65±0.35	<0.001*
HbA_1C	6.47±2.14	3.19±1.48	<0.001*
Cholesterol(mg/dl)	188.62±37.09	140.15±25.71	<0.001*
Triglycerides(mg/dl)	92.65±13.12	66.73±11.56	<0.001*
LDL(mg/dl)	116±7.o	111±5.2	<0.001*
HDL(mg/dl)	38±2.o	46±2.o	<0.001*
urea(mg/dl)	51.41±12.31	33.17±8.17	<0.001*
Creatinine(mg/dl)	0.88±0.8	0.26±0.1	<0.001*
RBP4(ug/ml)	59.88±13.92	30.20±5.94	<0.001*

*<0.001 highly significant

Insulin sensitivity. As expected, obese T2DM subjects were more insulin resistant than lean control as shown in (table 1)

Significant correlations were observed between the serum RBP4 concentration and fasting blood glucose(r=0.237, P=0.005) (Figure 1)

The elevation in RBP4 serum concentrations in type 2 diabetic than control was statistically significant (figure 2)

Correlation between insulin sensitivity and serum RBP4: In obese diabetic there was negative correlation between serum RBP4 concentration and insulin (r= -0.348, P = 0.845). No correlation was observed between HOMA-IR and serum RBP4 concentration) (r= - 0.344, P = 0.007) (figure 3)

There was no correlation between BMI and serum RBP4 concentration (r=0.217, P=0.96), waist/hip ratio (r=0.078, P=0.554) (figure 4).

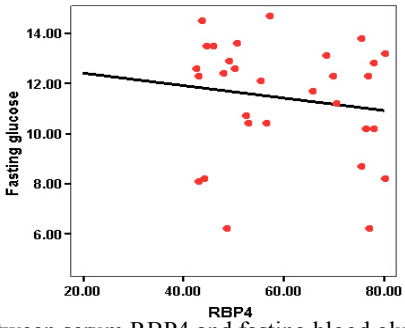

Fig. 1: The positive correlation between serum RBP4 and fasting blood glucose (r=0.237, P=0.005).

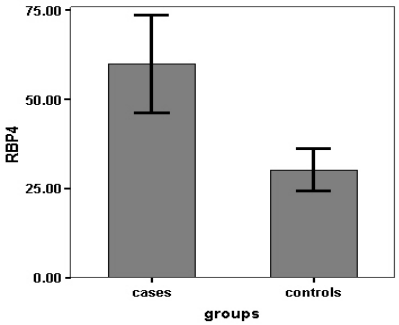

Fig. 2: The Serum concentration of RBP4 in patients and control subjects.

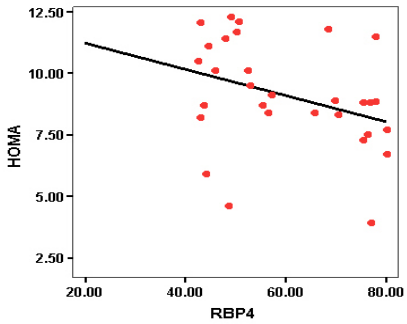

Fig. 3: The negative correlation between serum RBP4 and HOMA-IR (r=-0.344, P=0.007).

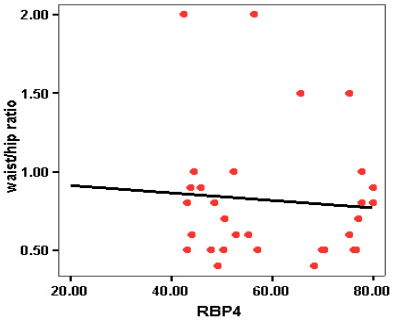

Fig. 4: No correlation between serum RBP4 and waist/hip ratio (r=0.078, P=0.554).

Discussion:

Our results demonstrate that serum RBP4 levels was elevated in T2DM subjects compared to control subjects and correlate with measures of glycemia, but not with insulin sensitivity.

Our results agreed with different studies (Alberto, 2009; Graham, 2006; Cho YM, 2007; Takebayashi, 2007; Weiping, 2006) that demonstrated elevated serum RBP4 levels in type 2 diabetic subjects.

Several mechanisms link RBP4 to insulin resistance and type 2 diabetes. Increase of hepatic gluconeogenesis by enhancing the expression of phosphoenolpyruvate carboxykinase in the liver and the attenuated insulin signaling in skeletal muscle (Yang, 2005). Insulin signaling in primary human adipocytes was affected by RBP4 through blocking the insulin-stimulated phosphorylation of insulin receptor substrate-1 at serine in position 307 (Ost A, 2007). Obesity is strongly associated with insulin resistance and impaired glucose mediated insulin secretion. Adipose tissue produces many cytokines and secretory factors termed adipokines from visceral adipose tissue in particular (Gauvreau, 2011).

The link between RBP4 and type 2 diabetes could also be mediated through impaired insulin secretion. Circulating RBP4 concentration was negatively associated with insulin secretion in humans. In fact, it is well known that retinol (the ligand for RBP4) is pathophysiologically linked to β-cell function. Retinol-binding protein circulates in serum, forming a complex with transthyretin, a transport protein for thyroxin. Borch *et al.,* 2007, disclosed that transthyretin constitutes a functional component in pancreatic β-cell stimulus-secretion coupling. The binding of RBP to the receptor is inhibited by transthyretin. Thus, it is possible that increased serum RBP4 prevents transthyretin from exerting its β-cell stimulus-secretion effects (Broch M, 2007).

We did not find any correlation between insulin sensitivity and serum RBP4 in our obese diabetic patients. Consistent with the latter observation, previous studies in different populations also failed to observe any association between circulating RBP4 levels and insulin sensitivity (Alberto, 2009; Promintzer, 2007; von Eynatten, 2007; Yao-Borengasser, 2007; Liu XH, 2010; Tajtakova, 2010). However, the mechanisms by which RBP4 induces insulin resistance are not well understood. However in contrary to different studies circulating RBP4 concentration correlated positively with insulin resistance in obese subjects (Yang, 2005; Graham, 2006; Liu XH, 2010; Stefan, 2007). Increased serum RBP4 levels have been reported in subjects with obesity, insulin resistance, and type 2 diabetes (Gavi S, 2007; Graham, 2007) and in other insulin-resistant states, such as nonalcoholic fatty liver disease and the metabolic syndrome. (Liu XH, 2010)**.**

In our study no correlation between serum RBP4 and BMI, waist, waist/hip ratio in contrary other studies showed association between circulating RBP4 concentration with waist circumference and percent trunk fat than with percent body fat (Graham, 2007). The association between RBP4 and visceral fat was stronger than its association with BMI, indicating a greater role of visceral than subcutaneous fat in the relationship between RBP4 and insulin resistance. This finding was confirmed by other authors who described that visceral adipose tissue accumulation was the stronger predictor of RBP4 levels (Lee JW, 2007). In this sense, increased RBP4 mRNA expression in visceral, compared with subcutaneous, adipose tissue has already been found (Klo¨ ting, 2007). Circulating RBP4 levels also correlated positively with ectopic fat accumulation at hepatic and skeletal muscles (Stefan, 2007).

In our study although serum RBP4 levels were increased in T2DM, but no association was observed between serum RBP4 and insulin resistance. It is possible that adipose tissue in humans is not the major source of RBP4 and, as in rodents; liver could be the major source of circulating RBP4 (Alberto, 2009).

It is possible that the correlation between plasma RBP4 and glucose concentrations represents a secondary, rather than a primary, phenomenon. One way to distinguish between acquired, i.e., hyperglycemia, vs. genetic etiologies is to study subjects at high risk of developing diabetes but who have normal glucose tolerance (NGT). Thus Graham *et al*., 2007, reported that NGT subjects with a family history of T2DM had elevated plasma RBP4 levels.

Several explanations could be postulated to explain the controversy of different studies: different ethnic groups in the studies, RBP4 genetic variation, different methods of measurement of RBP4, sex related levels of RBP4 (Alberto, 2009; Kovacs, 2007; Klein, 2010; von Eynatten, 2007; Janke, 2006).

The present findings are contrary to those of different ethnic backgrounds (Caucasian in the current study compared with Chinese and Japanese in earlier reports) could explain the observed differences in serum RBP4 levels. T2DM individuals of Mexican American decent reported that, in this ethnic population, serum RBP4 concentrations are elevated in T2DM and do not correlate with measures of insulin resistance or insulin secretion (Alberto, 2009).

The RBP4 genetic variations were also likely to affect measures of insulin resistance, BMI, waist-to-hip ratio, and circulating free fatty acids However, the associations among RBP4 levels, *RBP4* gene expression, and measures of obesity or insulin resistance have not been universally described (Promintzer, 2007). Kovacs *et al*., 2008, indicated a role for RBP4 genetic variation in susceptibility to type 2 diabetes and insulin resistance, possibly through an effect on RBP4 expression. The *RBP4* heliotypes were related to an increased risk of type 2 diabetes (Kovacs, 2007). Diabetes risk *RBP4* haplotype carriers had a higher mean visceral and subcutaneous *RBP4* mRNA expression, compared with no carriers (Kovacs, 2007). However, the associations among RBP4 levels, *RBP4* gene expression, and measures of obesity or insulin resistance have not been universally described (Promintzer, 2007).

Different assays have been used to measure RBP4 levels, and this could account for the varied results reported by different laboratories (Graham, 2007). A recent study reported a strong correlation between RBP4 measured by Western blot and by ELISA, but neither method was able to detect a difference in plasma RBP4

concentrations between insulin sensitive and insulin-resistant individuals (Stefan, 2007). In the present study, we did not employ Western blotting to measure serum RBP4 concentrations. However, based on the results of other studies (Klein, 2010; Kiernan, 2011), one would not expect to observe a difference between insulin-sensitive and insulin-resistant groups even if the Western blot method was employed. Different assays have been used to measure RBP4 levels, and this could account for the varied results reported by different laboratories (Graham, 2006). However, the serum RBP4 levels estimated by the current assay are well within the range of values reported in the literature (Alberto, 2009; Janke, 2006).

It is known that circulating RBP4 resides in multiple variants which may provide enhanced clinical utility, but conventional immunoassay methods are blind to such differences. A Mass spectrometric immunoassay (MSIA) technology that cans quantitative total RBP4 as well as individual isoforms may provide an enhanced analysis for this biomarker (Duncan, 2008; Kiernan, 2011).

Difference in our study may be explained by sex dimorphic pattern of RBP4 in our study 40 females, 20 males with mean age 52.27 years.

Many adipokines have been found to be sexually dimorphic. Both leptin and adiponectin are increased in serum of women compared with men. This observation has been explained on the basis of different fat quantities and the influence of sex hormones. Serum RBP4 concentrations, however, exhibit an opposite pattern. The median (range) for RBP4 in serum was higher in men than in women (Janke, 2006). Serum RBP4 levels in women over the age of 50 years were found to be significantly higher than those in women under the age of 50 years. No such age-associated difference in RBP4 serum levels was observed in men (Janke, 2006).

One limitation of our study is that we have not measured serum retinol concentrations, since RBP4 is the transport protein for retinol. However, studies in patients with T2DM have shown similar serum retinol concentrations compared with NGT subjects (Basualdo, 1997; Abahusain, 1999). Therefore, even though retinol concentrations are not measured in the current study, it is unlikely to affect the results.

In summary, our results indicate that, in obese type 2 DM, serumRBP4 levels are elevated but are not associated with insulin resistance or impaired insulin secretion. However, a correlation between serum RBP4 concentration and hyperglycemia was observed. The failure to observe any correlations among these variables suggests the presence of significant ethnic differences in the regulation of serum RBP4 levels.

We conclude that elevated serum RBP4 may not play a role in the development of insulin resistance in obese type 2 diabetes mellitus.

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