High Plasma Retinol-Binding Protein-4 Level is Associated with Impaired Glucose Metabolism in Obese Subjects

Mohamed M Awad* , Ashraf Taalat, Alsayed A.Alnahal*,Mohamed S Fawzy****.**
***** , Internal Medicine Department Zagazig University,
******Internal Medicine Department ,Banha University
******* Medical Biochemstry Department, Zagazig University.

ABSTRACT

Background: Retinol-binding protein 4 (RBP4) is a novel adipokine that induces insulin resistance in mice.**Objective:** Measurement of serum RBP4 concentration and relationship to insulin sensitivity and secretion in obese subjects with dysglycemia.**Design and Participants:** This case-control study consisted of 100 subjects, 20 of them were healthy lean subjects and 80 obese patients .They were divided into three groups : normal glucose tolerance (NGT; *n* = 20), impaired glucose tolerance (IGT; *n* = 20), and type 2 diabetes (*n* = 40). Laboratory and anthropometric measurements including, serum RBP4 levels, were assessed.**RESULTS**: Plasma RBP4 levels were higher in obese IGT and T2DM subjects compared with obese NGT subjects (23.7±10, 26.3±10 vs. 16.64±8 μg/ml, P<0.001). In obese IGT and T2DM subjects, a positive correlations were observed between the plasma RBP4 and SBP and DBP (r = 0.358, P<0.001 and r = 0.332, P<0.001, respectively) .A positive correlations were observed between the plasma RBP4, BMI (r = 0.618, P<0.001) , FBG (r = 0.506, P<0.001), 2-hour PBG (r= 0.485, P<0.001) during OGTT, HbA1C (r = 0.387, *P<0.001), fasting insulin (r = 0.417, P<0.001), HOMA-IR (r = 0.450, P<0.001), total* cholesterol (r = 0.283, P<0.01), LDL-C (r = 0.232, P<0.05) and triglyceride (r = 0.437, P<0.001). A negative correlations were observed between plasma RBP4 and HOMA-B (r = -0.215, P<0.05) and HDL-C (r = -0.250, P<0.05). **CONCLUSIONS**: RBP4 concentrations were elevated in obese subjects with IGT or type 2 diabetes and was related to clinical parameters of insulin resistance.

***Keywords*:** Adipokines, RBP4, Insulin resistance, *HOMA-IR, HOMA-B and Type 2 DM.*

INTRODUCTION

Insulin resistance (IR) is a characteristic feature of type 2 diabetes mellitus (T2DM), prediabetic (IGT) individuals, and normal glucose tolerant (NGT) subjects with a strong family history of diabetes.[(1)].

In addition to its main metabolic role of storing energy in the form of fat, adipose tissue is now considered an active endocrine organ that secretes a variety of bioactive peptides. These peptides are known as "adipokines" and they coordinate biological processes such as energy metabolism, immune and neuroendocrine functions.[(2)].

Obesity is a condition that is associated with low-level chronic inflammation, IR, hyperlipidaemia and other metabolic disorders. There is now growing evidence that adipokines are important in the etiopathology of obesity-related disorders, either through a traditional (circulating) hormonal effect or by a local action in the adipose tissue.[(3)]

Since the circulating levels of a recently identified adipokine that acts as a carrier of retinol (vitamin A) in the blood, retinol binding protein-4 (RBP4), have been positively correlated with obesity and IR in an adipocyte-specific glucose transporter 4 knock-out mouse model[(4)], it has been proposed that RBP4 may be behind the adipocyte-muscle connection that links obesity and IR. However, its role in human

obesity and IR physiopathology is still unclear.[5].

Some [6,7], but not all [5,8] studies in humans have shown that plasma RBP4 levels are elevated in T2DM patients, and an inverse correlation has observed between the plasma RBP4 concentration and insulin sensitivity. Insulin resistant subjects with T2DM, hypertension, and polycystic ovarian syndrome have increased serum RBP4 levels and elevated RBP4 levels predicts future diabetes.[7,9].

However, these data have not been replicated in all populations. Recent studies failed to demonstrate any correlation between RBP4 levels and IR in T2DM patients [10,11]. In lean NGT subjects, no difference in RBP4 levels was seen between insulin-resistant and insulin-sensitive subjects with and without a family history of T2DM.[12]. In obese women, neither plasma RBP4 levels nor RBP4 expression in adipose tissue was increased, and RBP4 levels did not correlate with insulin sensitivity.[13].

The main objective of this study was to examine whether plasma RBP4 levels was associated with IR in obese and/or glucose-intolerant nondiabetic and T2DM individuals.

SUBJECTS AND METHODS

Study population:

This case control study was conducted at diabetes and obesity outpatient clinic of Internal Medicine and Biochemistry Departments, Faculty of Medicine, Zagazig University Hospitals and Banha University Hospitals.

One hundred subjects were included in this study and received a 75-gm oral glucose tolerance test (OGTT). Subjects were divided into the following four groups: 20 lean subjects with normal glucose tolerance (NGT) (Lean NGT, BMI <25 kg./m^2) as control, 20 obese (BMI > 30 kg/m^2) IGT (IFG/IGT) subjects and 40 obese (BMI > 30 kg/m^2) subjects with T2DM according to American Diabetes Association Criteria[14]. All subjects had normal liver, cardiopulmonary, and kidney function as determined by medical history, physical examination, screening blood tests, electrocardiogram and urinalysis. No NGT or IGT subjects was taken any medication known to affect glucose tolerance such as β-blockers, thiazide diuretics or statin therapy for at least last two weeks.

Subjects with T2DM were treated with diet alone (n=5), metformin (n=10), sulfonylurea (n=10), or a combination of both (n=15). No T2DM patients were on thiazolidinedione or insulin. Body weight was stable (±1.5 kg) of at least 3 months before study in all subjects. No subjects participated in any excessively heavy exercise program. The study protocol was approved by the local ethics committee and informed written consent was obtained from all study participants before entering the study.

Anthropometrical measurements and analytical methods

Body height and weight were measured with the patient standing in light clothes and without shoes. BMI was calculated as body weight divided by height squared (kg/m^2). Blood pressure was measured in the supine position on the right arm after 10 min of rest. A standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals. The same physician performed all examination.

OGTT. A 75- gram glucose load was performed between 8.00 and 10.00 am after an 8-hour overnight fast and blood samples were collected for the measurement of fasting (FBG) and 2-hour post load plasma glucose (2-hour PBG) and fasting serum insulin levels.

Blood samples were drawn from each subjects after an overnight fasting

period. Serum was centrifugated at 4.000 g for 10 min, immediately divided into aliquots, and frozen at -80°C until analysis. The serum glucose and lipid profile parameters were determined using standard clinical biochemistry methods. The level of low-density lipoprotein (LDL)-cholesterol was estimated using the formula: total cholesterol-HDL-cholesterol-(Triglyceride÷ 5). Fasting serum insulin concentrations were measured in duplicate by a monoclonal immuno-radiometric assay (the bio source INS-EASIA). Intra-assay and interassay coefficients of variation were <7%. HbA_1C was measured by high-performance liquid chromatography by means of a fully automated glycated hemoglobin analyzer system (Hitachi L-9100). Fasting plasma RBP4 concentration was measured in duplicated by enzyme-linked immunoassay kit (R&D systems, Inc., Minneapolis, United State of America).

Analysis of insulin resistance parameters:

IR was estimated by homeostasis model assessment of IR (HOMA-IR). HOMA-IR was calculated using fasting glucose and insulin with the formula.

$$\text{HOMA-IR} = \frac{\text{glucose[mg/dl]} \times \text{insulin [uU/ml)}}{405}$$

The cut-off value of 1.77 for HOMA-IR, as defined for subjects with metabolic syndrome .[15], was also used in the present study. β-cell function was estimated by homeostasis model assessment of β-cell function (HOMA-B). HOMA%B was calculated using fasting glucose and insulin with the formula.[16]:

$$\text{HOMA\%B} = \frac{20 \times \text{insulin [uU/ml)}}{0.055 \times \text{glucose [mg/dl)} - 3.5}$$

Statistical analysis:

All collected data were first assessed before taken for analysis. The normality test of Kosmogorov-Smirnov was done to assess whether the data were normally distributed or not. Unless otherwise stated, data represent the means ±SD. Differences between parameters were tested using ANOVA or student's t-test, post hoc tests (least significant of difference) were performed for multiple comparisons between groups, while X^2 tests were used for categorical variable. Correlation between variables of interest was performed using Pearson's correlation. A multiple linear regression analysis were performed using statistical package of social sciences (SPSS) for windows version 17. A P-value ≤ 0.05 was considered statistically significant.

RESULTS

Patients characteristics

Lean/obese NGT, IGT and T2DM subjects were well matched for age and gender (Table 1). BMI was statistically higher in obese subjects with or without NGT as compared to lean subjects with NGT ($F = 158.29$, P-value<0.001). However, BMI was similar in obese NGT, IGT and T2DM subjects. Both systolic (SBP) and diastolic blood pressure (DBP) were statistically higher among obese subjects with or without NGT when compared to lean NGT subjects ($F = 5.50$, P-value<0.001 and $F = 4.27$, P-value<0.001, respectively). However, no significant difference as regard to both SBP and DBP, is observed in obese groups. Subjects with IGT and T2DM had higher FBG and 2-hour PBG levels than lean/obese NGT subjects ($F = 136.2$, P-value<0.001 and $F = 93.6$, P-value<0.001, respectively). In T2DM, both FBG and 2-hour PBG levels were significantly higher compared with IGT subjects (P-value<0.001).

Obese IGT and T2DM subjects had lower fasting serum insulin levels compared

to lean/obese NGT subjects (F = 17.84, P-value<0.001). However, no significant difference was found between obese IGT and obese T2DM subjects (P-value>0.05). In T2DM, HbA1c was 7.4±0.5%, indicating to lesser extent good glycemic control.

Obese NGT, IGT and T2DM subjects had higher plasma triglyceride concentrations compared to lean NGT subjects (F = 8.8, P-value<0.001), however, no significant difference was found between obese NGT and IGT (P-value>0.05). Subjects with T2DM had higher plasma triglyceride and lower plasma high-density lipoprotein cholesterol concentrations compared with lean/obese NGT subjects (P-value<0.001)(Table1).

Insulin sensitivity. As expected, obese NGT, IGT and T2DM subjects were more insulin resistant (measured by HOMA-IR) than lean NGT subjects (F = 46.3, P-value<0.001). Insulin sensitivity was decreased more in obese IGT and T2DM subjects compared with obese NGT subjects (Pvalue<0.001). Insulin secretion (measured by HOMA-B) was decreased in obese NGT, IGT and T2DM subjects compared with lean NGT subjects (F = 21.55, P-value<0.001). Obese T2DM subjects had lower insulin secretory function compared with obese NGT and IGT subjects (P-value<0.001) (Table1).

Circulating RBP4 concentrations in obese subjects with or without NGT

Fasting plasma RBP4 levels were significantly higher in obese IGT and T2DM subjects compared with lean and obese NGT subjects (23.7±10, 26.3±10 vs. 6.1±1 and 16.64±8 μg/ml, F = 6.12, P-value<0.001). However, no significant difference was found between obese IGT and T2DM subjects (23.7±10 vs 26.3±10 μg/ml, P-value>0.05) (Table2).

Relationship between plasma RBP4, metabolic and anthropometric parameters

Correlation between plasma RBP4 and various parameter in all three obese groups collectively, there was no correlation between plasma RBP4 and age (r = 0.143, P-value>0.05) or gender (r=0.115, P-value>0.05). A highly significant positive correlation was observed between plasma RBP4 and BMI (r = 0.618, P-value<0.001) (Table 3).

In obese NGT and T2DM subjects, a highly significant positive correlations were observed between the plasma RBP4 concentration and systolic and diastolic blood pressure (r = 0.358, P-value<0.001 and r = 0.332, P-value<0.001, respectively) (Table 3).

Significant positive correlations were observed between the plasma RBP4 concentration and FBG (r = 0.506, P-value<0.001), 2-hour PBG (r= 0.485, P-value<0.001) during OGTT, HbA1C (r = 0.387, P-value<0.001), fasting serum insulin (r = 0.417, P-value<0.001), HOMA-IR (r = 0.450, P-value<0.001), serum total cholesterol (r = 0.283, P-value<0.01), serum LDL-C (r = 0.232, P-value<0.05) and serum triglyceride (r = 0.437, P-value<0.001). A negative correlations were observed between plasma RBP4 concentration and HOMA-B (r = -0.215, P-value<0.05) and HDL-C (r = -0.250, P-value<0.05) (Table 3 & Fig.1,2,3,4,5,6,7).

In the multivariate analysis, in a model including BMI and, as confounding factors, age, gender, FBG, fasting serum insulin, and HOMA-1R, we found that BMI (B = 0.868, P-value<0.001) and FBG level (B = 0.185, P-value<0.01) were independently associated with the RBP4 systemic levels (Table 4).

Table (1): Demographic data and clinical parameters of studies groups.

Variable	Lean NGT Group 1 (n=20)	Obese NGT Group 2 (n=20)	Obese IGT Group 3 (n=20)	Obese T2DM Group 4 (n=40)	F	P
• Gender (M/F)	10/10	5/15	7/13	4/26		
• Age (years)	39.3±9.5	43.1±8.8	46.4±8.39	45.9±11.1	2.43	>0.05
• BMI (kg/m^2)	23.05±1	35.3±2.7[a]	43.5±1.9[b]	34.8±2.4[c]	158.29	<0.001
• Blood pressure (mmHg)						
- Systolic	116.5±339	129.4±13[a]	134±13.5[b]	133.6±13[c]	5.50	<0.01
- Diastolic	74.25±0.37	81±6.198[a]	86.9±8.9[b]	86.7±9.5[c]	4.27	<0.001
• Glycemic parameters:						
- FBG (mg/dl)	87.9±3.5	92.6±4.5	107±11[bd]	176.9±2939[ce]	136.2	<0.001
- PBG (mg/dl)	117.9±7.6	119±8.6	145±17[bd]	211.5±36.8[ce]	93.6	<0.001
- HbA1C (%)	6.3±0.3	6.6±.4[a]	6.9±0.5[b]	7.4±0.5[ce]	25.8	<0.001
- Fasting insulin (u/U/ml)	17.10±6.2	14.52±4.6	11.09±3.5[bd]	8.15±1.6[c]	17.84	<0.001
• Fasting lipid profiles:						
- Total Cholesterol (mg/dl)	196.8±22.7	207.1±34	220.9±25	208.6±38.7	1.81	>0.05
- HDL-cholesterol (mg/dl)	41.95±6.3	42.50±6.5	41.15±6.75	34.78±5.08[ce]	11.3	<0.001
- LDL-cholesterol (mg/dl)	119.25±4.7	122.7±34.6	135.90±5.4	128.3±38	0.992	>0.05
- Triglycerides (mg/dl)	178±23.9	209.1±40.4[a]	219±29.9[b]	229±42.7[ce]	8.8	<0.001
• HOMA-IR	1.7±0.3	2.5±0.7[a]	3.8±1.3[bd]	7.4±3.0[ce]	46.3	<0.001
• HOMA-B	119±27.8	54.6±12[a]	48.8±10.9[b]	38.2±6[ce]	21.55	<0.001

Data are presented as mean±SD
a = P-value<0.001 in comparison with group1 and 2
b = P-value<0.05 in comparison between group 1 and 3.
c = P-value>0.05 in comparison between group 1 and 4.
d = P-value>0.05 in comparison between group 2 and 3
e = P-value>0.05 in comparison between group 3 and 4

Table (2): Study of plasma RBP-4 (ug/ml) among the studied groups.

Variable	Group 1 (n = 20)	Group 2 (n = 20)	Group 3 (n = 20)	Group 4 (n = 40)	F	P
RBP-4 (ug/ml)	6.1±1	16.64±8[a]	23.7±10[bd]	26.3±10[c]	6.12	<0.001

Data are presented as mean±SD
a = P-value<0.001 in comparison with group1 and 2
b = P-value<0.05 in comparison between group 1 and 3.
c = P-value>0.05 in comparison between group 1 and 4.
d = P-value>0.05 in comparison between group 2 and 3
e = P-value>0.05 in comparison between group 3 and 4

Table (3): Correlation of plasma RBP4 level with various metabolic variables in obese subjects with or without impaired glucose metabolism (n = 80).

Variable	Plasma RBP4 level	
	r	P
Age (years)	0.143	NS
Gender	0.115	NS
BMI (kg/m^2)	0.618	<0.001
Blood pressure (mmHg):		
- Systolic	0.358	<0.001
- Diastolic	0.332	<0.001
Glycemic parameters:		
- FBH (mg/dl)	0.506	<0.001
- PBG (mg/dl)	0.485	<0.001
- HbA1C (%)	0.387	<0.001
- Fasting insulin (u/U/ml)	0.417	<0.001
HOMA-1R	0.450	<0.001
HOMA-B	-0.215	<0.05
Fasting lipid profile		
- Total cholesterol (mg/dl)	0.283	<0.01
- LDL-cholesterol (mg/dl)	0.232	<0.05
- HDL-cholesterol (mg/dl)	-0.250	<0.05
- Triglycerides (mg/dl)	0.437	<0.001

Table (4): Multivariate analysis for the relationship between metabolic parameters and plasma RBP4 (ug/ml).

	Unstandardized coefficients		Standardized coefficients		P
	B	Std. error	B	t	
Constant	-25.008	10.117		-2.472	NS
Age	-8.053E-02	0.088	-0.075	-0.974	NS
Gender	-4.385	3.171	-0.114	-1.383	NS
FBG	0.185	0.060	0.721	3.087	<0.01
Fasting insulin	1.101	0.562	0.560	1.957	NS
BMI	0.868	0.204	0.392	4.253	<0.001
HOMA-1R	-2.948	1.498	-0.802	-1.969	NS

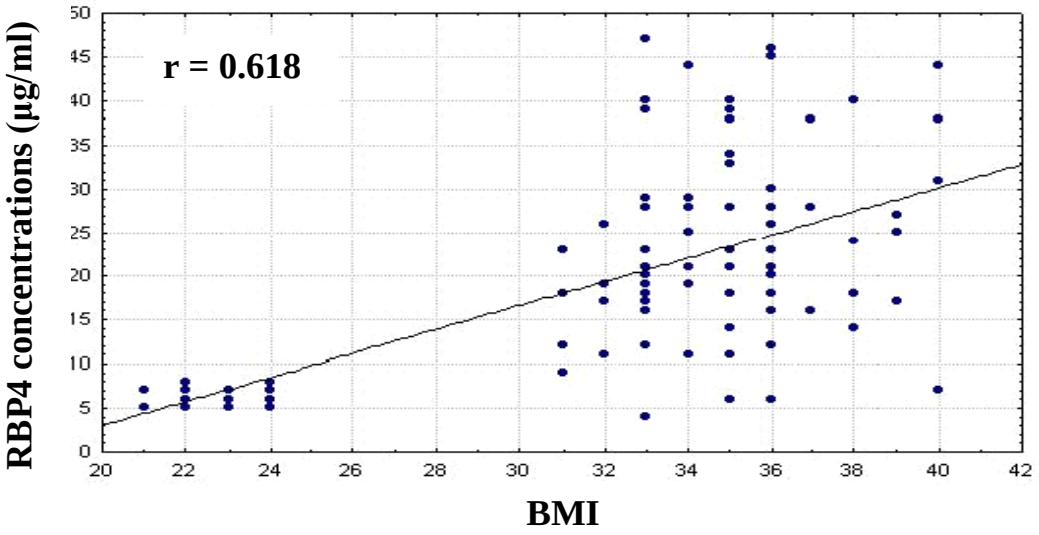

Fig (1): Plasma RBP4 concentrations (µg/ml) were highly correlated with BMI(kg/m^2).

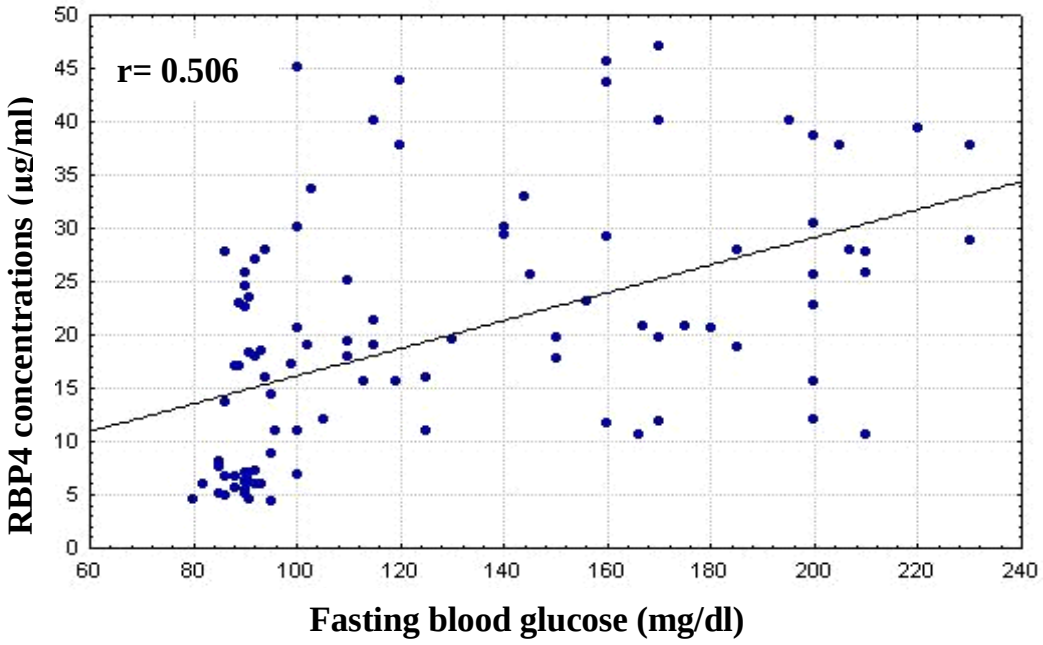

Fig (2): Plasma RBP4 concentrations (µg/ml) were highly correlated with fasting blood glucose (mg/dl).

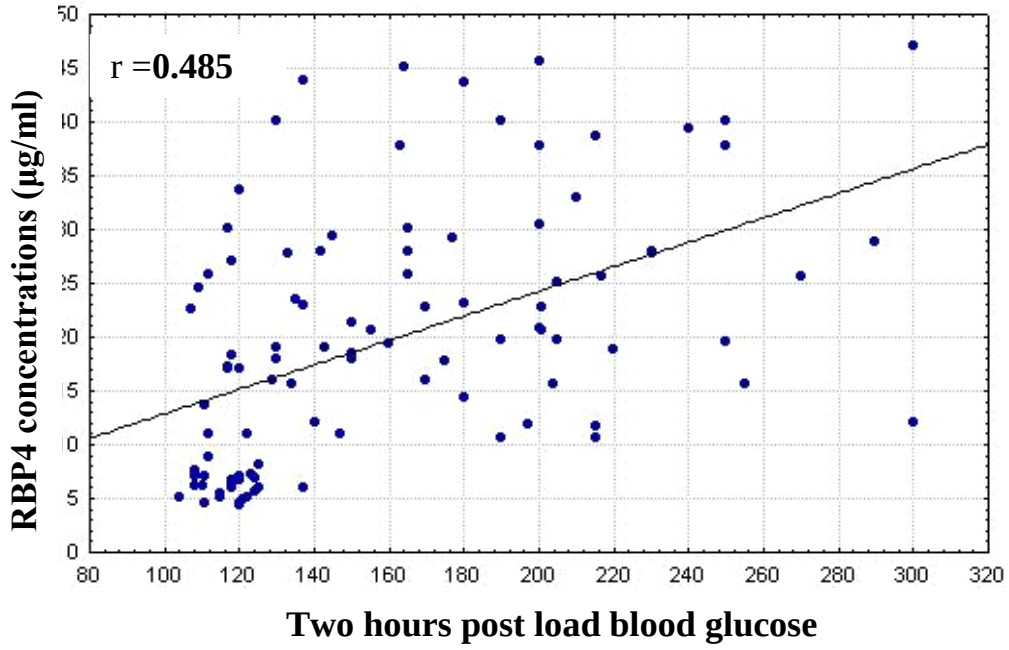

Fig (3): Plasma RBP4 concentrations (µg/ml) were highly correlated with two hours post load blood glucose (mg/dl).

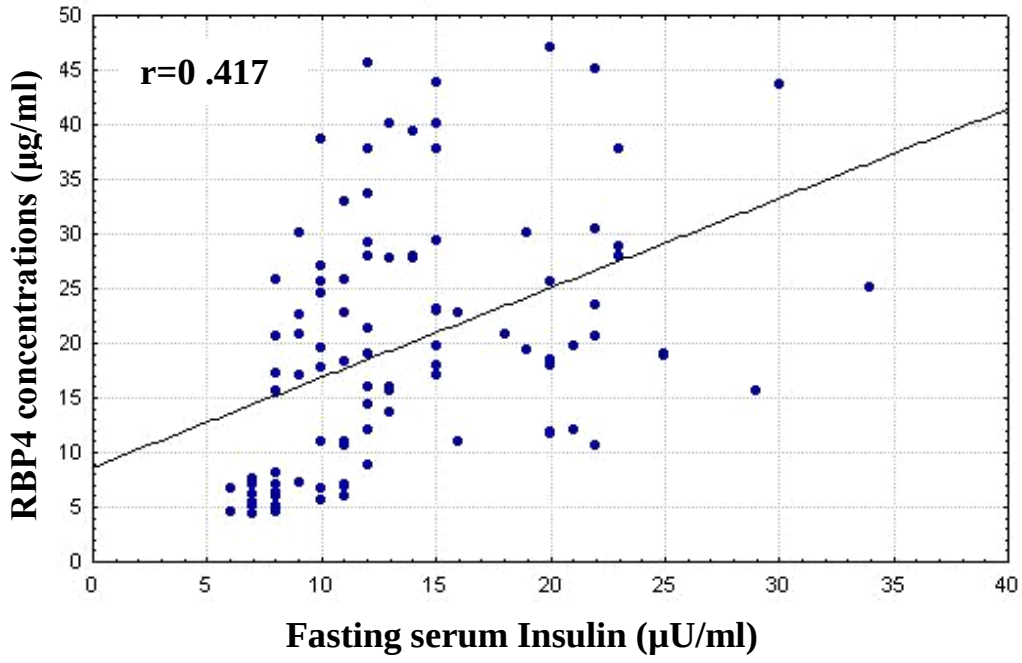

Fig. (4): Plasma RBP4 concentrations (µg/ml) were highly correlated with fasting serum Insulin (µU/ml).

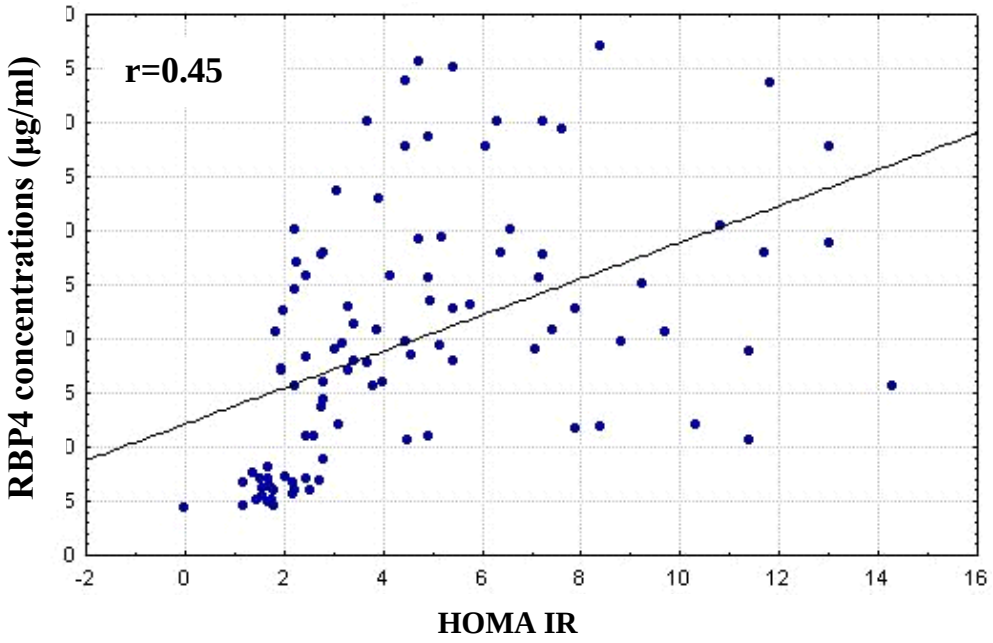

Fig (5): Plasma RBP4 concentrations (µg/ml) were highly correlated with HOMA .IR.

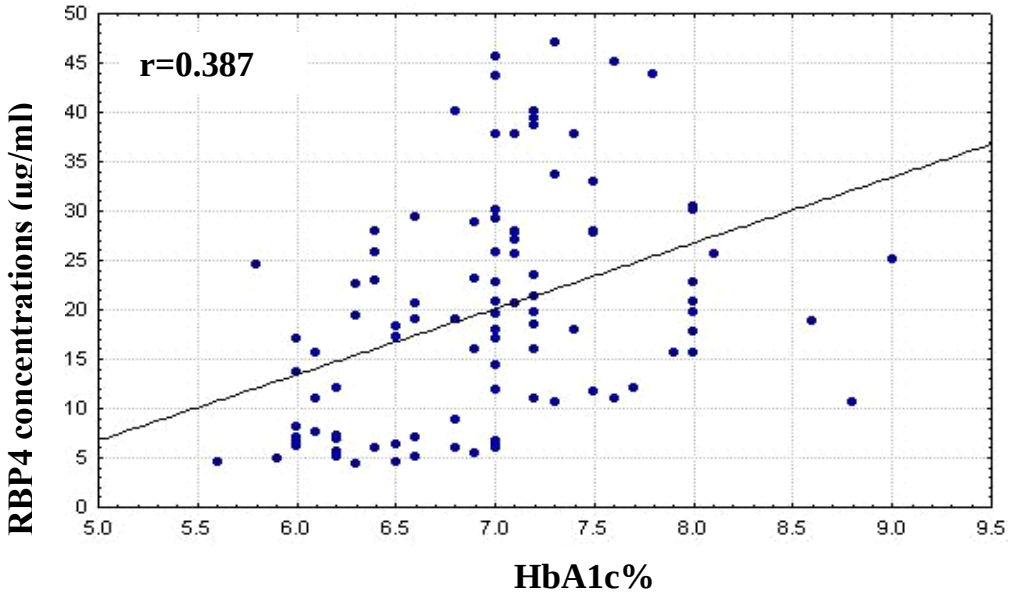

Fig (6): plasma RBP4 concentrations (µg/ml) were highly correlated with HbA1c.

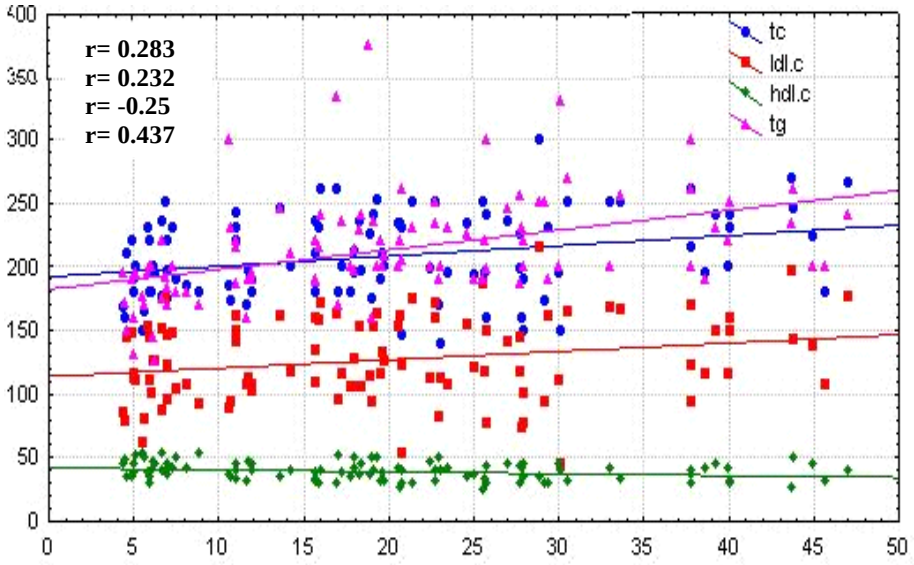

Fig (7): Plasma RBP4 concentrations (µg/ml) were highly correlated with serum triglycerides (mg/dl) and serum cholesterol (mg/dl), while plasma RBP4 concentrations were modestly correlated with serum LDL.C (mg/dl) and serum HDL-C (mg/dl).

DISCUSSION

In the present study, we demonstrated that serum RBP4 levels were elevated in obese IGT and T2DM subjects compared to learn and obese NGT subjects and correlate with both measures of glycemia and insulin sensitivity.

Our results agreed with different clinical studies [4,17] that demonstrated elevated serum RBP4 levels in subjects with impaired glucose tolerance, T2DM and correlate inversely with insulin sensitivity in non-diabetic subjects with a family history of T2DM. Circulating RBP4 levels correlate with the degree of insulin resistance in these subjects and the relationship is independent of obesity. [18]

Our analysis between the circulating RBP4 protein levels and parameters of obesity revealed an increase in serum RBP4 levels in obese subjects with impaired glucose tolerance and T2DM. This positive relationship between serum RBP4 and obesity has been reported by other studies. [4,18].

In addition to increased BMI and whole-body fat content, increased circulating RBP4 levels are linked to increased visceral adipose tissue content[19]. In a recent study, higher waist circumference and waist-to-hip ratio were associated with higher RBP4 levels and markers of systemic inflammation.[20].

Significant decrease in weight, achieved by diet, exercise, or bariatric surgery, leads to a decrease in circulating and/or adipose tissue RBP4 levels.[21]. A decrease in serum RBP4 levels predicts the improvement in insulin sensitivity with greater specificity than leptin, adiponectin, interleukin-6 or C-reactive protein.[18].

Obesity is strongly associated with insulin resistance and impaired glucose-mediated insulin secretion. Adipose tissue is an endocrine organ secreting biologically active factors called adipokines. RBP4 is a recently identified adipokine suggested to

link obesity with its comorbidities, especially insulin resistance and T2DM.[22].

Several mechanisms link RBP4 to IR and T2DM. Increasing serum RBP4 induces hepatic expression of the glyconeogenic enzyme phosphoenol-pyruvate carboxykinase and impairs insulin signaling in skeletal muscle.[4] Insulin signaling in primary human adiocytes was affected by RBP4 through blocking the insulin-stimulated phosphorylation of insulin receptor-1 at serine in position 307.[23]In addition to decreased insulin sensitivity, a negative effect of RBP4 on the secreting function of the beta cell in suggested. It is well known that retinol, the ligand for RBP4, in pathophysiologically linked to B-cell function. Retinol-binding protein circulates in serum forming a complex with transthyretin (TTR), a transport protein for thyroxine. A recent investigation disclosed that TTR constitutes a functional component in pancreatic B-cell stimulus-secretion coupling. TTR inhibites the binding of RBP to the receptor. It is thus possible that increased serum RBP4 prevents transthyretin from exerting its B-cell stimulus-secretion effects.[24].

Increased serum RBP4 levels have been reported in subjects with obesity, IR, and T2DM [18] and in other insulin-resistant states, such as non alcoholic fatty liver disease and the metabolic syndrome.[25].

In the present study, serum RBP4 were significantly higher in obese subjects with IGT and T2DM compared to lean and obese NGT subjects. However, no significant difference was found between IGT and T2DM subjects. Consistent with the latter observation, previous studies[18,26] in different populations also demonstrated elevated serum RBP4 levels in subjects with IGT and T2DM.

However, in contrary to our results, previous studies in different populations failed to observe any association between circulating RBP4 levels, insulin sensitivity and type 2DM. [27,28]

In our study, there is a correlation between serum RBP4 and different parameters of metabolic syndrome such as BMI, blood pressure and fasting serum lipids in agreement with other studies.[29] In contrary other studies showed no association between circulating RBP4 concentration and parameters of IR.[10,24] 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.[30]

It is possible that the correlation between RBP4, glucose concentrations and HOMA-B could suggest that over-secretion of RBP4 may negatively affect B-cell function in agreement to **Broch et al.,**[24] who concluded that RBP4 could be one signal from insulin-resistant tissues that impacts on β-cell secretion and this mechanism could be behind the association between increased circulating RBP4 and type 2 diabetes.

However, **Cho et al.,**[6] reported no association between circulating RBP4, glucose concentrations and B-cell function.

Several explanations could be postulated to explain the controversy of different studies: different ethnic populations in the studies, RBP4 genetic variation, different methods of measurement of RBP4, and sex-specific dimorphism of RBP4.

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 and do not correlate with measures of insulin resistance or insulin secretion.[30]

Several RBP4 genetic variants are associated with insulin resistance and type 2 DM, possibly through an effect on RBP4 expression. The RBP4 haplotypes were

related to an increased risk of type 2DM and are associated with a higher visceral and subcutaneous RBP4 mRNA expression. However, the association among RBP4 levels, RBP4 gene expression, and measures of obesity or insulin resistance have not been universally described.[31]

Different assays have been used to measure RBP4 levels, and this could account for the varied results reported by different laboratories. 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.[10] In the present study, we did not employ western blotting to measure serum RBP4 concentrations. However, based on the results of other studies,[32] one would not except to observe a difference between insulin- sensitive and insulin-resistant groups even if the western blot method was employed. The serum RBP4 levels estimated by the current assay are well within the range of values reported in the literature.[30]

It is known that certain adipokines are expressed differently depending on the gender. Higher circulating levels of leptin and adiponectin are found in women. [33] On the contrary, higher levels of circulating RBP4 are described in men.[34] This dimorphism was, however, not shown in all studies.[10,35]

It is suggested that sex-specific dimorphisms in circulating levels of adipokines can be related to direct effects of sex hormones on adipocyte expression and secretion. In case of RBP4, this possible mechanism is not supported by the fact that serum RBP4 levels are similar in pubertal and prepubertal subjects that significantly differ in levels of circulating sex hormones.[36]A possible direct role of gonadotropins on the expression of RBP4 is also proposed, as circulating RBP4 levels are higher in post menopausal compared with premenopausal women and in women older than 50 years when compared with those younger than 50 years.[6]

One limitation of our study is that we have not measured serum retinol concentration, since RBP4 is the transport protein for retinol. However, studies in patients with T2DM have shown similar serum retinol concentrations compared with NGT subjects.[37] 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 serum RBP4 levels are elevated in obese IGT and T2DM subjects**,** compared with lean subject and are associated with metabolic parameters of IR. Therefore, the elevated serum RBP4 is related to decreased insulin sensitivity. Our data indicate that over-secretion of RBP4 may negatively affect B-cell function and this mechanism could be behind the association between increased circulating RBP4 and type 2 diabetes.

We conclude that elevated serum RBP4 may play a role in the development of IR and B-cell dysfunction in obese subjects with abnormal glucose metabolism.

REFERENCES

1- **DeFronzo RA.** Pathogenesis of type 2 diabetes mellitus. Med. Clin. North Am 2004; 88: 787–835.

2- **Lago F, Dieguez C, Gomez-Reino J & Gualillo O.** The emerging role of adipokines as a mediators of inflammation and immune resposes. Cytokine and Growth Factor Reviews 2007; 18: 313–325.

3- **Fantuzzi G.** Adipose tissue, adipokines, and inflammation. Journal of Allergy and Clinical Immunology 2005; 115: 911–919.

4- **Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L & Kahn**

BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes.Nature 2005; 436: 356–362.

5- **Broch M, Auguet MT, Ramı´rez R, Olona M, Aguilar C, Megia A, Alcaide MJ, Pastor R, Martı´nez S, Caubet E, Garcia-Espan˜a A & Richart C.** Parallel downregulation of retinol-binding protein-4 and adiponectin expression in subcutaneous adipose tissue of non-morbidly obese subjects. European Journal of Endocrinology 2009; 161: 87–94.

6- **Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK & Park KS.** Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 2006; 29: 2457–2461.

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

8- **Krzyzanowska K, Zemany L, Krugluger W, Schernthaner GH, Mittermayer F, Schnack C, Rahman R, Brix J, Kahn BB & Schernthaner G.** Serum concentrations of retinol-binding protein 4 in women with and without gestational diabetes. Diabetologia 2008; 51: 1115–1122.

9- **Weiping L, Qingfeng C, Shikun M, Xiurong L, Hua Q, Xiaoshu B,Suhua Z & Qifu L.** Elevated serum RBP4 is associated with insulin resistance in women with polycystic ovary syndrome. Endocrine 2006; 30: 283–288.

10- **Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Schleicher E,Fritsche A & Haring HU.** High circulating retinol-binding protein 4 is associated with elevated liver fat, but not with total-, subcutaneous-,visceral-, or intramyocellular fat in humans. Diabetes Care 2007; 30: 1173–1178.

11- **Yao-Borengasser A, Varma V, Boodles AM, Rasouli N,Phanavanh B, Lee MJ, Starks T, Kern LM, Spencer HJ III, Rashidi AA, McGehee RE Jr, Fried SK & Kern PA.** Retinol binding protein 4 expression in humans. Relationship to insulin resistance,inflammation, and resposte to pioglitazone. Journal of Clinical Endocrinology and Metabolism 2007; 92: 2590–2597.

12- **Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S,Cline GW, Befroy D, Zemany L, Kahn BB, Papademetris X, Rothman DL & Shulman GI.** The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci USA* 2007; 104:12587–12594.

13- **Janke J, Engeli S, Boschmann M, Adams F, Bohnke J, Luft FC, Sharma AM & Jordan J.** Retinol-binding protein 4 in human obesity. Diabetes 2006; 55: 2805–2810.

14- **Matsuda M & DeFronzo RA.** Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22: 1462–1470.

15- **Esteghamati A, Ashraf H, Khalilzadeh O, Zandieh A, Nakhjavani M, Rashidi A, Haghazali M & Asgari F.** Optimal cut-off of homeostasis model assessment of insulin resistance (HOMA-IR) for the diagnosis of metabolic syndrome :third national surveillance of risk factors of non-communicable diseases in Iran (SuREFNCD-2007). Nutr Metab 2010; 7: 26.

16- **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC.** Homeostasis model assessment: Insulin resistance and beta-

cell function from plasma glucose and insulin concentration in man. Diabetologia 1985; 28: 412-419.

17- **Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon MR, Kern M, Stumvoll M & Bluher M.** Insulin-sensitive obesity. Am J Physiol Endocrinol Metab 2010; 299: E506–E515.

18- **Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U & Kahn BB.** Retinol-binding protein 4 and insulin resistance in lean,obese, and diabetic subjects. New England Journal of Medicine 2006; 354: 2552–2563.

19- **Kelly KR, Kashyap SR, O'Leary VB, Major J, Schauer PR & Kirwan JP.** Retinol-binding protein 4 (RBP4) protein expression is increased in omental adipose tissue of severely obese patients. Obesity 2010; 18: 663–666.

20- **Hermsdorff HH, Zulet MA, Puchau B & Martinez JA.** Central adiposity rather than total adiposity measurements are specifically involved in the inflammatory status from healthy young adults. Inflammation 2010; 34: 161–170.

21- **Lee JW, Lee HR, Shim JY, Im JA & Lee DC.** Abdominal visceral fat reduction is associated with favorable changes of serum retinol binding protein-4 in nondiabetic subjects. Endocrine Journal 2008; 55: 811–818.

22- **Kotnik P, Fischer-Posovszky P & Wabitsch M.** RBP4: a controversial adipokine. Eur J Endocrinology 2011; 16.

23- **Ost A, Danielsson A, Lide´n M, Eriksson U, Nystrom FH & Strålfors P.** Retinol-binding protein-4 attenuates insulin-induced phosphorylation of IRS1 and ERK1/2 in primary human adipocytes. FASEB J 2007; 21: 3696-3704.

24- **Broch M, Vendrell J, Ricart W, Richart C & Ferna´ndez-Real JM.** Circulating retinol-binding protein-4, insulin sensitivity, insulin secretion, and insulin disposition index in obese and nonobese subjects. Diabetes Care 2007; 30: 1802–1806.

25- **Tönjes A, Blüher M & Stumvoll M.** Retinol-binding protein 4 and new adipocytokines in nonalcoholic fatty liver disease. Curr Pharm Des 2010; 16(17):1921-8.

26- **Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon MR, Kern M,Stumvoll M & Bluher M.** Insulin-sensitive obesity. American Journal of Physiology. Endocrinology and Metabolism 2010; 299: E506–E515.

27-**Lewis JG, Shand BI, Frampton CM, Elder PA & Scott RS.** Plasma retinol binding protein is not a marker of insulin resistance in overweight subjects: a three year longitudinal study. Clinical Biochemistry 2008; 41: 1034–1038.

28-**Tajtakova M, Petrasova D, Pidanicova A, Gallovicova A, Blanarova C & Petrovicova J.** Serum levels of leptin, adiponectin, retinol binding protein 4 and leptin/adiponectin molar ratio as another possible marker of insulin resistance in obese. Bratisl Lek Listy 2010; 111(4): 212-215.

29-**Graham TE, Wason CJ, Bluher M & Kahn BB.** Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetologia 2007; 50: 814-823.

30- **Alberto O, Dawn K, Subhash K, Cromack T, Franco F & Devjit T.** 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: 758-764.

31- **Promintzer M, Krebs M, Todoric J, Luger A, Bischof MG, Nowotny P, Wagner O, Esterbauer H & Anderwald C.** Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor.J Clin Endocrinol Metab 2007; 92: 4306-4312.

32- **Kiernan UA, Philips DA, Trenchevska O & Nedelkov D.** Quantitative mass spectrometry evaluation of human retinol binding protein 4 and related variants. PLoS One 2011 30; 6(3): 17-28.

33- **Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H,Nagaretani H, Matsuda M, Kondo H, Furuyama N, Kihara S, Nakamura T, Tochino Y, Funahashi T & Matsuzawa Y.** Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte derived protein. Diabetes 2002; 51: 2734–2741.

34- **Kim IK, Lee HJ, Kang JH & Song J.** Relationship of serum retinol-binding protein 4 with weight status and lipid profile among Korean children and adults. European Journal of Clinical Nutrition 2011; 65: 226–233.

35- **Kos K, Wong S, Tan B, Kerrigan D, Randeva H, Pinkney J & Wilding J.** Human RBP4 adipose tissue expression is gender specific and influenced by leptin. Clinical Endocrinology 2010; 74: 197–205.

36- **Santoro N, Perrone L, Cirillo G, Brienza C, Grandone A, Cresta N & Miraglia del GE.** Variations of retinol binding protein 4 levels are not associated with changes in insulin resistance during puberty. Journal of Endocrinological Investigation 2009; 32: 411–414.

37-**Abahusain MA, Wright J, Dickerson JW & deVol EB.** Retinol alphatocopherol and carotenoids in diabetes. Eur J Clin Nutr 1999; 53: 630-635.

 ISSN 2047-3745