ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study of Retinol and the Retinol Binding protein 4 in cases of overweight and obese Adolescents.

Medhat H. Shehata[1], Hamed A. Elkayat[2], Adel N. Hanna[3], Zakaria A. El-Khiyat[4], Iman H. Kamel[5], and Alyaa H Kamhawy[6].

[1]Professor of Pediatrics, Institute of Postgraduate Childhood Studies, Ain Shams University, Egypt.
[2]Professor of Pediatrics, Faculty of Medicine Ain Shams University, Egypt.
[3] Research Professor of child health, National Research Centre, Egypt.
[4]Research Professor Biochemistry, National Research Centre, Egypt.
[5]Assistant Research Professor of Child Health, National Research Centre, Egypt.
[6] Researcher of Child Health, National Research Centre, Egypt.

ABSTRACT

Obesity is a leading cause of morbidity and mortality worldwide and is known to arise from an imbalance between energy intake and expenditure. Study of Retinol ,the Retinol Binding protein 4 in cases of overweight and obese Adolescents to study the relationships between RBP4, insulin resistance and weight status in overweight and obese adolescents. This study is a case-control study included 88 children classified as forty five overweight and obese children and young adolescents attending nutrition Clinic, Children's Hospital, Ain shams University From January 2013 to November 2013. Forty three healthy children and young adolescents age and sex matched were included as control subjects. Serum retinol and RBP4 levels of obese and overweight group were higher than those of the control group. There was a positive correlation between fasting serum retinol,RBP4 and anthropometric and clinical data (weight SDS, BMI SDS, waist/hip ratio, systolic, and diastolic blood pressure), laboratory data (Fasting serum insulin, HOMA-IR, total cholesterol, LDL-c), body composition data (body fat percent, fat mass and fat free mass), RBP4 is positively correlated to serum insulin level, HOMA/IR, and lipid profile, so RBP4 can be used as a marker for insulin resistance and obesity. Retinol(vitamin A)concentration was positively associated with measures of obesity and that vitamins A have a role in lipid metabolism.

Keywords: Childhood obesity, Insulin resistance, RBP4, Retinol.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Childhood obesity is one of the most serious public health challenges of the 21st century. The problem is global and is steadily affecting many low- and middle-income countries, particularly in urban settings. The prevalence has increased at an alarming rate [19]. Childhood obesity is a major risk factor for chronic diseases and plays a central role in the insulin resistance The growing worldwide prevalence of type 2 diabetes mellitus in the young has contributed to increased prevalence of childhood obesity [30].

Overweight and obese children are likely to stay obese into adulthood and more likely to develop non-communicable diseases like diabetes and cardiovascular diseases, hyperlipidemia, liver and renal disease, and reproductive dysfunction at a younger age. Overweight and obesity, as well as their related diseases, are largely preventable. Prevention of childhood obesity therefore needs high priority [4].

In last years, white adipose tissue (WAT) has been considered as an endocrine organ because of its capacity to secrete hormones and cytokines.Thus, adipose tissue is not only known for its capacity to store the excess of dietary energy in the form of triglycerides, but also is now recognized as a fundamental participant in the control of energy metabolism by secreting many proteins called adipocytokines such as retinol binding protein 4 (RBP4), resistin, tumor necrosis factor a (TNF-a), interleukin 6 (IL-6), leptin, vaspin, visfatin, omentin, chemerin, apelin, etc. [33-40].

Retinol binding protein 4 (RBP4) is a recently identified adipokines suggested to link obesity with its comorbidities, especially insulin resistance, type 2 diabetes (T2D), and certain components of the metabolic syndrome [29].

Retinol Binding Protein 4 (RBP4) is considered to be the primary carrier of retinol to the tissues. RBP4 secretion in the liver is strongly influenced by the abundance of retinol. In the fasted state, vitamin A circulates primarily as retinol bound to RBP (*holo*-RBP) in approximately a 1:1 molar ratio, and the free RBP is termed as *apo*-RBP [2].

Retinol, the alcohol form of vitamin A, is an essential nutrient for growth, development, reproduction, and vision and is transported in plasma to target cells bound to a specific carrier protein, retinol-binding protein (RBP) [31].Retinol (vitA) regulates obesity, insulin resistance, inflammation, dyslipidemia, and hemostasis through its metabolites retinaldehyde (Rald) and retinoic acid (RA) produced in endogenous enzymatic reactions [24].

PATIENTS AND METHODS

This case-control study included 88 children classified as 45overweight and obese children compared to 43 healthy control who were recruited from the outpatient clinic of the Nutrition clinic Ain shams university from January 2013 to November 2013. This includes full personal, past history of systemic diseases, drug administration (as corticosteroids), and symptoms covering various systems, and family history of chronic non-communicable diseases (obesity, diabetes, cardiovascular diseases and hypertension).

All anthropometric measurements have been obtained using standardized equipment, and following the recommendations of the International Biological program [17].

Assessment of BMI was done using categories reported by the World Health Organization (WHO) Child Growth Charts Standards for age and sex [42]. Obesity considered when BMI exceeds 95th percentile while overweight considered when BMI exceeds 85th percentile [37].

Waist Circumference was measured using inelastic insertion tape to the nearest 0.1 cm, with the subject in a standing position; the tape was applied horizontally midway between the lowest rib margin and the iliac crest. Assessment of waist circumference was done using categories reported by [10]. Thorough medical general examination (head & neck, chest, heart, abdomen, upper &lower limbs) including measurement of blood pressure and comparing it to age specific blood pressure percentiles reported by[26].

Blood samples were withdrawn from patients and controls after overnight fasting (>12 hours). Fasting venous blood samples were collected in heparinized centrifuge tubes. Plasma was separated by centrifugation (3000 rpm, 15 min). Separated plasma aliquots were removed and stored frozen at - 32° C until further analyses were carried out, following tests were performed: Fasting serum glucose, fasting serum insulin, serum retinol,RBP4 level, Cholesterol, Triglyserides, HDL-cholesterol, LDL-cholesterol.

Insulin resistance was estimated by using the Homeostasis Model Assessment (HOMA), which calculated according to the known formula, Insulin resistance being defined as a HOMA index > 3.16). The greater the HOMA value the greater the level of insulin resistance [22].

Statistical analysis

Data management and analysis were performed using the Statistical Package for Social Sciences (SPSS) vs. 21.

Numerical data were summarized using means and standard deviations or medians and ranges. Categorical data were summarized as percentages. Comparisons between groups for normally distributed numeric variables were done using the Student's t-test while for non normally distributed numeric variables were done by Mann-Whitney test. Chi square test or Fisher's exact test were used to compare between the groups with respect to categorical data. To measure the strength of association between numeric variables, Spearman's correlation coefficients were computed. All p-values are two-sided. P-values < 0.05 were considered significant.

RESULTS

Comparing studied sample as regard their anthropometric measurements, as shown in table 1, mean weight was 85.1±19.1Kg in cases and 44.0±12.8Kg in control, mean BMI was 33.9±4.7in cases and 19.5±2.9 in control while the mean waist circumference was 102.3±14.3cm in cases and 69.2±9.4cm in control.

There was a significant difference in weight, BMI, Waist circumference, Hip circumference and waist hip ratio where p <0.001*, while there was no significant difference as the regard height measurement between cases and controls.

Table (1):Comparison of anthropometric data in obese children and control.

Parameter	Measures	Cases (N=45)	Control (N=43)	Case/ Cont
Weight (kg)	Mean±SD	85.1±19.1	44.0±12.8	<0.001*#
	Range	56.8–169.1	19.0–80.0	
Weight sds	Med (IQR)	2.7 (2.1–3.0)	3.3 (3.1–3.4)	<0.001*#
	Range	0.7–5.2	2.8–4.2	
Height (cm)	Mean±SD	156.2±9.8	156.7±11.2	^ 0.810
	Range	132.0–172.0	130.0–173.0	
Height sds	Med (IQR)	-0.6 (-1.4–0.1)	2.6 (2.5–2.8)	0.202 #
	Range	-2.6–1.3	2.0–3.2	
BMI	Mean±SD	33.9±4.7	19.5±2.9	<0.001*#
	Range	28.0–49.1	15.3–24.8	
BMI sds	Med (IQR)	3.0 (2.6–3.3)	0.1 (-1.0–1.1)	<0.001*#
WC (cm)	Mean±SD	102.3±14.3	69.2±9.4	<0.001*
	Range	80.0–155.0	54.0–86.0	
HC	Mean±SD	117.1±13.9	86.3±10.8	<0.001*

(cm)	Range	97.0–185.6	68.0–105.0	
WH ratio	Mean±SD	0.87±0.07	0.80±0.06	<0.001*
	Range	0.72–1.09	0.66–0.94	

* P value is significant if < 0.05
†BMI = Body Mass Index
$t^{\#}$: Independent t-test, **$\chi^{2\&}$:** Chi square test, *Significant at p **<0.001**

Table (2):Comparison of the laboratory parameters of cases and controls.

Parameter	Measures	Cases (N=45)	Control (N=43)	Case/ Cont
Cholesterol (mg/dL)	Mean±SD	249.4±88.7	183.5±54.0	0.010 *
	Range	105.9–503.5	58.6–288.2	
Triglycerides (mg/dL)	Mean±SD	243.8±34.7	64.4±37.9	<0.001*
	Range	196.0–325.0	13.5–158.6	
LDL-c (mg/dL)	Mean±SD	173.6±89.6	156.1±66.2	0.303
	Range	13.2–426.8	47.1–300.6	
HDL-c (mg/dL)	Mean±SD	27.1±8.5	36.5±9.7	<0.001*
SGOT (IU/L)	Mean±SD	11.8±4.7	11.4±3.8	0.625
	Range	4.0–24.0	4.0–19.0	
SGPT (IU/L)	Mean±SD	5.3±2.2	5.5±2.0	0.625
	Range	4.0–14.0	3.0–10.0	
Glucose (mg/dL)	Mean±SD	81.5±8.0	81.6±9.0	0.979
	Range	70.0–100.0	70.0–110.0	
Insulin (µIU/mL)	Mean±SD	16.4±7.5	9.0±4.5	<0.001*
	Range	3.5–40.6	1.1–21.5	
HOMA-IR	Mean±SD	5.9±2.7	3.3±1.7	<0.001*
	Range	11.0–14.4	0.4–7.6	

#Independent t-test, *Significant

Table (2)shows that: Cholesterol, Triglycerides were siginificantly higher incase group than in control group. HDL was siginificantly lower in case group than in the control group.No significant difference between the two groups regarding LDL-c. also Serum insulin and insulin resistence was siginificantly higher in case group than in the control group. No significant difference between study groups regarding serum SGOT, SGPT and glucose.

Table (3): Comparison between study groups regarding retinol and RBP4.

Parameter	Measures	Cases (N=45)	Control (N=43)	Case/ Cont
Retinol (µmol/L)	Mean±SD	49.1±13.4	33.7±16.9	<0.001*
	Range	22.9–76.5	10.0–69.7	
RBP4 (µmol/L)	Mean±SD	54.1±17.5	39.6±11.3	<0.001*
	Range	22.0–92.6	11.7–59.8	

#Independent t-test, *Significant

Table (3)shows that: Retinol and RBP4 were siginificantly higher in the obese group than in the control group

ISSN: 0975-8585

Table (4): Comparison between study groups regarding blood pressure.

Parameter	Measures	Cases (N=45)	Control (N=43)	Case/ Cont
SBP (mmHg)	Mean±SD	115.7±9.4	107.8±6.8	0.009*
	Range	100.0–130.0	90.0–120.0	
DBP (mmHg)	Mean±SD	73.8±6.5	68.6±5.2	0.005*
	Range	60.0–90.0	60.0–75.0	

#Independent t-test, *Significant

Table(4)shows that: SBP and DBP were siginificantly higher in the case group than in the control group.

Table (5): Correlation between, retinol and RBP4 in study groups.

	Cases		Control	
	r	p	r	p
	Retinol			
RBP4	0.908	**<0.001***	0.911	**<0.001***

r: Pearson correlation, *Significant

Table (5)shows that:There were significant positive correlations between retinol and RBP4 in the study groups.

Table (6): Correlation between Retinol and other variables in study groups.

	Cases		Control	
	r	p	r	p
Age	0.106	0.326	0.408	0.060
Tanner^	0.064	0.555	-0.004	0.978
Wtsds^	0.596	**<0.001***	0.406	**0.061**
Htsds^	0.181	**0.091**	0.092	0.685
BMIsds^	0.623	**<0.001***	0.765	**<0.001***
WHratio	0.405	**<0.001***	0.287	0.196
Cholesterol	0.488	**<0.001***	0.693	**<0.001***
Triglyc.	0.539	**<0.001***	0.645	**<0.001***
LDL	0.434	**0.032**	0.131	0.401
HDL	-0.537	**0.048**	-0.633	**0.013**
SGOT	0.023	0.828	0.223	0.319
SGPT	-0.025	0.818	0.123	0.584
Glucose	0.039	0.720	0.055	0.809
Insulin	0.521	**<0.001***	0.728	**<0.001***
HOMA-IR	0.512	**<0.001***	0.189	0.224
TBW %	-0.520	**<0.001***	-0.103	0.510
TBF %	0.540	**<0.001***	0.145	0.355
BMR	0.480	**<0.001***	0.183	0.414

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SBP	0.218	**0.041**	-0.129	0.566
DBP	0.190	**0.077**	-0.125	0.580

r: Pearson correlation, ^Spearman correlation, *Significant

Table (5) and figure (1) show that: There were significant positive correlations between retinol and weight sds ,height sds,BMI sds,WH ratio, cholesterol,triglycerides and TBF% insulin&HOMA-IR,SBP,DBP in the obese group. There were significant negative correlations between retinol and HDL and TBW% in the obese group.

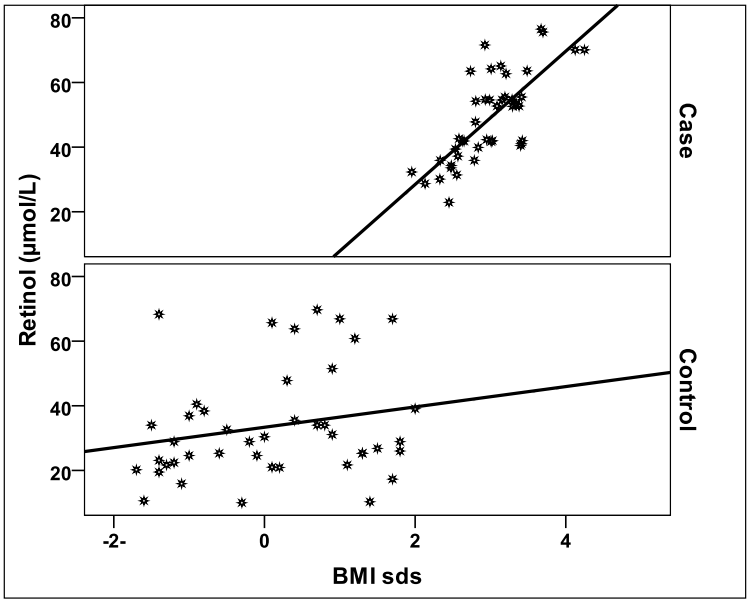

Figure (1): Correlation between Retinol and BMI sds

Table (6): Correlation between RBP4 and other variables in study groups.

	Case		Control	
	r	p	r	p
Age	0.209	**0.050**	0.017	0.916
Tanner^	0.104	0.335	0.026	0.870
Wtsds^	0.508	**<0.001***	0.237	0.127
Htsds^	0.158	0.141	0.209	0.179
BMIsds^	0.551	**<0.001***	0.268	**0.082**
WHratio	0.380	**<0.001***	0.221	0.154
Cholesterol	0.608	**<0.001***	0.187	0.229
Triglyc.	0.572	**<0.001***	0.166	0.289
LDL	0.555	**<0.001***	0.187	**<0.001***
HDL	-0.584	**<0.001***	-0.280	**0.069**
SGOT	0.065	0.550	0.017	0.912
SGPT	0.019	0.859	0.181	0.246
Glucose	-0.016	0.882	0.013	0.935
Insulin	0.559	**<0.001***	0.105	0.501
HOMA-IR	0.538	**<0.001***	0.114	0.466

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TBW %	-0.569	**<0.001***	-0.199	0.201
TBF %	0.562	**<0.001***	0.225	0.148
BMR	0.502	**<0.001***	0.198	0.204
SBP	0.198	**0.065**	-0.217	0.163
DBP	0.189	**0.078**	-0.258	0.095

r: Pearson correlation, ^Spearman correlation, *Significant

Table (6) and figure (2) show that: There were significant positive correlations between RBP4 and Wt sds,BMI sds,WH ratio cholesterol, triglycerides,LDL and TBF%, insulin& HOMA-IR SBP,DBP in the obese group. There were significant negative correlations between retinol and HDL and TBW% in the obese group

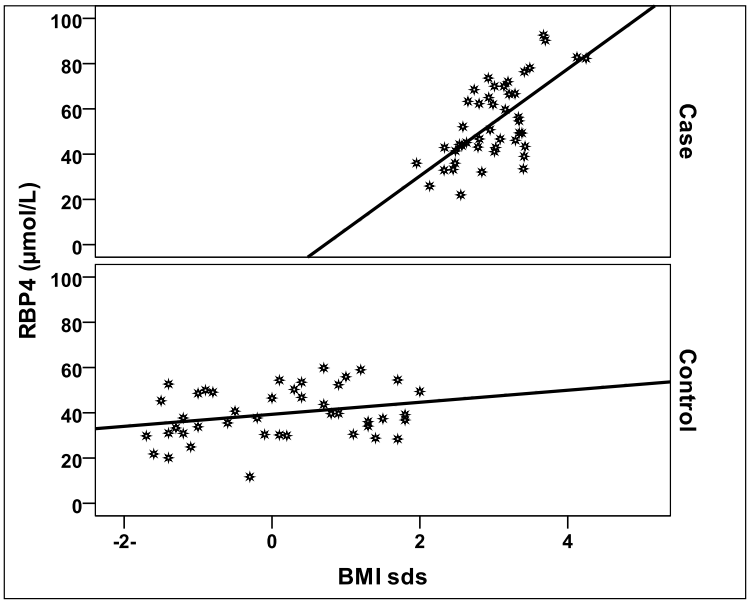

Figure (2): Correlation between RBP4 and BMI sds

DISCUSSION

In this study Waist circumference standard deviation (WC-SDs) in the case group is significantly higher than (102±14.3*Vs* 69.2±9.4) in the control group,Hip circumference standard deviation(HC-SDs) in the case group is significantly higher than (117.1±13.9 *Vs* 86.3±10.8) in the control group,WH ratio –SDs in the case group is significantly higher than (0.87±0.07 *VS 0.80±0.06)* in the control group. And this agree with the study of Young [43] on Korean obese children and adolescence.

In our study total cholesterol(P-value=0.010) ,TG(Pvalue=0.001) and HOMA-IR(Pvalue=0.001) were significantly increased in case group in comparison to controls . While HDL-c was significantly decreased in case group in comparison to controls (P value = 0.001). Our results were consistent with the findings of Himah [18]; who reported significant high triglycerides and low HDL-c in 53 obese children aged 10-12 years in comparison to 53 non-obese children. Also Friedland [11] reported significant high total cholesterol and triglycerides in 89 obese children in comparison to 53 non-obese children.AlsoReinehr [34] found significantly higher triglycerides, and lower HDL-c concentrations in obese children in comparison to non-obese children.

The findings in the present study revealed a high prevalence of high systolic and diastolic blood pressure in case group , these results were in the same range of results of [32-38-12] who found a considerable percentage of hypertensive obese children. Galhotra , stated that long-term epidemiological studies support the concept that atherosclerosis has its inception in childhood, and its risk factors, which accelerate it, originate in this age group. Attention should be given for monitoring blood pressure in obese and

overweight children(12).

In the present study fasting insulin and insulin resistance as expressed by HOMA is significantly increased in the case group than the control group,However this agrees with Kanaka-Gantenbein [21] who found that insulin resistance as expressed in the HOMA index, was significantly increased in the obese and morbidly obese individuals compared with the control individuals (*P* _ 0.04, and *P* _ 0.02,respectively). This relationship is similar to that found in adult populations [8-15].

In this study Serum retinol levels of case group were higher than those of a control group , The means and corresponding ranges of plasma concentrations of retinol were: 49.1±13.4 µmol/L, in the case group versus in the control group were: 33.7±16.9 µmol/L In our study serum retinol levels are positively correlated with BMI, WC,WH ratio,total cholesterol,triglyceride, LDL, this agrees with a study done by Olga [28] who found that Retinol (vitamin A) concentration was positively associated with BMI, BMI-for-age, waist circumference, waist/hip ratio and abdominal fat ($p < 0.05$) concentrations of total cholesterol, triglycerides and LDL were significantly and positively associated with vitamin A.Thus vitamin A concentration was positively associated with measures of obesity.This also agrees with a study in women from the same rural areas in Mexico, vitamin A was positively related to measures of obesity, but only on the lowest terciles of BMI (<30 kg/m^2), waist circumference (<84.6 cm) and body fat (<36%) [13]

In our study Retinol (Vitamins A) concentrations were positively related to triglycerides and total cholesterol concentration. Our results agree with those of Obeid [27] where higher vitamin A concentrations were associated with higher concentrations of total cholesterol, LDL and triglycerides in a Lebanese adult population.

These results differ from Viroonudomphol *et al.* [41], who found that low concentrations of vitamin A in Thai adults who were overweight and obese were associated with higher weight, BMI and hip circumference.

In our study, serum RBP4 levels of obese and overweight group were significantly higher than those of control group.The means and corresponding ranges of plasma concentrations of RBP4were: 54.1±17.5µmol/L in the case group versus in the control group were: 39.6±11.3µmol/L,in this study no statistical significant difference in RBP4 between both sexes , this agree with a study done by Young , on Sixty-one boys and forty-two girls between the ages of 6 and 18 yr were included in this study. There was no significant difference in serum RBP4 between male and female (54.94 ± 20.18 vs 49.67 ± 16.66 mg/L, *P* = 0.166)[43].

In our study RBP4 positively correlated with BMI, WH ratio , this agrees with a study done by Chin-Jung [7] on a total of 1082 adolescents were enrolled and categorized based on their body mass index (521 boys and 561 girls) with a mean (range) age of 13.7 (13-15) years were included in the final analyses. In this large adolescent population, we found that the RBP4 levels were positively correlated with most of the obesity indices.

This agrees with several previous studies in adolescents suggested that RBP4 levels had an important effect on obesity indices, as measured by BMI, [9- 14].WC, waist-to-hip ratio, and body fat percentage/ fat mass [36] also this is consistent with a study done by Santanam [35] who found that RBP4 (p=0.016) was significantly higher in obese children and were positively correlated with body mass index (p<0.001), BMI--SDS (Standard--Deviation Score) (p<0.001) and waist circumference (p=0.03).

This disagrees with other studies who suggested that in adults, RBP4 is associated with visceral fat amount rather than body weight or BMI.[20], also this disagree with studies in adults and adolescents by [36]who found that RBP4 levels were better correlated with WC (a rough proxy for visceral fat) rather than BMI. Another prospective study suggested that baseline RBP4 levels predict subsequent increase in WC in a Korean adolescent population [9].

In our study there is a significant positive correlation between RBP4 and HOMA-IR($r = 0.538$; $p < 0.001$),W\H ratio($r = 0.380$; $p < 0.001$) TG($r = 0.572$; $p < 0.001$) systolic($r = 0.198$; $p= 0.065$) and diastolic blood pressure($r = 0.189$; $p =0.078$) and inversely correlated with HDL cholesterol($r =- 0.584$; $p < 0.001$). This agrees

with the study done by Mehmet(25), in a study done on 148 nondiabetic pubertal obese subjects. who found that RBP4 concentrations were significantly directly correlated with HOMA-IR ($r = 0.653$; $p < 0.001$), followed by W\H ($r = 0.247$; $p < 0.001$), TG level ($r = 0.390$; $p < 0.001$), diastolic blood pressure ($r = 0.279$; $p < 0.001$) and systolic blood pressure ($r = 0.419$; $p < 0.001$), and were inversely correlated with HDL cholesterol ($r = -0.275$; $p < 0.001$)and this consistent with a study done by Ansar[3]in a case-control study on 73 obese and 90 non-obese participants were assessed RBP4 following an overnight fasting for RMR by means of indirect calorimetry. Circulating RBP4 level correlated positively with log insulin ($r=0.278$, $p=0.04$) in obese subjects. Our results agree with Choi[9] in a study done in obese Korean boys and also agree with Goodman[14] in a study done on overweight black adolescence who found that RBP4 positively correlates with HOMA-IR

Also this agrees with a study done by Chin-Jung[7] who found that RBP4 levels were positively correlated with TG in which RBP4 was positively associated with BP,[1-9] lipid profiles [23] .

This Agrees with Bobbert[5] found that circulating RBP4 levels were correlated positively with cholesterol, triglyceride, body mass index and waist circumference. Also, Circulating RBP4 levels were positively associated with increase intima media thickness. So, they suggested that RBP4 might be a possible predictor of atherosclerosis.

Also, Haider [16] found an association of increased circulating RBP4 levels with insulin resistance, and the metabolic syndrome. They also found that improving insulin sensitivity by interventions such as exercise training, lifestyle modification, or gastric banding surgery reduced serum RBP4 levels. This doesn’tagree with a cross sectional study byThiruvengadam [39] in a tertiary care children's hospital where in 98 obese children were included and their metabolic parameters analysed with regards to insulin resistance and RBP4 levels. High RBP4 levels were observed in 69.6 %.But there was no significant association between insulin resistance and RBP4 levels (p 0.8) detecting that RBP4, the sole retinol transporter in blood, secreted from adipocytes and liver has been implicated in insulin resistance. The index study however, did not show a significant positive association.

On the other hand, Broch[6] failed to establish an association of RBP4 levels with obesity, insulin resistance, type 2 diabetes or components of the metabolic syndrome.

CONCLUSION

RBP4 is positivity correlated to serum insulin level, HOMA/IR, and lipid profile, so RBP4 can be used as a marker for insulin resistance and obesity. Retinol (vitamin A) concentration was positively associated with measures of obesity and that vitamins A have a role on lipid metabolism

Recommendations

Further studies may be needed to detect whether RBP4 may be used to identify subgroups of individuals at risk for T2D or to early diagnose patients with insulin resistance and adipose tissue dysfunction

Further studies may be needed to detect the importance of retinol (vitamins A) in lipids metabolism, public health strategies should focus on the impact of vitamin A deficiencies on an abnormal lipid profile

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