ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Serum Retinol Binding Protein-4 to Probe Insulin Resistance and Atherogenic Dyslipidemia in Metabolic Syndrome Patients Following a Diet Therapy.

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ABSTRACT

The objective was to find a relationship between the metabolic syndrome (MetS) criteria (obesity, insulin resistance and serum lipid disorders) and the vitamin A-transport protein secreted by adipocytes known as retinol binding protein-4 (RBP-4), and to explores the role of using special form of the dietary therapy in women suffering from MetS. Seventy four obese volunteers women suffering from MetS, shared in this study which lasted for 2 months. Anthropometric measurements, biochemical variables and systemic RBP4 levels were determined to all participants in three separate visits. The results showed that the anthropometric parameters, the MetS criteria and the RBP-4 levels improved significantly after using the dietary therapy. Positive significant correlations were detected between RBP-4 and fasting blood glucose, triglycerides, atherogenic non-high density lipoprotein and insulin resistance at $p<0.001$.Based on these current data, the role of RBP-4 as an adipokine exerting metabolic effects on glucose and lipid metabolism in the MetS patients was confirmed; in addition usingspecial dietary therapy showed satisfactory healthy impact on both the MetS criteria and the serum RBP-4 levels.

Keywords: Metabolic syndrome, Retinol binding protein-4, Dietary therapy.

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ISSN: 0975-8585

INTRODUCTION

Retinol-binding protein-4 (RBP-4), a 21-kDa protein, has been described as a murine adipokine [1]. The RBP-4 is secreted primarily by the liver and adipocytes apparently linked to obesity and its co-morbidities in humans, especially insulin resistance, type 2 diabetes mellitus (T2D), and certain components of metabolic syndrome (MetS) [2,3]. Circulating RBP4 levels have been shown to rise and to positively correlate with body mass index (BMI) [2,4], and to be associated with insulin resistance [2,5], while there have been contrasting reports of similar RBP4 levels regardless of BMI and no significant correlation between RPB-4 and insulin resistance [6,7].

Dyslipidemia is known as a danger risk factor to atherosclerosis, what's more may be usually interfaced will insulin resistance, obesity, MetS and T2D [8]. The two real quantitative lipid abnormalities are abnormal triglyceride levels and low high density lipoprotein (HDL) levels. When higher proportion of small and dense low-density lipoprotein particles (sdLDL) are associated with high triglyceride levels and low HDL levels atherogenic dyslipidemia will be diagnosed [9]. This condition is a paramount marker of cardiovascular disease risk in patient complaining of obesity, insulin resistance, and MetS [10]. It has been proved that increase RBP-4 lead to endothelial inflammation and therefore may play a causative role in the progression of vascular inflammation during cardiovascular disease and microvascular complications of diabetes [11, 12].

Beta glucan (β-glucan) is a soluble fiber readily available from barly and oat grains that has several bioactive and functional properties. It has a positive effect in insulin resistance, dyslipidemia, hypertension, and obesity. The fermentability of β-glucans also their capability to structure profoundly viscous results in the human gut may constitute the basis of their health benefits. Consequently, the applicability of β-glucan as a food ingredient is being widely considered with the dual purposes of increasing the fiber content of food products and enhancing their health properties [13].

Aim

The objective of this study was to explore the possible involvement of the adipokine RBP-4 in the pathogenesis of the different metabolic disorders of the metabolic syndrome. In addition to using special dietary therapy with two slimming supplements, consisting of oat *(Avena Sativa)* flour mixed with two different wheat *(Triticuma Estivum)* grain extracts, in the treatment of the obese metabolic syndrome patients to clarify their effects on the different parameters including the RBP-4.

MATERIALS

Wheat grains (Giza 168) was purchased from Wheat Research Department, Field Research Institute, Agric. Res. Center, Giza, Egypt. Wheat grains (Giza 168) were cleaned, tempered (15% moisture) and milled (Quadrumat Junior flour mill) to 100 % extraction flour. Wheat flour 72% extraction was purchased from the North Cairo Flour Mills Company, Egypt. Oat flour was obtained from local shop, Dokki, Egypt. Skimmed milk, shortening, corn oil, baking powder, emulsifier, vanilla and bread improver were purchased from the local market, Dokki, Egypt. Basic and modified formulae were prepared by mixing Whole meal wheat flour (WMWF) 100 % extraction (biscuit 1) and wheat flour (WF) (72% extraction) (Biscuit 2) with oat flour (OF) at the levels of 50 % (of the flours' weight) with other ingredients according to **Table (1).** Then a suitable amount of water was added according to (AOAC, 2000) [14]. These formulae were baked in a special oven at 200 °C for about 15 minutes. Weight, volume, specific volume, diameter, thickness and spread ratio of the snacks were recorded.

Table 1: Composition of the two different biscuits (in g/ 100 g)

Items	biscuit (1)	biscuit (2)
WMWF	38.7	-
WF (72%)	-	38.7
OF	38.7	38.7
Corn oil	10.8	10.8
Skimmed milk	10.8	10.8
Baking powder	0.5	0.5
Flavors	0.5	0.5

WMWF: Whole meal wheat flour **WF:** Wheat flour **OF:** Oat flour

ISSN: 0975-8585

SUBJECTS AND METHODS

Subjects

Seventy four obese women suffering from MetS shared as volunteers in this study which lasted for 8 weeks. The study was divided into two phases, phase (1) and phase (2); each one lasted for 4 weeks. The patients were divided into two groups, group (1) with mean age of 48.24± 1.17 years and mean BMI of 38.53± 0.69 kg/m², and group (2) with mean age of 48.63±1.28 years and mean BMI of 39.11±1.01 kg/m². At phase (1), group (1) followed a low caloric balanced diet supply about 1000 K Calories/ day (21% from protein, 29% from fat and 48% from carbohydrate). The diet was supplemented by the 50% OF plus 50% WMWF biscuits (Biscuit 1), that was consumed before breakfast (2 biscuits) and before dinner (1biscuit), each biscuit weighing 20 g, while group (2) consumed the biscuits made from 72% WF (50%) and OF (50%) (Biscuit 2) according to the same protocol. Phase (2) lasted for 4 weeks in which the volunteers were following only the same low caloric balanced diet. All women were subjected to thorough clinical examination. Blood pressure was recorded. The protocol of the study was approved by the National Research Center Ethics Committee. In addition, informed consent was obtained from each participant to be included in the study.

Anthropometric parameters and blood pressure measurements

Relevant anthropometric measurements were recorded including height, weight and minimal waist circumference using standard methods [15]. BMI was calculated (weight in kg/ height² in meter). Blood pressure for each patient was measured 3 times and the mean was recorded.

Blood sampling and Biochemical analysis

Fasting blood samples (after 12 hour fasting) were drawn from the patients. Fasting blood glucose was determined on fresh samples; other biochemical parameters were performed on fasting sera that were stored at -70 C° until used. Fasting blood glucose (FBG) was determined in fresh samples using glucose oxidase method [16]. Serum total cholesterol (TC), High density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were done using; cholesterol proceed No 1010, Stanbio Liquicolor [17], HDL-C proceed No 0599 Stanio Liquicolor [18], and triglycerides proceed No 2100, (Enzymatic method) [19], respectively. Low density lipoprotein- cholesterol (LDL-C) was calculated according to Friedewald equation [20]. Non-HDL-C (TC-HDL-C) and risk factor (TC/HDL-C) were calculated. Serum C peptide was done by ELISA kit. PR. Code=2725-300A. Lot#EIA-27K2G1.Monobind, Inc. Lake Forest, CA (92630) USA [21]. Modified homeostatic model assessment of insulin resistance (M.HOMA-IR) was calculated, where M.HOMA-IR=1.5+fasting blood glucose x fasting c-peptide/2800 [22]. Retinol binding protein-4 was estimated using sandwich enzyme-linked immune-sorbent assay "The Assay Max Human RBP 4 ELISA" kitcatalog no. ER3005-1 provided by Assay Pro LLC, St. Charles, Missouri, USA [23].

Statistical Analysis

All values were expressed as mean value ±SE. Two tailed student t-test was used to compare between different phases in the same group. Correlation between the different parameters was tested by Pearson test. P values <0.05 were considered statistically significant. SPSS window software version 17.0 (SPSS Inc. Chicago, IL, USA, 2008) was used.

RESULTS

Table (2) shows the mean± SE of anthropometric parameters and blood pressure of obese women at the baseline and at the end of the two phases of the dietary therapy. All the anthropometric measurements of the two groups decreased significantly at p<0.05-0.01 at the end of phase (1). Significant reduction of all the anthropometric measurements reported in group (1) at the end of phase (2), blood pressure values decreased numerically. Patients of group (2) showed significant increase in the anthropometric measurement by the end of the second phase. Group (2) showed significant decrease in SBP and DBP by the end of the phase 1, while significant decrease in SBP only was detected by the end of phase 2.

ISSN: 0975-8585

Table 2: Mean± SE of anthropometric parameters and blood pressure of the two groups at the baseline and at the end of the two phases of the dietary therapy.

Parameters	Group (1) (no.=42)					Group (2) (no.=32)				
	Baseline (1st visit)	Mid (2nd visit)	Last (3rd visit)	Percent changes		Baseline (1st visit)	Mid (2nd visit)	Last (3rd visit)	Percent changes	
				B vs. M	M vs. L				B vs. M	M vs. L
Age (year)	48.24± 1.17					48.63±1.28				
Height (cm)	154.75±0.84					154.88±0.95				
Weight (Kg)	92.12± 1.59	89.01±1.49**a	85.49±1.71**b	-3.24	-3.94	93.13±2.05	90.04±1.79**a	90.37±2.48**b	-3.32	0.37
BMI (Kg/m^2)	38.53± 0.69	37.25±0.67**a	35.73±0.78**b	-3.32	4.08	39.11±1.01	37.80±0.89**a	38.19±1.19**b	-3.34	1.03
Body fat (%)	47.83±0.65	46.37±0.73**a	44.73±1.09**b	-3.05	-3.54	48.83±0.84	46.94±0.84**a	48.31±1.01	-3.87	2.92
MWC (cm)	97.12± 1.06	92.33±0.99**a	89.53±1.25**b	-4.93	-3.03	98.03±1.52	92.90±1.57**a	93.50±1.71	-5.23	0.65
Hip (cm)	121.21± 1.44	117.27±1.19**a	114.17±1.62**b	-3.25	-2.64	122.48±1.92	116.13±1.76**a	116.30±2.31**b	-5.19	0.14
WHR (cm/cm)	0.80±0.01	0.79±0.01**a	0.79±0.01	-1.25	0	0.80±0.01	0.80±0.01	0.81±0.01	0	1.25
SBP (mmHg)	120.24±2.80	116.43±1.79	114.64±2.80	-3.17	-1.54	130.86±3.28	125.36±2.73*a,	114.44±3.26**b	-4.20	-9.54
DBP (mmHg)	79.05±1.55	76.67±1.43	71.07±1.59	-3.01	-7.30	81.43±2.17	76.43±1.56*a	74.44±2.39	-6.14	-2.60

BMI: Body mass index, MWC: Minimal waist circumference, **WHR:** Waist hip ratio, **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure.
*P<0.05 **P<0.01 **a:** Base vs. Mid **b:** Mid vs. Last of the same group

Table 3: Mean± SE of the biochemical parameters of the two groups at the baseline and at the end of the two phases of the dietary therapy

Parameters	Group (1) (no.=42)					Group (2) (no.=32)				
	Baseline (1st visit)	Mid (2nd visit)	Last (3rd visit)	% change		Baseline (1st visit)	Mid (2nd visit)	Last (3rd visit)	% change	
				B vs. M	M vs. L				B vs. M	M vs. L
FBG (mg/dl)	95.42±2.84	79.87±1.72**a	88.34±2.99**b	-16.29	28.41	90.72±1.84	85.52±1.53**a	83.13±2.01	-5.73	2.80
TC (mg/dl)	230.08±8.15	183.58±5.64**a	186.70±6.90*b	-20.21	10.61	208.76±6.66*c	184.54±4.99**a	205.68±7.24**b	-13.13	11.46
LDL-C (mg/dl)	148.52±8.25	97.98±6.29**a	125.82±8.52**b	-34.09	1.70	130.39±7.34*c	101.47±5.00**a	125.85±8.88**b	-22.18	24.03
HDL-C (mg/dl)	50.03±1.57	61.41±1.94**a	57.04±1.70**b	22.75	-7.12	49.23±1.61	60.61±2.24**a	53.39±2.61**b	23.12	-11.91
Non HDL-C (mg/dl)	180.05±8.85	122.18±6.72**a	148.97±9.64**b	-32.14	21.93	159.52±7.59*c	123.93±4.91**a	152.28±8.62**b	-22.31	22.88
Risk factor	4.87±0.29	3.17±0.17**a	3.82±0.26**b	-34.91	20.51	4.43±0.23	3.13±0.11**a	4.05±0.27**b	-29.35	29.39
TG (mg/dl)	157.63±7.64	120.95±4.96**a	115.76±8.41	-23.27	-4.29	145.68±6.71	112.29±5.61**a	132.16±10.40	-22.92	17.70
C-peptide (ng/dl)	4.73±0.64	2.53±0.45**a	3.02±0.43	-46.51	19.37	5.08±0.56	2.13±0.28**a	2.65±0.42	-58.07	24.41
M.HOMA-IR	1.66±0.03	1.58±0.02**a	1.60±0.02**b	-4.82	1.27	1.67±0.18	1.57±0.01**a	1.58±0.01	-6.0	0.64
RBP-4 (ng/ml)	31.99±1.28	26.24±1.05**a	25.20±0.87**b	-17.97	-3.96	32.59±1.93	27.02±1.52**a	25.58±1.01	-17.09	-5.33

FBG: Fasting blood glucose, **TC:** Total cholesterol, **LDL-C:** Low density lipoprotein- cholesterol, **HDL-C:** High density lipoprotein cholesterol, **TG:** triglycerides, **M.HOMA-IR:** Modified homeostatic model assessment of insulin resistance, **RBP-4:** Retinol bounded protein-4
*P<0.05 **P<0.01 **a:** Baseline vs. Mid **b:** Mid vs. Last of the same group **c:** Baseline of group (1) vs. Baseline group (2).

Table 4: Correlation coefficient between retinol binding protein-4 and the most important anthropometric and biochemical parameters, among the whole sample at the baseline and at the end of the two phases of the dietary therapy

Parameters	Retinol Bounded Protein-4		
	Baseline	Mid	Last
Weight	-0.001**	-0.001**	-0.124
BMI	0.156	0.349	0.644
MWC	0.266	0.512	-0.733
SBP	0.703	-0.081	-0.086
DBP	0.610	-0.013*	-0.047*
FBG	0.009**	0.007**	0.058
TG	0.002**	0.003**	0.053
HDL-C	-0.000**	0.858	0.117
Non-HDL-C	0.001**	0.001**	0.068
C-peptide	0.142	0.009**	0.220
M.HOMA-IR	0.039*	0.005**	0.197

BMI: Body mass index, MWC: Minimal waist circumference, **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure. **FBG:** Fasting blood glucose, **TG:** triglycerides, **HDL-C:** High density lipoprotein cholesterol, **M.HOMA-IR:** Modified homeostatic model assessment of insulin resistance.

Table (3) shows the mean± SE of the biochemical parameters of obese women at the baseline and at the end of the two phases of the dietary therapy. At the end of phase (1) the FBG and the lipid profile levels decreased significantly at p< 0.01, the percent decrease was high in group (1) in case of TC, LDL-C and non HDL-C concentration, while equal significant increase was found in the level of HDL-C between the two groups. Significant decrease in the C-peptide concentration and M.HOMA-IR level were detected. At the end of phase (2) most of the mentioned parameters significantly increased in the two groups, with significant decrease in the HDL-C at <0.05-0.01. The mean of C-peptide numerically increased in both groups. The mean level of RBP-4 concentration showed high significant decrease at p<0.001 in group (1) in both phases, while in group (2) it was significantly decrease in phase (1) and numerically decrease in phase (2).

Table (4) shows the correlation coefficient between retinol binding protein-4 and the most important anthropometric and biochemical parameters, among the whole sample. At the baseline negative significant correlations were found between RBP-4 and both of the body weight and HDL-C, while positive significant correlations were detected between RBP-4 and FBG, TG, non HDL-C and M.HOMA-IR at p<0.001. At the end of the mid phase significant correlations were detected between RBP-4 and body weight at p<0.001, and with DPB at p< 0.05, while significant positive correlations were found between RBP-4 and FBG, TG, non HDL-C, C-peptide and M.HOMA-IR at p<0.001. At the end of phase (2) only significant negative correlation was found between RBP-4 and DBP at p< 0.05.

DISCUSSION

Data of this study showed the healthy beneficial effects of the two dietary therapies on both the anthropometric and biochemical parameters especially with group (1), which was clear in improvement of the level of both FBG and lipid profile. Among soluble fibers, β-glucan is the most frequently consumed and is associated with reduced presence of insulin resistance, dyslipidemia, hypertension, and obesity [13]. However, we have found that in spite of the decrease in the mean concentration of the circulating RBP-4 with the decrease in the mean values of the anthropometric parameters indicating obesity, yet no significant correlation between RBP-4, BMI and MWC could be reported. In this context, the circulating levels of RBP-4 in obese individuals may not be merely a consequence of excess adipose tissue. In contrast to the animal data, RBP-4 mRNA was down regulated in subcutaneous adipose tissue of obese women, and circulating RBP-4 concentrations were similar in normal weight, overweight, and obese women [6, 12].

In both baseline and mid assessments, our current data demonstrated high significant positive association between RBP-4 and the biochemical criteria of the metabolic syndrome namely FBG, triglycerides, non-HDL-C and M.HOMA-IR, and a negative significant association with HDL-C. Wolf [24], reported that insulin resistance occurs under conditions of obesity, metabolic syndrome, and T2D. It was found to be accompanied by down-regulation of the insulin-responsive glucose transporter-4 (GLUT-4). Decreased adipocyte GLUT-4 caused secretion of the serum RBP-4 by adipocytes. Enhanced levels of serum RBP-4 appeared to be the signal for the development of systemic insulin resistance both in experimental animals and in humans. Furthermore,

the author stated that in mice, increased levels of serum RBP-4 led to impaired glucose uptake into skeletal muscle and increased glucose production by liver. Increased serum RBP-4 levels induced hepatic expression of the gluconeogenic enzyme phosphor-enol-pyruvate carboxykinase and impaired insulin signaling in the muscle [2], whereas lowered serum RBP-4 levels greatly enhanced insulin sensitivity. Von-Eynatten et al. [7] stated that experimental clinical approaches in humans confirmed the correlations of RBP-4 with insulin resistance. Serum RBP-4 levels are increased in subjects with impaired glucose tolerance, T2D, and correlate inversely with insulin sensitivity in non-diabetic subjects with a family history of T2D [2, 25]. Circulating RBP-4 levels correlate with the degree of insulin resistance in these subjects and relationship is independent of obesity [4].

Data of this study revealed significant associations between RBP-4 and the atherogenic lipids mainly the triglycerides and the non- HDL-C which influence atherosclerosis and related complications. Many human studies have found a strong relationship between RBP-4 and triglycerides, as they show the possible involvement of RBP-4 in the lipid metabolism in obese patients with varying degrees of insulin resistance [4, 26], and others failing to do so [2, 28]. Given the basic function of RBP-4 as a retinol binding protein, it is reasonable to speculate that RBP-4 serves as a link between retinol metabolism and activation of nuclear receptors and may be implicated in the regulation of lipid homeostasis. Thus, retinoids and retinol-binding proteins can modulate lipid activities, such as the expression of several genes involved in hepatic and intestinal triglyceride production and secretion, the hepatic production of very low density lipoprotein (VLDL), and the regulation of Apo C-III production –a protein that delays the catabolism of VLDL particles- and β-oxidation [29]. In fact, Vergès et al. [28] had demonstrated that RBP-4 is involved in VLDL catabolism.

CONCLUSION

Based on these current data, the function of RBP-4 as an adipokine exerting metabolic effects on glucose and lipid metabolism in the MetS patients was confirmed. In addition, the effect of the two dietary therapies that were consumed by the patients proved to be beneficial in the fundamental therapeutic implications.

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