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Retinol-binding protein 4 in obese and obese-diabetic postmenopausal women in Montenegro

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ABSTRACT

Introduction: Menopause is associated with an increase in visceral fat and obesity is the leading risk factor for insulin resistance (IR) and type 2 diabetes mellitus (T2DM). Recent evidence suggests that retinol-binding protein 4 (RBP4) is not an independent determinant of IR and its role in human glucose metabolism is not well clarified. We examined RBP4 and its association with IR, cardiometabolic and kidney parameters in obese postmenopausal women with and without T2DM.

Methods: Basic anthropometric, biochemical parameters, and blood pressure (BP) were determined in 50 obese diabetic and 50 obese non-diabetic sedentary postmenopausal women, and compared with 50 healthy normal weight controls.

Results: Higher levels of RBP4 were observed in obese individuals, as compared with normal weight group (p=0.033). However, we did not find significant difference between obese non-diabetic and obese-diabetic individuals (p=0.583). Serum RBP4 did not correlate with anthropometric measurements or any indicator of glucose metabolism in diabetic group, whereas RBP4 correlated with creatinine (r=0.416, p=0.003), eGFR (r= -0.304, p=0.032) and triglycerides (r=0.484, p<0.001). In obese non-diabetic group, correlations were observed with fasting glucose (r=0.346, p=0.014), insulin (r=0.292, p=0.038), HOMA-IR (r=0.329 p=0.020), HbA1c (r=0.326, p=0.021), creatinine (r=0.399, p=0.004), eGFR (r= -0.389, p=0.005), HDL-c (r= -0.316, p=0.025), triglycerides (r=0.461, p<0.001), and systolic BP (r=0.286, p=0.044). In multiple regression analysis, triglycerides (Beta=0.302, p<0.001) and eGFR (Beta= -0.188, p=0.015) were independent predictors of RBP4.

Conclusions: Serum RBP4 is not increased in obese type 2 diabetic postmenopausal women, but is associated with triglycerides and eGFR independently of diabetes.

Keywords: retinol-binding protein 4; postmenopausal women; obesity; type 2 diabetes mellitus

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INTRODUCTION

Menopause per se is associated with an increase in total body fat and visceral fat (1). Recent study found 49% increase in abdominal fat and 22% increment of the subcutaneous fat in women in the postmenopausal stage, compared to premenopausal

women (2). Obesity is the leading risk factor for insulin resistance (IR) and type 2 diabetes mellitus (T2DM). Women with a body mass index (BMI) of 30 kg/m² have about 28 times greater risk of developing diabetes than do women of normal weight (3).

Although considered as a part of Mediterranean basin, epidemiological data in Montenegro reported that 55.1% adults were overweight or obese (4) whereas the prevalence of diabetes in adults was 10.1% (5). However, the International Diabetes Federation estimates that these figures are not exactly in line with national statistics. According to local research and analyses, the number of persons with "latent" diabetes is 2-3 times higher than the estimated number of people with diabetes (6).

Obesity is characterized by changes in adipocytokines and activation of low-grade inflammation (7). Retinol-binding protein 4 (RBP4) is a recently discovered adipokine, which is potentially associated with IR and obesity (8). However, some other studies indicated that it is more likely linked with renal function (9).

Recent evidence suggests that RBP4 is not an independent determinant of IR (10-13). However, discrepant results are reported in the literature, and its role in human glucose metabolism is not well clarified (13). The purpose of the current study was to examine the association of RBP4 with parameters of glycemic control and IR, cardiometabolic and renal parameters in obese non-diabetic and obese diabetic postmenopausal women in Montenegro.

METHODS

Study population

We enrolled a total of 150 sedentary postmenopausal women (mean age 57.2±5.9 years) who volunteered to participate in the study. Menopause was defined as the absence of menstrual bleeding for more than one year. The participants were divided into three groups: normal weight (n=50), obese (n=50) and obese diabetic ones (n=50). Participants were consecutively recruited in the study when seeking gynecologic healthcare in the Primary Health Care Center in Podgorica, in a period from October 2012 to June 2013. All the participants

completed a questionnaire including demographic characteristics, somatic illnesses, medications use, and lifestyle habits. Specific eligibility criteria included postmenopausal, sedentary (< 90 min of weekly exercise), normoalbuminuric women (urinary albumin excretion, UAE < 30mg/24h) with creatinine-based estimated glomerular filtration rate (eGFR) ≥ 60 ml/min/1.73 m², without acute inflammatory disease, CVD, urinary infection, with no history or the presence of malignancy, hypo- and hyperthyroidism. For non-diabetic groups inclusion criterion was also women who were not taking any medicament therapy. Participants who had kidney dysfunction (eGFR < 60ml/min/1.73m² or UAE ≥ 30mg/24h), Type 1 Diabetes Mellitus, morbid obesity (BMI) > 40 kg/m², hepatic dysfunction, pregnancy, alcohol abuse, were excluded from the study, as well as diabetic patients on insulin therapy and those on anti-inflammatory medications. For non-diabetic groups, exclusion criteria were also fasting glucose ≥ 6.1mmol/L, glycosylated hemoglobin (HbA1c) > 6% and any medication use.

Basic anthropometric measurements: body height (cm), body weight (kg) and waist circumference (WC) (cm) were recorded. Weight was measured to the nearest 0.1 kg on a balance beam scale, with the subjects barefoot and with light clothing. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, without shoes. Waist circumference was measured with the non-stretchable tape, over the unclothed abdomen at the midpoint between the lowest rib and the iliac crest. Measurements were made at the end of normal expiration. The tape was parallel to the floor and did not compress the skin. Body mass index was calculated as weight in kilograms divided by height in meters squared. Although our obese population consisted of slightly obese (overweight, 25 ≤ BMI < 30 kg/m²) and moderately obese (30 ≤ BMI < 40 kg/m²), for simplicity reasons, we referred to them as the obese participants. Blood pressure (BP) was measured with a sphygmomanometer after the subject had been seated for 15 minutes. Average of three measurements taken on the right arm was recorded. All measurements were taken by the same trained evaluator.

All the participants provided written informed consent. The study protocol was approved by the

Ethical Committee of Primary Health Care Center in Podgorica and the research was carried out in compliance with the Declaration of Helsinki.

Biochemical analyses

Serum samples were divided into aliquots and stored at -20º C, without prior thawing and re-freezing before analyses, except for fasting glucose which was determined immediately after blood was drawn. Another aliquot was collecting as a whole blood in K_2EDTA for determination of glycosylated hemoglobin (HbA1c). Serum levels of glucose, total cholesterol, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were measured spectrophotometrically using enzymatic procedures, while HbA1c and UAE were measured with immunoturbidimetric assay (Roche Cobas 400, Mannheim, Germany). RBP4 was determined using an immunonephelometric assay (Behring Nephelometer Analyzer, BN II, Marburg, Germany). Insulin was measured by chemiluminescent immunometric assay (AxSYM, Abbott, Abbott Park, Illinois, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated: HOMA-IR=Fasting glucose (mmol/L) x fasting insulin (µIU/L)/22.5. GFR was estimated using the using the Chronic Kidney Disease Epidemiology Collaboration Equation, CKD-EPI (14).

Statistical analysis

Statistical analyses were performed using SPSS statistical package (version 15.0 for Windows, SPSS, Chicago, IL, USA). Data are presented as mean ± standard deviation, or median (interquartile range), or as counts and percentages. Differences between groups were evaluated with ANOVA (for normally distributed data) or Kruskall-Wallis test (for non-normally distributed data). A correlation analysis by Pearson's correlation coefficient was used to determine the relationship between RBP4 levels and other variables. Due to skewed distribution, log transformed insulin, HOMA-IR, HbA1c, triglycerides and UAE were used. Multiple regression analysis was performed to identify independent factors affecting RBP4 and to estimate the final predictors of its variability. A p value of <0.05 was considered as statistically significant.

RESULTS

Table 1 shows general clinical and biochemical characteristics of the study participants divided into three groups: normal weight, obese and obese diabetic postmenopausal women. There was significant increase in waist circumference, insulin resistance and triglycerides, as well as in blood pressure, but decrease in HDL-c across the study groups (p<0.001). Moreover, serum RBP4 level was increased in obese groups as compared with the lean group (p=0.033). However, there was no difference in serum RBP4 levels between obese non-diabetic and obese-diabetic women (p=0.583).

The relationship between RBP4 and clinical and biochemical characteristics in obese non-diabetic and obese diabetic participants is presented in Table 2. Serum RBP4 did not correlate with anthropometric measurements or any indicator of glucose metabolism in diabetic group, whereas RBP4 correlated with creatinine (r=0.416, p=0.003), eGFR (r=-0.304, p=0.032) and triglycerides (r=0.484, p<0.001). In obese non-diabetic group, correlations were observed with fasting glucose (r=0.346, p=0.014), insulin (r=0.292, p=0.038), HOMA-IR (r=0.329 p=0.020), HbA1c (r=0.326, p=0.021), HDL-c (r=-0.316, p=0.025), triglycerides (r=0.461, p<0.001), systolic BP (r=0.286, p=0.044), creatinine (r=0.399, p=0.004), and eGFR (r=-0.389, p=0.005).

To determine which of the examined parameters were significantly associated with serum RBP4, we performed multiple linear regression analysis between RBP4 and all parameters in the whole group of obese participants. In multiple linear regression analysis only eGFR (Beta= -0.188, p= 0.015) and triglycerides (Beta=0.302, p<0.001) remained significant independent predictors of higher RBP4 levels (Table 3). These associations were independent of diabetes.

DISCUSSION

We reported higher levels of RBP4 in obese postmenopausal women, as compared with normal weight group. However, we did not find significant difference between obese non-diabetic and obese-diabetic women. Moreover, despite the observed correlation between RBP4 and glycemic control and IR

TABLE 1. Demographic and general characteristics of studied women

Variable	Normal weight (n=50)	Obese (n=50)	Obese diabetic (n=50)	p
Age (years)	56.4±4.89	57.7±6.34	58.3±5.98	0.240
BMI (kg/m²)	21.9±2.01	30.1±3.35[aa]	29.9±2.75[aa]	<0.001
WC (cm)	75.5±3.89	99.5±7.80[aa]	102.7±9.64[aa]	<0.001
Fasting glucose (mmol/L)	5.15±0.39	5.45±0.41[aa]	8.05±2.44[aa,bb]	<0.001
Fasting insulin (μIU/L)	4.92 (4.00-5.97)	9.02 (6.90-12.90)[aa]	10.70 (6.30-18.20)[aa]	<0.001
HOMA-IR	1.09 (0.98-1.34)	2.20 (1.66-3.09)[aa]	3.33 (2.23-6.87)[aa,bb]	<0.001
HbA1c (%)	4.95 (4.80-5.10)	5.50 (5.30-5.60)	6.70 (6.30-7.30)[aa,bb]	<0.001
TC (mmol/L)	6.39±0.98	6.29±1.16	6.13±1.07	0.470
HDL-c (mmol/L)	1.94±0.42	1.47±0.365[aa]	1.31±0.37[aa,bb]	<0.001
LDL-c (mmol/L)	4.09±0.95	4.15±1.24	3.73±0.94	0.098
TG (mmol/L)	1.06 (0.78-1.40)	1.46 (1.07-1.97)[aa]	1.81 (1.42-2.49)[aa,b]	<0.001
SBP (mm Hg)	120±26.1	145±15.0[aa]	145±15.9[aa]	<0.001
DBP (mm Hg)	79.3±15.83	91.6±9.81[aa]	89.6±13.62[aa]	<0.001
Creatinine (μmol/L)	52.8±6.06	60.3±10.15[aa]	56.8±12.17[aa]	0.001
eGFR (mL/min/1.73m²)	102 (97-107)	94 (90-103)[aa]	99 (95-104)[aa]	<0.001
UAE (mg/24h)	5.90 (4.85-7.48)	6.10 (4.91-8.32)	7.35 (4.96-10.46)[a]	0.055
RBP4 (μg/mL)	38.60±9.91	42.30±7.77[a]	43.30±10.23[a]	0.033
Antihypertensive drugs % (n)	0	0	86 (43)	-
Lipid lowering drugs (n) % (n)	0	0	72 (36)	-
Hypoglycemic drugs –metformin % (n)	0	0	100 (50)	-
Current smokers % (n)	0	10 (5)	14 (7)	-
Duration of diabetes (years)	-	-	4.11±0.93	-

Data are presented as mean±standard deviation or median (interquartile range), or counts and percentages; ANOVA (for normally distributed data) or Kruskall-Wallis test (for non-normally distributed data) were used for testing the difference between groups; BMI-Body mass index; WC-Waist circumference; HOMA-IR-Homeostasis model of insulin resistance; HbA1c-glycosilated hemoglobin; TC-total cholesterol; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG-Triglycerides, [aa]p<0.001 compared with normal weight group, [a]p<0.05 compared with normal weight group, [bb]p<0.001 compared with obese non-diabetic group, [b]p<0.05 compared with obese non-diabetic group

in obese non-diabetic participants, triglycerides and eGFR were found to be the best predictors of RBP4 levels.

Namely, the mechanism of the effect of diabetes on the RBP4 concentration remains unclear since discrepant results were obtained from many studies. Results in the present study are in concordance with results by An et al. (15) who did not find the difference in serum RBP4 concentration between postmenopausal women with T2DM and healthy postmenopausal control, after adjustment for age and BMI. They also demonstrated that serum RBP4 levels even had no correlation with the prevalence of T2DM in postmenopausal women like some previous studies, which have failed to report the involvement of RBP4 in the development of T2DM in humans (16,17).

Comucci et al. (18) also did not find the difference between obese and obese-diabetic participants, but unexpectedly, they reported lower RBP4 levels in obese and obese diabetic subjects compared to control, lean group. On the contrary, higher levels of RBP4 in obese diabetic subjects as compared with obese non-diabetic ones were reported by some other colleagues (19), showing that serum RBP4 levels were highly associated with IR (20) and were increased in diabetic patients (21,22).

Lack of significant difference in serum RBP4 between obese non-diabetic and obese-diabetic women found in our present study may partially be explained by the fact that diabetic participants were not able to exclude metformin, which may underestimate RBP4 levels. Moreover, antihypertensive medications were not excluded in diabetic

TABLE 2. Pearson's correlation coefficients (r) of RBP4 and studied parameters among obese non-diabetic and obese diabetic postmenopausal women

Variable	Obese (n=50)		Obese diabetic (n=50)	
	r	p	r	p
Age (years)	0.180	0.212	−0.022	0.879
BMI (kg/m²)	−0.055	0.706	0.070	0.630
WC (cm)	0.056	0.699	−0.137	0.343
Fasting glucose (mmol/L)	0.346	0.014	0.092	0.525
Log insulin (μIU/L)	0.294	0.038	0.016	0.910
Log HOMA-IR	0.329	0.020	0.083	0.568
Log HbA1c (%)	0.326	0.021	−0.114	0.430
TC (mmol/L)	0.232	0.104	0.179	0.215
HDL-c (mmol/L)	−0.316	0.025	−0.107	0.459
LDL-c (mmol/L)	0.191	0.183	0.218	0.127
Log TG (mmol/L)	0.461	<0.001	0.484	<0.001
SBP (mm Hg)	0.286	0.044	0.180	0.211
DBP (mm Hg)	0.188	0.191	0.117	0.416
Creatinine (μmol/L)	0.399	0.004	0.416	0.003
eGFR (mL/min/1.73 m²)	−0.389	0.005	−0.304	0.032
Log UAE (mg/24 h)	0.134	0.352	0.049	0.735

BMI-Body mass index; WC-Waist circumference; log HOMA-IR-Logarithmically transformed Homeostasis model assessment of insulin resistance; log HbA1c-Logarithmically transformed glycosylated hemoglobin; TC-Total cholesterol; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; log TG-Logarithmically transformed triglycerides; SBP-Systolic blood pressure; DBP-Diastolic blood pressure; eGFR-Estimated glomerular filtration rate; log UAE- Logarithmically transformed urinary albumin excretion

TABLE 3. Multiple regression analysis with RBP4 as dependent variable in all obese women

Independent variable	B	Std Beta	p
logHOMA-IR	0.952	0.111	0.166
logTG	13.637	0.302	<0.001
eGFR	−0.225	−0.188	0.015
SBP	0.058	0.150	0.089

RBP4-retinol-binding protein 4; LogHOMA-IR-Logarithmically transformed homeostasis model assessment of insulin resistance; LogTG-Logarithmically transformed triglycerides; eGFR-Estimated glomerular filtration rate; SBP-Systolic blood pressure

individuals, which can also influence serum RBP4 levels (23, 24) and IR (25-27). Nevertheless, An et al. (15) also found insignificant difference in serum RBP4 between newly diagnosed T2DM postmenopausal women who were not under any medicament therapy and healthy postmenopausal control. On the other hand, our results are discrepant with Shaker and colleagues (28), who reported higher plasma RBP4 levels in obese T2DM even with the treatment with oral hypoglycemic agents. However, this elevation may partially be due to longer duration of diabetes and potential renal impairment in diabetic individuals, since they did not take into account renal function in their study. Higher RBP4 levels in T2DM were also reported by Takebayashi et al. (21), but these patients had higher BMI as compared to the non-diabetic group, which may contribute the higher RBP4 levels in diabetic individuals, too. Their participants also had longer duration of diabetes than our study participants. As far as the current study is concerned, we included in our study only patients with eGFR ≥60 mL/min/1.73 m2, and did not record significant difference in BMI and eGFR between obese diabetic and obese non-diabetic group.

Furthermore, in the current study we did not record significant correlation between RBP4 and anthropometric parameters in obese individuals. This may be explained by the fact that we did not use the precise imaging techniques to measure adipose tissue depots, but we took simple anthropometric measurements, like the majority of the previous studies. Indeed, Lee et al. (29) showed that obese subjects without visceral adiposity had lower serum RBP4 levels than lean subjects with visceral adiposity. As our study groups were consisted of only postmenopausal women, and the postmenopausal status is characterized with adipose tissue distribution towards visceral one (2), central obesity as measured by WC seemed to be better indicator of obesity, rather than the overall one, as measured by BMI. Namely, visceral adipose tissue is a source of variety adipocitokines which reach the liver in high concentrations via the portal vein and directly impacts on hepatic insulin sensitivity which progressively increases hepatic and adipose-tissue insulin resistance (7).

However, the association of RBP4 with HOMA-IR and glycemic control disappeared after multiple regression analysis, whereas only eGFR and triglycerides remained independent predictors of RBP4. It is in accordance with some other studies (30), thus suggesting that previously reported associations between RBP4 and insulin resistance

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may be influenced by triglycerides and free fatty acid serum concentrations.

RBP4 independently correlated with triglycerides even in lean and healthy adults, as shown by Ebert et al. (31).

Sun et al. (32) found a significant positive association between plasma RBP4 concentrations and risk of developing T2DM after 6-y follow-up. They also observed that incidence of type 2 diabetes was elevated in the top category of RBP4 regardless of the amounts of HOMA-IR, suggesting that RBP4 may increase the risk of T2DM through pathway(s) not largely overlapping with insulin resistance.

The results on the associations of RBP4 with markers of kidney function were also observed in some previous studies (9), indicating that kidney dysfunction might be determinant of higher RBP4 levels. This suggests the necessity of taking into account renal function when examining RBP4 in obesity and diabetes, knowing that kidneys play an important role in retinol homeostasis, which is regulated by glomerular filtration and subsequent reabsorption of RBP4 into the proximal tubular cells (33).

Our study has some limitations. Due to its cross-sectional design, the causal relationship between serum RBP4 and examined parameters in postmenopausal women could not be established. Furthermore, as our study was based on small sample size, selection bias might have affected the outcome of the study. Thus, larger sample size in general population may be required to confirm our results. Also, diabetic postmenopausal women were not asked to stop taking their medications, such as antihypertensive, lipid-lowering drugs and metformin which might affect the outcome of our study.

CONCLUSION

We reported higher levels of RBP4 in obese postmenopausal women, as compared with normal weight group. However, serum RBP4 was not increased in obese type 2 diabetic postmenopausal women as compared with obese non-diabetic women, but is associated with eGFR and triglycerides independently of diabetes. Prospective studies and further analyses are needed to clarify the underlying mechanism of the association between RBP4, obesity and T2DM.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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