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MENOPAUZA KAO NEZAVISNI PREDIKTOR POVIŠENIH VREDNOSTI RETINOL-VEZUJUĆEG PROTEINA 4 U SERUMU

MENOPAUSAL STATUS AS AN INDEPENDENT PREDICTOR OF HIGH SERUM RETINOL-BINDING PROTEIN 4 LEVELS

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Sažetak: Cilj: Retinol-vezujući protein 4 (RBP4) je novi adipokin, usko povezan sa insulinskom rezistencijom. Međutim, nema dovoljno podataka u literaturi o uticaju menopauze na vrednosti ovog proteina u serumu. Zato je cilj ove studije bio da se ispita da li je povezanost menopauze i RBP4 nezavisna ili je posredovana insulinskom rezistencijom. Metode: Ukupno 30 žena u premenopauzi i 100 žena u postmenopauzi, koje nisu na terapiji su uključene u studiju preseka. Mereni su antropometrijski i biohemijski parametri, kao i krvni pritisak (KP), HOMA indeksi, procenjena jačina glomerularne filtracije (JGF) i izračunati su. Rezultati: Kod žena u postmenopauzi zabeležene su veće vrednosti RBP4 i nepovoljniji kardiometabolički profil, u poređenju sa ženama u premenopauzi. Višestruka linearnaregresiona analiza je pokazala da su više vrednosti triglicerida (beta=0,315; p=0,002), smanjena JGF (beta=-0,258; p=0,004), više vrednosti sistolnog KP (beta=0,418; p=0,028), i menopauza (beta=0,240; p=0,016), nezavisni prediktori povišenih vrednosti RBP4 u serumu (R2-adjusted=0,310; F=6,522; p<0,001). Zaključak: Menopauza utiče na vrednosti RBP4 u serumu, što treba uzeti u obzir prilikom ispitivanja uloge ovog adipokina u pojavi kardiometaboličkih poremećaja.
Ključne reči: adipokini, insulinska rezistencija, gojaznost, postmenopauza, retinol-vezujući protein 4

Abstract: Aim: Retinol-binding protein 4 (RBP4) is a novel adipokine closely related to insulin resistance. However, data on the influence of menopausal status on serum RBP4 are scarce. Therefore, the aim of the current study was to examine whether RBP4 levels are associated with menopausal status per se, independently of insulin resistance. Methods: A total of 30 premenopausal and 100 postmenopausal women non-treated with medications were included in the cross-sectional study. Anthropometric and biochemical parameters, as well as blood pressure (BP) were obtained. The homeostasis model assessment of insulin resistance (HOMA-IR) and estimated glomerular filtration rate (eGFR) were calculated. Results: Postmenopausal women displayed higher RBP4 and an unfavorable cardiometabolic profile, compared to premenopausal ones. Multiple linear regression analysis showed that in addition to high triglycerides level (beta=0.315; p=0.002), decreased eGFR (beta=-0.258; p=0.004) and high systolic BP (beta=0.418; p=0.028), menopause per se is an independent predictor of higher RBP4 levels (beta=0.240; p=0.016), (R2-adjusted=0.310; F=6,522; p<0.001). Conclusions: Serum RBP4 levels are dependent of menopausal status, which should be taken into account when examining the role of this adipokine in cardiometabolic diseases' occurrence.
Keywords: adipokines, insulin resistance, obesity, postmenopausal, retinol-binding protein 4

INTRODUCTION

Women at reproductive age are at a lower risk of obesity-related comorbidities, compared with postmenopausal women [1]. However, a plethora of metabolic disturbances accompanied with an increased visceral obesity, such as dyslipidemia, insulin resistance, hypertension are observed in women after menopause [2-5].

Having in mind that adipose tissue redistributionhas been increasing towards

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visceral region, along with an increased adipokines secretion, it is speculated that postmenopausal women display higher level of adipokines than premenopausal ones [6-9]. Many of these adipokines may impair insulin signaling leading to metabolic disorders, making them as one of the major culprits for diabetes mellitus type 2 and cardiovascular diseases [6, 10, 11].

One such adipokine is retinol-binding protein 4 (RBP4), which has been widely studied in the recent years [10-12]. Although primarily secreted by the liver, adipose tissue also represents the highest expression of this protein [6]. A majority of studies confirmed an independent relationship between RBP4 and cardiometabolic states closely related to insulin resistance (IR) [9, 10, 12, 13], thus suggesting obesity-induced IR as a hallmark of increased RBP4 levels.The proposed mechanism of such relationship may act through down-regulation of the insulin responsive glucose transporter-4, which represents the trigger for RBP4 adipocytes secretion [6, 11].

Additionally, a sexual dimorphism is typical for RBP4 secretion, which can be partly explained by the influence of sex hormones and indirectly, by body fat distribution [14].

On the other hand, _our knowledge about the influence of menopausal status on RBP4 is scarce and the underlying mechanism of this relationship is not well elucidated. In line with this, the aim of the current study was to examine whether RBP4 levels are associated with menopausal status, independent of insulin resistance.

MATERIALS AND METHODS
Study population
The current research derived from our previous studies examining the utility of inflammation and metabolic markers in postmenopausal women [1-5, 11, 15, 16].

A total of 30 premenopausal and 100 postmenopausal women non-treated with medications were included in the current cross-sectional study. All women volunteered for biochemical analyses check-up in the Center of Laboratory Diagnostics of the Primary Health Care Center in Podgorica, Montenegro, in a period from October 2012 to May 2013. Women were considered to be premenopausal if they self-reported regular menstrual cycle, while the self-reported absence of menstrual bleeding for

more than one year was the criterion for postmenopausal status.

Inclusion criteria for participants to enter the study were: premenopausal and postmenopausal otherwise healthy women, with no signs and symptoms of acute inflammatory disease.

Exclusion criteria were: diabetes mellitus, hypothyroidism or hyperthyroidism, liver disease other than steatosis, renal dysfunction, cardiovascular disorders, malignant diseases, high sensitivity C-reactive protein (hsCRP) >10 mg/L, smoking, hormone replacement therapy or any other medicament therapy used in the last six months.

All women that entered the study provided written informed consent. The research was carried out in compliance with the Declaration of Helsinki, and with approval of the Ethical Committee of Primary Health Care Center in Podgorica, Montenegro.

Anthropometric measurements
Basic anthropometric measurements, such as waist circumference (WC) and body mass index (BMI) were obtained, as described previously [3].

Biochemical analyses
Blood samples were taken and biochemical parameterswere measured after an overnight fast of at least 8 hours, as described previously [3]. Serum levels of glucose, creatinine, and lipid parameters [e.g., total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), triglycerides (TG)], were determined spectrophotometrically (Roche Cobas 400, Mannheim, Germany). Levels of hsCRP were determined using an immunonephelometric assay (Behring Nephelometer Analyzer, BN II, Marburg, Germany).Insulin concentration was determined by chemiluminescent assay (Immulite 2000, Siemens, Muenchen, Germany). HOMA-IR and estimated glomerular filtration rate (eGFR) were calculated, as described elsewhere [2, 11].

Blood pressure was measured as described elsewhere [2].

Statistical analysis
Testing distributions of examined variables were performed by Kolmogorov-Smirnov test. Data were shown as a

mean±standard deviation for normally distributed variables. Log-normally distributed variables were presented as geometric mean (95% Confidence Interval) [17]. A comparison of normal and log-normal continuous variables were done by Student's t-test. Skewed distributed data were given as median (interquartile range) and compared by Mann-Whitney test. Spearman's correlation analysis was used to estimate correlations between the examined parameters in pre- and postmenopausal women. Data from correlation analysis were presented as coefficient correlation, rho (ρ). If probability values (p) for ρ were less than 0.1, those variables (independent) were tested in further multiple linear regression analysis. As well, skewed distributed data were not included as independent variables. Multiple linear regression analysis was performed to estimate the independent contribution of clinical parameters, and presence of menopausal status on RBP4 level. Categorical data referring to menopausal status were included in the Model and coded as 1-premenopausal status and 2-postmenopausal status. The F-ratio of the ANOVA test in multiple linear regression analysis was used to determine whether the overall regression model is a good fit of the data. Multicollinearity among independent variables was also tested. Statistical analyses were performed using PASW® Statistic version 18 (Chicago, Illinois, USA) and MedCalc version 15.8 softwares. All statistical tests were considered significant when p was less than 0.05.

RESULTS

Baseline clinical and laboratorycharacteristics according to menopausal status are summarized in Table 1. Postmenopausal women were older and displayed higher DBP than premenopausal. There were none significant differences in BMI, WC and SBP between tested groups (Table 1).

Furthermore, postmenopausal women had higher TC, LDL-c, TG concentrations and HOMA-IR than premenopausal women (Table 1). Also, statistically higher levels of RBP4 were determined in postmenopausal women. Estimated GFR was significantly lower in postmenopausal women, but as it can be seen from the Table 1, both groups of women had preserved kidney function.

Table 1. Baseline clinical and laboratory characteristics of women according to menopausal status
Tabela 1. Klinički i laboratorijski parametri ispitanica u odnosu na menopauzu

	Premenopausal women	Postmenopausal women	p
N	30	100	
Age, years	47.56 (46.74-48.39)	56.52 (55.68-57.38)	<0.001
BMI, kg/m2	25.52 (23.49-27.72)	26.41 (25.65-27.19)	0.342
WC, cm	85.40 (79.78-91.43)	89.25 (87.12-91.43)	0.146
SBP, mmHg	125.00 (118.00-132.00)	128.00 (124.00-134.00)	0.480
DBP, mmHg	76.00 (73.00-81.00)	83.00 (80.00-86.00)	0.021
HDL-c, mmol/L	1.68±0.42	1.66±0.42	0.820
LDL-c, mmol/L	3.59 ±1.17	4.41 ±1.05	<0.001
TG, mmol/L	1.09 (0.94-1.26)	1.37 (1.26-1.49)	0.015
Glucose, mmol/L*	5.40 (5.10-5.80)	5.30 (5.00-5.70)	0.606
Insulin, μIU/L**	6.40 (5.05-8.10)	6.71 (6.13-7.35)	0.668
HOMA-IR**	1.53 (1.20-1.97)	1.61 (1.46-1.77)	0.001
Creatinine, μmol/L*	56.50 (53.36-59.57)	57.00 (51.00-62.00)	0.861
eGFR, mL/min/1.73 m^2**	105.65 (100.58-10.97)	99.72 (98.50-100.96)	0.001
HsCRP, mg/L**	0.80 (0.52-1.23)	0.99 (0.83-1.18)	0.311
RBP4, mg/L*	34.47 (35.16-39.94)	43.24 (41.95-44.58)	<0.001

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Data are presented as an arithmetic mean ± SD and compared to Student's t-test
*Skewed distributed data are presented as median (interquartile range) and compared with the Mann-Whitney U test
**Log-normal distributed data are presented as geometric mean (95% CI) and compared with Student's t-test after logarithmic transformation
BMI-Body mass index; WC-Waist circumference; SBP-Systolic blood pressure; DBP-Diastolic blood pressure; TC-Total cholesterol; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG-Triglycerides; HOMA-IR-Homeostasis model assessment of insulin resistance; eGFR-Estimated glomerular filtration rate; hsCRP-High-sensitivity C-reactive protein; RBP4-Retinol-binding protein 4

Associations of examined parameters with RBP4 were tested with Spearman's correlation analysis. Significant positive correlations were determined between RBP4 and age, BMI, WC, SBP, DBP, TC, LDL-c, TG, glucose, insulin, HOMA-IR, creatinine (Table 2). On the contrary, RBP4 significantly negatively correlated with HDL-c and eGFR.

Table 2. Spearman's correlation analysis between RBP4 and clinical and laboratory parameters in all women
Tabela 2. Spirmanova korelacija između RBP4, kliničkih i laboratorijskih parametara u celoj grupi ispitanica

	RBP4, mg/L	
	ρ	p
Age, years	0.255	0.002
BMI, kg/m2	0.252	0.002
WC, cm	0.255	0.002
SBP, mmHg	0.389	<0.001
DBP, mmHg	0.381	<0.001
TC, mmol/L	0.212	0.009
HDL-c, mmol/L	-0.290	<0.001
LDL-c, mmol/L	0.256	0.002
TG, mmol/L	0.452	<0.001
Glucose, mmol/L	0.215	0.008
Insulin, μIU/L	0.165	0.041
HOMA-IR	0.188	0.022
Creatinine, μmol/L	0.250	0.002
eGFR, mL/min/1.73 m^2	-0.361	<0.001
HsCRP, mg/L	0.081	0.324

Data are presented as correlation coefficient Rho (ρ)
BMI-Body mass index; WC-Waist circumference; SBP-Systolic blood pressure; DBP-Diastolic blood pressure; TC-Total cholesterol; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG-Triglycerides; HOMA-IR-Homeostasis model assessment of insulin resistance; eGFR-Estimated glomerular filtration rate; hsCRP-High-sensitivity C-reactive protein; RBP4-Retinol-binding protein 4

Further statistical testing included multiple linear regression analysis in order to identify the demographic and clinical parameters independently associated with RBP4 (Table 3). Independent variables that correlated with RBP4 levels in Spearman's correlation analysis with the significance of p<0.1 (Table 2) and which were not skewed distributed after tested by Kolmogorov-Smirnov test, entered into the Model. Although, insulin concentration was used in HOMA-IR calculation, it was not included into the Model. Also, because of glucose and WC

skewed distributions, these parameters were not included into the Model. According to the ANOVA test of multiple linear regression analysis, tested independent variables statistically significantly predicted RBP4 concentration, F=6,522, p<0.001. This also demonstrated the Model is a good fit of the data. An adjusted R2=0.310 for the Model demonstrated that 31% variation in RBP4 concentration could be explained by this Model. SBP was independently associated with an increase in RBP4 levels (β=0.418, p=0.028), as well as TG levels (β=0.315, p=0.002). On the other hand, eGFR was independently associated with decrease in RBP4 levels (β= - 0.258, p=0.004). The independent positive association of menopausal status and RBP4 concentration, with standardized coefficient β=0.240, p=0.016 was of the greatest importance.The multiple regression unstandardized coefficient (B), its standard error (SE), standardized coefficient (β) and p levels are presented in Table 3.

Table 3.Multiple linear regression analysis of the association of RBP4 with examined parameters
Tabela 3. Višestruka linearna regresija povezanosti RBP4 sa ostalim ispitivanim parametrima

Predictors	Unstandardized		Standardized coefficient	
	B	Standard error	β	p
Age, years	-0.155	0.175	-0.091	0.376
BMI, kg/m2	-0.073	0.096	-0.072	0.446
SBP, mmHg	0.385	0.174	0.418	0.028
DBP, mmHg	-0.299	0.199	-0.291	0.135
HDL-c, mmol/L	-0.004	0.018	-0.020	0.843
LDL-c, mmol/L	0.002	0.005	0.024	0.760
TG, mmol/L	0.123	0.040	0.315	0.002
HOMA-IR	-0.007	0.029	-0.021	0.817
eGFR, mL/min/1.73 m^2	-0.536	0.181	-0.258	0.004
Menopausal status	0.047	0.019	0.240	0.016

BMI-Body mass index; SBP-Systolic blood pressure; DBP-Diastolic blood pressure; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG-Triglycerides; HOMA-IR-Homeostasis model assessment of insulin resistance; eGFR-Estimated glomerular filtration rate; RBP4-Retinol-binding protein 4

DISCUSSION

The main result of our study is that menopause per se has an independent influence on higher RBP4 levels (Table 3). Also, postmenopausal women in the current study displayed significantly higher levels of this adipokine than premenopausal counterparts (Table 1). Similarly, some previous researchers also reported higher RBP4 in postmenopausal women [7-9].

The higher RBP4 concentration in postmenopausal women in our study may in part be explained by estrogen deficiency. In line with this, an inverse relationship between serum RBP4 and estradiol levels in a cohort of obese women, was recently reported [18]. The indirect effect of estrogen may be exerted through the influenceof the body fat distribution on serum RBP4. Estrogen levels are related to reduced fat storage and increasedenergy expenditure[14]. In line with that, it was shown that ovariectomy was accompanied with an increase weight gain in response to a high-fat diet in female mice in comparison with mice on a normal-fat diet [14]. On the contrary, supplementation of 17-β estradiol (E2) had no effect on male mice, but only on female mice [19], suggesting sex difference in body fat distribution, mainly due to estrogen influence.

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In addition, animal and human studies showed that lack of endogenous estrogen production leads to IR [14]. Furthermore, E2 replacement therapy in postmenopausal women is related to enhanced insulin signaling through suppression of lipolysis [20]. Namely, it is well established that obesity is accompanied with increased IR and unfavorable lipid profile [15]. Although in the current study there were no significant difference in anthropometric parameters between pre- and postmenopausal women, the latter ones showed higher HOMA-IR, TC, LDL-c, and TG than premenopausal ones (Table 1).

In our study, in addition to SBP, TG and eGFR, menopausal status exerted the independent influence on RBP4 (Table 3). This may in part be explained by diminished effect of estrogen to suppress the lipolysis through activation of estrogen receptor alpha (ER-α) in adipose tissue [19], thus suggesting the contribution of increased lipolysis and subsequent free fatty acid secretion from adipose tissue in insulin resistance occurrence and progression in postmenopausal women.

Additionally, in Spearman's non-parametric correlation analysis, RBP4 correlated with anthropometric and cardiometabolic parameters (Table 2). However, we failed to show the direct influence of anthropometric parameters on RBP4 levels. Our results are in line with the study of Tan et al. [21] who showed that RBP4 mRNA expression was significantly increased in human adipocytes of overweight women with polycystic ovary syndrome, but they excluded the influence of anthropometric indices on higher RBP4 mRNA expression and higher RBP4 levels in circulation.

Our study has some limitations, such as the relatively small sample size of examined cohorts and its cross-sectional design. In addition, we were not able to determine sex hormones in our study group. However, our cohort comprised of premenopausal and postmenopausal otherwise healthy women, without hormone replacement therapy or any medication usage in the last six months, so we excluded such confounding factors when estimating cardiometabolic profile of examined groups. In addition, we also excluded cardiometabolic diseases that may also affect serum RBP4 level. Future longitudinal studies with larger sample size are needed to further

explore pathophysiological mechanisms concerning the impact of menopause on RBP4 level in order to find the best therapeutic target approach for the decrease of this adipokine in postmenopause.

CONCLUSION

Postmenopausal women displayed higher retinol-binding protein 4 and unfavorable cardiometabolic profile, compared to premenopausal ones. Menopause per se is an independent predictor of high serum retinol-binding protein 4levels which should be taken into account when examining the role of this adipokine in cardiometabolic disease occurrence.

Conflict of Interest Statement

The authors have declared no conflicts of interest.

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