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Plasma concentration of Retinol Binding Protein 4 (RBP4) in relation to nutritional status and kidney function in older population of PolSenior Study

Piotr Kocełak[a], Aleksander Owczarek[b], Maria Bożentowicz-Wikarek[c], Aniceta Brzozowska[a], Małgorzata Mossakowska[d], Tomasz Grodzicki[e], Andrzej Więcek[f], Jerzy Chudek[c,g], Magdalena Olszanecka-Glinianowicz[a,*]

[a] Health Promotion and Obesity Management Unit, Department of Pathophysiology, Medical Faculty in Katowice, Medical University of Silesia, Katowice, Poland
[b] Department of Statistics, Department of Instrumental Analysis, Faculty of Pharmacy and Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland
[c] Pathophysiology Unit, Department of Pathophysiology, Medical Faculty in Katowice, Medical University of Silesia, Katowice, Poland
[d] International Institute of Molecular and Cell Biology, Warsaw, Poland
[e] Department of Internal Medicine and Gerontology, Jagiellonian University Medical College, Krakow, Poland
[f] Department of Nephrology, Transplantation and Internal Medicine, Medical University of Silesia, Katowice, Poland
[g] Department of Internal Medicine and Oncological Chemotherapy, Medical Faculty in Katowice, Medical University of Silesia, Katowice, Poland

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ABSTRACT

Purpose: The aim of the study was to assess plasma RBP4 concentration in elderly subjects in relation to nutritional status and kidney function in the population of the PolSenior Study.

Material and methods: We assessed RBP4, glucose, insulin, albumin, lipid profile, C-reactive protein, (hsCRP) and creatinine concentrations in 2614 PolSenior Study participants (1235 women and 1379 men). The study group was divided based on BMI and HOMA-IR values, and the occurrence of diabetes.

Results: Plasma RBP4 concentration was similar in normal weight, overweight, and obese subgroups, both in women (40.4 *vs* 40.8 *vs* 41.8 ng/ml, respectively), and men (41.2 *vs* 40.3 *vs* 42.9 ng/ml, respectively). Similar values were found in subjects with HOMA-IR < 2.5; ≥ 2.5 and diabetes, while those with decreased eGFR (< 60 ml/min/1.73 m^2) were characterized by increased RBP4 levels [46.0 (32.0–64.8) *vs* 39.4 (28.2–54.9) ng/ml; $p < 0.001$]. Plasma RBP4 level variability was explained by: age, waist circumference or BMI, and eGFR, but not HOMA-IR and/or hsCRP. The standardized coefficients β (slopes) for BMI and waist circumference were similar.

Conclusions: The results revealed that in older subjects, circulating RBP4 levels are mostly affected by kidney function and modestly by age, gender, and nutritional status, but not insulin resistance.

1. Introduction

One of the adipokines possibly involved in the development of insulin resistance is retinol binding protein 4 (RBP4). The increased expression of RBP4 was shown in adipose tissue of obese glucose transporter 4 (*GLUT4*) knockout mice [1]. The major sources of RBP4 in humans are adipocytes and hepatocytes [2], and it's only known physiological function is to transport retinol from the liver to the peripheral tissues [3]. It has been shown that RBP4 in adipose tissue is produced by both mature adipocytes [4] and activated macrophages [5]. Plasma RBP4 level is proportional to fat deposit in adults and children [4,5], and partially reflects severity of adipose tissue inflammation [6].

Primarily, it has been shown that RBP4 attenuates insulin induced phosphorylation of tyrosine in insulin receptor substrate 1 and insulin receptor signaling *via* inhibition of phosphatidylinositol 3 kinase (PI3K) [7]. Furthermore, the expression of *GLUT4* in muscle cells in subjects with impaired glucose metabolism and in adipose tissue of obese subjects was inversely related to RBP4 level [1,8]. Moreover, RBP4 enhances hepatic gluconeogenesis [1] and alters glucose metabolism [8].

The proinflammatory properties of both, RBP4 individually and RBP4 with TNF-α co-expression in immune cells, has also been found [1].

Some previously published studies revealed a strong association between the RBP4 levels and the components of metabolic syndrome in

* Corresponding author at: Health Promotion and Obesity Management Unit, Department of Pathophysiology, Medical University of Silesia, Medyków 18, 40-752 Katowice, Poland.
E-mail address: magols@esculap.pl (M. Olszanecka-Glinianowicz).

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humans [9]. However, the results of studies that assessed a relationship between the circulating RBP4 levels and insulin resistance are inconsistent. Some studies show an association [10], but more recent studies do not confirm it [11]. In addition, similar circulating RBP4 levels in nondiabetic, prediabetic, and diabetic subjects with obesity were shown [12].

So far published studies suggested that RBP4 is a marker of metabolic complications of obesity [13], and low grade systemic inflammation [14]. However, it should be noted that numerous factors may influence circulating RBP4 and retinol level. Examples include: formation of a complex with transthyretin that prevents glomerular filtration of RBP4 and its subsequent extraction by the kidneys, as well as iron and ferritin status [15]. Renal RBP4 biodegradation includes transportation of RBP-retinol complex to lysosomes by megalin and degradation of RBP leading to retinol liberation, which combines with newly synthesized RBP, and subsequently, this complex is secreted into the bloodstream. [16]. In addition, increased urine RBP4 level is arguably a sensitive biomarker of proximal renal tubule dysfunction [17].

There is a lack of studies assessing RBP4 levels in relation to gender, nutritional status, insulin resistance, and type 2 diabetes in large populations of older people. The impact of kidney excretory function on RBP4 levels might bias these associations, and have to be included in the analysis [18]. Therefore, the aim of this study was to assess the relationships between circulating RBP4 levels, nutritional status, insulin resistance, and kidney function in a large population-based elderly cohort.

2. Patients and methods

2.1. Study design and setting

The study group consisted of 1235 women and 1379 men, participants of the PolSenior study, aged 65 years and older, with available plasma samples stored at −70 °C, that remained after protocol based and later, additional assessments of plasma RBP4 levels. The PolSenior study design and methodology was described in detail elsewhere [19].

Six, similar sized, age cohorts (65–69 yrs, 70–74 yrs, 75–79 yrs, 80–84 yrs, 85–89 yrs, 90 yrs and older) were recruited from the population using a three stage stratified, proportional draw with the response rate of 43%. During three visits performed by nurses trained for this purpose, a questionnaire survey, comprehensive geriatric assessment, body mass, height, and waist circumference measurements were performed; also, blood and urine samples were collected.

Study protocol was approved by the Ethics Committee of Medical University of Silesia (KNW/0022/KB1/38/II08/10 and KNW-6501-38/I/08).

2.2. Biochemical measurements

Serum total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, fasting glucose, albumin (also in urine), creatinine, and C reactive protein concentrations were previously assessed by an automated system (Modular PPE, Roche Diagnostics GmbH, Mannheim, Germany) in a single certified laboratory with inter-assay coefficients of variability below 1.7%, 1.2%, 1.3%, 1.8%, 1.7%, 1.7%, 2.3%, and 5.7%, respectively.

Serum insulin levels were assessed by electrochemiluminescence method on a Cobas E411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), with inter-assay coefficients of variability below 3.8%.

Plasma RBP4 concentrations were measured by ELISA (Immundiagnostik AG, Bensheim, Germany) with a low limit of sensitivity of 0.9 pg/ml, and with mean intra- and inter-assay coefficients < 9.8% and < 5.0%, respectively. The normal range in adults reported by the manufacturer is 20–75 μg/ml. We followed the methods of our previous paper [20].

2.3. Data analysis

Obesity was diagnosed according to the WHO criteria [21], and visceral obesity was diagnosed according to the International Diabetes Federation criteria [22].

Diagnosis of diabetes was established based on medical history, medications, or fasting serum glucose ≥126 mg/dl. Insulin resistance in a group of nondiabetic subjects was determined on the basis of homeostasis model assessment of insulin resistance (HOMA-IR) calculated by the standard formula: HOMA-IR = fasting serum insulin (μIU/ml) × fasting glucose (mg/dl)/405. The cut-off value for insulin resistance was ≥ 2.5.

Estimated glomerular filtration rate (eGFR) was calculated according to the MDRD short formula [23]. eGFR below 60 ml/min/1.73 m^2 was considered as decreased. eGRF stages (G1-4) were scored according to the recommendations of Kidney Disease Improving Global Outcomes (KDIGO) [24].

2.4. Statistical analysis

Statistical analysis was performed using STATISTICA 10.0 PL (StatSoft, Cracow, Poland), StataSE 12.0 (StataCorp LP, TX, U.S.) and R software. Statistical significance was set at a p value below 0.05. All tests were two-tailed. Nominal and ordinal data were expressed as percentages, whilst interval data were expressed as mean value ± standard deviation in the case of normal distribution, or as median (lower quartile – upper quartile) in the case of data with skewed or non-normal distribution. Distribution of variables was evaluated by the Shapiro-Wilk test and homogeneity of variances was assessed by the Levene test. For comparison of data between normal weight, overweight, and obese groups as well as between insulin sensitive, insulin resistant, and diabetes groups, the one-way ANOVA analysis was used with RIR Tukey *post-hoc* test. Categorical variables were compared using either $\chi 2$ tests or Armitage $\chi 2$ tests for trend.

In order to assess the relationship between the RBP4 plasma levels and other variables, the least angle regression analysis was used.

3. Results

3.1. Characteristics of participants

The analysis included 2614 subjects (52.8% participants of PolSenior study), 1235 females and 1379 males with available stored plasma samples for RBP4 and nutritional status and insulin assessment, without missing data for body mass, height, and waist circumference (Table 1).

Overweight was diagnosed, on the basis of WHO criteria in 442 (35.8%) women and 639 (46.3%) men and obesity in 509 (41.2%) women and 369 (26.7%) men.

There were significant associations between the nutritional status and the prevalence of diabetes. We observed, significantly higher triglycerides, LDL cholesterol, hsCRP, fasting glucose, insulin levels, HOMA-IR values, and significantly lower serum HDL cholesterol concentrations (in subjects non-treated with statins) in the overweight and obese patients in contrast to normal weight subgroups (Table 1). Similar plasma RBP4 levels, though not related to nutritional status, were observed in men and women (Table 1, Fig. 1).

Furthermore, there was no difference in RBP4 levels between nondiabetic subjects with HOMA-IR values < 2.5, ≥2.5 and diabetics (Table 2).

The prevalence of diabetes was 24.1% in women and 20.7% in men. Additionally, among nondiabetics, 53.0% of women and 44.4% of men were insulin resistant (Table 2). The plasma RBP4 levels were similar in all the study subgroups in both males and females.

One thousand, one hundred, and fourteen (1114) subjects (46.5%) were 80 years old or more. Median RBP4 levels were 41.4 (IQR:

Table 1
The characteristics and comparison of subgroups according to nutritional status (mean value ± SD or median/interquartile range).

	All	Normal weight	Overweight	Obese	p
Women					
	N = 1235	**N = 284**	**N = 442**	**N = 509**	
Age [years]	78 ± 8	81 ± 9	77 ± 8#	76 ± 8#	< **0.001**
Waist circumference [cm]	96.7 ± 13.1	83.3 ± 8.8	93.8 ± 8.2#	106.8 ± 10.2#	< **0.001**
Diabetes [(%)]	298 (24.1%)	39 (13.7%)	91 (20.6%)	168 (33.0%)	< **0.001***
$eGFR_{MDRD}$ [ml/min/1.73 m^2]	67.8 ± 17.7	65.7 ± 18.8	68.3 ± 17	68.6 ± 17.6	0.08
hsCRP [mg/l]	2.4 (1.2–4.8)	1.5 (0.8–3.4)	2.2 (1.2–4.2)	3.3 (1.6–5.6)	< **0.001**
Total cholesterol [mg/dl]@	223 ± 45.5	222.3 ± 45.5	225.5 ± 45.5	221.1 ± 45.6	0.44
LDL cholesterol [mg/dl]@	136.3 ± 38.8	136.8 ± 40.2	138.5 ± 38.9	133.8 ± 37.8	0.27
HDL cholesterol [mg/dl]@	53.3 ± 14.3	57.1 ± 15.7	52.7 ± 13.7	51.3 ± 13.4	< **0.001**
Triglicerydes [mg/dl]	123.4 (93.1–161)	106.0 (84.4–137.9)	124.0 (90.2–165.2)	130.2 (103.2–170.8)	< **0.001**
Glucose [mg/dl]	94.7 (86–107.3)	89.4 (82.3–98.7)	94.1 (85.8–105.9)	98.8 (89.8–113.5)	< **0.001**
Insulin [μIU/ml]	12.2 (8.4–17.7)	9.3 (6.5–13)	11.2 (8–15.1)	15.9 (10.9–20.8)	< **0.001**
HOMA-IR	2.9 (1.9–4.4)	2.1 (1.3–3.0)	2.7 (1.8–3.8)	3.9 (2.6–5.6)	< **0.001**
RBP4 [μg/ml]	41.1 (29.6–57.1)	40.4 (28.8–53.8)	40.8 (30–57.6)	41.8 (29.9–58.5)	0.59
Men					
	N = 1379	**N = 371**	**N = 639**	**N = 369**	**p**
Age [years]	78 ± 8	81 ± 9	79 ± 8#	76 ± 8#	< **0.001**
Waist circumference [cm]	101.3 ± 12.5	89.5 ± 9.5	100.9 ± 7.6#	114 ± 9.7#	< **0.001**
Diabetes	285 (20.7%)	34 (9.2%)	122 (19.1%)	129 (35.0%)	< **0.001***
$eGFR_{MDRD}$[ml/min/1.73 m^2]	70.3 ± 17.2	70.4 ± 17.2	69 ± 17.3	72.4 ± 16.8	< **0.05**
hsCRP [mg/l]	2.2 (1.0–4.9)	1.9 (0.8–4.3)	2.1 (1.0–4.7)	2.8 (1.4–5.8)	< **0.001**
Total cholesterol [mg/dl]@	201.9 ± 40.8	200 ± 39.5	199.7 ± 39.3	208.9 ± 44.3	< **0.01**
LDL cholesterol [mg/dl]@	123.3 ± 34.8	121.1 ± 34.7	122.2 ± 32.8	128.1 ± 38.3	< **0.05**
HDL cholesterol [mg/dl]@	48.7 ± 13.6	54.3 ± 14.7	47.9 ± 12.9	43.3 ± 10.6	< **0.001**
Triglicerydes [mg/dl]	105.5 (82.0–143.4)	92.8 (72.0–118.8)	105.0 (81.6–142)	125.0 (98.7–67.6)	< **0.001**
Glucose [mg/dl]	95.8 (87.1–108.0)	90.2 (83.0–99.8)	96.2 (88.4–107.3)	102.9 (92.2–122.2)	< **0.001**
Insulin [μIU/ml]	10.6 (7.2–16.1)	7.3 (5.0–10.6)	10.9 (7.7–15.7)	14.8 (10.0–21.9)	< **0.001**
HOMA-IR	2.5 (1.6–4.2)	1.6 (1.1–2.5)	2.6 (1.7–4.1)	3.7 (2.4–6.5)	< **0.001**
RBP4 [μg/ml]	41.1 (29.2–57.9)	41.2 (28.9–57.8)	40.3 (28.8–57.5)	42.9 (30.3–59.2)	0.20

* – χ^2 test for trend; # – p < 0.001 in comparison to normal weight; @ – for subjects without statins treatment.
eGFR – estimated glomerular filtration rate.
hsCRP – high-sensitivity C-reactive protein.
HOMA-IR – homeostasis model assessment of insulin resistance.
RBP4 – retinol binding protein type 4.
SD – standard deviation.

30.1–57.4) in the younger subgroup and 40.7 (IQR: 28.3–58.2) in the older subgroup.

The analyzed group, comprised of 381 (30.8%) women and 369 (26.8%) men with eGFR < 60 ml/min/1.73 m^2. Subjects with decreased eGFR were characterized by significantly higher plasma RBP4 levels (Me = 46.0, IQR: 32.0–64.8 *vs.* Me = 39.4, IQR: 28.2–54.9; p < 0.001). There was a progressive increase in the plasma RBP4 levels along G stages of eGFR (Fig. 2).

The two factor analysis of variance showed no significant main effect for the gender factor, F = 1.10, p = 0.29, but a significant main effect for the eGFR subgroups, F = 14.21, p < 0.001. There was no significant interaction between the gender and the eGFR subgroups,

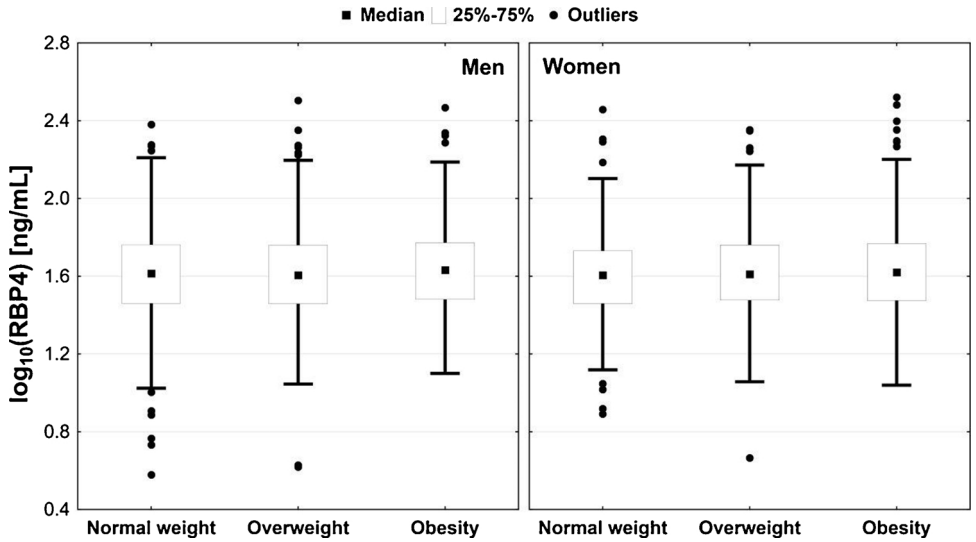


Fig. 1. Plasma retinol binding protein 4 (RBP4) concentrations in relation to nutritional status.
RBP4 – retinol binding protein type 4.

Table 2
Comparison of subgroups of insulin sensitive, insulin resistant, and diagnosed with diabetes (mean value ± SD or median with interquartile range).

	HOMA < 2.5 DM −	HOMA ≥ 2.5 DM −	DM +	p
Women				
	N = 441	**N = 496**	**N = 298**	
Age [years]	79 ± 9	76 ± 8$^{\#}$	78 ± 8	< **0.001**
Waist circumference [cm]	91.1 ± 11.7	98.4 ± 12.0$^{\#}$	102.4 ± 13.5$^{\#}$	< **0.001**
$eGFR_{MDRD}$ [ml/min/1.73 m^2]	67.9 ± 17.6	69.4 ± 17.5	65.0 ± 17.9$^{\$}$	< **0.01**
hsCRP [mg/l]	2.0 (1.0–4.1)	2.8 (1.4–5.3) $^{\#}$	2.5 (1.2–5.1)$^{\wedge}$	< **0.001**
Total cholesterol [mg/dl]$^{@}$	225.1 ± 43.0	224.7 ± 43.8	216.2 ± 53.2	0.07
LDL cholesterol [mg/dl]$^{@}$	139.0 ± 37.6	137.4 ± 38.7	128.2 ± 41.1$^{\wedge}$	< **0.01**
HDL cholesterol [mg/dl]$^{@}$	56.3 ± 14.9	52.5 ± 13.7$^{\#}$	48.9 ± 13.0$^{\#}$	< **0.001**
Triglicerydes [mg/dl]	104.3 (83.1–138.6)	129.1$^{\#}$ (102.9–165.0)	137.3$^{\#}$ (103.9–181.8)	< **0.001**
Glucose [mg/dl]	86.5 (80.8–93.1)	97.0$^{\#}$(90.4–104.3)	121.6$^{\#}$ (100.1–150.3)	< **0.001**
Insulin [μIU/ml]	7.7 (6.0–9.4)	15.6$^{\#}$ (12.7–19.6)	16.1$^{\#}$ (10.7–23.7)	< **0.001**
HOMA-IR	1.67 (1.23–2.05)	3.73$^{\#}$ (2.97–4.68)	5.04$^{\#}$ (3.04–8.68)	< **0.001**
RBP4 [μg/ml]	40.4 (30.5–55.0)	40.4 (28.3–57.6)	42.8 (31.1–59.2)	0.20
Men				
	N = 606	**N = 488**	**N = 285**	**p**
Age [years]	80 ± 9	77 ± 8$^{\#}$	77 ± 8$^{\#}$	< **0.001**
Waist circumference [cm]	96.1 ± 11.4	104.6 ± 11.3$^{\#}$	106.8 ± 12.5$^{\#}$	< **0.001**
$eGFR_{MDRDI}$ [ml/min/1.73 m^2]	71.2 ± 17	69.3 ± 16.7	70.1 ± 18.3	0.18
hsCRP [mg/l]	1.9 (0.9–4.3)	2.5 (1.2–5.9)$^{\#}$	2.1 (1.0–4.5)	< **0.001**
Total cholesterol [mg/dl]$^{@}$	200.6 ± 41.3	206.6 ± 37.6$^{\$}$	197.0 ± 44.2	< **0.05**
LDL cholesterol [mg/dl]$^{@}$	123.5 ± 35.7	127.2 ± 33.5	115.6 ± 33.7$^{\$}$	< **0.001**
HDL cholesterol [mg/dl]$^{@}$	52.2 ± 13.8	45.8 ± 12.4$^{\#}$	45.1 ± 12.9$^{\#}$	< **0.001**
Triglicerydes [mg/dl]	93.4 (73.3–117.9)	121.5 (95.0–162.4)$^{\#}$	119.7 (88.1–166.6)$^{\#}$	< **0.001**
Glucose [mg/dl]	89 (82.2–94.7)	100.1 (93.0–107.9) $^{\#}$	129.6 (104.5–156.0) $^{\#}$	< **0.001**
Insulin [μIU/ml]	7.2 (5.4–8.9)	15.6 (12.8–20.6) $^{\#}$	13.3 (8.9–22.7)$^{\#}$	< **0.001**
HOMA-IR	1.5 (1.1–2.0)	3.7 (3.1–5.1)$^{\#}$	4.5 (2.4–8.4)$^{\#}$	< **0.001**
RBP4 [μg/ml]	39.7 (28.1–58.2)	42.5 (29.8–59.6)	41.8 (30.2–56.5)	0.17

* – χ^2 test for trend; $^{\$}$ – $p < 0.05$ in comparison to the first group, $^{\wedge}$ – $p < 0.01$ in comparison to the first group, $^{\#}$ – $p < 0.001$ in comparison to the first group; @ – for subjects without statins treatment.
DM – diabetes mellitus.
eGFR – estimated glomerular filtration rate.
hsCRP – high-sensitivity C-reactive protein.
HOMA-IR – homeostasis model assessment of insulin resistance.
RBP4 – retinol binding protein type 4.
SD – standard deviation.

F = 0.13, p = 0.97. In younger subjects, the RBP4 serum levels were significantly higher for G3a (eGFR 45–60 ml/min/1.73 m^2), and G3b (eGFR 30–44 ml/min/1.73 m^2) in comparison with G1 (eGFR > 90 ml/min/1.73 m^2) subgroups ($p < 0.001$). In older subjects, plasma RBP4 levels were statistically significantly higher for G3a ($p < 0.01$), G3b ($p < 0.001$) and G4 (eGFR 15–29 ml/min/1.73 m^2; $p < 0.001$) in comparison with G1 subgroup.

3.2. Least angle regression analysis

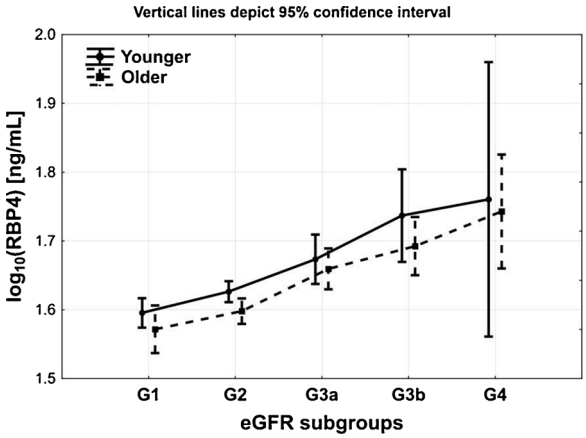
The results of the LARS regression of log10(RBP4) demonstrate that eGFR values, age, and female gender had opposite effects on plasma RBP4 levels, while the waist circumference or BMI values, are factors associated with higher plasma RPB4 level. Insulin resistance and serum hsCRP level demonstrate no influence on the plasma RPB4 level (Table 3). The most important factors for plasma RBP4 level are eGFR, age, waist circumference, and gender for model 1, and eGFR, age, gender and BMI for model 2. The standardized coefficients β (slopes) for BMI and waist circumference were similar (0.0487 *vs.* 0.0485).

4. Discussion

Our study revealed that plasma RBP4 level variability is mostly explained by impaired kidney function and to a lesser extent by waist circumference or BMI values, age, and female gender but not by insulin resistance.

Renal function was rarely assessed in previous studies, as the factor influencing plasma RBP4 levels. Our results are in accordance with the previously published data [18] describing an inverse association between eGFR and plasma RBP4 levels in diabetic subjects and general middle- aged population [25]. In addition, markedly increased RBP4 isoforms including apo-RBP4 (RBP4 not bound to retinol) and RBP4 truncated at the C-terminus (RBP4-L, RBP4-LL) were found in patients with end stage kidney disease [26]. These observations suggest the impaired biodegradation of RBP4 by the injured kidneys. This is a well-known mechanism explaining the accumulation of numerous proteins, including some hormones and adipokines, in the circulation. However, as was mentioned above, the urine RBP4 level is a highly sensitive biomarker of proximal renal tubule dysfunction with increased protein excretion [17]. We hypothesized that the increased RBP4 level is related to an impaired kidney function and as the result of increased transthyretin concentration in the circulation and formation of complexes with RBP4 that prevent glomerular filtration of RBP4 [15]. On the other hand, the results of the experimental studies have shown that megalin levels decrease with age, while the number of its receptors increase [27]. However, there is a lack of studies assessing the urine RBP4 levels and the levels of transthyretin and megalin in older subjects. Therefore, further studies are necessary to explain the role of kidney injury in RBP4 degradation and its accumulation in the circulation.

Contrary to previously published studies in various age groups [4,5], but in accordance with the data from the ARIC study [25], we did not observe any differences between the RBP4 levels in the normal weight, overweight, or obese subgroups. Only the results of a multivariate analysis have shown modest positive relation between the RBP4 levels and the waist circumference or BMI values. It is in accordance



	Age < 80		Age ≥ 80	
CKD subgroups	N	Me (Q_1 – Q_3)	N	Me (Q_1 – Q_3)
G1	436	38.5 (28.4 – 51.9)	167	34.0 (24.9 – 53.0)
G2	861	41.1 (30.3 – 57.9)	581	39.1 (27.4 – 53.8)
G3a	154	45.9 (34.5 – 61.7)	225	45.7 (30.7 – 64.3)
G3b	44	55.6 (39.1 – 74.3)	112	51.2 (34.6 – 66.1)
G4	5	74.3 (35.5 – 97.9)	29	57.2 (40.4 – 74.4)

Fig. 2. Plasma retinol binding protein 4 (RBP4) concentrations in relation to glomerular filtration rate in younger (65–80 years) and older (≥80 years) subgroups.
RBP4 – retinol binding protein type 4.
eGFR – estimated glomerular filtration rate.
CKD – chronic kidney disease.
G1, G2, G3a, G3b, G4 – stages of chronic kidney disease.

with recently published studies which show that the RBP4 levels are proportional to waist circumference [28]. The data presented in this study suggests that this association could diminish with age, and thus with the duration of obesity, which was not assessed in our study. This supposition is supported by our previously published study showing that visceral adiposity related inflammation, reflected by an increase of plasma TNF-α level, is an early event in visceral fat accumulation while further fat mass gain did not enhance the circulating TNF-α levels [29]. However, the results obtained in the group of subjects with moderate to severe psoriasis treated with anti-TNF showed a correlation between the RBP4 and BMI and waist circumference after treatment but not before, when they had an active disease [30], contrary to this hypothesis. Experimental studies are needed to explain these discrepancies, however, cannot exclude the hypothesis. Thus, it seems that the lack of difference in the RBP4 levels between insulin sensitive, insulin resistant, and subjects with type 2 diabetes and association between the plasma RBP4 levels and insulin resistance observed in our study is the result of persistent hormonal disturbances of adipose tissue in long-lasting visceral obesity, not only TNF system activity. It should also be noted that the conditions linked to chronic inflammatory burden have also been associated with components of metabolic syndrome. However, no correlation between the RBP4 and insulin resistance was found in patients with moderate to severe psoriasis or rheumatoid arthritis [30,31]. Nevertheless, these studies and our data did not exclude the role of the RBP4 in the development of insulin resistance, β cell dysfunction, and type 2 diabetes shown in published studies [32,33] in middle-aged adults.

The inverse association between the RBP4 and age shown in our study, was also previously described in a small, mostly middle-aged cohort (30–70 years) [34]. The reason for the decreased RBP4 production in old age remains unclear, though it is potentially linked to the catabolic state [35]. However, the association between the RBP4 and age is biased in univariate analysis by the increasing with age occurrence of the so called chronic kidney disease. A multiple regression model used in our study confirmed this hypothesis.

Contrary to some previously published studies, that described higher RBP4 levels in men than in women [22,28], our crude data failed to find any differences in the plasma RBP4 levels between genders. It should be noted that gender differences in the plasma RBP4 levels was shown in populations younger than our own [25,34]. In our study, the multiple regression model shows a weak inverse association between the plasma RBP4 levels and female gender. This weak association, demonstrated only in multiple regression, and the lack of differences between gender in crude analysis, can be explained by more frequent

Table 3
Results from the least angle regression of $\log_{10}$(RBP4) [$\times 10^3$].

Model 1 with waist circumference			
Step	C_p	Action	Coefficient in final model
1	104.44	Intercept	–
2	54.34	+ eGFR	−2.286
3	15.24	+ Age	−2.781
4	8.71	+ Waist circumference	0.348
5	3.45	+ Female	−9.364
6	5.43	+ hsCRP	–
7	7.00	+ HOMA-IR	–
Model 2 with BMI			
Step	C_p	Action	Coefficient in final model
1	100.16	Intercept	–
2	47.96	+ eGFR	−2.304
3	8.65	+ Age	−2.653
4	8.88	+ Female	−12.655
5	3.44	+ BMI	0.659
6	5.07	+ hsCRP	–
7	7.00	+ HOMA-IR	–

BMI – body mass index.
eGFR – estimated glomerular filtration rate.
hsCRP – high-sensitivity C-reactive protein.
HOMA-IR – homeostasis model assessment of insulin resistance.
RBP4 – retinol binding protein type 4.

prevalence of chronic kidney disease among older women in our population, which was described previously [36].

4.1. Study limitations

The present study has some limitations. The distribution of body fat and its visceral deposits were directly assessed using DEXA only in a small subset of patients, and therefore the results were not included in the analysis. Additionally, systemic inflammation was determined on the basis of hsCRP and IL-6, only. While, adipose tissue is a source of some other important inflammatory cytokines, such as TNF-α. Our study has also some advantages related to the study size and comprehensive description of the Polish elderly random sample.

5. Conclusions

In conclusion, our study revealed that in older subjects, circulating RBP4 levels are mostly affected by kidney function and modestly by age, gender, and nutritional status, but not insulin resistance.

Conflicts of interest

The authors have declared no conflict of interest.

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Authors contribution

The Polsenior study was designed by Małgorzata Mossakowska, Tomasz Grodzicki, Andrzej Więcek, and Jerzy Chudek. The conception of RBP4 analysis in the PolSenior population was created by Magdalena Olszanecka-Glinianowicz and Jerzy Chudek. The acquisition of data was performed by Maria Bożentowicz-Wikarek and Aniceta Brzozowska. Statistical analysis of data was done by Aleksander Owczarek. The paper was drafted by Piotr Kocełak, Magdalena Olszanecka-Glinianowicz, and Jerzy Chudek. All authors participated in revising the paper, while its final version was approved by Magdalena Olszanecka-Glinianowicz and Jerzy Chudek.

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