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PREX PROTEINS LEVEL CORRELATION WITH INSULIN RESISTANCE MARKERS AND LIPID PROFILE IN OBESE AND OVERWEIGHT NON-DIABETIC PATIENTS

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Metabolic dysregulation and obesity are associated with many metabolic alterations, including impairment of insulin sensitivity and dyslipidemia. Recent studies highlight the key role of phosphatidylinositol 3,4,5-triphosphate-dependent Rac exchange proteins (PREX proteins) in the pathogenesis of obesity, advocating further elucidation of their potential therapeutic implications. The present study aimed to estimate the serum level of PREX proteins and its potential association with insulin resistance markers and plasma lipids level in obese and overweight non-diabetic patients. The study included 30 persons classified as obese, 30 as overweight, and 30 healthy individuals of similar age and gender. The levels of PREX1 and PREX2 were measured using ELISA kits, insulin, fasting glucose, glycosylated hemoglobin and total lipid profile were determined using appropriate photometric kits. HOMA-IR was used as a measure of insulin sensitivity. According to the obtained results, obese non-diabetic patients had higher serum PREX1 level compared to both overweight and normal-weight individuals. PREX1 correlated positively with the markers of insulin resistance and dyslipidemia. PREX2 level was shown to be lower both in obese compared to overweight patients and in overweight compared to normal-weight individuals. PREX2 correlated negatively with the markers of insulin resistance but not with the markers of dyslipidemia.

K e y w o r d s: PREX proteins, obesity, overweight, insulin resistance, dyslipidemia.

The Global Burden of Disease Research GBD has calculated that the worldwide occurrence of obesity is 5% in children (equivalent to 108 million individuals) and 12% in adults (equivalent to 604 million individuals). The data indicates that the age-standardized prevalence of obesity has doubled in over 70 nations analyzed since 1980, and there have been consistent rises in the majority of the other countries [1]. High body mass index BMI is associated with several well-known health conditions, including cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), musculoskeletal diseases, some malignancies, and asthma. These conditions can result in premature death and a decrease in the overall quality of life [2-5]. Metabolic dysregulation and obesity are associated with many metabolic alterations, including impairment of insulin sensitivity and dyslipidemia [6-8]. The PREX protein family, comprising PREX1 and PREX2, are guanine-nucleotide exchange factors GEFs that enhance the activation of the small G-protein Rac by relying on phosphatidylinositol 3,4,5-trisphosphate. They participate in numerous biological processes in both healthy and diseased states, including inflammation [9], endothelial cell function [10, 11], neuronal development and neuronal plasticity [12, 13], carcinogenesis [14], glucose homeostasis, and insulin sensitivity via modulating insulin signaling and glucose uptake [15, 16]. Recent research highlights the pivotal role of phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchanger proteins (PREX proteins) in the pathogenesis of obesity, especially in non-diabetic individuals [17]. Additionally, investigations in China linked genetic polymorphisms in the PREX gene with increased adiposity and insulin resistance [18]. In the United States, pharmacological inhibi-

tion of PREX proteins shows promise in improving adipose tissue inflammation and insulin sensitivity in obese nondiabetic mice [19]. These findings underscore the significance of PREX proteins in obesity and advocate for further research to elucidate their mechanisms and potential therapeutic implications. The present study aimed to estimate the serum levels of PREX proteins and investigate the possible association of these proteins with markers of insulin resistance and plasma lipids in obese and overweight non-diabetic Iraqi subjects.

Materials and Methods

Study design. The present cross-sectional study was conducted at the Obesity Center in Al Imam Al Sadiq Teaching Hospital in Hilla/Babel Governorate, Iraq, from November 12, 2020 to February 16, 2021. The research was approved by the University of Baghdad's College of Pharmacy's Ethics Committee and conducted in accordance with the Declaration of Helsinki [20]. Before obtaining participants' consent, they were given information about the objective and expected benefits of the study. The study included thirty persons who were classified as obese, thirty individuals who were classified as overweight, and thirty apparently healthy individuals of similar age and gender to act as a control group. The categorization of the participants into the study groups was according to the American Gastroenterology Association (AGA) based on the measurement of BMI under the supervision of a specialist physician. The participants were obese BMI ≥ 30, overweight BMI 25-29.9 and normal weight BMI 18.5-24.9 individuals, aged ≥ 18 years, of both genders and on an unrestricted diet. Individuals with overt diabetes or any endocrinopathy, restricted physical activity, renal or hepatic diseases associated with alteration of lipid or glucose metabolism, and those taking any medications that may interfere with insulin sensitivity or lipid metabolism were excluded. Participants were interviewed separately. Demographic and medical data were collected and recorded using a data collection sheet.

Inclusion criteria. For the case groups: Obese (BMI≥30) and overweight (BMI 25-29.9) individuals aged ≥ 18 years, of both genders, on unrestricted diet. For the control group: Normal healthy individuals with BMI 18.5-24.9, of both genders and on unrestricted diet.

Exclusion criteria. Individuals with overt diabetes or any endocrinopathy, restricted physical activity, renal or hepatic diseases associated with alteration of lipid or glucose metabolism, and those taking any medications that may interfere with insulin sensitivity or lipid metabolism, were excluded.

Samples collection. Venous blood specimens 8–10 ml were withdrawn from each participant after an overnight fast 10–12 h and placed in gel tubes. Two milliliters of whole blood samples were transferred to EDTA tubes for glycosylated hemoglobin HbA1c measurements that were measured within 8 h of sample collection. The remaining blood samples were allowed to clot at room temperature, then centrifuged at 4000 rpm for 5 min to obtain sera. Each serum sample was divided into aliquots in Eppendorf tubes and stored at -20°C until the time of analysis. HbA1c was measured using the corresponding kit for the D-10™ hemoglobin testing system (Bio-Rad Laboratories, Inc.; USA). Serum insulin was estimated using the corresponding kit for Cobas e411 (Roche, Germany). C-reactive protein CRP was measured using the corresponding kit (DIRUI Industrial Co. Ltd., China). Serum glucose, total cholesterol TC, triglyceride TG, and high-density lipoprotein cholesterol HDL levels were measured calorimetrically using the corresponding kits (BIOLABO SAS; France). Finally, human PREX1 and PREX2 ELISA kits (MyBiosource, Inc.; USA) were used for the estimation of serum levels of these proteins. Body mass index was measured by dividing the weight (kg) by the height squared (m^2). Homeostasis model assessment for insulin resistance HOMA-IR was used as a measure of insulin sensitivity; HOMA-IR was calculated using equation (1) [21, 22]:

$$\text{HOMA-IR} = (\text{FIL}\times\text{FGL})/404, \quad (1)$$

where FIL – fasting insulin levels, FGL – fasting glucose levels.

Low-density lipoprotein-cholesterol (LDL) was estimated using the Friedewald's equation [23]:

$$\text{LDL (mg/dl)} = (\text{TC-HDL-TG})/5, \quad (2)$$

where (TG/5) represents the VLDL in mg/dl.

Ethical approval. Written illustrative consent form was signed by all parents/caregivers of the participating patients. This study was performed according to the ethical rules for medical research involving human participants of the Declaration of Helsinki (1964). Ethical approval was received from the ethical and research committee of the University of Baghdad, College of Pharmacy Ethics Committee, Baghdad, Iraq (No. 872 on 06/02/2021).

Statistical analysis. The statistical analysis was performed using the statistical package for social science SPSS, version 25. The Shapiro-Wilk-Wilk was used to check the uniform distribution of the data. Continuous variables were presented as means ± SD. The comparisons among groups were performed using the analysis of variance (ANOVA) test if there was a significant difference; the post hoc test was performed to describe differences among different groups. Categorical variables were presented as frequency and percentage and were analyzed by the chi-square χ^2 test. The correlation between different parameters and PREX proteins was tested using Pearson's correlation. $P < 0.05$ was considered significant.

Results

The sociodemographic and biochemical characteristics of the participants are shown in Table 1. The participants' ages and sexes were similar. When comparing body mass index (BMI), the three research groups showed statistically significant differences. Both the overweight and control groups had lower body mass indexes than the obese group; conversely, the control group had the lowest BMI. Participants in the obese group exhibited markedly elevated serum insulin levels in comparison to those in the overweight and control groups. Furthermore, the overweight group exhibited markedly elevated serum insulin levels compared to the control group. Participants in the obese and overweight groups had considerably higher fasting serum glucose FSG levels compared to those in the control group. However, there was no significant difference in FSG levels between participants in the obese and overweight groups. The FSG levels of all participants in the study groups were within the normal range, indicating that they did not have diabetes. The levels of glycosylated hemoglobin HbA1c were significantly higher in participants from the obese group compared to those in the overweight and control groups. Furthermore, the group of individuals who were overweight demonstrated a greater level of HbA1c in comparison to the control group. The HbA1c readings of all patients in the study groups were within the normal range, indicating that they

Table 1. Characteristics of participants: sociodemographic, clinical, and biochemical

Variables	Obese, $n = 30$	Overweight, $n = 30$	Control, $n = 30$	P-value
Age, years	39.03 ± 10.34	35.57 ± 9.63	37.70 ± 9.50	0.056
Gender				
Male	13 (43.3%)	14 (46.7%)	13 (43.3%)	0.063
Female	17 (56.7%)	16 (53.3%)	17 (56.7%)	0.063
BMI, kg/m^2)	37.90 ± 6.19^a	27.79 ± 1.26^b	22.62 ± 2.13^c	<0.001*
Fasting insulin, µU/ml	22.92 ± 19.15^a	15.56 ±14.83^b	7.63 ± 3.52^c	<0.001*
FSG, mg/dl	103.93 ± 17.18^a	98.60 ± 18.01^a	89.53 ± 14.13^b	0.004*
HbA1c, %	5.22 ± 0.66^a	4.90 ± 0.47^b	4.49 ± 0.48^c	<0.001*
HOMA-IR	6.01 ± 5.56^a	3.27 ± 2.47^b	1.70 ± 0.83^b	<0.001*
CRP, mg/dl	7.57 ± 5.90^a	2.95 ± 2.54^b	2.27 ± 1.23^b	<0.001*
TC, mg/dl	178.81 ± 44.69	161.16 ± 50.39	162.32 ± 43.70	0.262
TG, mg/dl	184.40 ± 95.83^a	139.01 ± 70.71^b	111.47 ± 56.87^b	0.002*
HDL, mg/dl	32.86 ± 7.91	33.06 ± 6.14	34.55 ± 6.14	0.576
LDL, mg/dl	110.43 ± 36.92	105.07 ± 47.53	105.48 ± 38.74	0.856
VLDL, mg/dl	36.87 ± 19.16^a	26.66 ± 14.44^b	22.29 ± 11.37^b	0.001*

Note. BMI – body mass index; WHR – weight to hip ratio; FSG – fasting serum glucose; HbA1c – glycated hemoglobin; HOMA-IR – homeostatic model assessment for insulin resistance; CRP – C-reactive protein; TC – total cholesterol; TG – triglyceride; HDL – high density lipoprotein; LDL – low density lipoprotein; VLDL – very low-density lipoprotein. *Refers to significant difference, and superscripts (a, b, c) among different groups refer to the difference among groups, such that (a) means the level is significantly higher than the level in (b) and the last is significantly higher than the level in (c)

did not have diabetes. The obese group exhibited significantly elevated HOMA-IR levels compared to both the overweight and control groups. However, there was no notable disparity in HOMA-IR levels between participants in the overweight group and the control group. Similarly, individuals in the obese group exhibited markedly higher levels of CRP compared to those in the overweight and control groups. Nevertheless, no substantial disparity in CRP levels was observed among participants in the overweight group and the control group. Finally, serum levels of TC, LDL, and HDL did not show any significant difference among participants in the study groups. However, participants in the obese group had significantly elevated serum TG and VLDL levels compared to those in the overweight and control groups. However, there was no significant difference in TG and VLDL levels between participants in the overweight and control groups. Table 2 displays the serum levels of PREX1 and PREX2 proteins in the participants. The obese group had significantly elevated serum levels of PREX1 protein compared to both the overweight and control groups. However, there was no significant difference in PREX1 protein levels between the overweight and control groups. On the other hand, participants in the obese group exhibited considerably reduced blood levels of PREX2 protein compared to both the overweight and control groups. Additionally, the overweight group had significantly lower serum levels of PREX2 protein compared to the control group.

Table 3 displays the connection between the levels of blood PREX1 and PREX2 proteins and the factors examined in the entire sample of participants $n = 90$. There was a direct association between serum PREX1 protein levels and BMI, insulin, HbA1c, HOMA-IR, TC, TG, and VLDL. However, Obesity, fasting serum glucose, haemoglobin A1c, C-reactive protein, and HOMA-IR levels were inversely related to blood PREX1 protein levels.

Figure shows that the levels of the PREX1 and PREX2 proteins in the serum were negatively correlated $r = -0.229$, $P = 0.03$.

Discussion

The current analysis suggests that the glucose regulation in obese and overweight patients is impaired compared to the control participants. Individuals classified as obese or overweight demonstrated elevated levels of fasting blood glucose FSG, glycated hemoglobin HbA1c, and homeostatic model assessment of insulin resistance HOMA-IR. It is important to mention that both FSG and HbA1c values remained within the normal range for those who do not have diabetes. Nevertheless, the HOMA-IR value fell within the range, indicating insulin resistance [24,25]. Furthermore, the obese individuals had elevated levels of HbA1c and HOMA-IR compared to the overweight individuals, showing that glucose metabolism decreased with increasing body mass index. Extensive research demonstrated that insulin resistance plays a pivotal role in the disruption of glucose regulation that occurs in obesity [26-28]. Obese people develop insulin resistance as a result of increased production of adipokines and proinflammatory cytokines, together with reduced production of adiponectin. As a result, leukocytes build up in adipose tissue, leading to persistent, mild inflammation. Moreover, there is an atypical accumulation of adipose tissue in the liver and skeletal muscles, which hinders the liver's ability to release glucose and interferes with insulin signaling, the occurrence was noted in 2013 [29-32]. Our findings concerning CRP, an inflammatory marker, are in agreement with this finding. As can be seen in Table 1, there was a statistically significant rise among the obese when contrasted with the overweight and control groups. There is a clear and direct relationship between levels of C-reactive protein CRP and insulin

Table 2. Serum PREX1 and PREX2 proteins levels in participants

Variables	Obese, $n = 30$	Overweight, $n = 30$	Control, $n = 30$	P-value
PREX1, ng/ml	4.73 ± 1.77^{a}	3.69 ± 1.44^{b}	3.32 ± 1.53^{b}	0.003*
PREX2, pg/ml	912.20 ± 273.63^{c}	1192.60 ± 330.93^{b}	1442.20 ± 469.58^{a}	<0.001*

Note. PREX1, Phosphatidylinositol (3,4,5)-trisphosphate (PIP3)-dependent Rac exchanger 1 is referred to as PREX1, whereas Phosphatidylinositol (3,4,5)-trisphosphate (PIP3)-dependent Rac exchanger 2 is referred to as PREX2. *Refers to significant difference, The use of superscripts (a, b, c) inside separate groups indicates substantial differences between the groups. Specifically, (a) denotes a significantly higher level compared to (b), while the last group has a significantly higher level than (c)

Table 3. The relationship between the serum levels of PREX1 and PREX2 proteins and the variables evaluated in all subjects was examined

Variables	PREX1 (ng/ml), *n* = 90		PREX2 (pg/ml), *n* = 90	
	r	*P*-value	*r*	*P*-value
Age, years	0.189	0.074	-0.150	0.157
BMI, kg/m^2	0.368	<0.001*	-0.445	<0.001*
Insulin, µU/ml	0.289	0.006*	-0.171	0.107
FSG, mg/dl	0.13	0.222	-0.207	0.05*
HbA1c, %	0.455	<0.001*	-0.397	<0.001*
HOMA-IR	0.376	<0.001*	-0.209	0.048*
CRP, mg/dl	0.199	0.06	-0.212	0.045*
TC, mg/dl	0.296	0.005*	0.046	0.668
TG, mg/dl	0.30	0.004*	-0.025	0.814
HDL, mg/dl	0.029	0.789	0.083	0.437
LDL, mg/dl	0.204	0.054	0.015	0.891
VLDL, mg/dl	0.301	0.004*	-0.033	0.76

Note. BMI – body mass index; FSG – fasting serum glucose; HbA1c – glycated hemoglobin; HOMA-IR – homeostatic model assessment for insulin resistance; CRP – C-reactive protein; TC – total cholesterol; TG – triglyceride level; HDL – high density lipoprotein; LDL – low density lipoprotein; VLDL – very low-density lipoprotein; PREX1 – Phosphatidylinositol (3,4,5)-trisphosphate (PIP3)-dependent Rac exchanger 1; PREX2 – Phosphatidylinositol (3,4,5)-trisphosphate (PIP3)-dependent Rac exchanger 2. *Refers to significant correlation

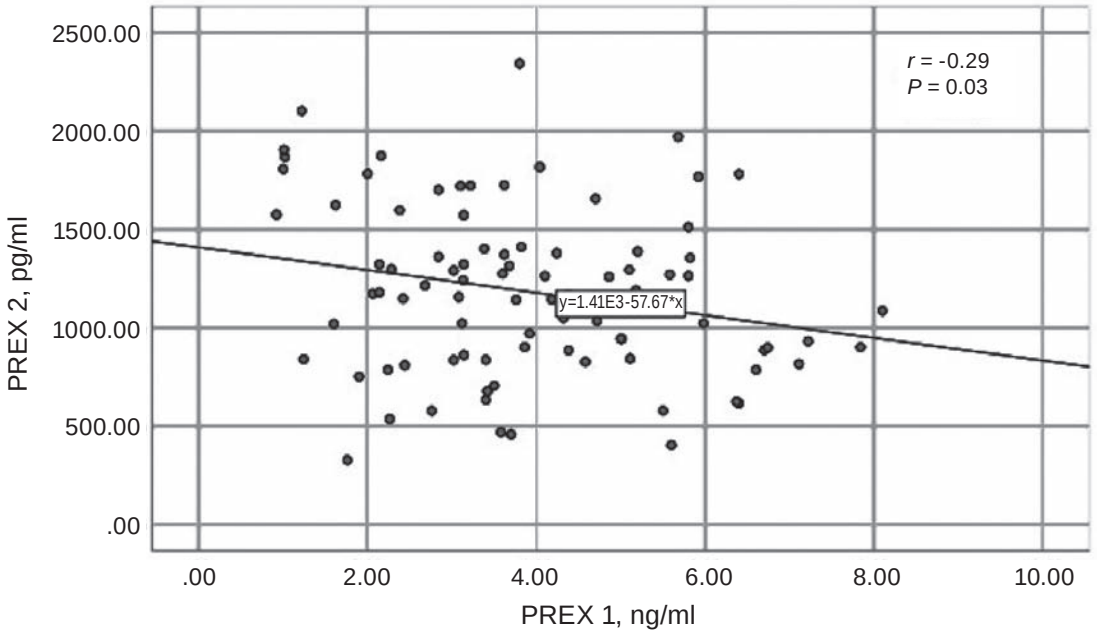

Fig. A scatter plot showing the correlation between serum PREX1 and PREX2 levels

resistance [33]. Elevated levels of high-sensitivity CRP was suggested to indicate future insulin resistance in individuals without diabetes [34]. In addition to being produced by the liver and pancreas during inflammation, CRP has also been observed to be created and secreted by adipocytes [35, 36]. A favorable correlation has been shown between CRP levels and BMI in both healthy persons and those who are

fat or overweight [37]. There is evidence that both healthy individuals and women with RA experience higher C-reactive protein levels when they are overweight. Most importantly, this correlation holds true irrespective of how bad the disease gets [38]. The current study found that compared to the overweight and control groups, the obese group had significantly higher levels of serum triglyceride TG and very low-density lipoprotein VLDL. Nevertheless, Neither the overweight nor the control group showed a significantly different pattern of triglyceride TG or very low-density lipoprotein VLDL levels. Similarly, the control and overweight groups had lower levels of HDL, whereas the obese group had higher levels of all other lipid profile markers; however, this difference was not statistically significant, the lipid profile levels were within the typical reference ranges. Lipid profile abnormalities are common in obese patients [39, 40]. Factors contributing to the dysregulation of lipid levels in obese people include an upregulation of lipid breakdown in adipose tissue, which releases free fatty acids that the liver uses to make cholesterol-rich particles, an increase in the production of very low-density lipoproteins VLDL by the liver, and a decrease in the breakdown of triglycerides TG in the bloodstream. Another factor is the impaired removal of free fatty acids from the circulation. Many of these mechanisms are greatly aided by insulin resistance [41]. The study found that obese individuals had greater levels of serum PREX1 compared to both overweight and normal-weight individuals. Obese individuals had lower levels of serum PREX2 compared to overweight individuals, while overweight individuals had lower levels compared to individuals with normal weight. Moreover, serum PREX1 levels were positively correlated, and serum PREX2 levels were negatively correlated with markers of poor glucose homeostasis and insulin resistance. These results occur in accordance with growing evidence of the metabolic effects of PREX proteins. Both PREX1 and PREX2 were shown to be involved in insulin signaling in *in vitro* and *in vivo* studies [42]. PREX1 studies have demonstrated that it plays a part in the release of insulin from pancreatic β cells in response to glucose stimulation. Additionally, it is involved in the movement of the glucose transporter, GLUT4, to the outer surface of adipocytes, which is dependent on insulin suggested that PREX2 gene deletion in mice is associated with reduced insulin signaling and thus reduced glucose uptake in liver and adipose tissue. Also, reduced PREX2 expression in the adipose tissue of humans with insulin resistance was reported [43]. In the present study, serum levels of PREX1 were negatively correlated with those of PREX2 that mostly reflects their levels of tissue expression.

Conclusions. Obese individuals from Iraq who do not have diabetes exhibited elevated levels of PREX1 in their bloodstream compared to both overweight and normal-weight individuals. In contrast, the obese subjects had lower levels of serum PREX2 compared to the overweight subjects, and the overweight subjects had lower levels than the normal-weight subjects. PREX1 correlated positively with the markers of insulin resistance and dyslipidemia, while PREX2 correlated negatively with the markers of insulin resistance but not with the markers of dyslipidemia.

Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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КОРЕЛЯЦІЯ МІЖ РІВНЕМ ПРОТЕЇНІВ PREX ТА МАРКЕРАМИ ІНСУЛІНОРЕЗИСТЕНТНОСТІ ТА ЛІПІДНИМ ПРОФІЛЕМ У ПАЦІЄНТІВ ІЗ ОЖИРІННЯМ ТА НАДМІРНОЮ ВАГОЮ БЕЗ ЦУКРОВОГО ДІАБЕТУ

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Метаболічні порушення та ожиріння пов'язані з багатьма метаболічними змінами, зокрема, з порушенням чутливості до інсуліну та дисліпідемією. Останні дослідження підкреслюють ключову роль фосфатидилінозитол 3,4,5-трифосфат-залежних протеїнів обміну Rac (PREX-протеїнів) у патогенезі ожиріння, що спонукає до подальшого з'ясування їх потенційних терапевтич-

них ефектів. Метою даного дослідження було оцінити рівень протеїнів PREX у сироватці крові та його потенційний зв'язок із маркерами інсулінорезистентності та рівнем ліпідів у плазмі крові пацієнтів із ожирінням та надмірною вагою, які не хворіють на цукровий діабет. У дослідженні взяли участь 30 осіб із ожирінням, 30 – з надмірною вагою і 30 здорових осіб аналогічного віку і статі. Рівні PREX1 і PREX2 визначали за допомогою ELISA. Рівні інсуліну, глюкози, глікозильованого гемоглобіну, загального ліпідного профілю визначали за допомогою відповідних фотометричних наборів. Як показник чутливості до інсуліну використовували індекс HOMA-IR. Показано, що пацієнти з ожирінням без діабету мали вищий рівень PREX1 у сироватці крові порівняно з особами з надмірною та нормальною вагою. PREX1 позитивно корелював із маркерами інсулінорезистентності та дисліпідемії. Рівень PREX2 виявився нижчим як у пацієнтів із ожирінням порівняно з пацієнтами з надмірною вагою, так і у пацієнтів із надмірною вагою порівняно з особами з нормальною вагою. PREX2 негативно корелював із маркерами інсулінорезистентності, але не з маркерами дисліпідемії.

Ключові слова: протеїни PREX, ожиріння, надмірна вага, інсулінорезистентність, дисліпідемія.

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