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Authors: Katarzyna Skrypnik, Dagmara Pluta, Mariusz Wojtowicz, Tahar Ben Rhaiem, Joanna Suliburska

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Association between serum levels of calcium, magnesium, iron and copper and insulin resistance in women with full blown and not-full blown phenotypes of polycystic ovary syndrome

Katarzyna Skrypnik[1], Dagmara Pluta[2], Mariusz Wojtowicz[3], Tahar Ben Rhaiem[4], Joanna Suliburska[5]

[1]*Department of Human Nutrition and Dietetics, Faculty of Food Science and Nutrition, Poznan University of Life Sciences, Poznan, Poland*
[2]*Department of Gynecological Endocrinology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland*
[3]*Department of Gynecological and Obstetrics, Women's and Child Health Center, Medical University of Silesia, Zabrze, Poland*
[4]*Clinical Department of Obstetrics, Gynecology and Oncological Gynecology in Kedzierzyn-Kozle, Poland*
[5]*Department of Human Nutrition and Dietetics, Faculty of Food Science and Nutrition, Poznan University of Life Sciences, Poznan, Poland*

Corresponding author:
Dagmara Pluta
Department of Gynecological Endocrinology, School of Medicine in Katowice, Medical University of Silesia, Medykow St., 44–752 Katowice, Poland
e-mail: dpluta@sum.edu.pl

ABSTRACT

The aim of present study was to investigate the association between serum calcium, iron, magnesium, copper levels and insulin resistance in women with full blown phenotype of polycystic ovary syndrome (PCOS) compared to women with not-full blown phenotype.
104 women, aged 18–39, in the first phase of menstrual cycle, diagnosed with PCOS based on the Rotterdam Criteria, were qualified for the study. Patients were divided into two groups:

group I contained women with full blown PCOS (phenotype A) and group II contained women with not-full blown PCOS (phenotypes B, C and D). Whole study population was divided on group X containing women with proper insulin sensitivity and group Y containing women with insulin resistance. The study found that women with full blown PCOS had lower level of magnesium compared with not-full blown phenotypes. Also, the level of copper was lower in group with proper insulin sensitivity compared to group with insulin resistance. Serum cooper content showed a negative correlation with Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) in group with full blown phenotype. Magnesium level showed positive correlation with level of calcium and cooper in group with proper insulin sensitivity. Level of iron content showed a negative correlation with sex hormone binding globulin (SHBG) and HOMA-IR showed a positive correlation with age and body mass index (BMI) in group with insulin resistance. Either level of calcium showed positive correlation with iron and cooper in group with insulin resistance.
Keywords: insulin resistance; polycystic ovary syndrome; phenotypes; micronutrients

INTRODUCTION

Polycystic ovary syndrome (PCOS) affects approximately 15% of women in reproductive age [1]. Etiology of PCOS seems to be complex, however genetic, metabolic and environmental factors are involved. According to Rotterdam Criteria, PCOS should be diagnosed, when two of the following criteria are met: oligoovulation or anovulation [ovulatory dysfunction (OD)]; hyperandrogenism (HA) and/or polycystic ovaries (PCO). Importantly, other disorders with clinical presentation similar to PCOS should be excluded, in this range: Cushing's syndrome, congenital adrenal hyperplasia and androgen-secreting tumors. Hyperandrogenism should be presented clinically — in a form of hirsutism, acne or androgenic alopecia, and/or in biochemical investigations, of which the measurement of free testosterone or the free testosterone (free androgen) index seems to be the most reliable [2]. Polycystic ovaries are defined as “presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (> 10 mL) in ultrasonographic (USG) examination [2]. According to National Institute of Health (NIH), there are four phenotypes of PCOS: full blown PCOS (phenotype A; all three criteria met); non-PCO PCOS (phenotype B; hyperandrogenism and ovulatory dysfunction); ovulatory PCOS (phenotype C; hyperandrogenism and polycystic ovaries) and non-hyperandrogenic PCOS (phenotype D; ovulatory dysfunction and polycystic ovaries) [2]. In most studies, phenotype A predominates and constitutes more than a half of cases of PCOS [3]. PCOS development is tightly

associated with insulin-resistance (IR) and studies show, that from 40% up to 70% of women with PCOS are affected by IR [4]. Mechanisms leading to IR in women with PCOS include epigenetic and genetic alterations, hyperandrogenaemia, disruption of insulin signal transduction, inflammation and excessive body mas increase [5]. In significant range of PCOS patients IR takes part in further development of diabetes type 2 and obesity [4].

Glucose and insulin metabolism are strongly related to minerals: calcium (Ca), iron (Fe), magnesium (Mg) and copper (Cu) [5–8]. Insulin secretion is a process dependent on calcium [6]. Studies suggests that improper calcium metabolism, especially elevated level of parathormone, may be associated with the pathogenesis of PCOS [6]. In healthy population, serum calcium level positively correlates with insulin level and insulin resistance [9]. In females with PCOS, especially in those with insulin-resistance, serum calcium level is also elevated [10]. In women with PCOS insulin resistance, morphology of ovaries and androgen levels associate to serum concentration of some calcium-regulating hormones: lower level of osteocalcin and higher level of its carboxylated form [11].

There is a broad range of reliable evidences on cross-talk between iron metabolism and glucose and insulin metabolism [8, 12–14].

Magnesium is a co-factor of a range of enzymes responsible for glucose metabolism and enhances dependent from insulin glucose uptake in adipose cells. Magnesium deficiency may contribute to IR [15]. It has been shown that serum magnesium level is diminished in women with PCOS, especially in those with insulin-resistance, compared to healthy females [10]. However, in the light of discrepant results showing no association between magnesium deficiency and IR in women with PCOS [16].

There are evidences linking copper blood concentration with the development of IR. Elevated serum copper levels are associated with increased secretion of insulin in β-cells, especially in patients in hyperglycemic condition [17]. Most studies show that serum copper level in patients with type 2 diabetes is higher than in healthy persons [7, 18, 19]. However, some contradictory findings are also available [7, 20]. A recent meta-analysis on over 1160 women with PCOS and more than 1100 controls shown, that PCOS is strongly associated with increased circulating copper levels [21], but opposite results are also available [22].

Insulin sensitivity varies across different phenotypes of PCOS. Metabolic abnormalities are the strongest in women with anovulation and hyperandrogenism, independently from PCO; mild or absent in patients with PCO and anovulation without hyperandrogenism or with hyperandrogenism and PCO with ovulatory cycles; and absent in women with PCO and regular cycles, even if subtle hormonal disturbances may be present

[23]. However, the link between IR and calcium, iron, magnesium and copper serum level in different PCOS phenotypes remains unknown. The aim of our paper was to investigate the association between selected micronutrients levels and insulin resistance in women with full blown phenotype of PCOS compared to women with not-full blown phenotype.

MATERIAL AND METHODS

The study was designed as a clinical trial. Study protocol was approved by Ethics Committee (no. KNW/0022/KB1/140/II/15/16). Women with PCOS diagnosed according to Rotterdam Criteria [3] were enrolled. After enrolment patients were divided into two groups: group I contained women with full blown PCOS (phenotype A) and group II contained women with not-full blown PCOS (phenotypes B, C and D). Also, for further analysis, the whole study population was divided on group X containing women with proper insulin sensitivity [Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) < 2.5] and group Y containing women with insulin resistance (HOMA-ID ≥ 2.5). After enrolment anthropometric measurements were performed. Also, after enrolment fasting blood samples from forearm vein, in the follicular phase between the 2^{nd} and 5^{th} day of the menstrual cycle, were collected to whole-blood and serum-separate tubes after overnight fast. Serum samples were stored at -80 degrees Celsius for further biochemical and mineral analyses.

One hundred four women aged 18–39, in the first phase of menstrual cycle were initially screened at the Department of Gynecological Endocrinology, Medical University of Silesia in Katowice in Poland. Patients who met all the down-mentioned inclusion criteria were enrolled: informed consent in writing, PCOS diagnosed according to Rotterdam Criteria, right age, the right phase of the cycle. Exclusion criteria were hyperprolactinemia, hypercortisolemia, thyroid disorders, hormonal contraceptive therapy, steroids intake, antiandrogens intake, dietary supplements intake in the last three months, viral and autoimmune liver diseases, focal changes in the liver, alcohol consumption over 20 g per day and any fluctuations of body weight in the last six months. Subjects who met any of the exclusion criteria were not included into the study. The occurrence of any of these exclusion criteria during the study would cause immediate withdrawal of the patient from the trial.

All anthropometric measurements were performed after overnight fast and rested in conditions of metabolic laboratory with subjects wearing light clothes, without shoes. Body mass was measured with electronic scales to the nearest 0.1 kg. Height was measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as mass divided by height squared (kg/m^2). Waist circumference (WC) was estimated to the nearest 0.5 cm at the end of a normal

expiration between the iliac crest and the lower rib. The waist hip ratio (WHR) was calculated by dividing waist measurement by hip measurement.

The ultrasound examination was performed with the Voluson 730 Expert.

The evaluation of insulin resistance was conducted using the indirect method with the HOMA-IR index.

Using the colorimetric method, the markings in the lipid profile serum and glucose [analyzer AU 680 with reagents from Beckman Coulter (Brea, CA, USA)] were made. Using the method of chemiluminescence [with microparticles and chemiluminescence marker (CMIA) and reagents by Abbott (Architect i2000SR; Chicago, IL, USA)], the following serum concentrations were marked: estradiol, follicle-stimulating hormone (FSH), luteinising hormone (LH), testosterone, 17-OH-progesterone, androstenedione, sex hormone binding globulin (SHBG), and insulin. Liver parameters were marked using a COBAS c501 chemistry analyzer from Roche.

The Ca, Mg, Fe and Cu contents in serum were determined after digestion in 65% (w/w) spectra pure HNO_3 (Merck, Kenilworth, NJ, USA) with the use of Microwave Digestion system (Mars 5, CEM, Matthews, NC, USA). Upon digestion and dilution with deionized water, the concentrations of Ca, Mg, Fe and Cu in the mineral solutions were measured with the use of flame atomic absorption spectrometry (AAS-3, Carl Zeiss, Jena, Germany). The mineral contents of serum were determined at wavelengths of 248.3 nm for Fe, 324.8 nm for Cu, 422.7 nm for Ca and 285.2 for Mg. The accuracy of the method was verified with certified reference materials (HUM ASY CONTROL 2, Sero, Billingstad, Norway) and was 95% for Fe, 103% for Cu, 91% for Ca, 98% for Mg.

The patients' randomization codes were blinded until the statistical analysis. Data are shown as means ± standard deviations (SDs). All statistics were calculated using Statistica 10.0 software (StatSoft, Krakow, Poland). The Shapiro–Wilk test was performed to check the normal distribution. Comparisons between groups were performed with the use of Mann-Whitney U Test. The correlation coefficients were calculated with Spearman's rank analysis. It was calculated that a sample size of at least XX subjects in each group would yield at least 80% power of detecting a difference that was statistically significant at the .05 α level. A p-value of less than 0.5 was regarded as significant. No important changes to methods after trial commencement have been implemented.

RESULTS

A random cohort of 150 patients from the 118 with PCOS diagnosed according to Rotterdam Criteria [2] was screened. Eight patients were not included in the study due to the presence of exclusion criteria or lack of inclusion criteria. A total of 110 subjects were enrolled and allocated into groups I (n = 65) and II (n = 45). All participants from both groups underwent data collection, blood sample collection and measurement procedures. Three women from the group I and three women from group II were excluded from the trial after completion of data collection, blood sample collection and measurement procedures because of low blood samples quality and low data quality. A total of 104 participants — 62 from group I and 42 from group II — underwent statistical analysis. All patients in group I had full blown phenotype of PCOS (phenotype A) and II group had not full-blown phenotype (phenotype B, C and D). In group II there were 16 patients with phenotype B of PCOS, 14 patients with phenotype C and 12 patients with phenotype D. The trial was completed when data collection, blood sample collection and measurement procedures from all women from both groups was completed.

The baseline characteristics of the study groups are shown in Table 1. Patients' body mass and fasting blood insulin were higher in group I. There were no differences between the study groups in patients' age, waist circumference, fasting blood glucose, and day of the menstrual cycle.

Table 1. Study population characteristics

Parameter	**Group I**	**Group II**	**p value**
Participants	62	42	–
Age [years]	25.73 ± 5.00	25.36 ± 3.75	0.6855
Body mass [kg]	74.65 ± 18.32	65.96 ± 14.05	0.0107
WC [cm]	85.77 ± 18.13	79.98 ± 11.87	0.0739
Glucose [mg/dL]	88.97 ± 8.17	87.21 ± 8.20	0.2879

Insulina 0' [uIU/mL]	9.26 ± 5.97	6.45 ± 3.03	0.0060
Day of the menstrual cycle	5.89 ± 1.64	8.24 ± 14.73	0.2148

Parameters are shown as mean ± standard deviation (SD); WC — waist circumference

The results of anthropometric parameters are presented in Table 2. There were no differences between study groups in height, WC and WHR. Women in group I had higher body mass, BMI and hip circumference (HC) compared to group II.

Table 2. Anthropometric results

Parameter	**Group I (n = 62)**		**Group II (n = 42)**		**p value**
	Mean ± SD	**Median [Q1; Q3]**	**Mean ± SD**	**Median [Q1; Q3]**	
Height [cm]	166.56 ± 5.91	165.00 [162.00; 170.00]	165.76 ± 7.69	166.00 [162.00; 170.00]	0.5490
Body mass [kg]	74.65 ± 18.32	67.50 [60.25; 89.00]	65.96 ± 14.05	65.50 [55.50; 73.00]	0.0107
BMI [kg/m^2]	26.96 ± 6.68	25.12 [21.60; 31.99]	23.86 ± 4.04	23.80 [20.75; 24.77]	0.0084
WC [cm]	85.77 ± 18.13	80.00 [76.00; 98.00]	79.98 ± 11.87	77.00 [70.75; 82.50]	0.0739
HC [cm]	101.56 ± 11.75	98.00 [94.00; 107.50]	94.61 ± 9.86	94.00 [89.00; 98.50]	0.0023
WHR	0.85 ± 0.12	0.84 [0.78; 0.91]	0.82 ± 0.08	0.80 [0.77; 0.86]	0.2329

SD — standard deviation; Q1 — first quartile; Q3 — third quartile; BMI — body mass index; WC — waist circumference; HC — hip circumference; WHR — waist hip ratio

Serum concentrations of biochemical parameters are shown in Table 3. There were no differences between study groups in serum levels of total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), fasting glucose, insulin in 30 min. of oral glucose tolerance test (OGTT), alanine transaminase (Alat), and gamma-glutamyl transferase

(GGT). Serum levels of triglycerides (TG), glucose in 120 min. of OGTT, fasting insulin, insulin in 60 min. and 120 min. of OGTT, aspartate transaminase (Aspat) and HOMA-IR were higher in group I compared to group II.

Table 3. Serum concentration of biochemical parameters

Parameter	Group I (n = 62)		Group II (n = 42)		p value
	Mean ± SD	Median [Q1; Q3]	Mean ± SD	Median [Q1; Q3]	
TC [mg/dL]	178.84 ± 32.97	175.00 [159.25; 192.50]	172.48 ± 31.30	172.50 [142.25; 192.00]	0.3268
HDL [mg/dL]	51.69 ± 12.81	51.00 [42.25; 58.00]	56.31 ± 11.51	55.00 [46.75; 64.00]	0.0633
LDL [mg/dL]	103.35 ± 26.30	100.50 [87.75; 115.75]	99.14 ± 24.85	99.50 [80.50; 111.00]	0.4147
TG [mg/dL]	124.63 ± 74.92	100.00 [6900; 169.50]	88.38 ± 34.86	81.00 [64.25; 105.00]	0.0042
Glucose [mg/dL]	88.97 ± 8.17	87.00 [84.00; 92.00]	87.21 ± 8.20	87.50 [82.00; 91.00]	0.2879
Glucose 120 min [mg/dL]	110.30 ± 32.83	107.00 [91.00; 130.00]	91.99 ± 21.30	90.50 [82.25; 104.00]	0.0019
Insulina 0' [uIU/mL]	9.26 ± 5.97	7.10 [5.10; 11.64]	6.45 ± 3.03	5.82 [4.46; 8.09]	0.0060
HOMA-IR	2.07 ± 1.59	1.62 [1.05; 0.95]	1.39 ± 0.68	1.26 [1.05; 1.76]	0.0000
ALAT [U/L]	25.56 ± 23.92	18.50 [15.00; 24.75]	18.45 ± 14.07	15.00 [13.00; 19.00]	0.0861
Aspat [U/L]	25.40 ± 13.90	21.00 [19.00; 25.00]	19.88 ± 5.93	19.00 [17.00; 22.00]	0.0170
GGT [U/L]	27.47 ± 32.78	17.00 [13.00; 24.25]	17.98 ± 15.42	14.00 [11.00; 17.25]	0.0835

SD — standard deviation; Q1 — first quartile; Q3 — third quartile; TC — total cholesterol; HDL — high density lipoprotein; LDL — low density lipoprotein; TG — triglycerides; HOMA-IR — Homeostasis Model Assessment of Insulin Resistance; ALAT — alanine transaminase; Aspat — aspartate transaminase; GGT — gamma-glutamyl transferase

Serum levels of calcium, iron, magnesium and copper are depicted in Table 4. Serum level of magnesium was lower in group I compared to group II. There were no other differences between study groups in the range of blood mineral concentrations.

Table 4. Serum concentration of minerals

Parameter	Group I (n = 62)		Group II (n = 42)		p value
	Mean ± SD	Median [Q1; Q3]	Mean ± SD	Median [Q1; Q3]	
Ca [ug/mL]	115.02 ± 18.81	113.84 [108.33; 120.34]	116.85 ± 14.04	118.32 [109.81; 122.38]	0.5928
Mg [ug/mL]	21.08 ± 2.45	21.37 [19.52; 23.08]	22.28 ± 2.29	21.71 [21.02; 23.58]	0.0135
Fe [ug/mL]	1.18 ± 0.47	1.14 [0.88; 1.45]	1.30 ± 0.50	1.27 [0.96; 1.63]	0.2342
Cu [ug/mL]	0.75 ± 0.30	0.77 [0.51; 0.97]	0.80 ± 0.25	0.84 [0.64; 1.00]	0.4376

SD — standard deviation; Q1 — first quartile; Q3 — third quartile

Serum concentration of investigated hormones is shown in Table 5. Serum level of LH and testosterone were higher in group I compared to group II, while serum concentration of SHBG was lower in group I compared to group II. There were no differences in blood concentrations of estradiol, FSH, 17-OH progesterone and androstenedione.

Table 5. Serum concentration of hormones

Parameter	Group I (n = 62)		Group II (n = 42)		p value
	Mean ± SD	Median [Q1; Q3]	Mean ± SD	Median [Q1; Q3]	
Estradiol [pg/mL]	41.05 ± 19.29	34.10 [29.80; 46.88]	40.72 ± 24.82	30.45 [26.23; 40.55]	0.9480
LH [IU/L]	10.05 ± 6.13	8.95 [5.85; 13.48]	7.31 ± 4.16	6.45 [4.93; 8.50]	0.0114
FSH [IU/L]	6.51 ± 5.43	5.90 [5.03; 6.68]	5.95 ± 1.61	5.90 [5.00; 7.00]	0.5233
SHBG [ng/mL]	48.40 ± 34.11	47.20 [26.70; 63.60]	70.86 ± 25.61	65.65 [50.63; 84.25]	0.0003
Testo [ng/mL]	0.49 ± 0.17	0.48 [0.34; 0.66]	0.38 ±	0.37 [0.29; 0.50]	0.0036

(0.084–0.481)			0.16		
17-OH progesteron [ng/mL] (0.2–1.3)	1.81 ± 0.70	1.64 [1.35; 2.19]	1.67 ± 0.40	1.56 [1.39; 1.88]	0.2364
Androstendion [ng/mL] (0.3–3.3)	22.58 ± 1.63	3.68 [2.65; 4.36]	21.52 ± 1.23	2.64 [1.95; 3.42]	0.8925

SD — standard deviation; Q1 — first quartile; Q3 — third quartile; LH — luteinising hormone; FSH — follicle-stimulating hormone; SHBG — sex hormone binding globulin

Blood morphological parameters are presented in Table 6. Red blood cells (RBC), white blood cells (WBC) and PCT levels were higher, and P-LCR was lower in group I compared to group II. Levels of HB, PLT, MCV, MCH, MCHC, HCT, RDW-SD, RDW-CV, MPV, and PDW were comparable between groups.

Table 6. Blood morphological parameters

Parameter	**Group I (n = 62)**		**Group II (n = 42)**		**p value**
	Mean ± SD	**Median [Q1; Q3]**	**Mean ± SD**	**Median [Q1; Q3]**	
HB [g/dL] (11.5–15.0)	13.57 ± 1.06	13.60 [12.83; 14.18]	13.32 ± 0.93	13.45 [12.65; 14.00]	0.2235
RBC [10e6/uL] (3.7–5.0)	4.56 ± 0.39	4.59 [4.30; 4.80]	4.39 ± 0.32	4.36 [4.13; 4.69]	0.0227
WBC [10e3/uL] (4.0–10.0)	6.99 ± 1.84	6.30 [5.53; 8.48]	5.69 ± 1.06	5.75 [4.81; 6.40]	0.0001
PLT [10e3/uL] (130–400)	255.40 ± 61.46	260.00 [201.25; 296.50]	241.64 ± 46.01	243.00 [216.25; 278.00]	0.2198
MCV [fL] (84–98)	88.43 ± 5.37	88.00 [85.63; 91.00]	89.13 ± 3.96	88.90 [87.00; 92.00]	0.4694
MCH [pg] (27–31)	29.87 ± 1.93	29.70 [28.83; 30.90]	30.30 ± 1.61	30.20 [29.23; 31.60]	0.2330
MCHC [g/dL] (32–36)	33.71 ± 0.71	33.80 [33.30; 34.18]	33.92 ± 0.71	34.00 [33.40; 34.40]	0.1459
HCT [%] (36–46)	40.03 ± 2.91	39.95 [38.13; 41.80]	39.20 ± 2.47	39.35 [37.43; 41.20]	0.1305
PCT [%] (0.17–0.35)	0.24 ± 0.05	0.23 [0.20; 0.27]	0.22 ± 0.04	0.22 [0.20; 0.24]	0.0213
RDW-SD [fL] (37–54)	43.52 ± 2.67	43.00 [42.00; 45.00]	43.60 ± 2.44	44.00 [42.00; 45.00]	0.8809
RDW-CV [%] (11–16)	13.14 ± 1.01	13.05 [12.53; 13.78]	13.00 ± 0.75	12.90 [12.53; 13.30]	0.4346

MPV [fL] (7–15)	9.38 ± 0.94	9.20 [8.80; 9.90]	9.08 ± 0.86	9.15 [8.60; 9.60]	0.0977
P-LCR [%] (13–43)	86.28 ± 29.51	28.30 [23.80; 34.25]	97.46 ± 15.64	30.20 [28.90; 31.50]	0.0306
PDW [fL] (9–17)	14.81 ± 2.41	14.50 [13.35; 16.00]	14.74 ± 2.40	14.40 [13.30; 16.50]	0.8778

SD — standard deviation; Q1 — first quartile; Q3 — third quartile; RBC — red blood cells; WBC — white blood cells

The comparison of anthropometric parameters between groups X and Y are presented in Table 7. Women in group X had lower body mass, BMI, WC and WHR compared to group Y.

Table 7. Anthropometric results

Parameter	**Group X (n = 72)**		**Group Y (n = 31)**		**p value**
	Mean ± SD	**Median [Q1; Q3]**	**Mean ± SD**	**Median [Q1; Q3]**	
Age [years]	26.17 ± 4.85	25 [23; 28.25]	24.32 ± 3.41	24 [22; 26]	0.1074
Height [cm]	166.31 ± 7.18	165.50 [162; 170]	166.32 ± 5.33	166.00 [162.50; 170]	0.9283
Body mass [kg]	66.29 ± 13.24	63.00[58; 71.50]	82.90 ± 19.81	80.00 [69; 97.50]	0.0000
BMI [kg/m^2]	23.96 ± 4.61	23.23 [20.67; 25.18]	29.88 ± 6.69	30.11 [25.11; 35.04]	0.0000
WC [cm]	79.99 ± 10.52	78.00 [73.25; 84.50]	90.65 ± 23.18	89.00 [76.50; 105.50]	0.0034
HC [cm]	96.86 ± 9.67	96.00 [91; 100]	103.26 ± 14.32	100.00 [92; 114.50]	0.0568
WHR	0.80 ± 0.08	0.80 [0.73; 0.86]	0.92 ± 0.11	0.90 [0.84; 0.98]	0.0000

SD — standard deviation; Q1 — first quartile; Q3 — third quartile; BMI — body mass index; WC — waist circumference; HC — hip circumference; WHR — waist hip ratio

Serum levels of selected minerals in group X and Y are shown in Table 8. Serum level of copper was lower in group X compared to group Y. There were no other differences between study groups in the range of blood mineral concentrations.

Table 8. Serum concentration of minerals

Parameter	Group X (n = 72)		Group Y (n = 31)		p value
	Mean ± SD	Median [Q1; Q3]	Mean ± SD	Median [Q1; Q3]	
Ca [ug/mL]	114.69 ± 19.06	114.62 [106.68; 121.05]	118.09 ± 11.09	116.66 [111.48; 124.74]	0.1204
Mg [ug/mL]	21.55 ± 2.55	21.47 [19.77; 23.41]	21.61 ± 2.28	21.56 [20.03; 22.74]	0.9618
Fe [ug/mL]	1.26 ± 0.49	1.19 [0.93; 1.55]	1.15 ± 0.46	1.06 [0.83; 1.32]	0.1483
Cu [ug/mL]	0.73 ± 0.28	0.76 [051; 0.93]	0.86 ± 0.26	0.92 [0.66; 1.08]	0.0196

SD — standard deviation; Q1 — first quartile; Q3 — third quartile

Serum Cu content showed a negative correlation with SHBG and HOMA-IR in group I. SHBG showed a negative correlation with HOMA-IR in group I. Also, SHBG showed a negative correlation with HOMA-IR in group II. Other significant correlations of study parameters in group I and group II are depicted in Table 9.

Table 9. Significant correlations of study parameters in group I and group II

Group I		**Group II**	
Serum Cu & SHBG	–0.29	SHBG & HOMA-IR	–0.55
Serum Cu & HOMA-IR	–0.44	17-OH progesteron & HOMA-IR	0.33
SHBG & HOMA-IR	–0.43	BMI & HOMA-IR	0.51
Testosteron & BMI	0.32	Estradiol & serum Ca	0.48
Testosteron & HOMA-IR	0.34	Serum Mg & serum Ca	0.35
Androstendion & HOMA-IR	0.37	Serum Mg & serum Cu	0.51

BMI & HOMA-IR	0.48	Serum Fe & serum Ca	0.39
Serum Cu & BMI	0.28		
Serum Cu & serum Mg	0.59		
Serum Cu & serum Ca	0.46		
Serum Mg & serum Ca	0.52		
Serum Fe & wiek	0.38		

Data presented as the Spearman correlation coefficient R value; SHBG — sex hormone binding globulin; HOMA-IR — Homeostasis Model Assessment of Insulin Resistance; BMI — body mass index

Significant correlations of study parameters in group X and group Y are presented in Table 10. Homeostasis Model Assessment of Insulin Resistance showed a negative correlation with SHBG and positive correlation with 17-OH progesterone and BMI in group X. Also, serum Mg showed positive correlation with serum Ca and serum Cu as well as serum Fe showed positive correlation with age in group X. Serum Fe content showed a negative correlation with SHBG and HOMA-IR showed a positive correlation with age and BMI in group Y. Either serum Ca showed positive correlation with serum Fe and Cu in group Y.

Table 10. Significant correlations of study parameters in group X and group Y

Group X		**Group Y**	
SHBG & HOMA-IR	-0.43	Serum Fe & SHBG	-0.41
17-OH progesteron & HOMA-IR	0.32	BMI & HOMA-IR	0.41
BMI & HOMA-IR	0.26	Age & HOMA-IR	0.42
Serum Mg & serum Ca	0.25	Serum Ca & serum Fe	0.43
Serum Mg & serum Cu	0.32	Serum Ca & serum Cu	0.44
Serum Fe & wiek	0.28		

Data presented as the Spearman correlation coefficient R value; SHBG — sex hormone binding globulin; HOMA-IR — Homeostasis Model Assessment of Insulin Resistance; BMI — body mass index

DISCUSSION

The present work has shown correlation between patients' body mass, BMI, fasting blood insulin and hip circumference in group with full blown phenotype of PCOS (group I) compared to not full-blown phenotypes (group II). Higher values of these parameters have been demonstrated also in many other studies [3]. No significant differences were noted in the waist circumference, waist hip ratio, fasting blood glucose and day of the menstrual cycle.

This work confirms that women with the full blown phenotype were more insulin resistant than those without full blown phenotype [3]. However, several studies did not confirm these findings [24]. This may be due to several limitations related to the use of substitute measurement methods, the lack of measurement standards or the method of assigning to individual groups.

Another serum concentrations of biochemical parameters like TG, glucose in 120 min. of OGTT, fasting insulin, insulin in 60 min. and 120 min. of OGTT and Aspat were higher in group I compared to group II and there were no differences between study groups in serum levels of TC, LDL, HDL, fasting glucose, insulin in 30 min. of OGTT, Alat, and GGT. Other authors also point to the occurrence of disturbances in biochemical parameters. However, it is important to remember about other factors influencing the differences in results, such as the body mass of the study group, the presence of fatty liver or the division into phenotypes [25, 26].

This paper has shown a higher serum level of LH and testosterone were in group I compared to group II, while serum concentration of SHBG was lower in group I compared to group II. There were no differences in blood concentrations of estradiol, FSH, 17-OH progesterone and androstenedione. Higher levels of luteinizing hormone and total testosterone, were also confirmed in another study, but the authors did not take into account the division into phenotypes, but only into normal and excessive body mass [25]. However, Sachdeva et al. [27] noted, that the FSH, LH, LH-FSH ratio and 17-OHprogesterone, levels were not significantly different among various PCOS phenotypes.

Elevation level of WBC, RBC and PTC was found in blood morphological parameters and lowered level of P-LCR in group I compared to group II, which is in agreement with other authors. This also agrees with the statement that PCOS is a chronic low-grade inflammation in which androgens are also predictors of leukocyte count [28].

However, the correlation between blood mineral concentration and IR in the context of PCOS phenotype is a completely new direction of research.

In this work has been demonstrated lower serum level of magnesium in group I compared to group II and there were no other differences between study groups in the range of calcium, iron, and copper. When Mg was co-supplemented with other agents, it was found to improve the inflammatory response, insulin resistance and lipid metabolism in PCOS patients, but there was no benefit from supplementing Mg alone [29].

When considering insulin sensitivity, serum level of copper was lower in the group with proper insulin sensitivity (group X) compared to group with insulin resistance (group Y). There were no other differences between study groups in the range of blood mineral concentrations. In the present work serum Cu content showed a negative correlation with SHBG and HOMA-IR in group I. Many authors observe higher levels of this element in women with PCOS [30]. Prodarchuk et al. [31] found a tendency to increase serum copper concentration in women with PCOS, but the differences were not confirmed statistically and there no division into phenotypes was used.

In this paper serum magnesium showed positive correlation with serum calcium and serum Cu as well serum iron showed positive correlation with age in group X. Serum Fe content showed a negative correlation with SHBG and HOMA-IR showed a positive correlation with age and BMI in group Y. Either serum Ca showed positive correlation with serum Fe and Cu in group Y. The influence of Mg, Fe and Ca levels on pathology of PCOS remains unclear [32]. In many articles level of Mg and Fe was slightly higher or elevated in women with PCOS and Ca was reduced [30, 33, 34]. However, the vast majority of works have serious limitations, like lack of consistency in nutrient and formulations or dosages, different diagnostic criteria or cut-offs and outcome measures.

CONCLUSIONS -

The study found that women with full blown PCOS had lower serum level of magnesium compared with not-full blown phenotypes. Also, serum level of copper was lower in group with proper insulin sensitivity compared to group with insulin resistance in the study group of patients. Serum cooper content showed a negative correlation with HOMA-IR in group with full blown phenotype. The serum level of magnesium showed positive correlation with serum level of calcium and cooper in group with proper insulin sensitivity. Serum level of iron content showed a negative correlation with SHBG and HOMA-IR showed a positive correlation with age and BMI in group with insulin resistance. Either serum level of calcium showed positive correlation with serum iron and cooper in group with insulin resistance.

Article information and declarations

Data availability statement

The data used to support the findings of this study are included within the aricle.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee (no. KNW/0022/KB1/140/II/15/16). Informed consent was obtained from all subjects involved in the study.

Author contributions

Skrypnik Katarzyna — 40%: concept, article draft.

Pluta Dagmara — 30%: corresponding author, article draft.

Wójtowicz Dariusz — 5%: analysis and interpretation of data.

Ben Rhaiem Tahar — 10%: analysis and interpretation of data, software.

Suliburska Joanna — 15%: supervision, project administration.

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Conflict of interest

Authors nothing to declare conflict of interest.

Supplementary material

None.

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