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Proinsulin, adiponectin and hsCRP in reproductive age women with polycystic ovary syndrome (PCOS) — the effect of metformin treatment

Proinsulina, adiponektyna i hsCRP u kobiet w wieku rozrodczym z zespołem policystycznych jajników (PCOS) — wpływ leczenia metforminą

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Abstract

Introduction: Women with polycystic ovary syndrome (PCOS) often suffer from obesity and insulin resistance. The role of proinsulin, which is known to be an indicator of fertility outcomes in PCOS women, and that of adiponectin, in the pathogenesis of PCOS is not well elucidated. Our objective was to determine proinsulin, adiponectin, hsCRP and other hormonal and metabolic parameters in PCOS women before and after metformin treatment.

Material and methods: Two PCOS groups of patients of reproductive age (90 lean and 88 obese or overweight) with two control groups, adjusted for body mass index (BMI), were compared at baseline. 32 PCOS women were studied at baseline, after three and six months of metformin (1,000 mg/day) treatment. Clinical, anthropometric, biochemical and hormonal parameters were assessed.

Results: Proinsulin and hsCRP levels were the highest in obese PCOS women and were statistically different than in lean PCOS women (proinsulin: 11.4 *v.* 6.9 pmol/L; hsCRP 2.46 *v.* 0.47 mg/L, $p < 0.01$) and than in obese controls. Levels of adiponectin were dependant on BMI. Plasma proinsulin and androstenedione levels decreased after metformin treatment only in obese PCOS women.

Conclusions: PCOS, when accompanied by obesity, is associated with elevated proinsulin concentrations, which correlates with higher hsCRP and increased FAI. Proinsulin level decreases due to metformin treatment. Our results suggest that obese or overweight PCOS and lean PCOS are characterised by different hormonal and metabolic parameters and have a different response to metformin treatment. **(Endokrynol Pol 2014; 65 (1): 2–10)**

Key words: *PCOS; proinsulin, insulin resistance; adiponectin; C-reactive protein; obesity*

Streszczenie

Wstęp: Zespół policystycznych jajników (PCOS) często wiąże się ze współwystępowaniem otyłości i insulinooporności. Rola proinsuliny, której stężenie koreluje z płodnością w PCOS, oraz adiponektyny nie jest określona w patogenezie PCOS. Celem pracy było zbadanie stężeń proinsuliny, adiponektyny, hsCRP i innych hormonalnych i metabolicznych parametrów u kobiet z PCOS przed i po leczeniu metforminą.

Materiał i metody: Porównano 2 grupy kobiet w wieku rozrodczym z PCOS (90 z prawidłową masą ciała i 88 z nadwagą lub otyłością) z 2 grupami kontrolnymi dobranymi pod względem wskaźnika masy ciała (BMI). Trzydzieści dwie kobiety z PCOS, u których wdrożono leczenie metforminą w dawce 1000 mg/d, były zbadane w warunkach podstawowych, po 3 oraz 6 miesiącach leczenia. Oceniano parametry kliniczne, antropometryczne, biochemiczne i hormonalne.

Wyniki: Otyłe kobiety z PCOS charakteryzowały się najwyższymi stężeniami proinsuliny i hsCRP , które były statystycznie istotnie wyższe w porównaniu ze szczupłymi kobietami z PCOS (proinsulina: 11,4 *v.* 6,9 pmol/l; hsCRP 2,46 *v.* 0,47 mg/l, $p < 0,01$) i z otyłymi kobietami z grupy kontrolnej. Stężenia adiponektyny były zależne od BMI. Stosowanie metforminy spowodowało obniżenie stężeń proinsuliny i androstendionu tylko w grupie otyłych kobiet z PCOS.

Wnioski: Zespół PCOS przebiegający z nadwagą lub otyłością wiąże się ze zwiększonym stężeniem proinsuliny, które koreluje z podwyższonym hsCRP i zwiększonym wskaźnikiem FAI. Stężenie proinsuliny ulega obniżeniu podczas leczenia metforminą. Uzyskane wyniki sugerują, że kobiety szczupłe i otyłe z PCOS charakteryzują się różnymi parametrami hormonalnymi i metabolicznymi oraz różną odpowiedzią na działanie metforminy. **(Endokrynol Pol 2014; 65 (1): 2–10)**

Słowa kluczowe: *PCOS; proinsulina; insulinooporność; adiponektyna; białko C-reaktywne; otyłość*

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous condition affecting 5–10% of women of reproductive age [1]. Regardless of the diagnostic criteria used, many different phenotypes of PCOS may be identified. Despite the strong relationship of PCOS and obesity, insulin resistance, diabetes, chronic inflammation and other metabolic disturbances, neither elevated insulin resistance indices nor body mass index (BMI) are criteria for recognising PCOS. The pathogenesis of PCOS is still unknown. Many factors including genetics, intrauterine or environmental ones play a significant role.

There are two paths which could be taken in order to attempt an explanation of hormonal disturbances and etiology of PCOS: insulin resistance, hyperinsulinaemia and defects in insulin action on the one hand, and disturbances in gonadotropin action on the other.

Women with PCOS have a much higher risk of type 2 diabetes and metabolic syndrome [2, 3]. The American Diabetes Association treats PCOS as a risk factor for type 2 diabetes mellitus (T2DM) and recommends screening [4].

Looking for factors which could play a role in the pathogenesis of PCOS, especially when it is accompanied by obesity, we concentrate on proinsulin and adiponectin. High-sensitive C-reactive protein (hsCRP), as a marker of chronic inflammation in PCOS, could also be elevated.

Proinsulin, which is an insulin precursor produced in the pancreas, can be a marker of insulin resistance or beta-cell dysfunction [5, 6] and is suspected to be involved in the pathogenesis of macroangiopathy. Proinsulin concentration is an independent indicator of future type 2 diabetes development. It has been found that in diabetic patients proinsulin level correlates with insulin resistance better than homeostasis model assessment (HOMA) [7]. How the proinsulin level correlates with fertility in PCOS patients is still unclear, but, as Rausch et al. revealed [8] proinsulin, but not insulin concentration, correlates with ovulation, pregnancy rate and live birth. Higher proinsulin levels have been determined in women who have never been pregnant vs. fertile women [9].

Adiponectin is an adipocytokine produced in white adipose tissue (especially visceral fat tissue), but its concentration is reduced in obesity. The adiponectin level correlates negatively with insulin resistance, and hypoadiponectinaemia is also associated with T2DM, obesity, atherosclerosis and coronary vascular diseases (CVD). Thus adiponectin could be treated as a potential factor involved in the pathogenesis of PCOS.

Using available criteria for PCOS such as the Rotterdam or The Androgen Excess Society (AES) criteria, we can recognise many different phenotypes of PCOS [10–12]. Neither obesity nor insulin resistance are essential for a diagnosis of PCOS, but due to epidemiological data and suggestions of experts of AES, at least one of them should be crucial for the final diagnosis of PCOS.

In the present study, we investigated proinsulin, adiponectin and hsCRP levels in PCOS patients vs. controls, but normal or increased BMI was a criterion for assigning a subject to one of the examined groups. Many other hormonal and metabolic factors were determined, too. In the second part of the study, PCOS women were treated with a small dose of metformin (500 mg two times daily) for six months. We investigated the influence of metformin treatment on proinsulin, adiponectin, hsCRP and on clinical outcomes and hormonal parameters like androgen levels in the whole group of PCOS participants and within two subgroups: lean and overweight/obese PCOS patients.

Material and methods

Subjects

We investigated 178 PCOS women (aged 16–40 years): 90 lean (BMI < 25 kg/m^2) and 88 overweight or obese (BMI ≥ 25 kg/m^2) and 42 (aged 17–40) healthy women (31 with BMI < 25 kg/m^2 and 11 with BMI ≥ 25 kg/ m^2). A diagnosis of PCOS was made according to the Rotterdam criteria [13]. All women in the control groups were in good general health, had regular menstruations, no sign of hyperandrogenism and normal-appearing ovaries in ultrasonography. They had not used any drugs chronically, including oral contraceptive pills (OCP).

All subjects were classified into one of four groups:

— Group 1 — lean PCOS (BMI < 25 kg/m^2);
— Group 2 — obese or overweight PCOS (BMI ≥ 25 kg/m^2);
— Group 3 — lean controls (BMI < 25 kg/m^2);
— Group 4 — obese or overweight controls (BMI ≥ 25 kg/m^2).

In the second part of the study, 32 PCOS women (N = 16 with BMI < 25 kg/m^2 and N = 16 with BMI ≥ 25 kg/m^2) were treated with metformin in a dose of 1,000 mg daily. Clinical, hormonal and biochemical assessment was performed at baseline and after three and six months of treatment. In none of the 32 patients were impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or T2DM diagnosed at baseline. The full observational period was finished by 28 patients.

All the participants gave their informed consent before the study, which was approved by the institutional ethics committee.

Protocol

All subjects underwent a physical examination which included anthropometric measurements and detailed

past and present medical history including drugs, menstrual history and fertility. BMI was calculated.

All hormonal and biochemical measurements were performed in the follicular phase of spontaneous or progestin-induced menstrual cycle.

Measurements

After an overnight fast, basal levels of proinsulin, adiponectin, hsCRP, sex hormone binding globulin (SHBG), estradiol (E2), testosterone, androstenedione, and DHEA-S were determined. Free androgen index (FAI) and free testosterone (fT) were calculated as previously described [14, 15]. All subjects underwent an oral glucose tolerance test (OGTT) with a load of 75 g glucose; glucose and insulin at 0, 30, 60 and 120 min. were determined, and insulin resistance indices (HOMA, Quicki, Matsuda, SigmaIRI and area under the curve (AUC) for glucose and insulin) were calculated as previously described.

Assays

Proinsulin was determined by radioimmunoassay (RIA) kit, LINCO Res. (sensitivity — 2 pmol/L, range: 2–100 pmol/L, specifity — human proinsulin = 100%, human insulin < 0.1%, C-peptide < 0.1%) and adiponectin by RIA kit, LINCO Res. (sensitivity — 1ng/mL, specifity — human adiponectin = 100%, human C1q < 0.01%).

Insulin levels were determined by BI-INSULIN IRMA, CIS Bio International; (sensitivity — 0.2 μIU/mL, range: 0.2–500 μIU/mL, specifity — human proinsulin < 0.0001%, C-peptide < 0.003).

Plasma glucose concentrations were determined by an oxydase method (Integra 400);

HsCRP was measured using the immunoturbidimetric method (Integra 400).

Levels of serum total testosterone, androstenedione and DHEA-S were quantified by RIA (TESTO-CT2 Kit - CIS Bio International, France; Androstendione RIA DSL-3800, Diagnostic Products Corporation, USA, Spectria DHEAS RIA kit - Orion Diagnostica, Finland, respectively).

SHBG was determined by immunoradiometric assay (IRMA) method (Spectra SHBG IRMA test — Orion Diagnostica), estradiol by an automated chemiluminescence system (Immulite 2000; Diagnostic Products Corporation, USA).

Statistical analysis

Values are reported as mean ± SD and as a median in the brackets. Normality of distribution was assessed by Shapiro-Wilk test. Because of the absence of normality, nonparametric testing: U-Mann Whitney, ANOVA Kruskal-Wallis, ANOVA Friedmann were used. Spearman rank correlation was used to evaluate the relationship between analyzed data. For statistical analysis Statistica 5.1 PL and 9.0 PL programmes were used. The limit of statistical significance was set at $P < 0.05$.

Results

Clinical, hormonal and metabolic parameters at baseline

Baseline characteristics of all groups are presented in Table IA, B and C.

The 'obese PCOS' group was characterised by higher insulin resistance. There were statistically significant differences in all checked insulin-resistance indices between 'obese PCOS' and 'lean PCOS'. 'Obese PCOS' and 'obese Controls' differed in most insulin-resistance indices, too.

Hs-CRP levels were the highest in the group of 'obese PCOS'; there was statistically significant difference between this group vs. 'obese Controls' (2.46 *v.* 0.88 mg/L; $p < 0.05$) and *v.* 'lean PCOS' (2.46 *v.* 0.47 mg/L; $p < 0.05$), however there were no differences between 'lean PCOS' *v.* 'lean Controls' and 'lean Controls' *v.* 'obese Controls'. Data is presented in Table IC and Figure 1.

Proinsulin and adiponectin levels at baseline

Proinsulin concentrations were statistically different and the highest in the group of 'obese PCOS' women compared to 'lean PCOS' women (Me = 11.4 pM/L *v.* 6.95, $p < 0.001$) and to 'obese Controls' (Me = 11.4 pM/L *v.* 7.7, $p < 0.05$). There were no differences between 'obese Controls' vs. 'lean Controls' and 'lean PCOS' *v.* 'lean Controls'. Data is presented in Table II and Figure 2. Adiponectin concentrations differed between 'lean PCOS' *v.* 'obese PCOS' and 'lean Controls' *v.* 'obese Controls'; no other differences were found. Data is shown in Table II.

Correlations

Proinsulin concentrations were positively correlated with FAI ($r = 0.23$, $p < 0.05$) and hsCRP ($r = 0.4$, $p < 0.05$) only in the group of obese PCOS women. Data is shown in Table III.

Metformin treatment

Baseline characteristics of the group of 32 PCOS women who were treated with metformin are presented in Table IVA and Figure 3 (M1). In the whole group during the six-month period of treatment we observed statistically significant decrease in body mass, BMI, androstenedione and proinsulin levels (Table IVA, Fig. 3); there was no influence on adiponectin concentration. We did not observe any changes in other checked parameters (data not shown).

All determined parameters were checked in subgroups — lean and obese PCOS. This analysis revealed

Table I. *Comparison between four groups:* **A.** *clinical characteristics,* **B.** *hormonal characteristics,* **C.** *metabolic characteristics*

Tabela I. *Porównanie w czterech badanych grupach:* **A.** *charakterystyka kliniczna;* **B.** *badania hormonalne;* **C.** *badania metaboliczne*

	A. Clinical characteristics					
	Group 1	**Group 2**	**p**	**Group 3**	**Group 4**	**p**
	Lean PCOS	**Obese PCOS**		**Lean Controls**	**Obese Controls**	
Body mass [kg]	58,37 ± 6.14 [58] a	86.34 ± 18.1 [82.5] a	< 0.01	56.02 ± 6.91 [56.5] a	78.88 ± 18.37 [67.4] a	< 0.01
BMI	21.03 ± 1.83 [21.05] a	31.69 ± 5.81 [30.36] a	< 0.01	20.76 ± 2.09 [20.24] a	29.38 ± 5.99 [25.85] a	< 0.01
WHR	0.74 ± 0.04 [0.74] a	0.85 ± 0.1 [0.82] a	< 0.01	0.73 ± 0.04 [0.73] a	0.8 ± 0.04 [0.79] a	< 0.01
	B. Hormonal characteristics					
	Group 1	**Group 2**	**p**	**Group 3**	**Group 4**	**p**
	Lean PCOS	**Ob. PCOS**		**Lean Controls**	**Ob.Controls**	
Testosterone [ng/mL]	0.75 ± 0.3 [0.7] c	0.83 ± 0.35 [0.8] b	NS	0.55 ± 0.2 [0.6] c	0.56 ± 0.2 [0.6] b	NS
Androstenedione [ng/dL]	355 ± 121 [331] c	376 ± 163 [366] b	NS	230 ± 77 [225] c	265 ± 97 [275] b	NS
DHEA-S [ng/mL]	3.167 ± 1.191 [3.004] c	3.347 ± 1.432 [3.022] a	NS	2.185 ± 772 [2.038] c	2/.634 ± 784 [2.552] a	NS
SHBG [nmol/L]	56.01 ± 22.82 [53] a	31.77 ± 18.61 [27] a	< 0.01	64.44 ± 24.33 [60] a	42.03 ± 21.97 [35.3] a	< 0.05
FAI	5.3 ± 2.8 [4.5] c	11.1 ± 7.2 [8.8] b	< 0.01	3.1 ± 1.2 [4.0] c	4.8 ± 2.2 [5.1] b	< 0.05
fT [pmol/L]	33.94 ± 13.84 [31.85] c	44.64 ± 17.17 [41.77] b	< 0.001	22.87 ± 8.87 [21.45) c	28.73 ± 11.35 [23.68] b	NS
E2 [pg/mL]	52.06 ± 38.14 [41.15] c	52.46 ± 25.55 [47.00] a	NS	69.30 ± 42.30 [56.90] c	49.70 ± 16 [46.35] a	NS
	C. Metabolic characteristics					
	Group 1	**Group 2**	**p**	**Group 3**	**Group 4**	**p**
	Lean PCOS	**Obese PCOS**		**Lean Controls**	**Obese Controls**	
Fasting glucose [mg/dL]	83.52 ± 7.18 [83.40] a	87.23 ± 7.55 [87.00] a	< 0.01	82.78 ± 6.69 [82.00] a	85.46 ± 9.38 [85.00] a	NS
Fasting insulin [μIU/mL]	5.26 ± 2.85 [5] a	11.69 ± 6.75 [10] b	< 0.01	4.70 ± 1.58 [5] a	5.50 ± 2.27 [4,5] b	NS
HOMA	1.21 ± 0.9 [0.98] a	2.54 ± 1.53 [2.2] b	< 0.01	1.02 ± 0.46 [0.97] a	1.46 ± 1.01 [1.09] b	NS
Quicki	0.387 ± 0.038 [0.385] a	0.345 ± 0.037 [0.339] b	< 0.01	0.389 ± 0.027 [0.39] a	0.374 ± 0.034 [0.38] b	NS
AUC glucose	12.941 ± 2.422 [12.972] c	14.208 ± 2.642 [14.001] a	< 0.01	11.718 ± 2.243 [11.507] c	13.959 ± 3.627 [14.010] a	NS
AUC insulin	4.177 ± 2.284 [3.495] c	7.934 ± 4.787 [7.125] b	< 0.01	2.774 ± 1.014 [2.520] c	3.171 ± 1.122 [2.580] b	NS
Sigma IRI	118 ± 59 [100] c	230 ± 135 [211] b	< 0.01	81 ± 28 [74] c	129 ± 111 [86] b	NS
Matsuda index	10.71 ± 5.97 [9.83] c	5.64 ± 3.79 [4.26] b	< 0.01	12.39 ± 3.93 [12.19] c	9.12 ± 5.04 [10.41] b	NS
hsCRP [mg/L]	0.78 ± 0.69 a [0.47]	3,70 ± 3,77 b [2.46]	< 0.05	0.83 ± 0.68 a [0.56]	1.33 ± 1.16 b [0.88]	NS

a — $p > 0.05$ when comparing group 1 *v.* group 3 and group 2 *v.* group 4; b — significant difference between group 2 and group 4, $p < 0.05$; c — significant difference between group 1 and group 3, $p < 0.05$

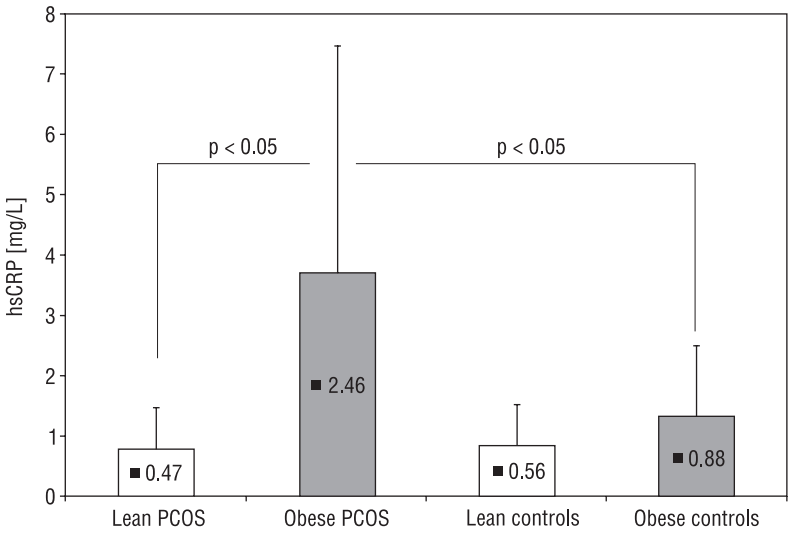

Figure 1. *High sensitive C-reactive protein (hsCRP) values. Comparison between four subgroups.* ■ *— median*
Rycina 1. *Stężenia hsCRP w 4 podgrupach.* ■ *— mediany*

Table II. ***Proinsulin and adiponectin levels in four groups***
Tabela II. ***Stężenia proinsuliny i adiponektyny w czterech badanych grupach***

	Group 1 Lean PCOS	Group 2 Obese PCOS	p	Group 3 Lean Controls	Group 4 Obese Controls	p
Proinsulin [pmol/L]	7.67 ± 3.47 [6.9] a	12.9 ± 7.23 [11.4] b	< 0.01	6.85 ± 2.65 [6.6] a	8.21 ± 2.86 [7.7] b	NS
Adiponectin [μg/mL]	12.13 ± 4.21 [11.5] a	9.52 ± 4.44 [8.3] a	< 0.01	13.30 ± 4.8 [12.1] a	9.52 ± 3.94 [10.4] a	0.06

a — $p > 0.05$ when comparing group 1 *v.* group 3 and group 2 *v.* group 4; b — significant difference between group 2 and group 4, $p < 0.05$; c — significant difference between group 1 and group 3, $p < 0.05$

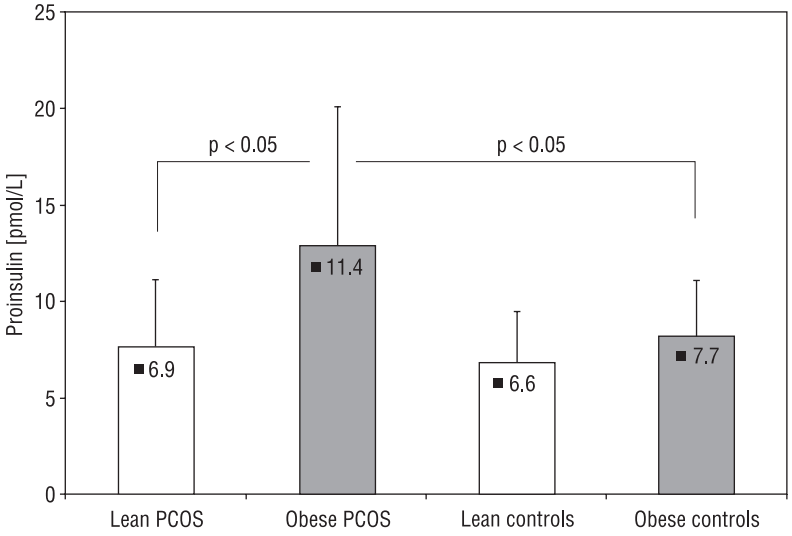

Figure 2. *Proinsulin concentrations. Comparison between four groups.* ■ *— median*
Rycina 2. *Stężenia proinsuliny w 4 podgrupach.* ■ *— mediany*

Table III. *Correlations within lean and obese PCOS groups*
Tabela III. *Korelacje w grupie szczupłych i otyłych kobiet z PCOS*

Proinsulin [pmol/L]	Lean PCOS (group 1)		Obese PCOS (group 2)	
	r	p	r	p
hsCRP [mg/L]	–0.052530	NS	**0.417037**	< 0.05
Androstenedione [ng/dL]	0.000287	NS	–0.034978	NS
Testosterone [ng/mL]	0.212666	NS	–0.053709	NS
FAI	0.122670	NS	**0.229280**	< 0.05

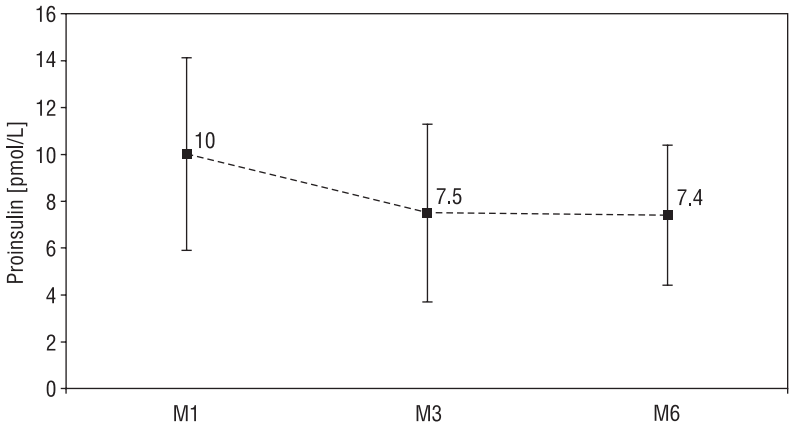

Figure 3. *The influence of metformin treatment in a group of 32 PCOS women on proinsulin concentration. M1 — baseline, M3 — 3 month treatment period, M6 — 6 month treatment period. Data are presented as medians and quartiles, p < 0.01*

Rycina 3. *Wpływ leczenia metforminą na stężenia proinsuliny w grupie 32 osób z PCOS. M1 — wyjściowo, M3 — po 3 miesiącach leczenia, M6 — po 6 miesiącach leczenia. Dane przedstawiono jako mediany i kwartyle, p < 0,01*

that the statistically significant decrease in the whole group was due to changes in the 'obese PCOS' group (Table IVB), whereas changes in 'lean PCOS' were not statistically significant. Figure 4 shows these findings for proinsulin concentrations. Similar results were found for body mass, BMI and androstenedione concentration.

Discussion

These results demonstrate that women with PCOS and excess body fat are characterised by different hormonal and metabolic parameters, including the highest proinsulin levels, compared to both lean PCOS patients and obese controls. Moreover, exclusively in this group correlations between proinsulin and hsCRP and proinsulin and FAI are observed and metformin causes significant decreases in proinsulin and androstenedione levels solely in obese/overweight PCOS patients.

It has been noted repeatedly that PCOS *per se* is an independent risk factor of insulin resistance [16–18] and that obese PCOS women have different metabolic and hormonal parameters than lean PCOS patients [19]. Pasquali et al. demonstrated [20] that obese PCOS women are characterised by worse insulin resistance than lean PCOS patients. As much as 30-70% of PCOS women are overweight or obese [21–23]. Therefore comparing this population to a healthy control group, without adjusting for BMI, could be misleading.

Our study confirmed different metabolic and hormonal profiles in PCOS women with increased BMI vs. lean PCOS patients. This could suggest that there is a different pathogenesis of this syndrome in patients with different phenotypes. The AES criteria [11] point clinical or biochemical hyperandrogenism as a *sine qua non* condition for the diagnosis of PCOS; the problem of many different phenotypes of PCOS could also be connected to the presence of obesity and/or insulin resistance.

The group of overweight/obese PCOS women was characterised by worse insulin resistance and the highest and statistically different concentrations of proinsulin, FAI, free testosterone and hsCRP than those in a group of lean PCOS and in obese controls. Correlations between these factors existing only in a group of overweight/obese PCOS, and the above-mentioned

Table IV. *The influence of metformin treatment in PCOS women on selected parameters.* **A.** *The influence of metformin treatment in a group of 32 PCOS women on selected parameters;* **B.** *The influence of metformin treatment in a group of 'obese PCOS' women on selected parameters. M1 — baseline, M3 — 3 month treatment period, M6 — 6 month treatment period*

Tabela IV. *Wpływ leczenia metforminą kobiet z PCOS na wybrane parametry.* **A.** *Wpływ leczenia metforminą na wybrane parametry w grupie 32 osób z PCOS;* **B.** *Wpływ leczenia metforminą na wybrane parametry w grupie osób z PCOS z nadwagą/otyłych. M1 — wyjściowo, M3 — po 3 miesiącach leczenia, M6 — po 6 miesiącach leczenia*

A. The influence of metformin treatment in a group of 32 PCOS women on selected parameters				
	M1	**M3**	**M6**	**p**
Body mass [kg]	71.33 ± 15.34 [67]	69.78 ± 14.33 [68.7]	69.43 ± 13.93 [67]	P < 0.05
BMI [kg/m^2]	26.26 ± 5.95 [24.20]	25.67 ± 5.54 [23.90]	25.54 ± 5.43 [23.91]	P < 0.05
Proinsulin [pmol/L]	10.0 ± 4.1 [9.0]	7.5 ± 3.8 [7.0]	7.4 ± 4.0 [6,6]	P < 0.001
Androstenedione [ng/dL]	380 ± 133 [375]	326 ± 131 [286]	279 ± 117 [237]	p < 0.05
Adiponectin [μg/mL]	9.68 ± 3.59 [8.6]	9.92 ± 3.31 [9.5]	9.51 ± 2.68 [9.1]	NS
B. The influence of metformin treatment in a group of 'obese PCOS' women on selected parameters				
	M1	**M3**	**M6**	**p**
Body mass [kg]	87.5 ± 14.58 [82.5]	85 ± 13.54 [82.8]	84.2 ± 13.38 [81.8]	< 0.05
BMI [kg/m^2]	31.19 ± 4.44 [30.52]	30.29 ± 4.06 [29.5]	30.02 ± 4.00 [29.57]	< 0.05
Proinsulin [pmol/L]	11.73 ± 4 [10.5]	8.55 ± 4 [8]	7.94 ± 3.2 [7,7]	< 0.05
Androstenedione [ng/dL]	387 ± 151 [358]	288 ± 120 [263]	268 ± 122 [233]	< 0.05
Adiponectin [μg/mL]	8.52 ± 2.8 [7.6]	8.63 ± 2.4 [8.2]	8.40 ± 2.0 [8.4]	NS

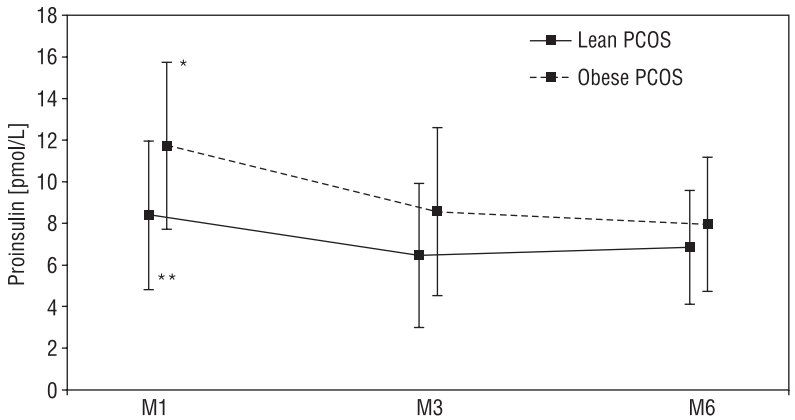

Figure 4. *The influence of metformin treatment in subgroups of lean and obese PCOS women on proinsulin. M1 — baseline, M3 — 3 month treatment period, M6 — 6 month treatment period.Data presented as means ± SD, * p < 0.05 ** p > 0.05 (NS)*

Rycina 4. *Wpływ leczenia metforminą na stężenia proinsuliny w podgrupie „PCO szczupłe' i „PCO otyłe'. M1 — wyjściowo, M3 — po 3 miesiącach leczenia, M6 — po 6 miesiącach leczenia. Dane przedstawiono jako średnie ± SD. *p < 0.05, **p > 0.05 (NS)*

differences, suggest that we should consider two different phenotypes of PCOS:
— phenotype with normal BMI,
— phenotype with increased BMI.

Proinsulin concentration seems to be not only a marker of insulin resistance, but also a factor directly involved in the development of metabolic and hormonal disturbances in PCOS in the phenotype with increased BMI. Proinsulin could also play a role in the fertility in PCOS, as revealed in the study of Rausch et al.: "We were surprised to find proinsulin as a marker of success in ovulation, conception, pregnancy, and live birth in PCOS women" [8].

Levels of adiponectin in obese or overweight PCOS patients did not differ in obese or overweight healthy women, nor in lean PCOS *v*. lean controls. It seems that adiponectin level was related rather to fat mass accumulation than to PCOS *per se.*

C-reactive protein is treated as a marker of early atherosclerosis. It is also an independent, sensitive marker of risk of CVD [3, 24, 25]. In our study obese/overweight PCOS women were characterised by the highest hsCRP concentrations and there were no differences in hsCRP levels between lean and obese controls and lean PCOS vs. lean controls. These differences between groups were analogous to those in proinsulin concentrations. In obese PCOS there was a correlation between hsCRP and proinsulin; such a correlation was not observed in lean PCOS women.

It is recommended to measure free testosterone or calculate it [15] in the process of establishing the diagnosis of PCOS. In our work there were statistically significant differences in the level of fT between groups, the highest value being determined in 'obese PCOS'. The concentration of androstenedione, the position of which in the diagnosis of PCOS is still not well established, seems to be a sensitive, well differentiating androgen, which could be helpful in the diagnosis of hyperandrogenaemia. Androstenedione was the only androgen which decreased due to metformin treatment only in 'obese PCOS'.

Low dose of metformin resulted in a statistically significant decrease in proinsulin concentration after only three months of treatment and persisted during the next three months. The very important observation is that such an effect in the PCOS group was dependant on the decrease in 'obese PCOS'. It is known [26] that obese PCOS women could benefit from pretreatment with metformin in ovulation induction. It has been shown that the lower the proinsulin concentration before pregnancy in PCOS women, the higher the chance of delivering a live birth [8]. Adiponectin concentration has not changed due to metformin treatment, although there was a decrease in body mass and BMI. In some previous studies [27, 28], the authors observed decrease in adiponectin, but the examined population of PCOS was characterised by very high BMI (mean 35 kg/m^2 and 43 kg/m^2, respectively) so the effect could be secondary to the significant body mass reduction. In our study we have not observed decrease in hsCRP concentration. Previous studies have shown such an effect of metformin, especially in obese PCOS women [29]. Our results could be due to the relatively short observation period or the low dose of metformin, whereas it was sufficient to cause proinsulin decrease because of more direct influence.

Metformin treatment in metabolically high risk patients with PCOS seems to be also beneficial because of the known influence of metformin on AMP-kinase (AMPK) and its potential antiatherogenic, antineoplasmatic and life-prolonging action, via target of rapamicine (TOR) mechanism [30–33].

The advantage of our study is a number of included patients and the comparison of four groups, which eliminates the possible influence of excess body mass, not PCOS itself, on investigated parameters. It has revealed that hyperproinsulinaemia is dependent on coexistence of PCOS and obesity, but adiponectin concentration is rather due to body mass.

To the best of our knowledge, what is new in this study is the recognition of a relationship between proinsulin concentration and hsCRP and FAI solely in obese PCOS patients and, finally, a decrease in proinsulin and androstenedione concentration after metformin treatment.

This study has some notable limitations. Firstly, there was a disproportion between PCOS and control groups. It was determined by the small number of healthy young women without any menstrual disturbances nor hyperandrogenic signs who wouldn't take any medicines, including OCP. Secondly, PCOS was diagnosed according to the Rotterdam Criteria, which have some widely known limitations and generate many different phenotypes of PCOS.

Conclusions

PCOS in obese women is associated with hyperproinsulinaemia, hyperandrogenaemia and increased hsCRP level, which correlate with each other.

Proinsulin seems to be a sensitive marker of insulin resistance in PCOS women.

The practical conclusion which can be drawn from this study is that in all PCOS patients both body fat and insulin resistance should be determined. The measurement of proinsulin level would be a practical index suggesting the phenotype of PCOS and potential usefulness of metformin treatment.

High priority ought to be placed on preventing the concurrent occurrence of PCOS and increased BMI.

Moreover, pharmacological treatment in PCOS should be attuned to the phenotype the patient presents. We suggest that, due to metabolic disarrangements and body fat, we can recognise different phenotypes of PCOS.

This leads to a question: does an obese PCOS woman suffer from the same syndrome as a lean PCOS woman?

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