

Leptin concentrations in patients with polycystic ovary syndrome before and after met-formin treatment depending on insulin resistance, body mass index and androgen concentrations – introductory report

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Abstract: Polycystic ovary syndrome (PCOS) is an endocrinological and metabolic disorder which may concern about 3-8 % of women. Some PCOS women have the increased leptin concentration in blood serum. Leptin concentration is higher in patients with high body mass index (BMI) and impaired tissue sensitivity to insulin. The aim of this study was to determine leptin concentrations in PCOS patients before and after metformin treatment depending on BMI, insulin resistance calculated on the basis of the Homeostasis Model Assessment (HOMA) index, as well as concentrations of androgens: testosterone and androstendion. Such values as BMI, insulin resistance according to the HOMA index, and concentrations of androstendion, testosterone and leptin were determined in 35 patients with PCOS before and after 3-month metformin treatment administered in daily doses of 2 x 850 mg. Increased leptin levels before the therapy were observed in 91.3 % (21 out of 23) of obese patients, in 75% (9 out of 12) non-obese patients, in 100% (8 patients) insulin resistance women, in 81.5% (22 out of 27) insulin sensitive patients, in 94.7 % (18 out of 19) women with elevated androstendion concentration and in 75% (12 out of 16) with normal androstendion concentration, in 93.7% (15 out of 16) patients with increased testosterone concentration and in 78.9% (15 out of 19) patients with testosterone concentrations within the normal range. After treatment statistically significant decrease in leptin concentration was obtained in the patients with BMI \leq 25, insulin-sensitive patients (HOMA $<$ 3.8) and patients whose testosterone and androstendion concentrations stayed within normal limits. Increased leptin concentrations may be one of the elements of PCOS clinical picture. Metformin treatment considerably reduces leptin concentration, if it is employed in non-obese PCOS patients, patients with normal androgen concentrations and those who not have an impaired glucose tolerance.

Key-words: PCOS, leptin, insulin resistance, BMI, androgens, metformin.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common reasons of menstrual disorders and infertility in women. According to different sources it can be observed in 5-8 % of women of reproductive age [1,2]. At present, PCOS is diagnosed on the basis of Rotterdam Sponsored Consensus Workshop Group criteria established at the conference of European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (ESHRE/ASRM)

in 2003 [3]. In order to diagnose polycystic ovary syndrome, 2 of the following symptoms must occur: rare or no ovulation, hyperandrogenism – either clinical one or that revealed in laboratory tests, or the ultrasound picture of polycystic ovaries. At the same time, other reasons for hyperandrogenism should be excluded.

It is accepted that PCOS is a genetic disease, and yet an influence of environmental and hormonal factors is also taken into consideration [4,5]. Clinical symptoms observed in polycystic ovary syndrome are mainly: menstrual disorders (oligomenorrhoea), infertility resulting from chronic anovulation, hirsutism, acne, seborrhea and obesity concerning 35 – 60% of patients. Ultrasonographic scanning reveals enlarged ovaries greater than 10cm³ with multiple small antral follicles and hormonal tests show increased androgen

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concentrations. Hyperandrogenism in PCOS is the consequence of greater than normal activity of theca cells chronically stimulated by acyclically secreted luteinizing hormone (LH). Relative follicle-stimulating hormone (FSH) deficiency, in turn, results in anovulation [5,6].

Apart from clinical and ultrasonographic symptoms as well as hormonal irregularities which are necessary to diagnose PCOS, there are also metabolic disturbances observed in some patients. Hyperinsulinemia resulting from tissue insulin resistance is found in 50% of patients with PCOS. If not treated, such disorders may lead to the development of type 2 diabetes in the future. It is therefore so important that all patients suspected of PCOS undergo a 75 g oral glucose tolerance test (OGTT) and have insulin concentrations determined so as carbohydrate metabolism disorders could be assessed [7,8].

According to latest recommendations of Androgen Excess Society (AES) all patients with PCOS, regardless of BMI, should undergo a 75 g oral glucose tolerance test. It is necessary to repeat this test after 2 years or frequently if there are any others risk factors of diabetes mellitus or every year if there are any symptoms of impaired glucose tolerance in 75 g oral glucose tolerance test [9].

Some authors report on increased serum leptin concentration in PCOS patients [1,2,10-12]. Leptin is a hormone produced predominantly in adipocytes. Presence of leptin receptors was observed in hypothalamus, liver, skeletal muscles, myocardium, ovaries, testicles, kidneys and adipose tissue [13]. The role of leptin in an organism has not been fully explained so far. It is assumed that it is involved in the regulation of appetite, pubescence and fat metabolism. After binding leptin to hypothalamus receptors, production and secretion of neuropeptide Y, responsible for appetite stimulation, become inhibited [14,15]. Leptin concentration in blood is directly proportional to BMI, but it also depends on tissue sensitivity to insulin. It is considerably higher in overweight and insulin-resistant people, than in individuals with proper body mass and normal sensitivity to insulin [16,17].

It is possible that leptin is an element linking mechanisms of energy balance with individual and sexual development. The situation when energetic resources of an organism diminish is a signal to increase food intake, and also to reduce fertility requiring considerable energy expenditure. Nevertheless, obese women whose leptin concentrations are high, often suffer from ovulatory disorders and infertility as well [18].

In case of women with PCOS and obesity, certain results suggest higher leptin concentration than it could be expected concerning BMI [19-22]. Perhaps in these cases, leptin can affect fertility through disturbing central hormonal regulation, which is mainly relat-

ed to the correlation between leptin, neuropeptide Y and LH, and indirectly also ovulation [18].

In people with the diagnosed impaired glucose tolerance and insulin resistance, it is justifiable to employ medicines which sensitize tissues to insulin. Patients usually receive metformin, an oral agent belonging to the biguanide group, which not only improves insulin sensitivity but also indirectly positively affects ovaries. About 40% of non-ovulating patients start to ovulate naturally [23,26]. It is also believed that metformin therapy significantly reduces leptin concentration both in obese and slim women [23,26-28].

The aim of this study was to assess leptin concentrations in the patients with the diagnosed polycystic ovary syndrome before and after metformin treatment depending on BMI, insulin resistance calculated on the basis of the Homeostasis Model Assessment (HOMA) index, as well as concentrations of androgens: testosterone and androstendion.

Material and methods

Patients. The research involved 35 patients with polycystic ovary syndrome diagnosed on the basis of Rotterdam criteria and treated in Department of Reproduction and Gynecology, Pomeranian Medical University. All the patients were carefully interviewed and information concerning menstrual disorders and fertility were collected. During the clinical examination, body mass and height were measured. On the basis of the obtained results, BMI (body mass index – the ratio of body mass in kg to the height in m raised to the second power) was calculated. The following normal ranges for BMI were accepted: ≤ 25 – normal weight; > 25 – overweight and obesity. Ultrasonographic examination of the reproductive organ was carried out using a 3.5 MHz intravaginal probe, Toshiba Tosbee.

Study protocol. After evaluation of liver and kidney activity (the concentrations of AspAT, AlAT, urea and creatinine were determined), patients were classified for the 3-month met-formin treatment administered in daily doses of 2×850 mg. Criteria excluding patients from the examination were: hypersensitivity to metformin, heart disease, hypertension, anaemia, increased serum concentrations of aminotransferases (norm: AST 10-31 U/L, ALT < 36.4 U/L), and elevated serum concentrations of urea and/or creatinine (norm: urea 17-34 mUg/dl, creatinine: 0.66-1.09 mg/dl), alcoholism and type 1 or type 2 diabetes. After this period of therapy, the women underwent all examinations performed at the beginning of treatment. Both before and after treatment, patients were divided into 2 groups: with BMI > 25 and ≤ 25 ; next the presence or lack of insulin resistance was determined in each patient by means of the HOMA index. Serum concentrations of testosterone, androstendion and fasting leptin were analysed in each patient.

Hormone concentration. Concentrations of hormones, namely androstendion (normal range: 0.5-3.5 ng/ml), testosterone (normal range: 0.06-0.82 ng/ml), FSH, LH, prolactin (PRL) and fasting leptin concentration were determined in blood serum. Leptin concentration < 8.2 ng/ml was accepted as normal – according to the local laboratory kit used to assess leptin concentration. All hormonal tests were performed in the fasted status (last meal at 6 pm on the day before the test) between 3rd and 5th day of menstrual period.

Oral glucose tolerance test. Additionally, in order to diagnose metabolic disorders, the patients were subjected to a 75 g oral glu-

cose tolerance test, during which concentrations of fasting glucose and insulin were determined and after 120 minutes from glucose administration. Using the obtained values of glucose and insulin concentrations, the Homeostasis Model Assessment (HOMA) index for insulin resistance assessment was calculated. Insulin resistance was diagnosed when it is ≥ 3.8 [29].

All blood tests were performed in Central Laboratory of Autonomous Public Clinical Hospital No. 1 of Pomeranian Medical University in Szczecin. To testosterone, androstendion, glucose and insulin concentrations assessments Roche laboratory kits were used. Glucose concentrations were evaluated by enzymatic method with hexokinase on Cobas Integra 400. Testosterone, androstendion and insulin concentrations were evaluated with electrochemoluminescence method (ECL) on Cobas e601. Leptin concentrations were evaluated by enzyme-linked immunosorbent assay method (ELISA) with Biowender kit.

Statistical analysis. Statistical analysis was performed using Statistica 6.0. The t-student test was used in case of normally distributed values, and in other case the Wilcoxon-Cox rank test was applied. $p < 0.05$ was accepted as statistically significant.

Results

From among all the patients with the diagnosed polycystic ovary syndrome, 30 women (85.7%) had the increased leptin concentrations. Before treatment in the group of 23 patients with BMI > 25 , the increased leptin levels were found in 21 (91.3%) patients. The mean leptin concentration in these women reduced after metformin treatment but the observed fall was not statistically significant. (Table 1). Before the therapy, 9 (75%) out of 12 patients with BMI ≤ 25 , had the elevated leptin levels. During metformin treatment, leptin concentration considerably decreased in this group. (Table 2). In the group of 8 patients with insulin resistance determined by means of the HOMA index, all the examined women had the increased leptin concentrations before the therapy. In this group the fall in leptin concentrations was noted after treatment, but it was not statistically significant. (Table 3). Conversely, in the group of 27 women with normal insulin sensitivity, the increased leptin concentrations were observed in 22 (81.5%) patients and the fall in leptin concentration in these women after metformin treatment was statistically significant. (Table 4).

Leptin concentrations were also analysed before and after treatment in the patients with the increased androstendion levels. In the group of 19 such women, 18 (94.7%) ones had leptin concentrations above normal range. In these women the fall in leptin concentration was observed, but it was not significant. (Table 5). On the contrary, considerable fall was noted in those women whose androstendion levels were within the normal range at the moment of PCOS diagnosis. From among 16 such patients, 12 (75%) ones had the increased leptin concentration before the therapy (Table 6).

Table 1. The changes and correlation between BMI and leptin concentration in the patients with BMI > 25 before and after metformin treatment (n=23).

	Before treatment	SD	After treatment	SD	p
Mean BMI (kg/m ²)	30.86	5.35	30.39	6.1	0.27
Mean leptin concentration (ng/ml)	31.63	19.64	27.71	18.15	0.11
r	0.83		0.87		
p	<0.001		<0.001		

Table 2. The changes and correlation between BMI and leptin concentration in the patients with BMI ≤ 25 before and after metformin treatment (n=12).

	Before treatment	SD	After treatment	SD	p
Mean BMI (kg/m ²)	20.99	1.87	20.75	1.79	0.26
Mean leptin concentration (ng/ml)	20.22	23	11.27	17.36	0.01
r	-0.23		0.87		
p	0.45		0.53		

Table 3. The changes and correlation between the HOMA index and leptin concentration in the patients with HOMA ≥ 3.8 before and after metformin treatment (n=8).

	Before treatment	SD	After treatment	SD	p
Mean HOMA	5.91	1.96	4.37	1.83	0.017
Mean leptin concentration (ng/ml)	41.29	17.6	32.51	19.38	0.11
r	0.15		-0.76		
p	0.71		0.05		

In the group of 16 women with the increased testosterone concentration at the moment of diagnosis, 15 (93.7%) ones had the elevated leptin levels before the therapy. After metformin treatment insignificant fall in leptin concentration was observed (Table 7). In the group of 19 patients with testosterone concentration within the normal range, hyperleptinemia was diagnosed in 15 (78.9%) ones and in these patients significant fall in leptin concentrations after metformin treatment was observed (Table 8).

In this study we also analysed how leptin concentrations depend on BMI, the HOMA index and androgen concentrations. The only group in which consider-

Table 4. The changes and correlation between the HOMA index and leptin concentration in the patients with HOMA<3.8 before and after metformin treatment (n=27).

	Before treatment	SD	After treatment	SD	p
Mean HOMA	1.93	0.9	1.81	1.11	0.58
Mean leptin concentration (ng/ml)	23.7	21.12	18.98	18.54	0.039
r	0.36		0.43		
p	0.06		0.02		

Table 5. The changes and correlation between the concentrations of androstendion (A) and leptin in the patients with the increased androstendion concentration before and after metformin treatment (n=19).

	Before treatment	SD	After treatment	SD	p
Mean A (ng/ml)	5.77	1.46	3.94	1.59	<0.001
Mean leptin concentration (ng/ml)	27.56	20.77	23.81	19.89	0.07
r	0.11		-0.24		
p	0.64		0.3		

Table 6. The changes and correlation between concentrations of androstendion (A) and leptin in the patients with androstendion concentration within the normal range before and after metformin treatment (n=16).

	Before treatment	SD	After treatment	SD	p
Mean A (ng/ml)	2.66	0.56	2.78	1	0.66
Mean leptin concentration (ng/ml)	27.9	22.95	20.01	19.07	0.02
r	0.008		-0.2		
p	0.97		0.45		

able correlation between leptin concentration and body mass index was confirmed both before and after treatment was the group of patients with BMI>25 (Table 1). Neither correlation was found between BMI and leptin concentration in the group of patients with BMI≤25, nor between insulin resistance expressed by the HOMA index and leptin concentration in the group of patients with HOMA≥3.8 and HOMA<3.8. No such correlation was found between concentrations of testosterone and androstendion and concentrations of leptin, which refers both to the group with increased concentrations of these hormones, and the groups in which testosterone and

Table 7. The changes and correlation between concentrations of testosterone (T) and leptin in the patients with the elevated testosterone concentration before and after metformin treatment (n=16).

	Before treatment	SD	After treatment	SD	p
Mean T (ng/ml)	1.00	0.12	0.81	0.21	0.02
Mean leptin concentration (ng/ml)	32.97	22.51	28.26	20.51	0.12
r	-0.19		0.02		
p	0.47		0.92		

Table 8. The changes and correlation between concentrations of testosterone (T) and leptin in the patients with testosterone level within the normal range before and after metformin treatment (n=19).

	Before treatment	SD	After treatment	SD	p
Mean T (ng/ml)	0.55	0.13	0.54	0.17	0.71
Mean leptin concentration (ng/ml)	23.29	20.08	16.87	17	0.02
r	0.03		0.23		
p	0.89		0.33		

androstendion levels stayed within the normal range (Tables 2-8).

Discussion

Polycystic ovary syndrome is a very heterogeneous condition. The diagnostic criteria are clear, and yet apart from menstrual disorders, hyperandrogenism – either clinical one or that revealed in laboratory tests, and the characteristic ultrasound picture of ovaries, there is a number of other complaints, mainly endocrinological and metabolic ones, which also belong to the clinical picture of this disease. Some of them are obesity and impaired glucose tolerance. It was the fact which persuaded us to make an assumption that increased concentration of leptin can be the one of PCOS laboratory symptoms, as well as it can play certain part in etiopathogenesis of this disease. It is possible because of the influence of this hormone on the production of ovarian hormones at the central level: in hypothalamus and pituitary gland, as well as due to the direct effect that it has on an ovary. In all these structures the presence of leptin receptors was confirmed [13,15,19,20].

Our study revealed that most patients with the diagnosed polycystic ovary syndrome had the elevated leptin concentration. These irregularities were observed both in

Table 9. Characteristic of the studied group.

	Mean value
Body mass (kg)	74,8
BMI (kg/m ²)	27,5
FSH (mIU/ml)	6,16
LH (mIU/ml)	7,86
T (ng/ml)	0,762
A (ng/ml)	4,21
Fasting glucose (mg/dl)	89,04
Fasting insulin (mIU/ml)	13,43
Leptin (ng/ml)	27,7

Abbreviations: BMI – body mass index; FSH – follicle stimulating hormone; LH – luteinizing hormone; T – testosterone; A – androstendion.

the group of obese and overweight patients, and in slim ones. Similar results were obtained while analysis of leptin concentration in the patients with confirmed insulin resistance – all the patients had hyperleptinemia. Also in the group of women with PCOS and tissue insulin sensitivity, the majority of patients had the increased leptin concentrations in blood serum. It suggests that hyperleptinemia in PCOS does not only depend on increased adipose tissue mass or insulin resistance.

Brzechffa *et al.* refer to the data which confirm that patients with polycystic ovary syndrome have higher leptin concentrations than it could be expected on the basis of their BMI or insulin resistance [19]. Similar results were reported by Panidis *et al.* who noticed that patients with PCOS and insulin resistance have considerably higher leptin concentrations, which means that leptin can play a part in etiopathogenesis of this syndrome [30]. Most authors confirm increased concentrations of leptin in blood of PCOS patients, but at the same time, they emphasize a lack of explicit evidence for its role in pathogenesis of this disease [10,12,31]. In our study, significant correlation between BMI and leptin concentrations was only proved in the group of obese and overweight women (BMI>25). Such results were reported by many other authors, as well [32-34].

We also assessed concentrations of androgens and leptin in PCOS patients. While analysing the groups of patients with PCOS and increased and normal androgen concentrations, we noticed the elevated leptin levels in most patients in both these groups. In none of the groups, however, we did manage to prove significant correlation between concentration of leptin and concentrations of androstendion and testosterone. Thus, influence of leptin on androgen concentrations in patients with polycystic ovary syndrome has not been ultimately confirmed. The literature gives different data on that issue. Some authors claim that such relation does not exist [32,33]. Others, in turn, prove the

correlation between concentrations of leptin and testosterone in PCOS [31].

In the presented study, we analysed how leptin concentration is affected by 3-month metformin treatment administered in daily doses of 2 x 850 mg. Metformin is an agent which reduces insulin resistance, decreases body mass index, and also, which is supported by many authors, causes the fall in leptin concentration in PCOS patients. Zhao mentions data which confirm the lowering of insulin concentrations measured in fasting status, the decrease in leptin levels, and hormonal normalization in women subjected to metformin treatment. Moreover, he noted that ovulation appeared naturally in these women, and that they better reacted to stimulation of ovulation after the therapy [27]. Also Kowalska *et al.* proved the efficiency of metformin therapy in PCOS patients. She noticed that metformin causes significant decrease in the concentrations of testosterone, insulin and leptin in obese PCOS patients [34]. In his research, Pasquali *et al.* compared influence of metformin and placebo on obese patients with PCOS. He confirmed therapeutic effect of this medicine, namely normalisation of testosterone and leptin levels and reduction of BMI [25]. In our study we observed that therapy with this agent leads to the fall in leptin concentrations mainly in non-obese women, and also those without impaired glucose tolerance. By contrast, it does not affect leptin concentration in women with PCOS and BMI>25 or patients with HOMA≥3.8. We found an evidence for the significant decrease in androgen concentrations in the patients treated with metformin, in whom these values went beyond the normal range before the therapy. Metformin treatment caused the decrease in leptin concentrations, but only in patients who had normal androstendion and testosterone concentrations at the moment of diagnosis.

The results may suggest that leptin fulfils an important role in pathogenesis of PCOS, irrespective of BMI, impaired glucose tolerance and hyperandrogenism. Perhaps achieved effect of metformin treatment also depends on the influence that it has on leptin concentration.

To conclude, increased leptin concentrations may be one of the elements of PCOS clinical picture and metformin treatment considerably reduces leptin concentration, if it is employed in non-obese PCOS patients, with normal androgen concentrations and do not have an impaired glucose tolerance.

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