CLINICAL STUDY

Insulin, leptin, IGF-I and insulin-dependent protein concentrations after insulin-sensitizing therapy in obese women with polycystic ovary syndrome

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Abstract

Objective: To determine the clinical, hormonal and biochemical effect of 4–5 months of insulin-sensitizing therapy (hypocaloric diet + metformin) in obese patients with polycystic ovary syndrome (PCOS).

Design: Prospective study.

Methods: Twenty-three obese patients with PCOS, 19 obese patients without menstrual disturbances and 11 healthy control women were recruited from the Department of Endocrinology and Endocrine Gynecology, Medical Academy, Białystok, Poland. Obese patients received 500 mg metformin together with hypocaloric diet three times daily for 4–5 months, after baseline study. The clinical parameters, menstrual pattern and serum concentrations of insulin, leptin, IGF-I, insulin-dependent proteins (sex hormone-binding protein (SHBG), insulin-like growth factor-binding protein-1 (IGFBP-1)), gonadotropins and sex steroids were determined before and after treatment.

Results: In the baseline study, obese patients with PCOS had significantly higher insulin, testosterone and LH concentrations in comparison with the other groups. The serum leptin, IGF-I, IGFBP-1 and SHBG were not different between the two groups of obese patients, but there was a significant difference in comparison with the control group. After metformin therapy a significant reduction in BMI, % of body fat and leptin concentration were observed in both groups of obese patients. Fasting insulin, testosterone and LH concentrations decreased significantly only in the PCOS group. Six out of 11 patients in the PCOS group had more regular menstrual cycles; two patients conceived.

Conclusions: Insulin-sensitizing therapy could be considered as an additional therapeutic option in obese women with PCOS.

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Introduction

Polycystic ovary syndrome (PCOS), which affects about 6–10% of women of reproductive age, is characterized by chronic anovulation and hyperandrogenism (1). Its etiology remains unknown but for the past few years it has been shown that hyperinsulinemia secondary to insulin resistance plays an important role in abnormalities in ovarian function (2, 3). The causes of these conditions are still unknown but probably the block in certain insulin receptor signaling pathways while others are preserved produces selective insulin resistance (4). Hyperinsulinemia enhances androgen concentration by direct stimulation of ovarian androgen synthesis (5), or by enhancing luteinizing hormone (LH) secretion (6) and lowering the concentration of sex hormone-binding protein (SHBG). Insulin in high

concentrations can also mimic insulin-like growth factor-I (IGF-I) actions by acting via IGF-I receptor (7). Some authors have suggested that this mechanism is responsible for insulin-mediated hyperandrogenism (8). Obesity, which is a common feature in women with PCOS, is also a well-recognized cause of hyperinsulinemia and insulin resistance in normal subjects (9). It is believed that insulin-sensitizing therapies could improve insulin resistance and hyperandrogenism and therefore metformin and hypocaloric diet have been proposed for the treatment of PCOS (10). The other potential implication for metformin therapy in this group of patients is the prevention of metabolic complications. There have been several studies on metformin therapy in patients with PCOS, but the results are inconsistent. In the studies described by Ehrmann and colleagues, hyperinsulinemia and hyperandrogenism did not

improve after metformin treatment in obese PCOS women (11). However, other authors observed improvement of insulin sensitivity associated with the decrease in androgen concentration after metformin therapy in PCOS patients (12–14).

The aim of this study was to test the hypothesis that metformin and hypocaloric diet improves insulin sensitivity in obese PCOS women through its influence on insulin and glucose concentrations, insulin-dependent protein concentrations (sex hormone-binding protein (SHBG) and insulin-like growth factor-binding protein-1 (IGFBP-1)) and insulin-like growth factor-I (IGF-I). Additionally, we checked if improving insulin sensitivity could influence leptin levels in obese PCOS women.

Subjects and methods

Patients

The study was carried out in 23 obese PCOS patients, 19 obese normally cycling women and 11 healthy controls. The protocol was reviewed and approved by the Institutional Review Board of Medical Academy in Białystok, Poland. All women gave an informed consent before their participation. The diagnosis of PCOS was made according to the characteristic clinical findings (the presence of oligo/amenorrhea and hirsutism), laboratory data (testosterone concentrations elevated or in the upper limit of normal) and all patients had polycystic ovaries shown by transvaginal ultrasonography (>8 subcapsular follicles of 3–8 mm diameter in one plane in one ovary and increased stroma) (15). We considered that patients had oligomenorrhea if they had fewer than six menstrual periods in the preceding year. Amenorrhea was considered as the absence of periods for >6 months. Hirsutism was evaluated using Ferriman–Gallwey scoring system, before the study. The patient was described as hirsute if the score was more than 10. Testosterone concentrations were determined in the local laboratory using chemiluminescence immunoassay. The range of normal values is from 0.2 to 0.8 ng/ml. We considered that a patient had elevated testosterone concentrations if the concentration exceeded 0.8 ng/ml (19/23 patients: 83% of studied group).

A patient was included in the PCOS group if she had ultrasound features of PCOS and fulfilled at least two of the following criteria: oligomenorrhea/amenorrhea, hirsutism and serum androgens in the upper limit of normal or elevated.

Obesity was defined as a body mass index (BMI) of more than 27.5 kg/m^2 . In the groups of obese patients the majority of patients had BMI $>30 \text{ kg/m}^2$ (PCOS—obese, 18/23: 78%; obese, 15/19: 79%). Obese women and the control group had regular menstrual cycles. Other reasons for menstrual disturbances (non-classical 21-hydroxylase deficiency, hyperprolactinemia,

androgen-secreting tumors and thyroid dysfunction) were excluded by appropriate tests before the study. None of the women was on a diet program or had been taking any drug known to affect carbohydrate metabolism for at least 2 months prior to the metabolic and endocrine investigations. Studies were conducted in regularly cycling women during the early follicular phase (3–5 days) of their menstrual cycle and in the PCOS group 3–5 days after spontaneous or progestin-induced menses.

Protocol of the study

In all subjects, a clinical examination and an evaluation of anthropometric parameters were performed (BMI, body fat distribution determined on the basis of the waist/hip ratio (WHR), and % body fat estimated using bioelectric impedance). After an overnight fast, each woman underwent the 2-h oral glucose tolerance test (OGTT) with 75 g of glucose load. Blood samples were taken 0, 60 and 120 min for assessing plasma insulin and glucose. Prior to OGTT, blood samples were drawn for serum leptin, testosterone, LH, follicle-stimulating hormone (FSH), estradiol, IGF-I, IGFBP-1 and SHBG estimations. Then 15 patients from the PCOS group and ten obese normally cycling patients were administered a hypocaloric diet (1200–1400 kcal/24 h) and metformin (Polfa, Kutno, Poland) therapy (500 mg three times a day) for a period of 4–5 months. After 4–5 months of therapy all pre-treatment studies were repeated. Menstrual pattern was also monitored during therapy. The clinical characteristics of the studied groups is given in Table 1.

Laboratory measurements

Blood samples were centrifuged, and the serum glucose and LH, FSH, testosterone and estradiol were measured immediately. The serum for insulin, leptin, SHBG, IGFBP-1 and IGF-I was stored at -20°C until assayed. Glucose was evaluated using the oxidative method (Cormay, Warsaw, Poland), LH, FSH, estradiol, testosterone by chemiluminescence method (ACS Chiron 180). RIA method was used for estimation of plasma leptin (Linco Research, St. Charles, MO, USA), IGF-I (Bio-Source, Nivelles, Belgium) and IGFBP-1 (Bio-Source), and the IRMA method was used for plasma insulin (Polatom, Świerk, Poland) and SHBG (Orion Diagnostica, Espoo, Finland).

Statistical analysis

The pre- and post-treatment data within the groups were compared using Wilcoxon rank sum test. The results between the groups were analyzed using the Mann–Whitney *U* test. Correlations were estimated using simple regression analysis. Data are expressed as

Table 1 Clinical characteristics of the studied groups.

	PCOS-obese (n = 23)	non-PCOS-obese (n = 19)	Control (n = 11)
Age (years)	25.3 ± 4.8	27.9 ± 7.3	30.4 ± 5.7
BMI (kg/m ²)	34.7 ± 6.0*	36.2 ± 6.0†	21.9 ± 2.0
% body fat	38.1 ± 8.2*	39.0 ± 7.3†	16.9 ± 4.3
Waist girth (cm)	98.1 ± 14.8*	102.3 ± 13.0†	72.4 ± 5.1
Hip girth (cm)	119.2 ± 13.0*	122.9 ± 13.1†	94.7 ± 4.8
WHR	0.83 ± 0.07*	0.83 ± 0.07†	0.77 ± 0.06
Testosterone (ng/ml)	1.03 ± 0.33*‡	0.67 ± 0.24	0.51 ± 0.24
FAI	14.49 ± 8.49*‡	9.97 ± 7.95	2.70 ± 1.49
LH (mIU/ml)	11.1 ± 5.0*‡	5.7 ± 2.0	3.5 ± 2.3
FSH (mIU/ml)	6.1 ± 1.4	7.7 ± 2.8	6.7 ± 1.5
LH/FSH	1.8 ± 0.76*‡	0.83 ± 0.4	0.61 ± 0.47
Estradiol (pg/ml)	66.9 ± 45.6	58.0 ± 31.5	43.3 ± 16.5
SHBG (nmol/l)	32.0 ± 18.3*	32.5 ± 16.5†	69.9 ± 16.6
IGF-I (ng/ml)	266.4 ± 115.8	257.8 ± 90.2	277.1 ± 105.6
IGFBP-1 (ng/ml)	9.7 ± 5.0*	12.0 ± 10.3†	34.1 ± 10.1
Leptin (ng/ml)	29.6 ± 14.0*	34.6 ± 15.0†	12.1 ± 5.6

* $P < 0.05$ obese-PCOS vs respective value in the control group.† $P < 0.05$ obese vs the respective value in the control group.‡ $P < 0.05$ obese-PCOS vs the respective value in the obese group.

FAI, free androgen index.

mean ± s.d. and $P < 0.05$ was considered statistically significant.

Results

Eleven patients out of 15 taking metformin in the PCOS-obese group and six patients from the obese regularly menstruating women completed the study. In the PCOS-obese group two patients conceived and delivered healthy children at term, two patients discontinued the study because of mild gastrointestinal side effects; in the group of obese patients without menstrual disturbances four patients discontinued the study due to mild gastrointestinal side effects (nausea, diarrhea).

Baseline results

There were no statistically significant differences in anthropometric parameters (BMI, % body fat, WHR, waist and hip girths) among the two groups of obese patients (Table 1). In both groups of obese patients all parameters were markedly higher in comparison with the control group (Table 1).

The hormonal profile was significantly different in the PCOS-obese group (Table 1). There were increased LH/FSH ratio, and LH and testosterone concentrations versus respective values in obese, regularly menstruating women. Also, fasting insulin concentration was highest in patients with PCOS and significantly different from other groups ($P < 0.05$ vs regularly menstruating obese patients and $P < 0.001$ vs control group) (Fig. 1). Leptin concentration was similar in both groups of the obese patients and markedly elevated in comparison with the control group

(Table 1). IGFBP-1 and SHBG were markedly diminished in both groups of the obese patients, but there was no significant difference between these two groups. The IGF-I level was not significantly different between the study groups (Table 1).

The fasting and post-load glucose concentrations were markedly elevated in the PCOS group (Fig. 1). Three patients fulfilled WHO criteria for diagnosis of impaired glucose tolerance.

Post-treatment results

Anthropometric parameters changed significantly in both groups of obese patients (Table 2). In the studied groups of obese patients on insulin-sensitizing therapy, most patients lost about 10% of their body weight. Only three patients from the PCOS-obese group lost more than 15% of initial body weight, which is 17% of all obese patients treated with metformin.

Fasting insulin concentrations decreased significantly only in PCOS-obese patients ($P = 0.0093$) (Table 2). Similar results were obtained for LH ($P = 0.01$) and testosterone concentrations ($P = 0.049$). Leptin concentrations decreased markedly only in PCOS-obese patients ($P = 0.005$). The IGF-I level remained unchanged while IGFBP-1 and SHBG concentrations showed the tendency to increase especially in the PCOS group – for SHBG the difference was statistically significant ($P = 0.037$) (Table 2).

Correlation

The baseline study has shown statistically significant correlation between insulin, leptin and anthropometric parameters in the whole group as well as within the

Figure 1 Glucose and insulin concentrations during OGTT before and after treatment. * $P < 0.05$ PCOS-obese vs respective value in the control group. ** $P < 0.05$ non-PCOS obese vs respective value in the control group. # $P < 0.05$ PCOS-obese vs respective value in non-PCOS obese group.

groups (Tables 3 and 4). We failed to find any correlation between insulin, leptin and other studied hormonal parameters in the group of obese women with PCOS (Tables 3 and 4). In the whole population studied, a significant correlation between fasting insulin and leptin ($P = 0.003$, $r = 0.408$) and inverse correlations between fasting insulin and SHBG

($P = 0.001$, $r = -0.447$), and IGFBP-I ($P = 0.0001$, $r = -0.5651$) were observed.

Treatment with metformin and weight loss significantly improved the menstrual pattern in PCOS-obese group. Six out of the 11 patients treated with metformin resumed regular menstruation; the other two patients conceived.

Table 2 Anthropometric and hormonal parameters after metformin treatment in both groups of obese patients.

	PCOS-obese (n = 11)		Non-PCOS-obese (n = 6)	
	Before treatment	After treatment	Before treatment	After treatment
BMI (kg/m ²)	34.9 ± 5.6	31.4 ± 4.8*	37.1 ± 7.5	35.8 ± 7.9*
% body fat	37.7 ± 8.7	30.9 ± 6.2	41.0 ± 11.8	39.2 ± 12.4
Waist girth (cm)	100.5 ± 13.1	93.4 ± 11.8*	103.5 ± 18	100 ± 19.5*
Hip girth (cm)	121.4 ± 13.4	112.3 ± 9.6*	126.8 ± 16	123.8 ± 16*
WHR	0.83 ± 0.08	0.83 ± 0.07	0.81 ± 0.06	0.80 ± 0.06
Testosterone (ng/ml)	1.03 ± 0.29	0.69 ± 0.32*	0.55 ± 0.12	0.49 ± 0.17
FAI	14.49 ± 8.49	9.31 ± 9.95	9.97 ± 7.95	5.49 ± 3.9
LH (mIU/ml)	10.5 ± 5.4	6.2 ± 2.8*	5.6 ± 2.0	5.1 ± 2.7
FSH (mIU/ml)	6.2 ± 0.93	5.1 ± 2.0	5.2 ± 1.64	6.2 ± 1.8
LH/FSH	1.70 ± 0.91	1.4 ± 0.89	1.14 ± 0.34	0.83 ± 0.3
Estradiol (pg/ml)	59.8 ± 32.7	50.8 ± 32	71.3 ± 51.9	46 ± 24.2
SHBG (nmol/l)	29.5 ± 16.7	38.6 ± 19.3*	31.3 ± 11.1	36.5 ± 13.3
IGF-I (ng/ml)	276.2 ± 103.9	263.4 ± 86.4	240 ± 127	241.9 ± 116.1
IGFBP-1 (ng/ml)	8.0 ± 3.44	11.1 ± 5.2	11.5 ± 13.3	9.9 ± 10.5
Leptin (ng/ml)	25.1 ± 10.7	15.6 ± 9.1*	34.6 ± 14.5	24.8 ± 13.3
Fasting insulin (IU/l)	26.2 ± 13.9	16.9 ± 9.1*	18.3 ± 14.2	20.0 ± 13.5

* $P < 0.05$ pre-treatment vs post-treatment value in studied groups.
FAI, free androgen index.

Table 3 Correlation between insulin and other parameters studied in the whole group and within groups (PCOS-obese, non-PCOS-obese).

	Whole group (n = 53)		PCOS-obese (n = 23)		Non-PCOS-obese (n = 19)	
	r	P	r	P	r	P
BMI	0.48	0.001	0.47	0.024	0.23	NS
% body fat	0.52	0.001	0.24	NS	0.55	0.014
Waist girth	0.58	0.001	0.51	0.018	0.51	0.025
Hip girth	0.47	0.001	0.45	0.039	0.21	NS
WHR	0.51	0.001	0.36	NS	0.58	0.009
SHBG	-0.45	0.001	-0.41	0.059	-0.19	NS
Leptin	0.41	0.003	0.20	NS	0.49	0.037
IGFBP-1	-0.56	0.001	-0.38	NS	-0.46	NS

Discussion

Burghen and colleagues first reported the role of insulin in the pathogenesis of PCOS (16). They observed that women with PCOS had basal and glucose-stimulated hyperinsulinemia as compared with weight- and age-matched control women. In our study we observed higher fasting and post-load glucose and insulin concentrations in PCOS women; three of them fulfilled WHO criteria for impaired glucose tolerance in spite of their young age. These data are in agreement with results obtained by Dunaif *et al.* showing a significantly higher glucose and insulin level during an oral glucose tolerance test in both obese and lean PCOS patients in comparison with the age- and weight-matched ovulatory women (17). Furthermore, we also observed a difference in the fasting insulin concentrations between the two groups of obese patients – the highest insulin level was observed in PCOS women. The BMI and WHR were similar in both groups, so this difference seems to be independent of obesity or fat distribution. The observed hyperinsulinemia can result from an increased insulin secretion or decreased hepatic clearance of insulin. In PCOS women both defects were noticed (18, 19).

Hypocaloric diet and metformin therapy improved glucose metabolism in the group of PCOS patients. We

also observed a reduction in fasting insulin concentrations after therapy. It is difficult to distinguish whether it is a direct effect of metformin action or an indirect result of the weight loss in this group of patients. After metformin therapy we also noted a significant reduction in body mass, BMI and % of body fat, whereas the WHR change did not reach statistical significance. Similar results are reported by Valazquez and colleagues (12). After 8 week of metformin therapy they observed the reduction of BMI, whereas WHR remained unchanged. Other authors did not observe any beneficial effect of metformin treatment on body weight (11). In addition, they had shown that metformin treatment had no effect of hyperinsulinemia and androgen excess, which is in contrast to our data. They concluded that there was no direct effect of metformin on gonadotropin or ovarian steroid production that could be independent of weight loss (11). Morin-Papunen and colleagues did not observe the significant change in BMI after 4–6 months of metformin treatment (13). However, contrary to Ehrmann *et al.*'s results (11), they observed a statistically significant decrease in fasting insulin concentrations and free testosterone levels (13). The other important question to be answered is whether insulin-sensitizing therapy (metformin) improves ovarian function and influences the ovulation rate. Some authors suggest that metformin may cause the resumption of ovulatory function. The indirect proof for that fact were two pregnancies observed during the study. Besides, more than 50% of our patients improved their menstrual pattern. This is in accordance with other studies in which the resumption of regular menses and pregnancies were observed (12, 13). Nestler and colleagues showed a marked increase in spontaneous or clomiphene-induced ovulation in PCOS-obese women during metformin therapy in comparison with a placebo-treated group (20). Interestingly, this effect was independent of obesity because there was no change in body weight during the study. Authors observed a decrease in the serum insulin response to oral glucose administration. It demonstrates that the reduction of hyperinsulinemia could influence the ovulation rate. The improvement in

Table 4 Correlation between leptin and other parameters studied in the whole group and within groups (PCOS-obese, non-PCOS-obese).

	Whole group (n = 53)		PCOS-obese (n = 23)		Non-PCOS-obese (n = 19)	
	r	P	r	P	r	P
BMI	0.76	0.001	0.62	0.002	0.65	0.003
% body fat	0.78	0.001	0.64	0.003	0.71	0.001
Waist girth	0.72	0.001	0.53	0.016	0.63	0.005
Hip girth	0.76	0.001	0.59	0.006	0.68	0.002
WHR	0.32	0.023	0.21	NS	0.17	NS
IGFBP-1	-0.60	0.001	-0.18	NS	-0.52	NS
SHBG	-0.50	0.001	-0.38	NS	-0.06	NS

ovulation rate in obese PCOS women was also observed by Pirwany *et al.* (21). The effect was also independent of the body weight (21).

It is postulated that insulin could influence ovarian steroidogenesis. *In vitro* studies showed that thecal cells from polycystic ovaries are more sensitive to insulin; the same insulin concentrations only slightly affect testosterone production in controls (5). We observed significantly higher testosterone concentrations in PCOS women, but we failed to show a correlation between insulin and testosterone in the PCOS group. It is well accepted that women with PCOS have significantly higher testosterone concentrations. As was mentioned earlier, insulin in high concentrations can also mimic IGF-I actions by acting via IGF-I receptor (7) and in this way could enhance ovarian steroidogenesis (8).

Another factor through which hyperinsulinemia could influence steroid levels is the synthesis of insulin-dependent proteins in the liver. Insulin strongly suppresses hepatic production of SHBG, resulting in increased levels of biologically available androgens (22). In our study we estimated the SHBG concentrations which were significantly decreased in both groups of obese patients. These data are consistent with the results obtained by other authors. After therapy there was a tendency in SHBG level to increase but the difference was statistically significant only for the PCOS group. IGFBP-1 concentrations, which regulate IGF-I bioavailability, followed the same pattern. The IGF-I level remained unchanged during the study. De Leo and colleagues observed a significant increase in IGFBP-1 concentrations in PCOS women after 30–32 days of metformin treatment. IGF-I concentrations did not change significantly during the study. They also calculated the IGF-I/IGFBP-1 ratio, which was significantly reduced after metformin treatment (23). One could speculate that, despite similar total IGF-I levels, the IGFBP-1 concentration is decreased in PCOS women, which in turn leads to an increase in the free fraction of IGF-I (24). Moreover, Duleba reported that insulin diminishes not only hepatic IGFBP-1 synthesis but also the intraovarian pool (25). It could promote higher local IGF-I activity. It was shown that IGF-I and insulin stimulate the proliferation of human theca interstitial cells (26). The increased activity of IGF-I could influence locally the selection of dominant follicle.

Another potential factor that could play a role in the pathogenesis of PCOS is leptin (27). Leptin is the hormone produced mainly by adipose tissue and is considered to be an indicator of body fat. Human obesity is accompanied by high leptin concentration (28). Since its discovery leptin has also been called a hormone of reproduction (29). In animal models, leptin has a direct stimulatory effect on gonadotropin production, ovarian follicle development and ovulation (30, 31). Recently, the expression of leptin and leptin

receptors has been found in the ovary (32). It is not known whether obesity is a state of central leptin resistance. To date it is possible that there is no resistance to leptin in the periphery and ovary could be exposed to higher leptin levels. In the first study to report on leptin levels in PCOS women, approximately 30% of PCOS patients had higher leptin concentrations (33). They also showed a significant relationship between leptin and insulin sensitivity, suggesting that leptin could play a role in pathogenesis of PCOS. In our study we also estimated leptin concentrations in all studied groups. Before treatment there was no difference in leptin levels between the two groups of obese patients. We found a significant correlation between leptin and BMI, % body fat, waist and hip girths but not WHR in the whole population studied and within the groups. This is in agreement with most studies on leptin concentrations in PCOS (34, 35). We were not able to demonstrate a correlation between leptin and insulin in the PCOS group; the correlation existed only in the whole group. The main predictor of serum leptin concentrations was the amount of body fat.

The limitation of our study is that most patients on metformin therapy lost weight; it is well accepted that weight loss alone also improves insulin sensitivity, but in view of studies discussed earlier, the effect of metformin seems to be independent of obesity. There are several studies that show that metformin therapy improves menstrual pattern and restores ovulation; some patients conceive, irrespective of whether or not they lose weight (12–14, 20, 21). Our results support the hypothesis that the beneficial effect of metformin and a hypocaloric diet depends on decreasing insulin concentrations. It suggests that insulin-sensitizing therapy could be useful in obese women with PCOS, but large randomized trials are needed.

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