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Effect of Metformin on Insulin-Like Growth Factor (IGF) I and IGF-Binding Protein I in Polycystic Ovary Syndrome

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ABSTRACT

The objective of the present study was to investigate whether metformin affected plasma concentrations of insulin-like growth factor (IGF) I and IGF-binding protein I (IGFBP-I) in polycystic ovary syndrome (PCOS) patients. This was an open study conducted by the Department of Obstetrics and Gynecology at the University of Siena, Italy. Seventeen women with PCOS participated in the study and were administered metformin at a dose of 500 mg three times a day. Treatment was continued for 30–32 days, after which the pretreatment evaluation was repeated. Plasma concentrations of LH, FSH, estradiol, free testosterone, IGF-I, IGFBP-I, sex hormone-binding globulin, and insulin were evaluated. Metformin led to a significant reduction in areas under the insulin curves (9310 ± 1509 *vs.* 6520 ± 1108 mU/mL·min; $P < 0.05$) and was associated with a decrease in plasma free testosterone levels (12.7 ± 1.7 *vs.* 10.3 ± 2 pg/mL; $P < 0.05$) and an increase in plasma sex hormone-binding globulin concentrations (62 ± 8 *vs.* 94 ± 13 nmol/L; $P < 0.05$). A nonsignificant increase in plasma IGF-I levels was observed after metformin (276 ± 48 *vs.* 291 ± 71 mcg/L), with a significant increase in plasma IGFBP-I levels (0.56 ± 0.2 *vs.* 0.98 ± 0.38 mcg/L; $P < 0.05$). The IGF-I/IGFBP-I ratio was significantly lower (492.8 ± 117 *vs.* 296.9 ± 82; $P < 0.05$) at the end of therapy than before treatment. In conclusion, it seems to be appropriate to intervene with an insulin-sensitizing agent such as metformin in an attempt to break the pathogenetic link between hyperinsulinemia and hormonal perturbations in PCOS. (*J Clin Endocrinol Metab* **85:** 1598–1600, 2000)

POLYCYSTIC OVARY SYNDROME (PCOS) is a common disorder characterized by hyperandrogenemia and anovulation. It is associated with major reproductive, as well as metabolic, derangements including hyperinsulinemia and insulin resistance, which may adversely affect folliculogenesis and ovulation.

Several studies suggest that insulin-like growth factor (IGF) I may play an important role in the regulation of ovarian follicular maturation (1–3) and steroidogenesis (4, 5). The action of IGF-I is modulated by IGF-binding protein I (IGFBP-I), a hepatic product the synthesis of which is inhibited by insulin (6, 7). IGFBP-I synthesis also takes place in ovarian granulosa cells (8) and endometrium (9), and in both sites IGFBP-I synthesis is inhibited by insulin.

Women with PCOS are reported to have lower plasma levels of IGFBP-I than normal women (10, 11), leading to increased ovarian bioavailability of IGF-I.

Insulin-lowering agents have recently been proposed as a new therapy for PCOS (12, 13). The biguanide metformin, for example, is normally used to treat noninsulin-dependent diabetes. It has multiple mechanisms of action, including inhibition of gluconeogenesis and stimulation of peripheral glucose uptake (14). In PCOS patients, metformin has also been shown to reduce plasma LH and ovarian androgen levels and to reverse hyperinsulinism (15).

Recently, we have demonstrated that by reducing hyperinsulinism, metformin reduced ovarian androgen levels, leading to a reduction in estradiol (E_2) levels that favored orderly follicular growth in response to exogenous gonadotropins (16). The aim of the present study was to investigate whether metformin affected plasma concentrations of IGF-I and IGFBP-I in PCOS patients.

Materials and Methods

Subjects

Seventeen women with PCOS were recruited. The clinical diagnosis of PCOS was based on hyperandrogenemia [plasma free testosterone (FT), >10 pg/mL] and oligomenorrhea (fewer than six menstrual periods in the previous year) or amenorrhea. A baseline ultrasound scan was performed to evaluate the uterus and ovaries. Ultrasonographic diagnosis of PCOS was based on the presence of 10 or more follicles (2–10 mm in diameter) in one or both ovaries.

None of the women had virilization or congenital adrenal hyperplasia (on the basis of normal levels of 17-hydroxyprogesterone). Before treatment, basal hormone levels revealed anovulatory cycles, increased serum concentrations of LH, an increased LH/FSH ratio with androstenedione, and testosterone levels at the upper limits of the normal range. All women were normoprolactinemic and had normal thyroid function.

All the women had fasting insulin levels above 8.4 mU/mL and accumulated insulin levels [area under the insulin curve ($AUC_{insulin}$) during a 2-h, 75-g oral glucose test) greater than 6700 mU/mL·min.

Study protocol

The patients entered the study during the follicular phase of the menstrual cycle (*i.e.* plasma levels of progesterone <1.8 ng/mL). Basal

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blood samples for LH, FSH, E_2, FT, sex hormone-binding globulin (SHBG), IGF-I, and IGFBP-I were obtained at 0800 h after overnight bed rest and fasting. On the following day, metformin (Metforal, Guidotti, Italy) was administered at a dose of 500 mg three times a day. Glucose plasma levels were not monitored during treatment. Treatment was continued for 30–32 days, after which basal blood samples for hormonal plasma levels evaluation were obtained. Spontaneous ovulation was detected by blood samples drawn 20 and 27 days after the beginning of metformin administration. Three women had serum progesterone values in the postovulatory range and were excluded from the study.

The study was approved by the Institutional Review Board of the University of Siena. Written informed consent was obtained from each subject.

Hormone assay

Plasma concentrations of LH, FSH, FT, IGF-I, IGFBP-I, and SHBG were measured by double-antibody RIA using Radim (Rome, Italy) kits for LH and FSH; DPC (Los Angeles, CA) kits for SHBG; DSL (Webster, TX) kits for IGF-I, IGFBP-I, and FT; and Biodata (Rome, Italy) kits for E_2. The samples were assayed in duplicate at two dilutions. All samples of a given subject were assayed together. Quality control pools at low, medium, and high hormone levels were included in each assay. The detection limit of the assay was 0.20 mIU/mL for LH, 0.18 mIU/mL for FSH, 0.15 pg/mL for FT, 2.06 ng/mL for IGF-I, 0.33 ng/mL for IGFBP-I, and 2.5 nmol/L for SHBG. Intra- and interassay variations were 7.8% and 8.2% for LH, 6.2% and 6.5% for FSH, 3.2% and 3.4% for FT, 3.9% and 3.8% for IGF-I, 4.6% and 3.6% for IGFBP-I, and 5.6% and 4.6% for SHBG.

Statistical analysis

$AUC_{insulin}$ were calculated by the trapezoidal method. Hormone basal levels were compared using Student's two-tailed *t* test for paired data. Differences were considered significant for $P < 0.05$.

Results

We were able to analyze complete data from 14 of the 17 women. The other three women ovulated after taking metformin.

The body mass index did not change significantly during the study period. As expected, metformin led to a significant reduction in $AUC_{insulin}$ (9310 ± 1509 *vs.* 6520 ± 1108 mU/mL·min; $P < 0.05$) and was associated with a decrease in plasma FT levels (12.7 ± 1.7 *vs.* 10.3 ± 2 pg/mL; $P < 0.05$) and an increase in plasma SHBG concentrations (62 ± 8 *vs.* 94 ± 13 nmol/L; $P < 0.05$).

No changes in plasma IGF-I levels were observed after metformin (276 ± 48 *vs.* 291 ± 71 mg/L), with a significant increase in plasma IGFBP-I levels (0.56 ± 0.2 *vs.* 0.98 ± 0.38 mg/L; $P < 0.05$). The IGF-I/IGFBP-I ratio was significantly lower (492.8 ± 117 *vs.* 296.9 ± 82; $P < 0.05$) at the end of therapy than before treatment (Fig. 1).

Diarrhea and meteorism were reported by six women. These side effects attenuated when the drug was taken at mealtime.

Discussion

In the present study, it is demonstrated for the first time that the reduction in insulin levels following metformin treatment in PCOS patients is associated with an increase in IGFBP-I and a decrease in the IGF-I/IGFBP-I ratio.

IGF-I may contribute to ovarian hyperandrogenemia in PCOS by autocrine and paracrine mechanisms. IGF-I has been shown to stimulate estrogen production by granulosa cells (4) and to act synergistically with FSH and LH in controlling granulosa cell aromatase levels (5, 17). It is also reported to synergize with LH to stimulate androgen production (18, 19), probably via its receptors on thecal cells (18). In PCOS, plasma IGF-I levels are within the normal range, whereas serum IGFBP-I levels are reported to be significantly lower than in normal women and inversely correlated with serum levels of insulin (6, 10, 11). This leads to an increased IGF-I/IGFBP-I ratio and an increase in bioavailability of IGF-I to the ovaries. In PCOS patients, plasma insulin and IGFBP-I levels are also known to influence ovarian response to exogenous gonadotropins (20). Homburg *et al.* (11) reported that the amount of gonadotropins required to induce ovulation was positively correlated with insulin and negatively related to IGFBP-I concentrations.

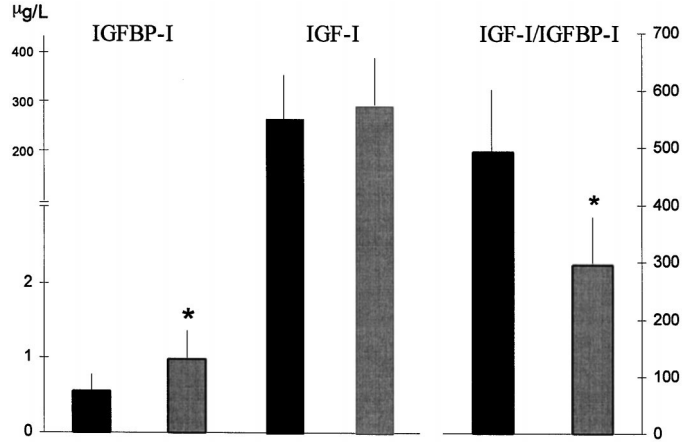

FIG. 1. Plasma IGF-I and IGFBP-I levels before (■) and after (▨) metformin treatment (*, $P < 0.05$; n = 14) and IGF-I/IGFBP-I ratio before (■) and after (▨) metformin treatment (*, $P < 0.05$; n = 14). Values are mean ± SD.

Various studies have investigated the role of circulating insulin excess on androgen concentrations in PCOS. Insulin may act through multiple sites to increase endogenous androgens in women. Insulin has been shown to directly dysregulate ovarian P450c17a (21), to inhibit hepatic SHBG secretion (22),and to reduce plasma IGFBP-I levels (23). Metformin treatment is reported to be associated with a reduction in androgen plasma levels due to a reduction in insulin levels (12, 15).

Our group recently demonstrated that metformin leads to orderly FSH-induced ovulation in PCOS patients. Pretreatment with metformin led to a reduction in the number of follicles greater than 15 mm in diameter, in E_2 levels on the day of human CG administration and in cycles in which human CG was withheld. This indicates a lower incidence of ovarian overstimulation and supports the hypothesis that insulin plays a role in the endocrine and paracrine control of the ovaries (16).

In this study, we demonstrated that metformin-induced insulin reduction is associated with an increase in SHBG, IGFBP-I, and a reduced IGF-I/IGFBP-I ratio, which may be partly responsible for the reduction in plasma androgen levels in PCOS patients. On the basis of these observations we may conclude that metformin reduces plasma insulin levels and IGF-I availability to the ovaries; this may modify the hyperandrogenic intrafollicular milieu recognized in PCOS,

TABLE 1. Clinical and hormonal data of 14 women with PCOS before and after metformin treatment

	Basal (n = 14)	After metformin (n = 14)
Age (yr)	26 ± 2	
BMI (kg/m^2)	30.3 ± 4.1	30.1 ± 3.9
LH (mIU/mL)	10.7 ± 1.3	10.6 ± 1.2
FSH (mIU/mL)	5.6 ± 0.7	5.9 ± 1
E_2 (pg/mL)	51.7 ± 11.7	49.6 ± 10
FT (pg/mL)	12.7 ± 1.7	10.3 ± 2[a]
SHBG (nmol/L)	62 ± 8	94 ± 13[a]
$AUC_{insulin}$ (μU/mL·min)	9310 ± 1509	6520 ± 1108[a]

Values are means ± SD.
[a] $P < 0.05$ *vs.* basal.
BMI, Body mass index.

partly explaining our previous finding of orderly follicular growth when ovulation is induced by exogenous gonadotropins.

Metformin has an antihyperglycemic action, and it is accepted that treatment with metformin is not associated with episodes of hypoglycemia (24).

In conclusion, it seems to be appropriate to intervene with an insulin-sensitizing agent such as metformin in an attempt to break the pathogenetic link between hyperinsulinemia and hormonal perturbations in PCOS.

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