Clomiphene citrate increases insulin-like growth factor binding protein-1 and reduces insulin-like growth factor-I without correcting insulin resistance associated with polycystic ovarian syndrome

Vincenzo De Leo1,2, Antonio la Marca1, Giuseppe Morgante1, Liliana Ciotta2, Luca Mencaglia3, Antonio Cianci3 and Felice Petraglia1

1Department Obstetrics and Gynecology, University of Study of Siena. 2Department Obstetrics and Gynecology, University of Study of Catania and 3Centro Florence, Firenze, Italy

To whom correspondence should be addressed at: Department Obstetrics and Gynecology, University of Siena, Policlinico Le Scotte, viale Bracci, 53100 Siena (SI), Italy. E-mail: deleo@unisi.it

The induction of ovulation by clomiphene could be the result of interaction of the drug at various levels: hypothalamus, pituitary and ovary. It was demonstrated that administration of clomiphene to women with polycystic ovarian syndrome (PCOS) is accompanied by a reduction in plasma concentrations of insulin-like growth factor-I (IGF-I). IGF-I seems to have an overall negative effect on normal folliculogenesis and ovulation. The aim of the present study was to evaluate the effect of clomiphene on plasma concentrations of IGF-I and IGF binding protein (IGFBP)-1 and on insulin resistance associated with PCOS. Fifteen patients diagnosed with PCOS were recruited. Clinical diagnosis was based on chronic oligomenorrhea or amenorrhea and hyperandrogenaemia. Clomiphene citrate was administered at a dose of 100 mg/day to all women from day 5 to day 9 of the spontaneous or medroxyprogesterone acetate (MAP)-induced menstrual cycle. Blood sampling and a 2 h oral glucose loading test (75 g) were performed the day before and after the course of clomiphene. Ovulation was confirmed in 13/15 PCOS patients. Plasma concentrations of IGF-I decreased by 31.5% (434 ± 84 versus 297 ± 71 ng/ml; P < 0.05) after 5 days of clomiphene therapy, whereas plasma concentrations of IGFBP-1 increased by ~28.1% (26.3 ± 4 versus 36.6 ± 7 ng/ml; P < 0.05). This gave a 56.5% reduction in the IGF-I:IGFBP-1 ratio (21.9 versus 9.53). No significant changes in basal plasma concentrations of fasting insulin or area under the insulin curve were observed in response to oral loading. The present results show that clomiphene does not cause changes in insulin resistance associated with PCOS but reduces plasma concentrations of IGF-I and increases those of IGFBP-1, with a consequent marked reduction in the IGF-I:IGFBP-1 ratio.

Key words: clomiphene/IGF-I/IGFBP-1/insulin/PCOS

Introduction

Clomiphene citrate is still the drug most widely used for inducing ovulation in women with polycystic ovarian syndrome (PCOS). Synthesized more than 40 years ago by F.P. Palopoli (Allen et al., 1959; Palopoli et al., 1967), it has mixed oestrogenic and anti-oestrogenic properties. Although it has been the subject of much research, its multiple mechanisms and sites of action are still to some extent unclear. The induction of ovulation by clomiphene could be the result of interaction of the drug at various levels: hypothalamus, pituitary and ovary (Adashi, 1984). It was demonstrated that administration of clomiphene to women with PCOS is accompanied by a reduction in plasma concentrations of insulin-like growth factor-I (IGF-I) and an increase in sex hormone binding globulin, leading to reduction in the free fraction of steroid hormones (Butzow et al., 1995). The importance of these observations lies in the fact that IGF-I and its binding proteins (IGFBP) affect follicular maturation by autocrine and/or paracrine mechanisms (Suikkari et al., 1991; Katz et al., 1993).

IGF-I seems to have an overall negative effect on normal folliculogenesis and ovulation (Barbieri et al., 1986; Cara and Rosenfield, 1988), favouring the production and accumulation of androgens in the ovary. Although plasma concentrations of total IGF-I are not reported to be significantly higher than those in women without PCOS (Homburg et al., 1992), the bioavailability of IGF-I may be raised by a decrease in IGFBP-1 plasma concentrations. A reduction in IGFBP-1 concentrations with respect to normal women has been demonstrated in women with PCOS (Suikkari et al., 1989). These lower concentrations of IGFBP-1 seem to be a consequence of hyperinsulinaemia, a recognized feature of PCOS (Suikkari et al., 1988). It was recently demonstrated that PCOS is associated with elevated serum concentrations of free IGF-I measured with a new direct immunoradiometric assay (Van Dessel et al., 1999).

The aim of the present study was to evaluate the effect of clomiphene on plasma concentrations of IGF-I and IGFBP-1 and on insulin-resistance associated with PCOS.

Materials and methods

The study was conducted at the Departments of Obstetrics and Gynecology of the Universities of Siena and Catania, Italy. Fifteen patients diagnosed with PCOS were recruited (Table 1). Clinical diagnosis was based on chronic oligomenorrhea (fewer than six menstrual periods in the previous 12 months) or amenorrhea and hyperandrogenaemia (elevated serum free testosterone concentrations). None of the women had virilization. Congenital adrenal hyperplasia was excluded by an adrenocorticotropic hormone (ACTH) stimulation test. Basal hormone concentrations assayed before treatment revealed anovulatory cycles, increased serum concentrations of LH, increased LH/FSH ratio and androstenedione and testosterone concentrations at the upper limits of the normal range.
A baseline ultrasound scan was performed to evaluate the uterus and ovaries. Ovarian volumes were calculated from the maximum longitudinal antero-posterior and transverse diameters. Ultrasonographic diagnosis of PCOS was based on the presence of 10 or more follicles (2–10 mm in diameter) in one or both ovaries.

All women were normoprolactinaemic, normotensive and without evidence of any other serious medical disorder. All had normal thyroid function.

Clomiphene citrate was administered at a dose of 100 mg/day to all women from day 5 to day 9 of the spontaneous or medroxyprogesterone acetate (MAP)-induced menstrual cycle. Blood sampling and a 2 h oral glucose loading test (75 g) were performed the day before (day 4, 08.00–10.00) and after the course of clomiphene (day 10, 08.00–10.00).

The study was approved by the institutional review board of Siena University. Written informed consent was obtained from the patients.

**Assays**

Plasma concentrations of LH, FSH, oestradiol, testosterone, free testosterone and 17-hydroxyprogesterone (17-OHP) were measured by double antibody radioimmunoassay using Radim kits (Rome, Italy) for LH and FSH, Sorin kits (Saluggia, Italy) for testosterone, DPC kits (Los Angeles, CA, USA) for 17-OHP and free testosterone, and Biodata kits (Rome, Italy) for oestradiol. Total IGF-I and IGFBP-1 were measured by immunoradiometric assay (IRMA) using DSL kits (Webster, TX, USA). 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 concentrations were included in each assay. The detection limits of the assays were 0.20 IU/l for LH, 0.18 IU/l for FSH, 2.06 ng/ml for IGF-I, 0.33 ng/ml for IGFBP-1, 18 pmol/l for oestradiol, 0.52 pmol/l for free testosterone, 277 pmol/l for testosterone and 0.21 nmol/l for 17-OHP.

Intra- and inter-assay variations were 7.8 and 8.2% for LH, 6.2 and 6.5% for FSH, 3.2 and 3.4% for free testosterone, 3.9 and 3.8% for IGF-I, 4.6 and 3.6% for IGFBP-1, 3.4 and 4.6% for testosterone, 4 and 4.8% for 17-OHP and 4.2 and 4.9% for oestradiol.

**Statistical analysis**

All values are mean ± SD. Areas under the insulin curves (AUC) were calculated by the trapezoidal method. Basal hormone concentrations were compared using Student’s t-test for paired data. Differences were considered significant for $P < 0.05$. Relationships between variables were sought by Pearson product-moment correlations (Glantz, 1988).

**Results**

Ovulation was confirmed in 13/15 PCOS patients by mid-luteal progesterone concentrations >35 nmol/l. Plasma concentrations of IGF-I decreased by 31.5% (434 ± 84 versus 297 ± 71 ng/ml; $P < 0.05$) after 5 days of clomiphene therapy, whereas plasma concentrations of IGFBP-1 increased by ~28.1% (26.3 ± 4.0 versus 36.6 ± 7.0 ng/ml; $P < 0.05$). This gave a 56.5% reduction in the IGF-I:IGFBP-1 ratio (21.9 versus 9.53) (Figure 1).

The oral glucose loading curves of 7/15 subjects are shown in Figure 2. All these women were hyperinsulinaemic with insulin after glucose above the normal range. Basal insulin concentrations were also above the normal range (36-78 pmol/l); in this group of patients, basal insulin concentration was 106 ± 12 pmol/l (mean ± SD). No significant changes in basal plasma concentrations of fasting insulin or area under the insulin curve (AUCinsulin) were observed in response to oral loading (fasting insulin: 108 ± 24 versus 103 ± 23 pmol/l; AUCinsulin: 65 850 ± 11 220 versus 65 180 ± 10 855 pmol/l/time). Glucose concentrations and fasting glucose:insulin ratio did not change after clomiphene treatment (4.29 ± 0.6 versus 4.19 ± 0.7 mmol/l and 0.039 ± 0.002 versus 0.04 ± 0.002, respectively).

No significant correlations were found between basal concentrations and variations after clomiphene therapy of IGF-I, IGFBP-1 and variations in steroids or gonadotrophins. The only significant correlation was between reduction in plasma concentrations of IGF-I after clomiphene and increase in IGFBP-1 concentrations ($r = 0.81; P = 0.015$).

**Discussion**

The results confirm the known reduction in plasma concentrations of IGF-I in response to clomiphene. The novelty of this study, however, consisted in studying changes in IGFBP-1 and insulin resistance associated with PCOS. Clomiphene treatment was associated with a 28.1% increase in plasma concentrations of IGFBP-1 and with no changes in fasting insulin, fasting glucose, AUCinsulin and glucose to insulin ratio. The absence of modifications in insulin concentrations after clomiphene treatment was expected. Other non-causal therapies for insulin-resistant states such as short-term caloric restriction and exercise do not improve insulin resistance (Mantzoros and Moses, 1997).

Although oral glucose tolerance test provides a convenient, readily available means of classifying individuals into normal, mild to moderate and severe insulin resistant, euglycaemic clamp is considered the gold standard in the assessment of insulin action (Del Prato, 1999).

The fasting glucose:insulin ratio has been reported to correlate with dynamic testing of insulin in PCOS (Legro et al., 1998). In the current study patients were their own controls and the comparison was made between before and
correlated with serum concentrations of insulin (Suikkari et al., 1989; Homburg et al., 1992). This leads to an increased IGF-I:IGFBP-1 ratio and an increase in bioavailability of IGF-I. This hypothesis is confirmed by the recent demonstration of elevated serum free IGF-I in women with PCOS (Van Dessel et al., 1999). Both high concentrations of insulin and IGF-I could amplify the effects of LH on granulosa cells, inducing terminal differentiation and leading to anovulation.

The reduced IGF-I:IGFBP-1 ratio after clomiphene administration could therefore play a role in the induction of ovulation by this drug. IGFBP-1 is a hepatic product, the synthesis of which is inhibited by insulin (Suikkari et al., 1989). IGFBP-1 synthesis also takes place in ovarian granulosa and endometrium (Koistinen et al., 1986), and in both sites IGFBP-1 synthesis is inhibited by insulin.

On the basis of the present results, the increase in IGFBP-1 cannot be explained by a reduction in insulin resistance, since no changes in the insulin curve were observed in response to oral glucose loading after clomiphene therapy. The increase in plasma concentrations of IGFBP-1 may therefore be a direct effect of clomiphene.

Other hypotheses show that IGF-I directly suppresses secretion of IGFBP-1 by granulosa cells (Dor et al., 1992), decidua (Thraikill et al., 1990) and HEpg2 cells (Conover and Lee, 1990) and thus a primary reduction in IGF-I could itself be responsible for the increase in IGFBP-1. It is therefore possible that clomiphene causes an increase in IGFBP-1 by reducing IGF-I.

However, a direct effect of clomiphene cannot be excluded, since this drug, being an oestrogen receptor probe, may theoretically act on all tissues that express receptors for oestrogens. In fact, about 30 years ago, Schultz et al. (1968) showed that [14C]clomiphene accumulates in oestrogen target tissues, such as the pituitary, hypothalamus, ovary, liver, uterus and adrenal gland. Since the number of clomiphene non-responders is so small in the current study, it is not possible to verify existing data on the predictivity of the response to clomiphene in terms of insulin resistance and IGFBP-1 (Tiitinen et al., 1993).

IGF-I has been shown to contribute to carbohydrate economy. Availability of recombinant IGF-I has made possible

![Figure 1](image1.png)

**Figure 1.** Insulin-like growth factor-I (IGF-I), IGF binding protein (BP)-1 and IGF-I/IGFBP-1 ratio in 15 polycystic ovarian syndrome patients before (grey) and after (black) clomiphene treatment (*P < 0.05).

![Figure 2](image2.png)

**Figure 2.** Fasting insulin and area under insulin curve (AUC insulin) before (grey) and after (black) clomiphene treatment in seven women (*: pmol/l/time×1000).
investigations on the regulatory relations among IGF-I and insulin. IGF-I suppresses insulin secretion and reduces fasting glucose, even under euglycemic conditions (Bondy et al., 1994); however, this effect is for supraphysiological doses of recombinant IGF-I. In the current study, fasting glucose did not change and IGF-I concentrations were in the normal range, hence it is unlikely that a reduction in bioavailable IGF-I for a few days might lead to changes in insulin concentrations.

It is difficult to evaluate the real contribution of the reduction in the IGF-I:IGFBP-1 ratio to induction of ovulation by clomiphene. It was recently shown that the insulin-sensitizing drug metformin reduces the IGF-I:IGFBP-1 ratio in PCOS (De Leo et al., 2000). This drug has recently been proposed to treat anovulation in PCOS and is reported to improve menstrual cycles (Velazquez et al., 1997) and the response to clomiphene (Nestler et al., 1998) and to normalize ovarian response to gonadotropins (De Leo et al., 1999). Hence the reduction in the IGF-I:IGFBP-1 ratio seems to be a fundamental element of initiation of ovulatory cycles in PCOS.

In conclusion, the present results show that clomiphene does not cause changes in insulin resistance associated with PCOS but reduces plasma concentrations of IGF-I and increases those of IGFBP-1, with a consequent marked reduction in the IGF-I:IGFBP-1 ratio. Reduction of this ratio could play a basic role in clomiphene-initiated ovulation, presumably by modifying the hyperandrogenic intrafollicular milieu recognized in PCOS. It can therefore be stated that clomiphene-induced ovulation in PCOS is the result of an action of the drug not only on the hypothalamus, pituitary and ovary, but also peripherally, as shown by changes in plasma concentrations of peptides of prevalently hepatic origin.

References

Received on April 27, 2000; accepted on July 31, 2000.