Insulin sensitivity in non-obese women with polycystic ovary syndrome during treatment with oral contraceptives containing low-androgenic progestin

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BACKGROUND: Combined oral contraceptives (COC) effectively suppress hyperandrogenism in women with polycystic ovary syndrome (PCOS), though deterioration of insulin sensitivity during treatment is assumed. The study aim was to investigate insulin action and androgen production during treatment with COC containing low-androgenic progestin.

METHODS: A total of 13 PCOS women and nine controls was enrolled into the study. Only non-obese women with a body mass index (BMI) ≤30 kg/m² were included. Hyperinsulinaemic euglycaemic clamp techniques were performed before and after 6 months of treatment with a monophasic COC containing norgestimate.

RESULTS: Anthropometric parameters [BMI, waist:hip ratio (WHR)] remained unaltered during the study in both groups. No deterioration in glucose disposal rate (M), insulin sensitivity index (ISI) or metabolic clearance rate of glucose (MCRG) was observed during treatment in PCOS subjects. Fasting glucose decreased significantly (P < 0.01), but fasting insulin remained unchanged. Significant decreases in concentrations of testosterone (P < 0.001), androstenedione (P < 0.01) and dihydroepiandrosterone (DHEA) (P < 0.001), a decrease in the free androgen index, and an increase in concentrations of sex hormone-binding globulin were found in PCOS subjects.

CONCLUSIONS: The norgestimate-containing COC significantly decreased androgen production and concentrations of free androgens, without reducing insulin sensitivity in non-obese PCOS subjects.

Keywords: androgens/insulin sensitivity/norgestimate/oral contraceptives/PCOS

Introduction

The polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women of reproductive age (Knochenhauer et al., 1998; Asuncion et al., 2000). It is the leading cause of menstrual cycle disturbances, skin androgenic complaints and anovulatory infertility. One of the principal endocrine abnormalities of the syndrome is overproduction of androgens in the ovaries, though many other endocrine and metabolic disturbances characterize the syndrome. PCOS women have been found to have lipid profile deterioration, increased concentrations of plasminogen activator inhibitor (PAI-1), and decreased concentrations of sex hormone-binding globulin (SHBG) (Dunaif et al., 1987; Holte et al., 1994; Sampson et al., 1996; Atiomo et al., 1998; Talbott et al., 1998). A frequent finding in non-obese and obese PCOS women is hyperinsulinaemia secondary both to increased peripheral insulin resistance and abnormal insulin secretion (Chang et al., 1983; Dunaif et al., 1989, 1992; Dunaif and Finegood, 1996). The major consequence of these abnormalities is a high incidence of impaired glucose tolerance and type 2 diabetes in these patients (Ehrmann et al., 1999; Legro et al., 1999). An increased risk of ischaemic heart disease and hypertension in the perimenopausal age has also been demonstrated (Dahlgren et al., 1992; Cibula et al., 2000).

Estrogen-progestogen products (combined oral contraceptives; COC) are an effective modality in the long-term treatment of hyperandrogenism in PCOS patients. They significantly suppress ovarian androgen synthesis and increase the binding capacity for circulating androgens by increasing SHBG concentrations (Falsetti and Pasinetti, 1995; Coenen et al., 1996). COC containing progestins with a low androgenic potency have a neutral or positive effect on the lipid profile, and sufficiently improve skin androgenic symptoms (Gevers Leuven et al., 1990; Kuhl et al., 1990). Apart from these beneficial effects a deterioration of insulin action is assumed. The impairment of insulin sensitivity in healthy women using COC has been demonstrated by several authors (Skouby et al., 1987; Kasdorf and Kalkhoff, 1988; Godsland et al., 1992).

To our knowledge, only one report has been published to date determining the changes in insulin action during COC
administration in PCOS patients, with insulin sensitivity being significantly decreased during three cycles of treatment in nine patients (Korytkowski et al., 1995). Norethindrone with a relatively high androgenic potency was used in this study as the progestogenic component. However, a less pronounced effect of agents with low-androgenic progestins on carbohydrate metabolism was demonstrated (Godslad et al., 1992). Hence, the present study was conducted in order to determine the effect of COC containing progestin with low androgenic potency on insulin sensitivity in PCOS women. The euglycaemic glucose clamp technique was used to study insulin action.

Materials and methods
Study group
A total of 14 patients meeting the diagnostic criteria of PCOS were enrolled into the study. PCOS was defined as follows: (i) oligomenorrhoea from menarche (menstrual cycle longer than 35 days); (ii) an increased concentration of at least one androgen above the upper reference limit [testosterone 0.5–2.63 nmol/l; androstenedione 1.57–5.4 nmol/l; dihydroepiandrosterone (DHEA) 0.8–10.5 nmol/l; dihydroepiandrosterone sulphate (DHEAS) 2.4–14.5 μmol/l]; and (iii) clinical manifestation of hyperandrogenism (acne, hirsutism, or both). Only non-obese women with a body mass index (BMI) <30 kg/m², aged >18 years, and who had not used hormonal therapy during the previous 6 months, were included. Women presenting with a secondary endocrine disorder, such as hyperprolactinaemia, thyroid dysfunction or a non-classical form of congenital adrenal hyperplasia, those wishing to conceive within the next 6 months, or women with contraindications to oral contraceptives use were excluded from the study. Normal glucose tolerance was established according to both the criteria of the World Health Organization and the revised criteria of the American Diabetes Association using the 2-h, 75-g oral glucose tolerance test (OGTT) (World Health Organization, 1985; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Only one patient did not complete the study protocol of COC treatment because of the occurrence of side effects (nausea, breast tenderness) requiring discontinuation. All patients were informed about the study protocol and provided their informed consent. The study was approved by the Local Ethics Committee of both institutions.

Control group
A control group was selected from women willing to use COC, who agreed with the study protocol. All subjects met the following inclusion criteria: (i) concentrations of androgens and SHBG within the reference limits; (ii) a regular menstrual cycle from menarche to the present; and (iii) absence of skin androgenic symptoms (hirsutism, persistent acne). Only non-obese women with a BMI <30 kg/m², age >18 years and who had not used hormonal therapy during the previous 6 months, were included. Patients with an established endocrine disorder or with contraindications to hormonal contraception use were excluded. Normal glucose tolerance was established before the study. All controls provided their informed consent.

Study protocol
All patients and controls received monophasic COC pills containing ethinyl estradiol (35 μg/day) and low-androgenic progestin (norgestimate 250 μg/day) for seven cycles. The COC agent was given in a cyclic regimen of 21 active pills followed by 7 days without hormonal use. All clinical investigations and laboratory tests were performed prior to treatment and after the sixth cycle of treatment.

Blood samples taken prior to treatment were withdrawn during the early follicular phase, i.e. between days 3 and 6 of the menstrual cycle. Provided that menstrual bleeding failed to occur until day 45 of the cycle, bleeding was induced by progesterone administration. The second blood sample was taken after six cycles of COC administration.

Euglycaemic hyperinsulinaemic clamp technique
The hyperinsulinaemic euglycaemic clamp was performed as described previously (Flier, 1992). A flexible cannula was inserted into the forearm vein to obtain blood samples for the determination of basal insulin, and plasma glucose and potassium concentrations. The cannula was then connected to an infusion module of a Biostator (GCSII; Elkhat, IN, USA) to administer an insulin solution (160 units of Actrapid HM®; Novo-Nordisk, in 500 ml 0.9% sodium chloride saline solution), 40% glucose solution, and wash-out sodium chloride saline solution (0.9% w/v). At the same time, 7.5% potassium chloride solution diluted with physiological saline solution 1:4 was delivered by perfusor (Infusor Secura FT; B. Braun, Germany) to another channel of the cannula at a rate of 0.1 ± 0.05 ml/min to maintain basal potassium concentrations. The rate of this infusion was adjusted according to the results of repeatedly determined serum potassium concentrations. A double-lumen catheter was inserted into the contralateral arm for continuous blood glucose determination. A third cannula placed into a wrist vein was used to collect blood samples for biochemical measurements. After a 30 min washout period, the hyperinsulinaemic euglycaemic clamp was performed using the Biostator (mode 7:1) over 120 min using a constant insulin infusion rate (1 mU/kg per min) (Fogt et al., 1978). The glucose solution (40% w/v) was sampled by the Biostator to maintain blood glucose concentration at baseline value. During the clamp, blood glucose concentrations were repeatedly determined using a glucose analyser (ESAT 6660-2; Melsungen, Germany). Two blood samples were collected for insulin determination during the last 20 min of clamping.

The following characteristics of insulin action were calculated: (i) glucose disposal rate (M, μmol/kg per min), defined as the amount of glucose supplied by the Biostator to maintain blood glucose concentrations during the clamps; (ii) the insulin sensitivity index (ISI, μmol/kg per min per mU/l×100), defined as the ratio of glucose disposal rate to insulin concentration at the end of the clamps; and (iii) metabolic clearance rate of glucose (MCRG, ml/kg per min), expressed as the ratio of glucose disposal rate to blood glucose concentration.

Analyses
All analyses were performed at the National Reference Laboratory. Serum LH, FSH and testosterone concentrations were measured with a chemiluminescence assay (ACS:180 auto-analyser; Bayer Diagnostics GmbH, Germany). The concentrations of DHEA, DHEAS and androstenedione were determined using radioimmunoassay methods (Immunootech, France). SHBG was measured using an IRMA kit (Orion, Finland). The intra- and interassay coefficients of variation (CV) were respectively: <3.7 and <6.7% for LH, <2.6 and <4.2% for FSH, <4.0 and <8.0% for testosterone, <7.9 and <11.9% for DHEA, <7.4 and <10.6% for DHEAS, <8.9 and <10.3% for androstenedione, and <5.5 and <6.9% for SHBG. The free androgen index (FAI) was calculated according to the following formula: FAI = 100×testosterone (nmol/l)/SHBG (nmol/l) (Carlstrom et al., 1987). Plasma glucose concentration was determined by the glucose oxidase method (Olympus Diagnostica GmbH, Germany). Plasma insulin concentrations were measured using radioimmunoassay kits (CIS Bio International, France). Normal range was 4–20 μIU/ml; interassay
Table I. Summary statistics of age, body mass index (BMI), waist:hip ratio (WHR), glucose, insulin, HbA1c, LH, FSH and steroids before treatment in controls and in PCOS patients

<table>
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<th>Parameter</th>
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<th>PCOS patients</th>
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<td></td>
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<td>BMI (kg/m²)</td>
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<td>WHR</td>
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<td>HbA1c (%)</td>
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<td>4.75</td>
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<tr>
<td>SHBG (nmol/l)</td>
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</tr>
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DHEA = dihydroepiandrosterone; DHEAS = dihydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin.

The most important consequence of endocrine and metabolic disturbances associated with PCOS is an increased risk for...
impaired glucose tolerance and type 2 diabetes mellitus (Dahlgren et al., 1992; Ehrmann et al., 1999; Legro et al., 1999; Cibula et al., 2000). Insulin resistance independent of obesity, abnormal insulin secretion and dyslipidaemia are risk factors participating in the pathogenesis of the above conditions (Dunaif et al., 1987, 1989, 1992; Holte et al., 1994; Talbott et al., 1998). New findings regarding the metabolic consequences have changed the concepts for the long-term treatment of PCOS. The optimal therapeutic modality should correct the increased androgen production, yet at the same time exert a beneficial, or at least neutral, effect on insulin action.

One of the most frequent treatment modalities—combined oral contraceptives—fulfilled almost all the criteria for optimal treatment. However, the impairment of insulin sensitivity, as described in healthy users, might be a significant weakness. The first study examining insulin sensitivity during COC use was published in 1987 (Skouby et al., 1987). These authors performed the euglycaemic clamp technique to demonstrate a decrease in insulin sensitivity in six healthy subjects after 6-month administration of a levonorgestrel-containing COC. Similar results were reported after 3 months of COC use; however, insulin sensitivity returned toward control values.

**Figure 1.** Changes in LH, FSH, LH/FSH, sex hormone-binding globulin (SHBG), steroids and the free androgen index (FAI) during treatment. Empty and full circles with error bars represent retransformed mean value ± SEM in controls and patients respectively. Crosses denote the significance of the differences in subjects between the stages of the study (before/after treatment) (+, $P < 0.05$; ++, $P < 0.01$; ++++, $P < 0.001$). Asterisks denote the significance of the differences between controls and patients (⋆, $P < 0.05$; **, $P < 0.01$). Closed circles in the frame denote the significance of interactions (oo, $P < 0.01$; ooo, $P < 0.001$). A = androstenedione; DHEA = dihydroepiandrosterone; DHES = dihydroepiandrosterone sulphate; T = testosterone.
after 6 months (Kasdorf and Kalkhoff, 1988). A decrease in insulin sensitivity was also demonstrated after application of a minimal model approach in 296 contraceptive users (Godsland et al., 1992).

To the best of our knowledge, only one report has been published to date addressing the changes in insulin sensitivity during COC administration in PCOS patients (Korytkowski et al., 1995), with insulin sensitivity being studied in nine women with PCOS, and in 10 controls. The hyperglycaemic clamp technique was performed before and after 3 months of therapy with triphasic oral contraceptives containing norethindrone, a progestogen with a relatively high androgenic potency. Despite the short time of intervention, a decline in insulin sensitivity was shown in both groups.

The present study is the first designed to investigate changes in insulin sensitivity in PCOS women receiving a COC containing a progestin with a low androgenic potency. The minimal androgenicity of progestins is reflected in a more pronounced increase in the concentrations of SHBG and a decrease in free testosterone concentrations in COC users (Hammond et al., 1984; van der Vange et al., 1990). Moreover, a number of reports have documented only slight changes in glucose tolerance, fasting insulin, fasting glucose and areas under glucose or insulin curves in users of COC with low-androgenic progestins (Petersen et al., 1988; Corson, 1990; Godsland et al., 1992; Crook et al., 1993). Studies with norgestimate, the progestin used in the present study, showed virtually no effect on carbohydrate metabolism. In two US multicentre trials, no significant changes in fasting blood glucose, or in the results of a 3-h glucose tolerance test, were demonstrated (Corson, 1990), whilst a 12-month German study documented no adverse effect of treatment on the insulin, glucose, or HbA1c concentrations (Becker, 1990). The effect of two products containing progestins with a low androgenic potency (norgestimate and gestodene) on insulin action was evaluated in a recent study (Petersen et al., 2000). Insulin sensitivity was determined by a frequently sampled intravenous glucose tolerance test. The users of either product showed increased concentrations of fasting plasma insulin and a decrease in insulin sensitivity. However, the relationship between the ISI and insulin response remained unchanged.

An important inclusion criteria in the present study was that of BMI. Only non-obese patients were eligible for inclusion, as it was well recognized that obesity is an independent risk factor worsening the parameters of insulin resistance (Acién et al., 1999; Ciampelli et al., 1999). Every minor shift in BMI during the study may dramatically influence insulin sensitivity in obese patients. On the other hand, impaired insulin action has been repeatedly documented in both non-obese and obese PCOS patients (Dunaif et al., 1989; Dunaif and Finegood, 1996).
Higher insulin concentrations in PCOS patients compared with controls were not observed prior to treatment. The value of the ISI was lower, though not significantly so, in the PCOS group. Importantly, no adverse effect of COC administration was observed on either insulin sensitivity, glucose disposal rate or the metabolic rate of glucose. It might be speculated therefore that COC could have had an adverse effect on ISI, but on the other hand there was a compensatory improvement in ISI due to a decrease in androgen concentrations. However, the control group—where no such marked effect on androgen concentrations was seen—also did not show any deterioration in insulin sensitivity. The only relevant effects of treatment on carbohydrate metabolism were significantly decreased concentrations of fasting glucose. Unlike the study of Petersen (Petersen et al., 2000), who used a frequently sampled intravenous glucose tolerance test, no deterioration of insulin sensitivity could be demonstrated in the group of healthy users.

The present study confirmed a marked suppressive effect of COC treatment on androgen concentrations that has been observed in other relevant investigations (Falsetti and Pasinetti, 1995; Coenen et al., 1996). In addition to reduced androgen production, the treatment significantly raised the serum androgen binding capacity. The significant increase in SHBG concentrations reflects the minimal androgenicity of the progestogenic component used in COC.

It can be concluded that, in the present study there was no deterioration of insulin sensitivity in non-obese PCOS women during administration of COC containing low-androgenic progestin. In addition, the beneficial effect of COC administration reducing the concentrations of total and free androgens was confirmed. It is clear that whilst the present study was of insufficient duration to draw any authoritative conclusions, the results obtained provide significant evidence supporting the use of COC containing low-androgenic progestins in the long-term treatment of PCOS patients.

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References


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