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The effect of combination therapy with metformin and combined oral contraceptives (COC) versus COC alone on insulin sensitivity, hyperandrogenaemia, SHBG and lipids in PCOS patients

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BACKGROUND: Neither oral contraceptives (COC) nor metformin are an optimal modality for the long-term treatment of polycystic ovary syndrome (PCOS). The aim of this study was to evaluate whether a combination of both is beneficial over COC monotherapy. METHODS: Altogether, 30 women were included in the study and 28 finished the protocol. The patients were randomly assigned to two groups treated with either COC (COC group) or COC and metformin (1500 mg/day) (METOC group) for 6 months. Anthropometric parameters, androgens, lipids, fasting insulin, glucose and sex hormone binding globulin (SHBG) concentrations were measured before and at the end of the sixth cycle of treatment. The insulin sensitivity index was evaluated using the euglycaemic clamp. RESULTS: There were no significant changes in anthropometric parameters, fasting glucose or insulin sensitivity in either group. Total testosterone, free androgen index, androstenedione and dehydroepiandrosterone decreased and SHBG increased significantly in both groups. When comparing the effect of both treatments, only a more pronounced decrease in free androgen index was found in the METOC group. CONCLUSIONS: Adding metformin slightly modified the treatment effect of COC, causing a more significant decrease in the free androgen index but having no additional positive impact on lipids, insulin sensitivity, SHBG or testosterone. The available data do not offer enough evidence to advocate the standard use of combined treatment in PCOS. Whether the combination might be beneficial for specific subgroups of patients is of further interest.

Key words: androgens/insulin sensitivity/metformin/oral contraceptives/polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome with a wide variety of endocrine and metabolic abnormalities and clinical symptoms. The optimal modality for long-term treatment should favourably influence androgen synthesis, sex hormone binding globulin (SHBG) production, the lipid profile, insulin sensitivity, and clinical symptoms including acne, hirsutism and irregular menstrual cycle. The above requirements are difficult to meet with a single form of treatment.

The two widely used options for long-term treatment, combined oral contraceptives (COC) and metformin, have different effects. There are data showing a direct comparison of metformin and COC in women with PCOS. Two studies separately evaluated the treatment effect in obese and non-obese patients, with comparable results (Morin-Papunen *et al.*, 2000; 2003). Metformin caused no changes in insulin sensitivity in non-obese patients, a slight improvement in obese patients and a significant decrease in fasting insulin in both groups. During COC treatment, no changes in insulin sensitivity or fasting insulin were found. The latter treatment caused a more significant decrease in total androgens and highly significant increase in SHBG in both subgroups of patients.

To date, only two studies have focused on treatment with COC and metformin in combination. Both included non-obese subjects only. Elter *et al.* (2002) found a greater decrease in androstenedione and more pronounced increase in SHBG in the group receiving combination treatment. In a recent study, COC was administered to adolescent PCOS women receiving continuous metformin and flutamide treatment (Ibáñez and Zegher, 2003). Addition of COC was followed by an increase in SHBG only. None of these studies directly evaluated insulin sensitivity.

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The aim of our study was to evaluate the effect of treatment with COC alone or in combination with metformin on insulin sensitivity, total androgens, SHBG and lipids. A priority of our study was the direct measurement of insulin sensitivity, which should be a key argument for adding metformin.

Materials and methods

Subjects

The subjects were recruited from the Unit of Reproductive Endocrinology. Patients fulfilling the diagnostic criteria for PCOS were consecutively enrolled in the study. PCOS was defined as follows: (i) oligomenorrhea from menarche (menstrual cycle >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, dehydroepiandrosterone (DHEA) 0.8–10.5 nmol/L]; and (iii) clinical manifestation of hyperandrogenism (acne, hirsutism or both). 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 contraceptive use were excluded from the study. The study was approved by the local ethics committees of the First Faculty of Medicine and General Teaching Hospital, and written informed consent was obtained from all subjects.

Protocol of the study

All patients were randomly assigned to two groups using a generator of random values with a uniform distribution within the interval 0 to 1 (statistical software NCSS 2002). The values obtained were transformed into rank values. The subjects with ranks 1–15 were assigned to the COC group and received a monophasic COC (EE 35 μg/NGM 250 μg) in a cyclic regimen (21 days of active pills followed by 7 days of pill-free interval) for 6 months. The remaining 15 subjects received an identical COC in combination with metformin (1500 mg/day) for 6 months (METOC group). All laboratory tests were performed prior to treatment and after the sixth cycle of treatment.

The weight and height of all women were taken to calculate the body mass index (BMI). The waist and hip circumferences were measured in the standing position at the levels of the umbilicus and spina iliaca anterior superior and the waist-to-hip ratio (WHR) was calculated. Blood samples were taken in the early follicular phase, i.e. between days 3 and 6 of the menstrual cycle.

Assays

All analytic determinations were performed at the National Reference Laboratory. Serum LH, FSH and testosterone concentrations were measured by chemiluminiscent assay using an ACS:180 Autoanalyzer (Bayer Diagnostics, GmbH, Germany). The concentrations of DHEA, dehydroepiandrosterone sulphate (DHEA-S) and androstenedione were determined by radioimmunoassay methods (Immunotech, Fullerton, CA, USA). SHBG was measured using IRMA kits (Orion, Finland). The free androgen index (FAI) was calculated according to the following formula: FAI = 100 × testosterone (nmol/l)/SHBG (nmol/l). Plasma glucose concentration was determined by the glucose oxidase method (Olympus Diagnostica, GmbH, Germany). Plasma insulin concentrations were measured by radioimmunoassay kits (CIS Bio International, France; normal range 4–20 mIU/l; inter-assay %CV <5; intra-assay %CV <8.5). Serum cholesterol and triglycerides were analysed using CHOD-PAP and GPO-PAP-based kits, respectively (Oxochrome; Lachema a.s., Czech Republic). High-density lipoprotein (HDL) cholesterol was determined by an immunoinhibition method (HDL-C Direct; Wako Chemicals GmbH, Neuss, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula (LDL cholesterol = total cholesterol-HDL cholesterol-triglycerides/2.19 mmol/l) (Friedewald *et al.*, 1972).

Euglycaemic hyperinsulinaemic clamp

The hyperinsulinaemic euglycaemic clamp was performed as described previously (De Fronzo *et al.*, 1979). Briefly, to obtain blood for biochemical analyses during the clamp, one cannule was inserted into the wrist vein. For continuous blood glucose determination, a double-lumen catheter was inserted into the cubital vein of the ipsilateral arm. A third cannule was inserted into the contralateral forearm vein for insulin and glucose administration by Biostator (GCIIS, Elkhart, IN, USA). After a 30-min washout period, a hyperinsulinaemic euglycaemic state was attained during the next 45 min and the clamp was then performed using a constant insulin infusion rate (1 mU/kg/min) over 120 min. The glucose solution (40% w/v) was sampled by Biostator (mode 7:1) to maintain blood glucose levels at baseline value. During the clamp, blood glucose levels were repeatedly determined by glucose analyser (ESAT 6660-2; PWG, Medingen, Germany). Two blood samples for insulin determination were collected in the last 20 min of the clamp.

The following characteristics of insulin action were calculated: glucose disposal rate (M), defined as the amount of glucose supplied by the Biostator to maintain blood glucose levels during the last 20 min of the clamps (in mmol/kg/min); the insulin sensitivity index (ISI), defined as the ratio of glucose disposal rate to insulin concentration at the end of the clamps (in mmol/kg/min/mU/l × 100); and the metabolic clearance rate of glucose (MCRg), expressed as the ratio of glucose disposal rate to blood glucose concentration (ml/kg/min).

Statistical analysis

With respect to deviations from the Gaussian distribution of data and the occurrence of severe non-homogeneities in some variables, the treatment effect was evaluated using the robust paired Wilcoxon's test. For the same reason, the differences between the groups were tested with the Mann–Whitney robust test. Spearman's robust correlations were used for evaluating the differences before and after treatment. Besides the Mann–Whitney test, a general linear model was applied with adjustment of FAI, DHEA and BMI to eliminate the differences in androgens and BMI between the groups found at the beginning of the experiment in relation to ISI. With respect to the skewed data distribution in FAI and DHEA, the variables were transformed by a power transformation to attain a Gaussian distribution. The minimum value of mean square error of the linear fit in the normal probability plot (the plot of experimental fractiles versus theoretical fractiles of Gaussian distribution) was used as a criterion for optimal transformation parameters.

Results

A total of 30 women were enrolled in the study and 28 completed the protocol. Two subjects were excluded from the study, both from the METOC group, for unacceptable adverse events (gastrointestinal problem in one case and for non-compliance with the study protocol in the other).

The characteristics of both groups before treatment as well as the changes in the parameters during treatment are

Table I. Summary statistics of anthropometric parameters, lipids, hormones and insulin sensitivity in the groups treated with combined oral contraceptives (COC) and COC with metformin (METOC)

Variable	Indices at the beginning of the experiment							Difference after treatment compared with before treatment								
	COC			METOC				COC				METOC				
	Mean	SD	95% CI of mean	Mean	SD	95% CI of mean	Differences between the groups	Mean	SD	95% CI of mean	Differences from zero	Mean	SD	95% CI of mean	Differences from zero	Differences between the groups
Age	23.2	4.6	20.7–25.7	23.8	5.4	20.3–27.2	NS	–	–	–	–	–	–	–	–	–
Waist	74.1	10.5	68.3–79.9	80.0	12.6	72.0–88.0	NS	–	–	–	–	–	–	–	–	–
WHR	0.752	0.079	0.708–0.796	0.794	0.086	0.740–0.849	NS	–	–	–	–	–	–	–	–	–
Weight	63.3	11.9	56.7–69.9	68.6	13.3	60.1–77.0	NS	1.8	3.5	−0.2–3.7	NS	−0.9	4.7	−3.9–2.1	NS	NS
BMI	22.1	3.1	20.4–23.8	24.7	4.9	21.6–27.8	NS	0.6	1.2	−0.1–1.3	NS	−0.3	1.7	−1.4–0.7	NS	NS
Io	9.4	6.7	4.9–13.9	11.2	4.9	7.7–14.7	NS	5.40	2.67	3.3–7.5	$P < 0.01$	3.90	6.31	−1.0–8.8	NS	NS
Go	4.60	0.45	4.35–4.85	4.68	0.50	4.36–5.00	NS	−0.22	0.67	−0.6–0.2	NS	−0.26	0.59	−0.63–0.12	NS	NS
ISI	59.2	29.7	42.0–76.3	44.4	27.6	25.8–62.9	NS	−6.6	37.3	−28.2–14.9	NS	−3.5	30.8	−24.2–17.2	NS	NS
MCRg	8.54	2.50	7.16–9.93	6.83	1.86	5.65–8.00	NS	0.66	3.96	−1.5–2.9	NS	0.43	3.41	−1.73–2.60	NS	NS
CHOL	4.63	0.70	4.24–5.02	4.81	0.80	4.30–5.32	NS	0.62	0.72	0.2–1.0	$P < 0.007$	0.71	0.67	0.29–1.14	$P < 0.006$	NS
TGD	0.941	0.420	0.708–1.173	1.168	0.480	0.863–1.473	NS	0.381	0.547	0.1–0.7	$P < 0.02$	0.29	0.65	−0.12–0.700	NS	NS
HDL	1.55	0.21	1.44–1.67	1.47	0.40	1.22–1.73	NS	0.13	0.40	−0.1–0.3	$P < 0.05$	0.29	0.24	0.14–0.44	$P < 0.008$	NS
LDL	2.67	0.58	2.34–2.99	2.81	0.58	2.44–3.18	NS	0.31	0.54	0.0–0.6	$P < 0.05$	0.29	0.67	−0.14–0.72	NS	NS
LH	6.82	3.96	4.63–9.02	6.88	5.56	3.35–10.41	NS	−4.77	4.37	−7.2−2.4	$P < 0.003$	−4.07	5.71	−7.69−0.44	$P < 0.003$	NS
FSH	4.83	1.57	3.96–5.70	4.65	1.92	3.43–5.88	NS	−1.47	3.97	−3.7–0.7	NS	−0.91	2.41	−2.44–0.62	NS	NS
PRL	11.8	5.5	8.7–14.8	11.7	5.6	8.2–15.3	NS	1.3	5.9	−2.0–4.5	NS	1.1	6.8	−3.2–5.4	NS	NS
T	3.94	1.49	3.11–4.76	4.84	1.16	4.10–5.57	NS	−0.45	1.01	−1.0–0.1	$P < 0.05$	–0.92	2.10	–2.25–0.42	$P < 0.05$	NS
FAI	11.8	7.9	7.5–16.2	19.2	6.9	14.6–23.8	$P < 0.02$	–9.0	7.2	−13.0−5.0	$P < 0.001$	−15.5	8.2	−21.1−10.0	$P < 0.004$	$P < 0.04$
A	11.1	5.8	7.8–14.3	12.6	3.5	10.4–14.9	NS	−4.0	6.4	−7.5−0.5	$P < 0.04$	−4.8	2.6	−6.4−3.1	$P < 0.003$	NS
DHEA	25.6	13.3	18.3–33.0	40.4	16.6	29.8–50.9	$P < 0.02$	−7.8	14.6	−13.9–2.3	$P < 0.05$	−14.3	12.1	−22.1−6.6	$P < 0.009$	NS
DHEA-S	10.5	2.2	9.3–11.8	12.2	3.8	9.8–14.6	NS	−4.1	3.0	−5.8−2.4	$P < 0.002$	−2.5	4.0	−5.0–0.1	NS	NS
Prog17	3.74	3.32	1.73–5.74	3.47	1.17	2.73–4.22	NS	−1.69	3.16	−3.6–0.2	$P < 0.02$	−0.62	1.79	−1.82–0.58	NS	NS
SHBG	32	13	31–58	27	9	21–33	NS	108	63	73.2–143.3	$P < 0.001$	116	71	68–164	$P < 0.004$	NS

CI = confidence interval; NS, not significant; –, not available; Io = fasting insulin; Go = fasting glucose; CHOL = total cholesterol; TGD = triglycerides; PRL = prolactin; T = testosterone; A = androstenedione; Prog17 = 17OH-progesterone.

demonstrated in Table I. There were no differences in anthropometric parameters, ISI, lipid profile, SHBG concentration and hormones, except for a higher DHEA concentration and higher free androgen index in the METOC group, of borderline significance. Owing to significant differences in androgens and an insignificant difference in BMI between both groups, a general linear model was applied with adjustment of FAI, DHEA and BMI to eliminate the effect of these differences on the change of ISI during treatment. Even after this adjustment, no differences were found in the change of ISI between the COC and METOC groups.

As demonstrated in Table I, there was a slight weight gain and increase of BMI in the COC group and the opposite tendency in the METOC group, although this was not significant in either group. Insulin sensitivity did not change significantly in either group, but fasting insulin increased in the COC group. Both treatment protocols caused an increase in total cholesterol, triglycerides, HDL and LDL cholesterol; changes in triglycerides and LDL cholesterol did not reach significance in the METOC group. During treatment, there was a significant decrease in testosterone, androstenedione, DHEA, DHEA-S and 17OH-progesterone in the COC group. More pronounced changes in androstenedione and DHEA and a lack of significance in changes of DHEA-S and 17OH-progesterone were found in the METOC group. Highly significant changes in SHBG were found in both groups. Comparing the effect of treatment in both groups, only a decrease in free androgen index was significantly different and more pronounced in the METOC group.

Discussion

There is an ongoing discussion in the literature concerning the role of metformin in the treatment of PCOS (Homburg, 2002; Legro, 2002). Promising results were not fully confirmed in prospective randomized studies (Harborne *et al.*, 2003). The most consistent effect of metformin is an improvement in the ovulation rate (Lord *et al.*, 2003). However, changes in insulin, glucose tolerance, BMI and androgens vary. Stimulation of SHBG production, which is one of the key mechanisms in acne and hirsutism improvement, is not seen or is insignificant in the vast majority of studies. Restoration of a regular menstrual cycle usually occurs in $<50\%$ of patients. Based on the above data it is difficult to consider metformin alone as a first-line option for the treatment of PCOS.

A combination of metformin with COC would seem to be a justifiable solution for many reasons. Treatment with COC enables significant inhibition of androgen production and a significant increase in SHBG synthesis (Cibula *et al.*, 2002; Elter *et al.*, 2002). As a consequence, it is successfully used in the treatment of acne and hirsutism (Redmond *et al.*, 1997). Restoration of a regular menstrual cycle is reliable. On the other hand, a beneficial influence on glucose metabolism or insulin action is unlikely, although some recent papers showed neutral or even positive effects of COC with low androgenic progestins on insulin sensitivity (Cibula *et al.*, 2002; Cagnacci *et al.*, 2003). The significant effect of COC on SHBG, androgen production, skin androgenic symptoms and irregular menstrual cycle might be successfully combined with the effects of metformin on anthropometric parameters, glucose tolerance and insulin sensitivity. An additional argument for combination therapy is the need for effective contraception in women while on metformin.

Two studies have been published to date that used combined treatment with COC and metformin in patients with PCOS. In the first study, from 2002, the authors found a significant decrease in BMI and WHR only in the group on combined treatment, and a more pronounced effect on androstenedione, SHBG and glucose-to-insulin ratio in the same group (Elter *et al.*, 2002). When comparing both groups, only changes in androstenedione and SHBG remained significantly higher in the group on combination treatment. The authors concluded that adding metformin to the COC treatment might improve insulin sensitivity and further suppress hyperandrogenaemia in non-obese women with PCOS. However, insulin sensitivity was not measured directly.

The second paper, from 2003, focused on adolescent girls with PCOS (Ibáñez and Zegher, 2003). The design of the study was different. COC was randomly added to a continuous treatment with metformin and flutamide. The additive effect of COC was investigated as such. The only significant change following addition of COC was an increase in SHBG and consequently a decrease in the free androgen index.

In our study, we compared COC monotherapy with combination therapy of COC with metformin for 6 months. This was the first study using the euglycaemic clamp for insulin sensitivity evaluation during combination treatment.

In agreement with the paper by Elter *et al.* (2002), we showed a slight decrease in fasting glucose in both groups, although the above changes did not reach significance in our study. A slight rise in fasting insulin was significant only in the COC group. While Elter and colleagues evaluated insulin sensitivity indirectly using a calculation of glucose-to-insulin ratio, which improved significantly in the group on combined treatment, we measured insulin sensitivity by the clamp technique and found no significant changes. It should be mentioned, however, that the effect of metformin on glucose metabolism is mostly exerted through the inhibition of glucose production, and this mechanism might be masked by the increased levels of insulin during the clamp. There were no differences between the two treatments in the effect on glucose or insulin in our study, as in the study by Elter *et al.* (2002). In conclusion, we were not able to show an expected improvement in insulin sensitivity while on combined treatment, and the trends in fasting insulin and glucose concentrations were comparable in both groups.

Besides a potential improvement in insulin sensitivity, another argument for metformin is its beneficial effect on anthropometric parameters. While BMI and weight increased in the COC group, these were decreased during combined treatment. This is in accordance with Elter *et al.* (2002), although the changes were not significant in our study in either group. However, it is difficult to conclude whether those changes are caused by direct metabolic effect of

metformin or by frequent gastrointestinal problems at the beginning of metformin treatment. The positive trend in weight and body fat distribution should be confirmed in a long-term follow-up.

Modification of the COC effect on androgens by metformin is difficult to interpret. Randomized prospective studies have documented a direct effect of metformin on ovarian steroidogenesis (Pasquali *et al.*, 2000; Ng *et al.*, 2001; Vrbíková *et al.*, 2001; Kocak *et al.*, 2002). A more pronounced decrease in androstenedione in the group on combined treatment was described previously by Elter and colleagues. In our study, comparable effects on testosterone and androstenedione were found in both groups, and a change in DHEA of higher significance in the women on combined treatment might rather be explained by higher basal concentrations at the beginning of the study.

Stimulation of SHBG production is one of the key mechanisms in the treatment of skin androgenic symptoms by COC. Elter and colleagues found a greater increase in SHBG in the group with combined treatment. This was not confirmed in our study. We showed a comparable significant increase in SHBG in both groups. Our results are in agreement with many papers that show insignificant changes or even a decrease in SHBG with metformin treatment (Nestler *et al.*, 1998; Pasquali *et al.*, 2000; Ng *et al.*, 2001; Fleming *et al.*, 2002).

In summary, our study confirmed a significant positive effect of COC on androgens and SHBG. Combination with metformin caused an additional decrease in FAI. The beneficial trends in anthropometric parameters in the METOC group are in accordance with other studies, but weight reduction and positive changes of body fat distribution should be confirmed with long-term follow-up. Besides a few positive trends, combined treatment with metformin did not cause added beneficial effects on lipids, insulin sensitivity, SHBG or testosterone. It should be emphasized, however, that for evaluation of insulin sensitivity the number of subjects needed to reach enough power is very high (>300 patients), and was not fulfilled in our study. We conclude that the available data do not offer enough evidence to advocate the standard use of COC in combination with metformin in the long-term treatment of PCOS due to unsatisfactory improvement of endocrine and metabolic abnormalities that characterize the syndrome. However, it might be argued that the value of metformin could be different in specific subgroups of PCOS patients, especially in obese ones. This can not be addressed by our study and remains an area of future interest.

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