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DISSERTATION

Effects of Insulin Sensitizing Drug Metformin on Clinical Features, Endocrine and Metabolic Profiles in Obese Women with Polycystic Ovary Syndrome: A Randomized, Double Blind, Placebo-Controlled Sixteen Weeks Trial

Zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
der Charité – Universitätsmedizin Berlin

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Datum der Promotion: 29.06.2006.

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1. INTRODUCTION

1.1. Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder that affects approximately 6-10% of women of reproductive age (Franks, 1995). Polycystic ovary syndrome is probably the most prevalent endocrinopathy in women and by far the most common cause for infertility. In fact, polycystic ovaries have been associated with 75% of cases of anovulation (Hull, 1987).

1.2. Presentation

The many features of this syndrome can be divided into three categories: clinical, endocrine and metabolic. The clinical features include menstrual abnormalities, hirsutism, acne, alopecia, anovulatory infertility and recurrent miscarriages. The endocrine features are presented with elevated androgens, luteinizing hormone, and oestrogen and prolactin levels. The metabolic aspects of this syndrome are insulin resistance, obesity, lipid abnormalities and an increased risk for impaired glucose tolerance and type 2 diabetes mellitus (type 2 DM).

Endocrine Abnormalities

The main endocrine features of PCOS are increased androgen production and disordered gonadotropin secretion. Both luteinizing hormone (LH) pulse frequency and amplitude are increased, whereas follicle-stimulating hormone (FSH) levels remain constant in the midfollicular range (Marshall et al., 1999). The frequency of gonadotropin-releasing hormone (GnRH) release is increased secondary to decreased sensitivity of the GnRH pulse generator to the negative feedback effects of estradiol and progesterone. This increased GnRH pulse frequency selectively increases LH release. The raised LH levels enhance thecal androgen production, and these androgens are incompletely aromatised into estrogens by the granulosa cells, because of arrested follicular development as a consequence of low-level cyclic FSH release. The so-called vicious cycle of PCOS is created, in which disordered gonadotropin secretion causes increased ovarian androgen production, which in turn alters gonadal steroid feedback,

perpetuating disordered gonadotropin release (Dunaif, 1997). Adrenal androgen production is also frequently increased in PCOS (Rosenfield, 1999). This finding might reflect a common defect in ovarian and adrenal androgen biosynthesis because adrenocorticotropin hormone (ACTH) release is not increased.

Metabolic Features

Insulin resistance is a prominent feature of PCOS, independent of obesity (Dunaif, 1992). Many but not all women with PCOS are insulin resistant. Obesity and PCOS have an additive deleterious effect on insulin sensitivity. The molecular mechanisms of this defect differ from those in other common insulin resistant conditions, such as Type 2 Diabetes mellitus and obesity, suggesting that PCOS-related insulin resistance has an individual genetic aetiology (Dunaif, 1997). Studies of PCOS adipocytes suggest that there is a post-binding defect in insulin receptor-mediated signal transduction, and this observation has recently been confirmed in skeletal muscle, the major site of insulin-mediated glucose uptake (Dunaif et al., 2001). Studies of insulin receptors isolated from PCOS-cultured skin fibroblasts suggested that the signalling defect results from a decrease in insulin receptor tyrosine kinase activity, secondary to a constitutive increase in receptor serine phosphorylation (Dunaif, 1997). The signalling defect produces selective insulin resistance, affecting metabolic but not mitogenic actions of insulin (Bock et al., 1999). Insulin acts through its cognate receptor, in synergy with LH, to stimulate theca cell steroidogenesis in PCOS (Nestler et al., 1998). Hence, it is possible that the selective insulin resistance of PCOS accounts for the continued actions of insulin on steroidogenesis, in spite of defects in insulin-mediated glucose metabolism. Insulin also contributes to increased adrenal androgen secretion in PCOS, in part by enhancing adrenal sensitivity to ACTH (Moghetti et al., 1996).

Ek and colleagues (2002) have identified fat depot-specific abnormalities in the regulation of lipolysis in PCOS. Isolated subcutaneous abdominal adipocytes are resistant to catecholamine-induced lipolysis in women with PCOS. The opposite phenomenon, markedly enhanced sensitivity, is observed in their visceral adipocytes (Large et al., 1998). The increase in visceral fat lipolysis could lead to an increase in free fatty acids (FFA) release that subsequently contributes to hepatic glucose production (Montague et al., 2000). Accordingly, enhanced visceral fat lipolysis could be

one mechanism for the increased risk for glucose intolerance in PCOS (Bergman, 1997).

1.3. Polycystic Ovaries

The classical ultrasound features of PCOS, which have been previously described (Adams et al., 1985) include an enlarged ovary with the presence of 10 or more cysts, 2-8 mm in diameter, arranged either peripherally around a dense core of stroma or scattered throughout an increased amount of stroma. However, up to 23% of normal women meet the sonographic criteria for polycystic ovaries. On the other hand, many investigators report that ovaries from women with PCOS may be normal (Timor-Tritsch et al., 1998) The presence of polycystic ovaries was not included in the definition of PCOS.

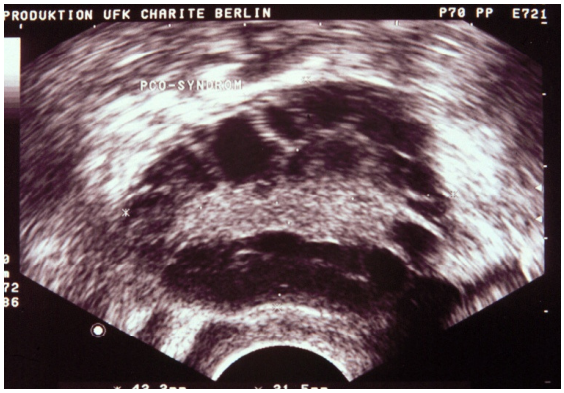

Figure I: Transvaginal ultrasound of the polycystic ovary

1.4. Pathogenesis Of PCOS

A series of investigations have emphasized a heterogeneous nature of PCOS with different combinations of features present in individual patients. The mechanisms involved in pathogenesis of this disorder remain up to now unclear and are multifactorial. There are several factors that contribute to the hyperandrogenemia and anovulation in this condition.

A primary Neuroendocrine Defect Leading To Exaggerated LH Pulse Frequency And Amplitude

A persistent finding in a majority of women with PCOS is abnormal gonadotropin secretion, particularly, elevated levels of LH. It was hypothesized that enhanced LH stimulation of the ovaries results in excess androgen secretion. This hypothesis was supported by studies using GnRH agonists which decreased serum LH, testosterone and androstendione, whereas DHEA-S and other adrenal androgens were unchained (Steingold et al., 1987). However, recent data from human and animal models suggests that the rapid GnRH pulse frequency is not a primary hypothalamic abnormality appreciating the effect of abnormal plasma levels of estrogen, insulin, or androgen (Poretsky et al., 1994; Dunaif et al., 1996; Dumesic et al., 1997).

A Defect Of Androgen Synthesis That Results In Enhanced Ovarian Androgen Production

PCOS theca cells show increased activity of multiple steroidogenic enzymes such as 17-hydroxylase and 17,20-lyase, resulting in raised androgen production, both basally and in response to LH (Franks et al., 1999; Nelson et al., 2001). These abnormalities are presented in both theca cells in women with polycystic ovaries and chronic anovulation or women with regular ovulatory cycles and polycystic ovaries (Franks et al., 1999).

Recent human and animal studies provided several mechanisms by which a primary defect resulting in hyperandrogenemia could cause PCOS. First, the decreased sensitivity of the GnRH pulse generator appears to be a consequence of raised circulating androgen levels, because androgen receptor blockade with flutamid abolishes this defect in PCOS (Eagleson et al., 2000). Second, studies on rhesus monkeys suggest that prenatal androgen exposure produces many features characteristic of the PCOS phenotype, such as increased LH secretion, ovarian hyperandrogenism, central obesity and defective insulin secretion (Eisner et al., 2000). Third, permanent alterations in LH secretion were demonstrated in women who were exposed to excess androgens during *in utero* development, such as women with congenital adrenal hyperplasia or with neonatal androgen-secreting neoplasm (Rosenfield et al., 1999). Fourth, Dörner et al., (1998) observed an approximately

fourfold increased prevalence of PCO in women born since 1955 in East Germany following the massive application of insecticide DDT. The DDT – metabolite o, p'-DDD is a strong inhibitor of 3β-hydxysteroid dehydrogenase, and DDT may induce 17,20 lyase activity, implying a possible connection between cases of PCOS in women born after 1955 and prenatal DDT exposure.

A Unique Defect In Insulin Action And Secretion

Peripheral insulin resistance (and associated hyperinsulinemia) plays a significant role for the pathogenesis of PCOS (Dunaif, 1997), and may be the primary abnormality in the aetiology of hormonal derangement. Insulin can directly stimulate testosterone synthesis in human theca cells (Nestler et al., 1998) and also contributes to increased adrenal secretion, in part by enhancing adrenal sensitivity to ACTH (Moghetti et al., 1996). Insulin decreases sex hormone-binding globulin production by the liver, subsequently increasing free serum testosterone (Nestler et al., 1991).

On the other hand, if insulin resistance and hyperinsulinemia have an important pathogenic role in PCOS, why are not all patients with hyperinsulinemia also hyperandrogenic, like many women with type 2 DM? Furthermore, how do ovaries appear to be insulin-responsive in an insulin-resistant state?

Conn et al., (2000) showed that although 82 % of women with type 2 DM had polycystic ovaries in ultrasound, only 52 % had hyperandrogenism and / or menstrual disturbances, suggesting that hyperinsulinemia alone is not sufficient for expression of this syndrome.

In another study in the group of Asian women, Rodin et al., (1998) reported that the effects of type 2 DM and polycystic ovaries on insulin sensitivity were independent, suggesting that these changes in insulin sensitivity involve different mechanisms. It is possible that the insulin resistance and the reproductive disturbances reflect separate genetic defects and that insulin resistance unmasks the syndrome in genetically susceptible women.

1.5. Long-Term Disease Risks

The abnormal hormonal milieu characteristics of PCOS may predispose to several conditions, which include type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease and some malignancies.

Type 2 Diabetes Mellitus

The association between hyperinsulinemia and hyperandrogenism was first described in 1980 by Burghen et al. and lead to the assumption that PCOS has associated metabolic risks. A recent analysis of glucose tolerance among a larger group of 249 women with PCOS aged 14 to 44 years indicated a 31% prevalence of impaired glucose tolerance and a 7% prevalence of type 2 diabetes mellitus, rates significantly higher than those seen in among normally cycling controls (Legro et al., 1999)

Cardiovascular Disease

Women with PCOS have higher cardiovascular risk than weight-matched controls with normal ovarian function (Wild, 2002) due to elevated androgen levels, body fat distribution and insulin resistance. A number of studies have shown that women with PCOS exhibit an abnormal lipoprotein profile characterized by raised concentrations of plasma triglycerids, marginally elevated low-density lipoprotein (LDL) cholesterol, and reduced high-density lipoprotein (HDL) cholesterol (Dejager et al., 2001: Pirwany et al., 2001). Furthermore, an increased hepatic lipase activity has been documented. A recent prospective study has linked menstrual irregularity, about 80% of which is due to PCOS, to increased risk of fatal coronary heart disease (Solomon et al., 2002).

Cancer

The prevalence of endometrial cancer (EM) appears to be increased in young women with PCOS (Niwa et al., 2000; Wild et al., 2000). Pierpont and co-workers (1998) in the retrospective study of 786 PCOS women and 1060 weight-matched control women in United Kingdom showed that women with PCOS were not at significantly increased risk of mortality or morbidity from breast cancer but were at increased risk of endometrial

cancer (Odds ratio 5.3). One possible mechanism is the associated high and unopposed level of estrogen; estrogen stimulation leads to endrometrial hyperplasia and subsequently to adenocarcinoma. Another theory suggests that hyperandrogenemia and hyperinsulinemia may increase the potential for neoplastic change in the endometrium through their effects on concentrations of SHBG, IGF-I and circulating estrogens (Meirow and Schenker, 1996).

The results concerning an association between PCOS and ovarian cancer are conflicting. One group (Coulam et al., 1983) showed no risk of ovarian carcinoma among anovulatory women, while others (Schildkraut et al.,1996) suggested a relative risk of 2.5 (95% CI 1.1–5.9) in the case-control study. In the large UK study the standardized mortality for ovarian cancer was 0.39 (95% CI 0.01-2.17).

1.6. Management of PCOS

Women with PCOS seek medical help in order to reduce a hair growth and/or acne, to restore a menstrual cyclicity and infertility. In addition, these patients are increasingly seeking treatment for the metabolic abnormalities such as insulin resistance and obesity. A “problem-oriented” approach to treatment of PCOS is the following:

Oral Contraceptives

Oral contraceptives are useful in patients with PCOS who do not desire pregnancy. Besides establishing regular menstrual cycle, the combined estrogen/progestin oral contraceptive pill inhibits endometrial proliferation and reduces ovarian androgen proliferation (Burkman RT, 1995). Hirsutism and acne respond well to oral contraceptive use. It is important to choose the appropriate oral contraceptive. Newer progestins such as desogestrel, as well as norgestimate and ethynodiol diacetate, have minimal androgenic potential and are considered to be superior to preparations, containing norgestrel or norethindrone, which have higher portal activity.

Glucocorticoids

At least fifty percent or more of women with PCOS have a significant adrenal component to their hyperandrogenism, as evidenced by elevated concentrations of DHEAS. Such women generally benefit from the use of glucocorticoid preparation such as dexamethasone (0.5 mg/d) and prednisolone (5 mg/d). Low doses of these drugs provide a good suppression of adrenal androgen secretion without significant cortisol suppression.

Clomiphene Citrate

The antiestrogen clomiphene citrate (CC) remains the first-line medical therapy for ovulation induction in women with PCOS. The standard regimen is 50 mg/day for 5 days beginning on cycle day 5 following spontaneous or progestine-induced bleeding. The dose can be increased (to a maximum dose of 250 mg/day) in subsequent cycles if serum progesterone in the luteal phase is less than 10 ng/ml. Clomiphene citrate induces ovulation in approximately 70% to 85% of patients, although only 33% to 45% conceive (Nasseri, 2001).

An improvement of the outcome in clomiphene citrate cycles is possible due to adjunct use of hCG. The dose of 5000 or 10000 U (intramuscularly) hCG triggers ovulation and results in LH surge in cases in which no spontaneous LH surge is detected.

Gonadotropins

The 15% of PCOS patients who fail clomiphene therapy are treated with gonadotropins.

The gonadotropin treatment of women with PCOS is relatively problematic because of high rates of multiple gestations and the occurrence of ovarian hyperstimulation syndrome. These problems can be avoided by use of low-dose, “step-up” regimens designed to result in the development of a single dominant follicle. The treatment starts at the dose of 37.5 U of FSH daily on day 3 after spontaneous or progestine–induced menses. The dose is increased every 7 days and an ovulatory hCG trigger is given when the lead follicle reaches a mean diameter of 18 mm. The “step-down” protocol involves higher starting FSH doses, followed by dose reduction when the leading follicle exceeds a mean diameter of 10 mm.

Ovarian Drilling

For patients who fail to respond to the use of injectable gonadotropin treatment, laparoscopic ovarian drilling is appropriate. The technique usually involves the laparoscopic cauterization of the ovarian surface (LCOS). A recent study of 1124 patients found spontaneous ovulation in 77% and pregnancy in 49% of women (Campo, 1998). In contrast to gonadotropins, the spontaneous abortion rate after a laparoscopic ovulation induction is only about 15% and multiple pregnancies are uncommon (2.5%). Even patients who fail to ovulate spontaneously after drilling may benefit due to improved response to gonadotropin treatment.

Given the importance of hyperinsulinemia in the pathogenesis of PCOS, it has been hypothesized that insulin-sensitizing agents may be useful in the restoration of normal endocrinological and metabolic parameters.

The most extensively studied insulin-sensitizing drug in the treatment of PCOS is metformin.

Metformin

Metformin is thought to have primary effects on peripheral glucose uptake in response to insulin, with some reduction in basal hepatic glucose production (Mehnert, 2001). It also lowers adipose-tissue lipolysis and improves insulin sensitivity in muscle (Witters, 2001). Its mechanism of action is not defined but recent findings suggest a unifying role of AMP-activated protein kinase in all the mechanisms of metformin action (Zhou et al., 2002).The drug does not provoke hyperinsulinemia and therefore does not cause hypoglycaemia. It is now recommended as first-line therapy in overweight patients with diabetes by most leading clinical associations. It is also inexpensive.

Non-Randomised Studies With Metformin

In PCOS the first pioneer study with metformin was conducted 1994 by Velasquez and co-workers. Most of following trials had cohort designs and showed an improvement in insulin metabolism and a reduction in circulating androgen concentrations (Crave et al.,

1995; Ehrmann et al., 1997; Morin-Papunen et al., 1998; Glueck et al., 1999). In most cases, small reductions were seen in body-mass index, waist/hip ratio, or both, and improvements in menstrual cyclicity (presumed ovulation) were also found. Only one of these trials (Crave et al., 1995) examined the effect of metformin on hirsutism, and there was no reported evidence on acne. In general, the results were encouraging, but all trials involved small numbers of patients, and most were of short duration and limited in design by not having a control or placebo group.

Concerning a menstrual cycle, the range of benefits in uncontrolled studies is wide. The normal menstrual frequency was achieved in 16% (four of 24 cases) (Crave et al., 1995) and in more than 90% (39 of 43 cases) (Glueck et al., 1999) in women with PCOS.

Results From Controlled Studies

There have been seven published studies on metformin that included some form of randomisation (control group with or without placebo), five of them were double-blind in design (Nestler et al., 1998; Moghetti et al., 2000; Morin-Papunen et al., 2000; Pasquali et al., 2000; Ng et al., 2001; Fleming et al., 2002; Kocak et al., 2002). The most consistent findings, were a decrease in body-mass index of around 4% and in androgen measures of around 20%, compared with placebo. The data on improvements in insulin concentrations and, in particular, SHBG are less convincing when considered together with placebo data. These observations show the potential for confounding effects during any prospective studies and re-emphasise the importance of control in study design.

For ovulation, the most important observations were that the interval from start of treatment to first ovulation was significantly shorter with metformin than with placebo, that menstrual or ovulation cyclicity was increased with metformin, and that these improvements were variable and modest.

Taken together, studies with metformin have controversial results concerning metabolic changes, androgen levels, ovulatory function and pregnancy. There were many variations in the patients examined and the methods of assessments used. Several points such as metformin dose and its relation to body mass remains unclear.

1.7. Aim Of The Trial

Therefore, the present randomised, double blind, placebo-controlled study was designed to compare the clinical, endocrine and metabolic effects of two treatment models for PCOS:

1) Metformin and lifestyle modification, including behavioural group therapy with aspects in nutrition and physical activity, and individual counselling by dietician.

2) Placebo and lifestyle modification, including behavioural group therapy with aspects in nutrition and physical activity, and individual counselling by dietician.

Changes in endocrinological and metabolic parameters such as FSH, LH, prolactin, estradiol, testosterone, free testosterone, DHEA-S and SHBG, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, insulin, glucose, HbA1c, leptin and IGF-I should be assessed before and after therapy. Modification in insulin and glucose levels should be evaluated by means of AUC (area under the curve).

The second purpose of this trial was to reveal an influence of metformin on the menstrual cycles and spontaneous pregnancy.

Forty six caucasian women with PCOS aged 21 to 39 were selected in the reproductive medicine department at the University Clinic Charité, Berlin, Germany.

The patients were divided into two groups. The first group received metformin and the second received a placebo during the sixteen weeks of the trial.

All laboratory investigations: FSH, LH, prolactin, progesterone, estradiol, testosterone, free testosterone, DHEA-S and SHBG, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, HbA1c, leptin and oral glucose tolerance test were performed every 8 weeks.

Body composition was studied by the bioelectrical impedance method at week 0, 8 and 16. Based on Resistance ® , Reactance (Xc) and the phase angle alpha measured at 5, 50 and 100 kHz body compartments (total body water, fat-free mass, body cell mass and fat mass) were calculated.

This study was approved by the Charité ethic commission, Berlin, Germany .

2. SUBJECTS AND METHODS

2.1. Patients

Forty six infertile women with PCOS, aged between 22 to 39 years, were recruited in the department of reproductive medicine at the University Clinic Charité, Berlin, Germany.

Criteria of PCOS were as follows:

1. Oligomenorrhea (less than four cycles in the last 6 months) or amenorrhoea (no menses in the last 4 months),
2. Hyperandrogenemia defined by the free androgen index (FAI) greater then 5.6
3. Presence of polycistic ovaries according to the ultrasound criteria of Adams and co-workers,1986.
4. Hirsutism (Ferriman-Gallwey Score > 6) (Ferriman & Gallwey, 1961)
5. LH/FSH ratio >2

Women with two or more criteria were considered as having PCOS.

All women had normal prolactin levels. The women were in good general health, euthyroid, and none had been taking hormonal medication or oral contraceptives during the three months before the initial investigation, nor during the study. None of the subjects had renal or liver dysfunction. The latter two criteria were determined to prevent risk of lactic acidosis due to metformin use.

Impaired glucose tolerance was not the obligate criteria for PCOS, therefore oGTT was not included in the screening programme.

Other exclusionary criteria comprised significant cardiovascular disease, bulimia nervosa, active cancer, intake of drugs affecting a body weight like diuretics, participation in dietary programs for weight reduction, and participation in another investigational study within the past 30 days.

Table 1 reflects the main characteristics of the patients.

Table 1: Clinical characteristics of PCOS women recruited in the trial

№	Age	BMI	WHR	Hirsutism score	Acne	Menstrual cycle	PCO in US	Involuntary Childlessness (years)
1	24	46.4	0.86	3	yes	Regular	yes	2
2	23	33.5	0,72	10	yes	Oligomenorrhea	yes	2
3	24	38.6	0.69	2	yes	Oligomenorrhea	yes	1
4	32	38.1	0.88	14	yes	Oligomenorrhea	yes	5
5	36	31.3	0.82	8	yes	Oligomenorrhea	yes	1
6	32	42.4	0.77	11	yes	Regular	yes	2
7	30	40.0	0.8	9	yes	Regular	no	2
8	39	39.2	0.8	12	yes	Regular	yes	7
9	35	45.7	0.78	10	yes	Oligomenorrhea	yes	3
10	29	42.0	0.87	9	yes	Oligomenorrhea	yes	7
11	31	45.0	0.83	12	yes	Regular	yes	7
12	36	46.0	0.82	11	no	Regular	yes	5
13	30	43.6	0.9	9	yes	Oligomenorrhea	yes	3
14	32	39.5	0.77	4	no	Oligomenorrhea	yes	3
15	32	49.9	0.78	2	no	Oligomenorrhea	yes	10
16	32	37.0	0.98	1	no	Oligomenorrhea	yes	4
17	33	44.0	0.95	8	yes	Oligomenorrhea	yes	4
18	30	38.8	0.78	4	yes	Amenorrhoea	yes	6
19	28	48.0	0.85	11	no	Oligomenorrhea	yes	7

№	Age	BMI	WHR	Hirsutism score	Acne	Menstrual cycle	PCO in US	Involuntary Childlessness (years)
20	23	37.0	0.85	10	no	Oligomenorrhea	yes	2
21	37	39.3	0.89	2	no	Oligomenorrhea	yes	15
22	29	41.3	0.8	12	yes	Regular	yes	4
23	24	46.2	0.88	23	yes	Amenorrhoea	yes	4
24	36	36.6	0.84	12	yes	Oligomenorrhea	yes	3
25	28	36.9	0.78	13	yes	Amenorrhoea	yes	3
26	27	33.5	0.84	10	yes	Amenorrhoea	yes	3
27	31	32.7	0.89	8	yes	Regular	yes	4
28	31	39.0	0.9	6	yes	Oligomenorrhea	yes	6
29	28	41.0	0.84	9	yes	Oligomenorrhea	yes	6
30	29	41.0	0.98	1	no	Oligomenorrhea	yes	6
31	21	44.1	0.8	3	no	Oligomenorrhea	yes	2
32	32	35.9	0.8	1	no	Regular	yes	3
33	24	42.7	0.8	12	no	Amenorrhoea	yes	4
34	28	42.1	0.9	10	yes	Oligomenorrhea	yes	4
35	36	30.2	0.82	10	yes	Regular	yes	7
36	28	36.3	0.89	8	yes	Oligomenorrhea	yes	1
37	30	37.6	0.81	3	no	Oligomenorrhea	yes	1
38	31	43.0	0.92	16	no	Amenorrhoea	yes	10
39	32	44.9	0.82	9	yes	Amenorrhoea	yes	5

№	Age	BMI	WHR	Hirsutism score	Acne	Menstrual cycle	PCO in US	Involuntary Childlessness (years)
40	27	31.0	0.88	2	yes	Oligomenorrhea	yes	1
41	28	48.6	0.84	2	no	Regular	yes	5
42	28	32.6	0.81	2	no	Oligomenorrhea	yes	1
43	29	37.7	0.89	3	no	Amenorrhoea	yes	1
44	37	36.8	0.86	9	no	Oligomenorrhea	yes	1
45	26	37.4	0.9	11	yes	Amenorrhoea	yes	5
46	33	45.0	0.8	9	no	Amenorrhoea	yes	10

The mean age of patients was 29.9 ± 4.2 years. All subjects were overweight (mean BMI 38.1; range 28.1 – 49.0 kg/m²) and the large majority of women (87.5%) had abdominal fat distribution defined by waist-to-hip ratio (WHR) greater than 0.8.

11 (23.9%) patients revealed regular menstrual cyclicity, 25 (54.3%) patients had less than four cycles in the last 6 months and 10 (21.7%) women had chronic amenorrhoea.

Polycistic ovaries were demonstrated by transvaginal ultrasound in all volunteers although its presence is not required for diagnosis of the polycystic ovary syndrome (Franks, 1995).

The study protocol was approved by the ethic commission of the University Clinic Charité, Berlin, Germany and written informed consent was obtained from each woman before study.

Metformin was helpfully granted by Berlin-Chemie, Germany.

2.2. Study Design

Therapy with metformin was initiated in a randomised double-blind trial of 1500 mg daily. Each woman took one 500 mg metformin tablet or an identical placebo tablet orally each morning, afternoon and evening for sixteen weeks. Patients were advised that they may have minor gastrointestinal side effects. These could be diarrhea, abdominal discomfort, anorexia, nausea, and rarely, a metallic taste in the mouth (Dandona et al., 1983).

The trial included five monthly visits. Participants came to the Division of Reproductive Medicine between 7.00 and 10.00 a.m. after a 12-hour overnight fast. During each visit (at week 0, 4, 8, 12 and 16) all the women underwent the following investigations:

- ultrasound scanning of the ovaries,
- recording of menstrual pattern,
- anthropometric and blood pressure measurements,
- recording of side-effects of metformin (if available).

Group therapy with aspects in nutrition and physical activity was conducted monthly. Each woman received individual counselling by a dietician.

At the first, third and fifth visits (at week 0, 8 ,16) blood for endocrine and metabolic measurements was obtained. Venous blood for the endocrine investigations was obtained before the start of the metabolic studies. A 75-g oral glucose was administered, and venous blood was obtained for glucose and insulin determination, basally, at 60 and 120 min through the catheter. The response of glucose and insulin to the oGTT was analysed by calculating the AUC (area under the curve).

Pill bottles with 90 metformin or placebo tablets for the next month were given at each visit.

As a monitor of general drug safety, a complete blood count with differential hepatic and renal characteristics was performed at baseline and after trial.

The study was conducted from September 2002 to July 2003 in the Department for Reproductive Endocrinology, University Hospital Charité, Berlin.

Limitation of the Study

All blood samples were obtained without regarding the day of menstrual cycle.

2.3. Methods

Anthropometric Measurements

BMI was calculated as weight (kg) divided by height (m) squared. Waist measurements were made with a soft tape midway between the lowest rib margin and the iliac crest in the standing position. The hip circumstance was measured over the widest part of the gluteal region.

Blood Pressure Measurements

Blood pressure was measured by a mercury sphygmomanometer in the sitting position, after a rest of at least 10 minutes.

Ultrasound Examination

Ultrasound assessments were conducted before the treatment, and at the first, fourth, eighth, twelfth and sixteenth week. Transvaginal ultrasonography (Logic TM 700,Probe 7.5 MHz, GE Medical Systems, USA) was performed to evaluate the ovaries, number of follicles and endometrium.

The ovaries were defined as “polycistic” when they were enlarged (diameter over 30 mm) with more than 8 small cysts, which are typically arranged peripherally around an increased echogenic stroma (Adams et al, 1986).

Endocrine Investigations

A determination of FSH, LH, DHEAS, SHBG, testosterone, estradiol, progesterone and metabolic parameters was done in the central laboratory of Charité Hospital.

A determination of insulin, free testosterone, leptin and IGF-I levels has been accomplished in the laboratory of Institute for Experimental Endocrinology, Charité Hospital.

Follicle–stimulating hormone (**FSH**), luteinizing hormone (**LSH**) and **prolactin** were measured by means of immunoassay sandwich method using direct chemiluminescence–technology (Firm Chiron Diagnostics ACS, USA). Results were expressed in IU/ml and µg/l (prolactin). Intraassay and interassay variations varied from 0.3% to 2.7% and 2.2% to 2.9% for FSH; 1.5% to 2.9% and 2.3% to 3.0% for LH; 2,7% to 3,3% and 1,4% to 4,7% for prolactin.

Testosterone (**T**) and dehydroepiandrosterone-sulfate (**DHEAS**) were determined by the competitive immunoassay method. ADVIA Centauer Testosterone-Assay (USA) was used for testosterone determination. IMMULITE Analyzer (USA) was used for DHEAS determination. Intraassay coefficients of variation were between 2.3% and 6.2%, for T, 6.8% and 9.5% for DHEAS. Interassay variations were between 1.4% and 4.7% for T , 10.8% and 16.6% for DHEAS.

Levels of sex hormone-binding globulin (**SHBG**) in serum were determined by the Immunometric Assay method (IMMULITE Analyzer, USA). Results were expressed in nmol/L. Intraassay and interassay variations varied from 4.1% to 7.7% and 5.5% to 8.9%, respectively.

Estradiol levels were determined by the competitive immunoassay method using direct chemiluminescence–technology (Chiron Diagnostics ACS, USA). Results were expressed in p/ml. Intraassay and interassay variations varied from 4.5% to 8.1% and from 6.3% to 12.1%, respectively.

Progesterone was determined in serum by the competitive immunoassay method using direct chemiluminescence–technology (Firm: ADVIA Centauer, USA). Intraassay and interassay variations varied from 1.9% to 5.7% and 3.7% to 12.7%, respectively.

Thyroid hormones were determined by the competitive immunoassay method using direct chemiluminescence–technology (Firm Chiron Diagnostics ACS, USA). Intraassay and interassay coefficients of variation were between 2.41%-2.48% and 2.05%-5.31% for TSH; 1.81%-2.57 and 1.48%-3.48% for fT3.

IGF-I was determined by the radioimmunassay with double antibodies technique (Adaltis, Italy) with intraassay coefficients of variation 2.9%-5.9% and interassay coefficients from 4.3% to 7.1%, respectively.

Serum **leptin** concentrations were determined by the human-Leptin–radioimmunassay (Mediagnost, Tübingen) with intraassay variation less than 5% and interassay variation less than 7.6%.

Free testosterone was determined by the RIA kits by firm Diagnostic Systems Laboratories GmbH (Sinsheim). Intraassay variations were between 3.7% to 6.2%, interassay variations were between 7.3% to 9.7%. The detection limit of the assay came to 0.18 pg/ml.

Metabolic Parameters

Oral Glucose Tolerance Test

Patients were asked to follow 300 g carbohydrate preparatory diet for 3 days before the test. An oral glucose tolerance test (oGGT) was performed in the morning after a 12-hours fast.

For determination of glucose and insulin levels blood samples were collected basally and after 60 and 120 min. A glycemic response to the OGTT was defined according to the criteria of the American Diabetes Association, 1997.

Table 2: Criteria for the diagnosis of diabetes mellitus

	Normal	Impaired glucose tolerance	Diabetes mellitus
Fasting glucose (mg/dl)	< 110	110-125	>125
120 min post 75g glucose load (mg/dl)	<140	140-200	>200

Levels of **glucose** were determined by the enzymatic test by Roche Company, Germany. Intraassay and interassay variations were 0.8% to 1.0% and 1.7% to 1.9%, respectively.

Insulin levels were determined by the radioimmunassay by Adaltis Italia S.p.A (Bologna, Italy). Intraassay variations were between 4.5% and 7.4%, interassay variations were between 4.2% and 8.0%.

Insulin resistance

The evaluation of insulin resistance in PCOS patients was assessed with Avignon insulin sensitivity index (SiM) (Avignon et al., 1999). Values of sensitivity index less than one characterize insulin resistance. Values of sensitivity index greater than one are supposed to be normal.

Cholesterol, triglycerids, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) were determined by the enzymatic colorimetric test by Firm Roche, Germany. Results were expressed in mg/dl for cholesterol, triglycerids, creatinine and in U/l for ALT, AST and GGT. Intraassay variations laid between 0.7% to 1% for cholesterol, 0.9% to 1.5% for triglycerids, 0.7%-0.9% for creatinine, 0.7%-2.9% for ALT, 1.1%-2.1% for AST and 0.3%-1.5% for GGT.

HbA1c was determined by the VARIANT analysis system using high performance liquid technology. Intraassay variations laid between 0.94% to 1.53%, interassay variations laid between 1.3% to 1.8%.

2.4. Free Androgen Index

The androgen status was assessed by calculating the ratio of the total testosterone (TT) concentration to the concentration (or binding capacity) of SHBG. It is typically calculated on a molar/molar basis and rescaled by a factor ten, or one hundred or one thousand (Wheeler MJ., 1995).

In this study the free androgen index was calculated as follows: (TT in nmol/L / SHBG in nmol/L) X 100.

2.5. Questionnaires

Patients answered questions relating to personal and family history, gynaecologic history like age of menarche, menstrual activity, contraception, miscarriages, extrauterine pregnancies, operations; weight reduction, smoking and sport activities. They were additionally asked to keep a nutritional protocol for seven days to estimate the nutritional status. Both questionnaires are attached to this paper.

2.6. Dietary Composition

Before treatment, all patients kept a nutrition diary for four days. The nutrition status of each patient was analysed by Nutrition software Diät 2000. Group therapy focussing on

nutrition and physical activity started at the first visit and was conducted monthly. After treatment, patients were asked to keep a second nutrition diary and nutrition status was further analysed.

Lifestyle recommendations for PCOS women included a low fat, high carbohydrates intake for breakfast and high protein intake in the evening. Increased consumption of fibre, wholegrain bread, fruit and vegetables was also recommended. It is suggested that such diets aid in increased weight loss owing to the increased satiating power of protein (Mikkelsen, 2002), and improve insulin sensitivity through maintenance of lean body mass.

2.7. Metformin Treatment

Metformin (Siofor®, Berlin-Chemie, Berlin, Germany) is a white crystalline compound with a molecular formula of C4H11N5▪HCl and a molecular weight of 165.63. Siofor tablets contain 500 mg or 850 mg of metformin hydrochloride. Each tablet contains the inactive ingredients povidone and magnesium stearate. The absolute bioavailability is approximately 50-60%. Following oral administration, 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours (Physicians' Desk Reference, 2000).

Metformin inhibits hepatic glucose production, decreases intestinal absorption and promotes glucose uptake and utilization by peripheral tissues at the post-receptor level. It increases the number of insulin receptors but not insulin concentrations and therefore does not cause hypoglycaemia in normoglycemic patients.

Common side effects include gastrointestinal symptoms such as nausea, vomiting, flatulence and diarrhea. Metformin cannot be used in patients with renal impairment (serum creatinine level > 1.4 mg/dL), congestive heart failure, or liver dysfunction.

The use of metformin increases the concentrations of lactic acid through induction of conversion of glucose to lactate by the intestinal wall. There is no evidence to date that metformin therapy is associated with an increased risk of lactic acidosis or with increased levels of lactate compared with other antihyperglycemic treatments if the drugs are prescribed under study conditions, taking into account contraindications

(Salpeter et al., 2003). The doses of metformin used for the treatment of type 2 diabetes mellitus are 1-2 x 500-850 mg/d (Herold et al., 2004).

2.8. Randomization

Randomization was conducted in a double blind fashion; patients received either placebo or metformin according to the code provided by the pharmacy department of the University Clinic Charité, Berlin, Germany. The randomization code was not broken until the last patient completed all observations.

2.9. Statistical Analysis

The integrated glucose (area under the curve glucose [AUCg]) and insulin (AUCi) responses during the OGTT were determined using the trapezoidal rule. Normal distribution of continuous variables were tested by the Kolmogorov-Smirnov test. Both groups were compared by the paired Wilcoxon nonparametric sign rank test. Correlation analysis was performed between variables using Spearman correlation coefficient. All statistical evaluations were performed by the SPSS (Statistical Package for the Social Sciences), Version 11.0. Differences were considered to be significant with P values less than 0.05, and data are reported as the mean ± SD.

Graphics are presented as box plots. The *heavy bars* represent the median values; the *lower and upper limits* of the boxes represent the interquartile range (25^{th} and 75^{th} percentiles); the *I bars* indicate the extreme values.

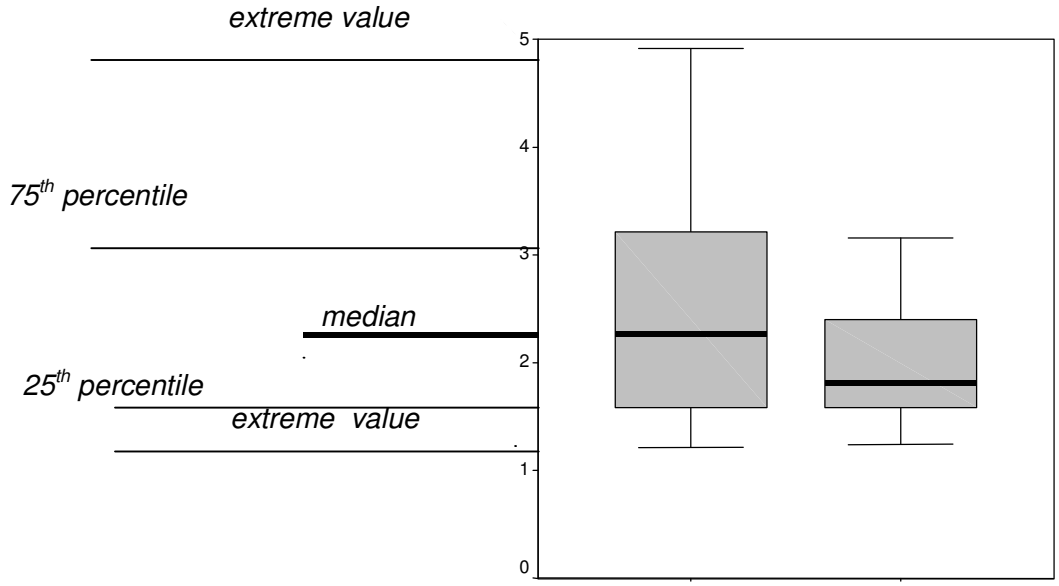

Figure II: Box plot

3. RESULTS

3.1. Recruitment And Pretreatment Assessments

A total of 46 women were recruited for the study and underwent the randomization, receiving metformin or placebo. Infertility was the reason for presentation for all patients in the both groups. Table 2 shows that the metformin and placebo groups were matched for age, BMI, testosterone, SHBG, fasting glucose and insulin, haemoglobin A1c, and circulating lipid fractions before treatment. According to Ferriman-Gallwey criteria for hirsutism,13 patients from placebo-treated group and 14 patients from metformin-treated group were hirsute. All women displayed a classical picture of PCOS on vaginal ultrasound scan.

Table 3: Characteristics of the patients randomized to receive placebo or metformin

Parameter	Placebo	Metformin
	mean ± SD	mean ± SD
Age (yr)	31.5 ± 5.3	31.7 ± 3.2
BMI (kg/m²)	40.1 ± 5.4	40.3 ± 4.4
Waist/hip ratio	0.84 ± 0.06	0.85 ± 0.07
LH/FSH	1.8 ± 0.7	1.7 ± 0.8
Total T (ng/ml)	2.7 ± 0.6	2.3 ± 0.4
free T (pmol/L)	12.0 ± 8.5	10.7 ± 5.3
SHBG (nmol/L)	29.8 ± 12.7	26.3 ± 10.2
Fasting insulin (µIU/L)	18.5 ± 10.4	21.4 ± 10.1
Fasting glucose (mg/dl)	76.7 ± 7.4	78.1 ± 14.9
Leptin (ng/ml)	41.2 ± 13.1	38.9 ± 12.8
Cholesterol (mg/dl)	218.9 ± 39,6	192.7 ± 40.4
Triglycerides (mg/dl)	153.4 ± 70.3	149.8 ± 64.1
HbA1c (%)	5.5 ± 0.4	5.6 ± 1.5

3.2. Treatment Compliance

There was no difference in the dropout rates between the placebo group (n=3) and metformin-treated group (n=3).

All metformin-treated patients experienced mild transient diarrhoea, nausea and headache and one woman has dropped out of the trial due to intolerable gastrointestinal side effects associated with metformin. One patient in the placebo group was excluded from the study because of noncompliance. Another five volunteers did not complete the study for personal reasons. Figure III shows the progress of these patients through the study.

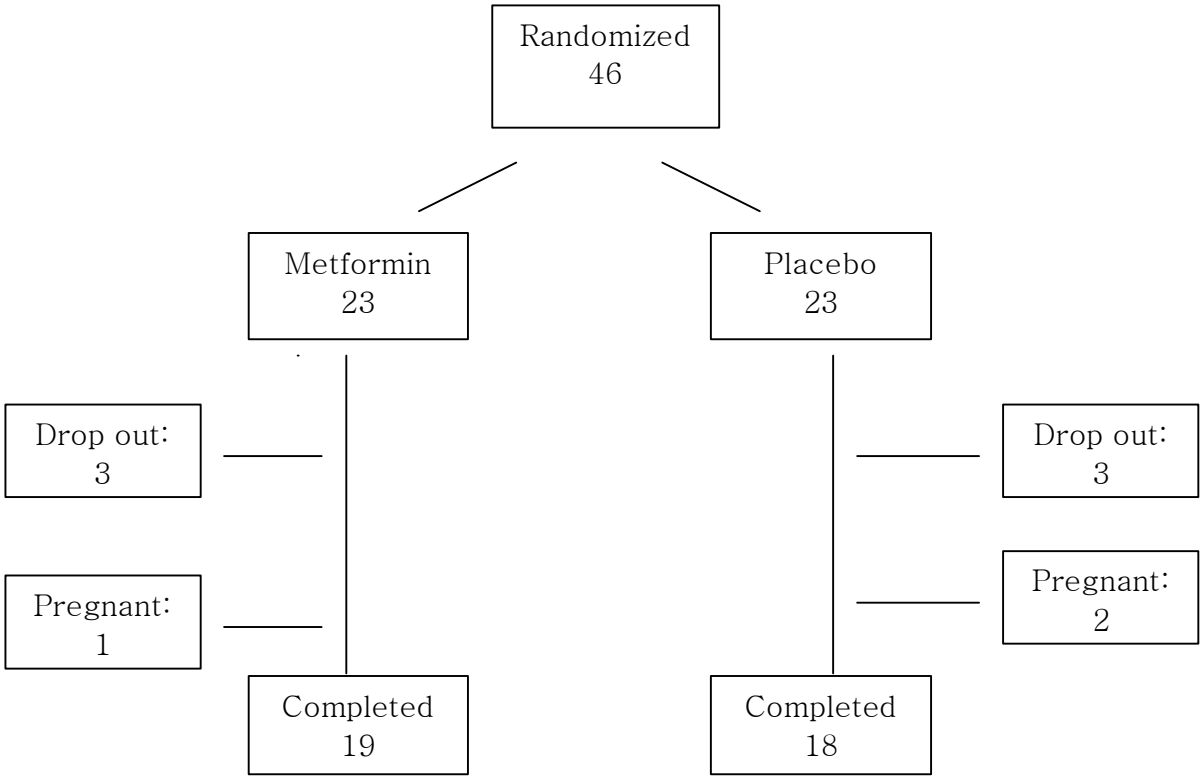

Figure III: Chart of patients progressing through the trial of placebo or metformin treatment.

3.3. Conception During Treatment

One patient in the metformin-treated group became pregnant in the fourteenth week of the treatment and gave a birth in due time. Two spontaneous pregnancies occurred in the placebo-treated group in the twelfth week of the study. One of the pregnancies in the placebo-taking group ended in sponataneous abortion after 7 weeks of gestation.

Pregnant patients didn't complete the study. Their data were not included into statistical analysis. The statistical analysis was conducted with the data of 19 patients in the metformin-treated group and the data of 18 patients in the placebo-treated group who completed the study (see Figure III).

3.4. Anthropometric Assessments

Body Mass Index

There was a reduction in the mean **BMI** in both groups although only in the metformin group body mass index and weight decreased significantly (-5.6 kg (metformin, P<0.001) vs. –2.2 kg (placebo, p NS)). The baseline BMI in the drug-treated group decreased from 40.6 ± 4.2 (kg/m²) to 38.9 ± 4.6kg/m² after 16 weeks. The pretreatment BMI in placebo-taking group was 39.5 ± 5.9 kg/m² and didn't change after treatment (38.9 ± 5.6 kg/m²).

Table 4: BMI measurements before and after treatment

	Placebo			**Metformin**		
		n = 18			n = 19	
Parameter	Pretreatment	16 wk	P	Pretreatment	16 wk	P
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
BMI	39.5 ± 5.9	38.9 ± 5.6	NS	40.6 ± 4.2	38.9 ± 4.6	**0.001**

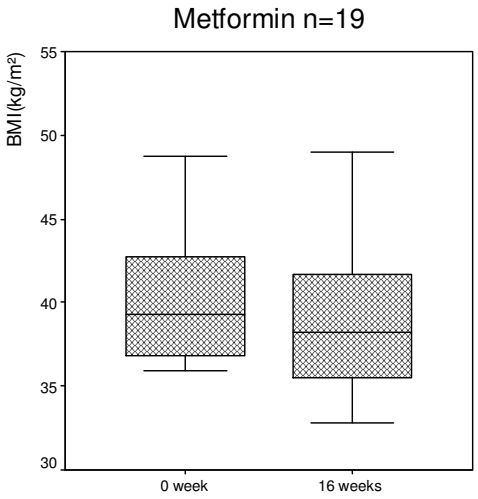

Figure IV: Box plot of BMI in women with PCOS before and after metformin treatment.

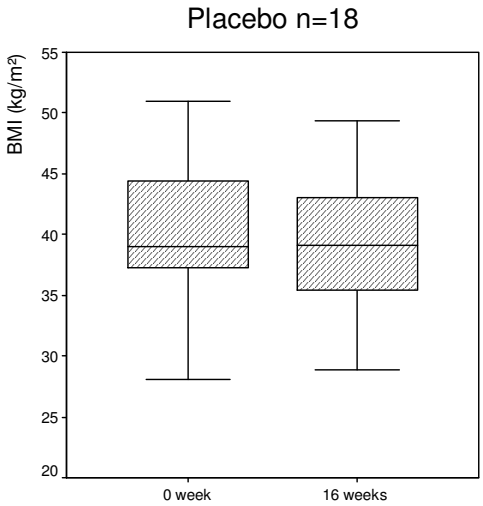

Figure V: Box plot of BMI in women with PCOS before and after placebo treatment.

Waist And Hip Circumferences

Table 5: Waist and hip measurements before and after metformin or placebo treatment

	Placebo			Metformin		
		n=18			n=19	
Parameter	Pretreatment	16 wk	P	Pretreatment	16 wk	P
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
Waist/hip ratio	0,84 ± 0,07	0,84 ± 0,06	NS	0,86 ± 0,06	0,86 ± 0,06	NS
Waist (cm)	108,7 ± 9,0	107,5 ± 10,1	NS	111,4 ± 9,0	108,2 ± 10,1	**0,005**
Hip (cm)	129,4 ± 12,1	127,8 ± 11,7	**0,02**	129,5 ± 9,5	125,4 ± 9,8	**0,0001**

After 16 weeks of metformin or placebo treatment, waist circumstances decreased considerably in the metformin–treated group (111.4 ± 9.0 vs. 108.2 ± 10.1, $p<0.005$). At the same time, hip circumstances changed significantly in both groups although a more considerable decrease was demonstrated in women treated with metformin (Table 5).

In spite of significant reduction of body mass index and both waist and hip circumferences in the metformin-treated patients, no changes in the pattern of body fat distribution were seen. The waist-to-hip ratio remained constant in the metformin-treated subjects (0.86 ± 0.06 (pretreatment) vs. 0.86 ± 0.06 (posttreatment) ($P=0.53$)).

3.5. Endocrine Assessments

Sex Hormones And SHBG

Table 6: Sex hormones and SHBG blood concentrations in PCOS women before and after placebo or metformin treatment

	Placebo			Metformin		
		n=18			n=19	
Parameter	Pretreatment	16 wk	P	Pretreatment	16 wk	P
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
LH/FSH	1.8 ± 0.9	1.78 ± 0.9	NS	1.6 ± 0.8	1.77 ± 0.8	NS
Prolaktin (ng/ml)	8.3 ± 2.8	10.4 ± 5.3	NS	9.9 ± 5.1	8.9 ± 4.4	NS
total testosterone (ng/ml)	2.6 ± 0.7	2.5 ± 1.1	NS	2.1 ± 0.9	2.0 ± 0.6	NS
free testosterone (pmol/L)	11.8 ± 8.7	8.8 ± 3.9	NS	10.3 ± 5.7	9.3 ± 5.6	NS
SHBG (nmol/L)	30.4 ± 12.8	33.6 ± 20.6	NS	26.4 ± 10.1	34.0 ± 16.7	0.04
DHEA-S (µmol/l)	6.0 ± 2.9	5.0 ± 2.3	**0,04**	6.5 ± 3.0	7.2 ± 2.9	NS
FAI	10.0 ± 4.9	11.1 ± 8.8	NS	8.3 ± 3.5	7.4 ± 4.2	NS

Total and Free Testosterone

The baseline total and free testosterone concentrations were higher in PCOS women taking placebo compared with those taking metformin: 2.6 ± 0.7 vs. 2.1 ± 0.9 for testosterone (in ng/ml) and 11.8 ± 8.7 vs. 10.3 ± 5.7 for free testosterone (in pmol/L). There was no significant reduction in androgen levels in both groups. Figure VI shows the minimal changes in free testosterone concentrations during the trial.

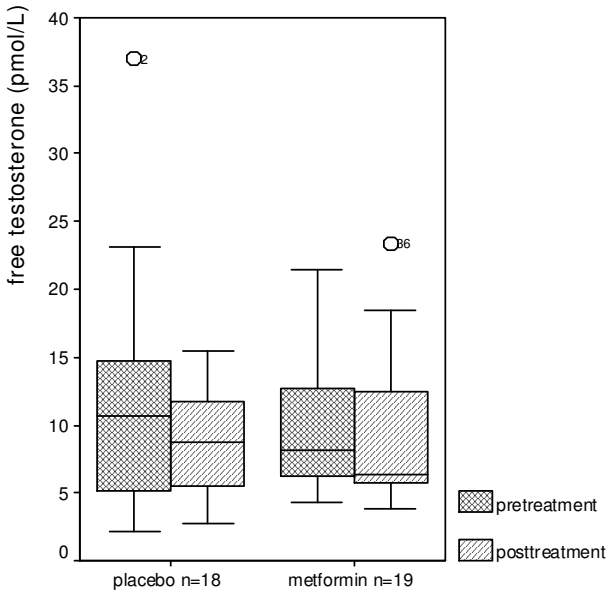

Figure VI: Box plot of free testosterone levels in women with PCOS before and after metformin or placebo treatment.

LH/FSH Ratio

Table 6 demonstrates that neither metformin nor placebo treatment significantly modified the LH/FSH ratio, FAI and prolactin parameters.

The LH/FSH ratio remained similar after treatment in the control group: 1.8 ± 0.9 vs. 1.78 ± 0.9 (P=0.81) and increased from 1.6 ± 0.8 to 1.77 ± 0.8 (P=0.28) in the metformin-taking group. The patients with random numbers 18 and 41 in the drug-treated group had higher LH/FSH ratios (4.0 for №18 ; 4.4 for №41) and are marked in the box plot as extreme values.

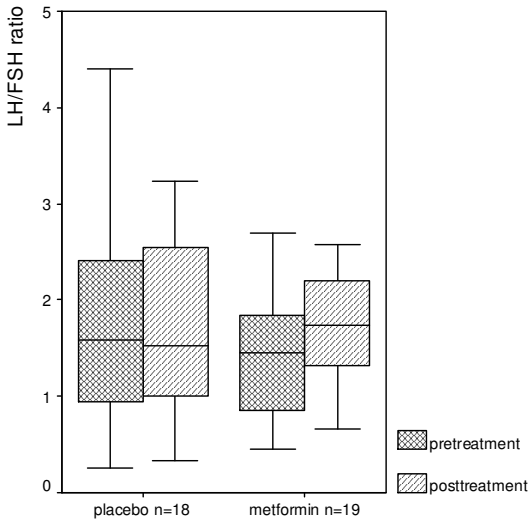

Figure VII: Box plot of LH/FSH ratio in women with PCOS before and after metformin or placebo treatment.

Free Androgen Index (FAI)

The free androgen index increased from 10.0 ± 4.9 before treatment to 11.1 ± 8.8 after 16 weeks, (P=NS) in placebo-taking women. There were no changes of free androgen index in the metformin treated group (8.3 ± 3.5 to 7.4 ± 4.2, (P=NS)).

Sex Hormone-Binding Globulin (SHBG)

Placebo treatment did not substantially modify SHBG concentrations in the controls (30,4 ± 12,8 (nmol/L) before study and 33,6 ± 20,6 (nmol/L) after study, P=NS). SHBG levels significantly increased in the metformin-treated group (26,4 ± 10,1 (nmol/L) before study and 34,0 ± 16,7 (nmol/L) after study, P=0,04).

Before treatment, PCOS women taking metformin seem to have slightly lower SHBG levels than women in the placebo-taking group. However, this difference was not significant (26.4 ± 10.1 vs. 30.4 ± 12.8 nmol/L, P=NS).

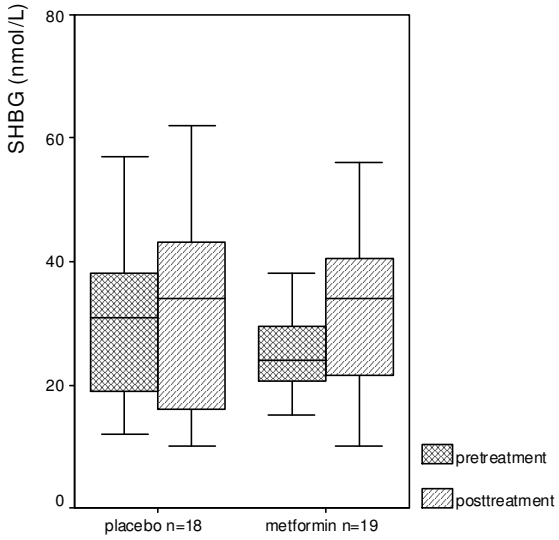

Figure VIII: SHBG plasma concentrations in women with PCOS before and after metformin or placebo treatment.

Glycemic Parameters

Table 7: Levels of glucose, insulin, Hb1Ac, leptin and IGF-I levels in PCOS women before and after placebo or metformin treatment.

	Placebo			Metformin		
	n=18			n=19		
Parameter	Pretreatment	16 wk	P	Pretreatment	16 wk	P
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
Fasting insulin (µIU/mL)	18.3 ± 10.8	26.9 ± 13.8	**0.006**	21.6 ± 10.0	26.2 ± 12.1	**0.04**
Fasting glucose (mg/dl)	76.8 ± 7.5	79.2 ± 8.8	NS	78.0 ± 15.3	77.11 ± 11.0	NS
120 min glucose (mg/dl)	109.5 ± 6.8	111.6 ± 5.0	NS	124.2 ± 9.3	119.8 ± 8.4	NS
120 min insulin (µIU/mL)	153.3 ± 26.8	112.8 ± 5.1	NS	138.9 ± 19.9	94.1 ± 14.1	**0.01**
AUC, insulin (µIU/mL·h)	261.2 ± 50.3	236.6 ± 31.1	NS	262.1 ± 34.0	211.4 ± 20.1	**0.03**
AUC, glucose (mmol/L·h)	12.8 ± 0.8	12.9 ± 0.6	NS	14.2 ± 0.7	14.8 ± 0.7	NS
SiM index	0.86 ± 0.8	0.8 ± 0.63	NS	0.64 ± 0.5	0.8 ± 0.53	NS
Leptin (ng/ml)	41.4 ± 13.3	43.8 ± 17.0	NS	38.5 ± 13.1	39.6 ± 20.8	NS
IGF-I (ng/ml)	165.6 ± 53.0	175.5 ± 47.1	NS	160.6 ± 84.5	149.1 ± 46.4	NS

Glucose And Insulin

Before treatment, fasting plasma glucose and insulin concentrations were not significantly different between two groups, however there was a tendency to higher fasting insulin levels in the metformin group.

At baseline, 2 hours post-75-g glucose load values were increased, but not significantly (P=0.2), in the metformin-treated women. Five patients in the metformin-treated group fulfilled ADA criteria for impaired glucose tolerance vs. two patients who took placebo.

After treatment, fasting plasma and 2 hours post-75-g glucose load glucose levels did not change substantially in either group. However, two patients with impaired glucose tolerance (one from the control, other from the metformin group) reverted to normal glucose tolerance.

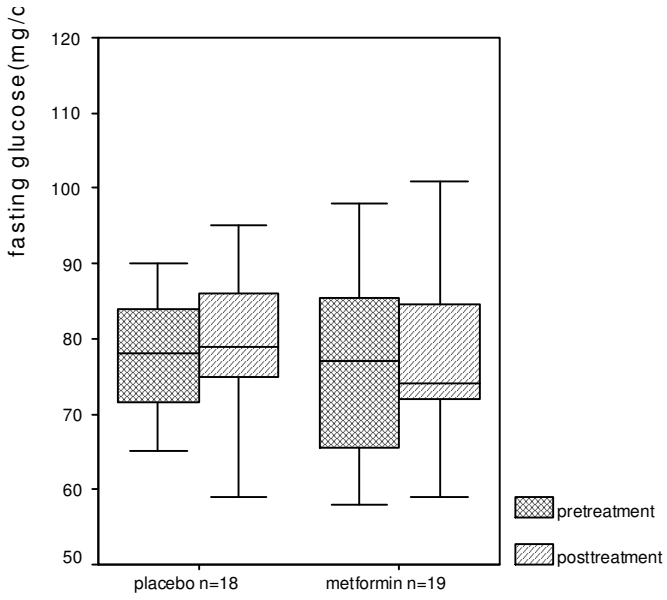

Figure IX: Fasting glucose levels in women with PCOS before and after metformin or placebo treatment.

It is interesting that fasting insulin levels increased significantly both in placebo- and metformin-treated patients. The elevation in the placebo-treated group was from 18.3 ± 10.8 (μIU/mL) to 26.9 ± 13.8 (μIU/mL), P=0.006. The elevation in the metformin-treated group was 21.6 ± 10.0 to 26.2 ± 12.1 (μIU/mL), P=0.04. The box plots demonstrate fasting insulin concentrations in placebo- and metformin-treated women with PCOS before and after treatment.

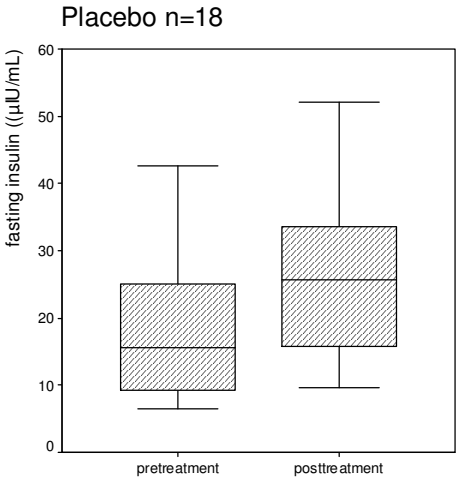

Figure X: Fasting insulin concentrations in women with PCOS before and after placebo treatment.

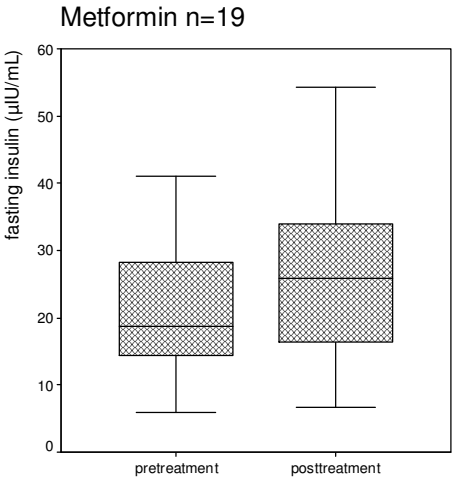

Figure XI: Fasting insulin concentrations in women with PCOS before and after metformin treatment.

2 hours post 75 g glucose load insulin concentrations declined significantly after 16 weeks of metformin treatment (138.9 ± 19.9 (µIU/mL) (pretreatment) vs. 94.1 ± 14.1 (µIU/mL) (posttreatment), P=0.01). The control group also revealed a decrease of 2 hours post 75 g glucose load insulin levels (153.3 ± 26.8 (µIU/mL) (pretreatment) vs. 112.8 ± 5.1 (µIU/mL) (posttreatment)) but P value did not achieve statistical significance (P=0.2).

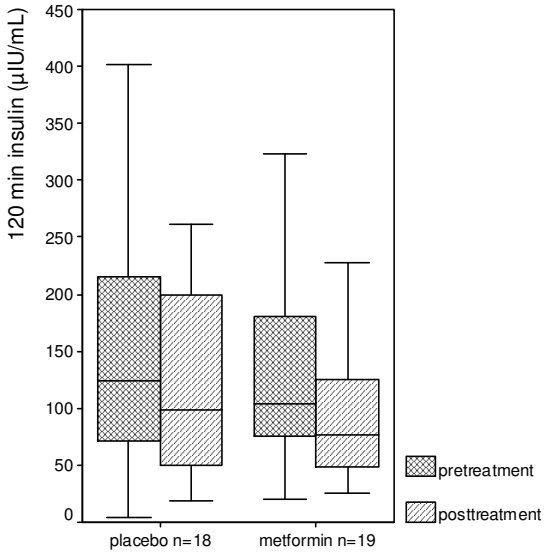

Figure XII: 2-hours postprandial insulin concentrations in women with PCOS before and after metformin or placebo treatment.

Area Under The Curve (Glucose And Insulin)

The integrated glucose response to the glucose load, AUC glucose, did not change in either group. AUC glucose was 12.8 ± 0.8 mmol/mL/h before treatment and 12.9 ± 0.6 mmol/mL/h after treatment, P=NS in the control group. In the metformin-treated group AUC glucose was 14.2 ± 0.7 mmol/mL/h before treatment and 14.8 ± 0.7 mmol/mL/h after treatment, P= NS.

Integrated insulin response, AUC insulin, decreased in the both groups. The decrease was from 262.1 ± 34.0 (µIU/mL·h) to 211.4 ± 20.1 (µIU/mL·h), P=0,03 for the drug-treated group. AUC insulin changed in the placebo-taking group 261.2 ± 50.3 (µIU/mL·h) vs. 236.6 ± 31.1(µIU/mL·h), but P value did not reach a significant rate.

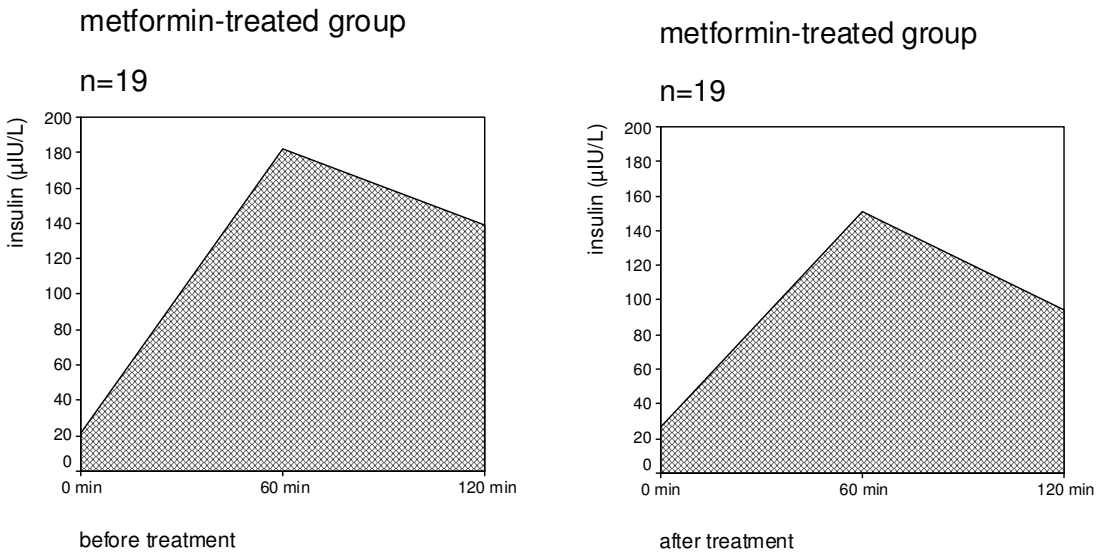

Figure XIII: Area Under The Curve insulin (AUC insulin) in metformin-treated women with PCOS before and after treatment.

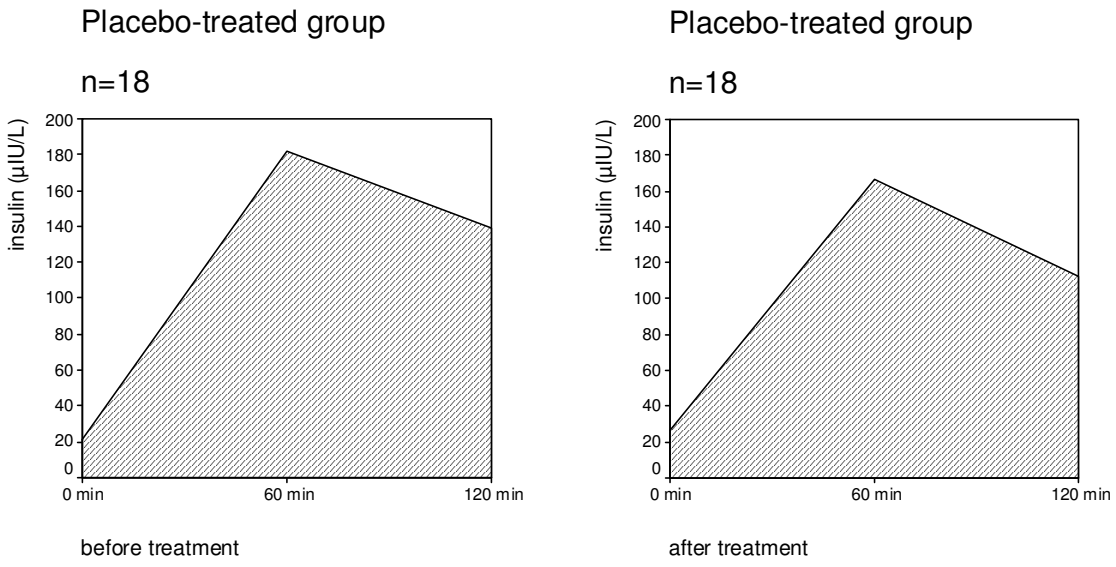

Figure XIV. Area Under The Curve insulin in placebo-treated women with PCOS before and after treatment

Leptin

There were no differences in baseline leptin concentrations between PCOS women and controls. In spite of significant weight reduction in the metformin-treated group, leptin levels did not decline. Moreover, a slight increase (38.5 ± 13.1 (pretreatment) vs. 39.6 ± 20.8 (posttreatment) ng/ml in the metformin and 41.4 ± 13.3 (pretreatment) vs. 43.8 ± 17.0 (posttreatment) ng/ml in the placebo-treated group) was shown in both groups, but this change was not significant.

IGF–I

IGF-I levels in the metformin-treated group demonstrated a tendency to decline (160.6 ± 84.5 (ng/ml) (pretreatment) vs. 149.1 ± 46.4 (ng/ml) (posttreatment), P= NS), difference came to –11,5 ng/ml. IGF-I levels demonstrated a tendency to augment in the control group (165.6 ± 53.0 (ng/ml) vs. 175.5 ± 47.1 (ng/ml), P=NS, difference was +9.9 ng/ml).

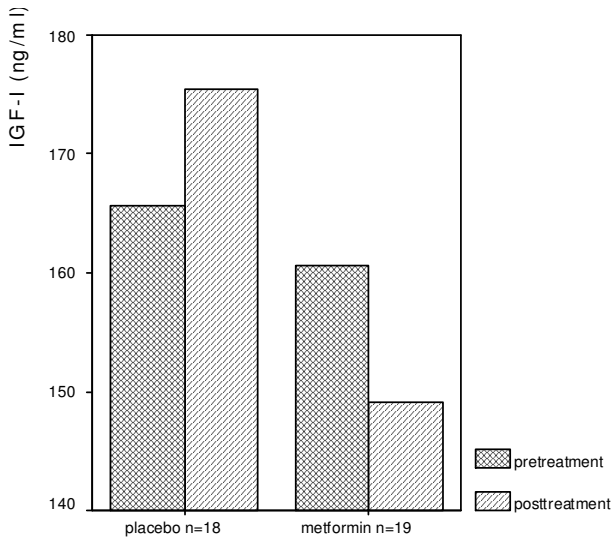

Figure XIV. IGF-I plasma concentrations in women with PCOS before and after metformin or placebo treatment

Cholesterol And Triglycerids

Table 8: Serum cholesterol and triglycerids concentrations in placebo- and metformin-treated women with PCOS before and after treatment.

	Placebo			Metformin		
		n=18			n=19	
Parameter	Pretreatment	16 wk	P	Pretreatment	16 wk	P
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
Total cholesterol (mg/dl)	216.7 ± 39.1	206.4 ± 32.7	NS	191.2 ± 40.1	196.8 ± 42.2	NS
HDL (mg/dl)	54.3 ± 14.2	52.6 ± 10.1	NS	47.3 ± 8.4	51.0 ± 10.1	**0.07**
LDL (mg/dl)	132.1± 28.6	123.4 ± 24.5	NS	114.2 ± 36.9	116.3 ± 38.5	NS
Triglycerides (mg/dl)	152.2 ± 71.1	159.4 ± 80.1	NS	149.0 ± 65.3	148.1 ± 58.2	NS

Table 8 shows that control women revealed higher baseline concentrations of total cholesterol compared to the metformin-treated group but this difference was not significant. Triglycerides, cholesterol and lipoprotein concentrations was in the upper bound of the normal range according to National Cholesterol Education Programme guidelines [total cholesterol ≥ 200 mg/dl; LDL-C ≥ 130 mg/dl; HDL-C <35 mg/dl;

TTG >200 mg/dl] in both groups. There was no significant reduction in these parameters after treatment. Total cholesterol levels didn't change in the metformin-treated group (191.2 ± 40.1 (pretreatment) vs. 196.8 ± 42.2 (posttreatment) mg/dl, PS=NS). Total cholesterol levels in the placebo-treated group were 216.7 ± 39.1 (pretreatment) vs. 206.4 ± 32.7 (posttreatment), P=NS.

At the same time, circulating HDL concentrations in the metformin-treated group showed a trend to rise (47.3 ± 8.4 (pretreatment) vs. 51.0 ± 10.1(posttreatment) mg/dl), however P value=0.07 did not achieve statistical significance.

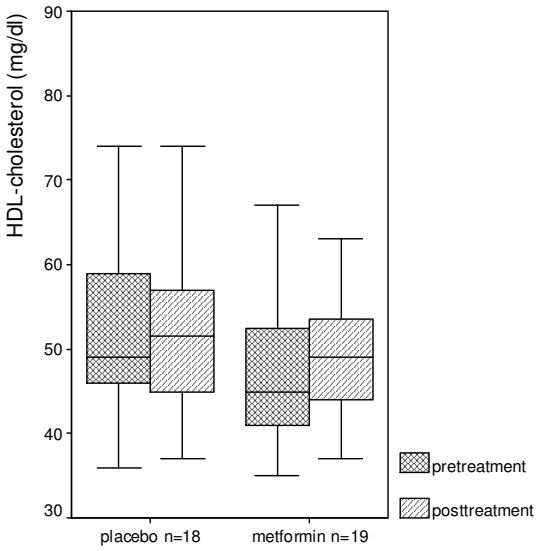

Figure XVI. HDL-cholesterol concentrations in women with PCOS before and after metformin or placebo treatment.

3.6. Menstrual Cycle

At the baseline, six (31.5 %) of the 19 women who took metformin had amenorrhea, eight (42.0 %) had oligomenorrhea and five (26.3%) patients had a regular menstrual cycle. In the placebo- treated group, four (22.2%) of the 18 women had amenorrhea, twelve (66.6%) women had chronic oligomenorrhea and two (11.1%) had a regular menstrual cycle before trial.

After metformin treatment, in two of six patients with amenorrhea, the cycle returned to normal, in one woman the cycle became oligomenorrheic and three patients remained amenorrheic. In three of eight oligomenorrheic women, the cycle became regular. There were 11 patients with a regular cycle after 16 weeks of metformin treatment.

Table 9: Menstrual cycle in metformin-treated women with PCOS before and after treatment, n=19

	0 week	16 weeks
Amenorrhea	6	3
Oligomenorrhea	8	5
Regular cycle	5	11

After placebo treatment, one of four amenorrheic patients became oligomenorrheic and three patients remained amenorrheic. In six of twelve oligomenorrheic women, the cycle turned normal. There were 9 patients with a regular cycle after 16 weeks of placebo treatment.

Table 10: Menstrual cycle in placebo-treated women with PCOS before and after treatment, n=18

	0 week	16 weeks
Amenorrhea	4	3
Oligomenorrhea	12	6
Regular cycle	2	9

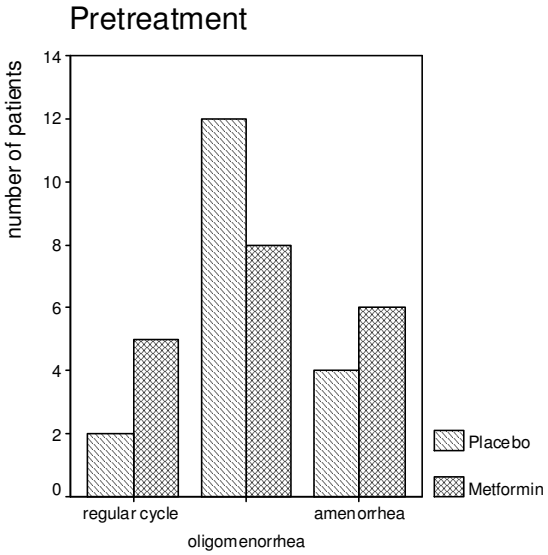

Figure XVII. Menstrual cycle in women with PCOS before metformin or placebo treatment.

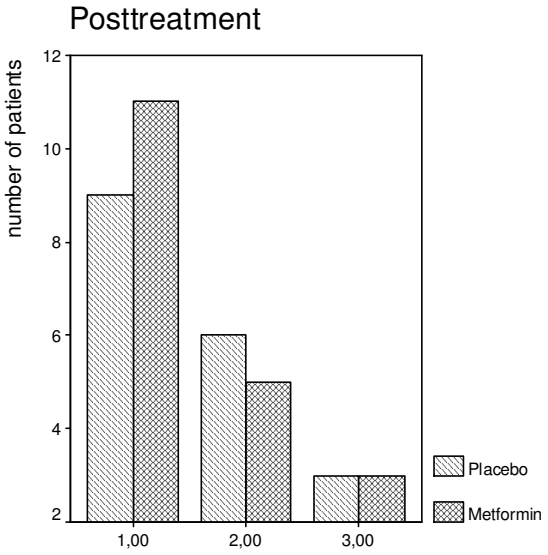

Figure XVIII. Menstrual cycle in women with PCOS after metformin or placebo treatment.

Table 11: Menstrual cycle in amenorrheic patients with PCOS treated with placebo or metformin.

	Metformin-treated group	***Placebo-treated group***
0 week	6 amenorrhea	4 amenorrhea
16 weeks	2 regular cycle	
	1 oligomenorrhea	1 oligomenorrhea
	3 amenorrhea	3 amenorrhea

Metformin-treated patients were divided into two groups according to menstrual changes: responders (n=7) and nonresponders (n=7). Baseline characteristics of responders and nonresponders were compared to identify any predictors of clinical response to metformin treatment. Women with regular cycle were excluded from analysis.

Table 12: Main baseline characteristics of metformin-treated patients divided into responders and nonresponders according to efficacy of treatment of menstrual disturbances

	RESPONDERS n=7	***NONRESPONDERS n=7***	P
BMI	41.5±4.2	38.4±3.1	NS
total testosterone (ng/ml)	1.9±0.6	2.5±1.1	NS
SHBG(nmol/L)	21.7±4.7	26.0±11.3	NS
FAI	8.9±3.2	10.0±3.4	NS
DHEAS(µmol/l)	8.6±3.1	4.4±1.3	**0.005**
fasting insulin(µIU/mL)	18.5±4.8	28.2±11.5	**0.06**

	RESPONDERS n=7	***NONRESPONDERS n=7***	
fasting glucose(mg/dl)	75.6±11.2	83.3±20.9	NS
AUC insulin (µIU/mL·h)	218,9±83.1	370.8±170.2	**0.05**
SIM index	0.58±0.23	0.41±0.27	NS

Analysis of baseline features showed that the responders had significantly lower insulin levels in response to oGTT (AUC insulin) and significantly higher DHEAS levels. Fasting insulin levels tended to be lower in responders. There was no difference in BMI, total testosterone and SHBG concentrations.

3.7. Subgroup Analysis

An additional aspect of this study was an analysis of the group treated with metformin. The patients were divided into two groups according to the body mass index. The commonly accepted definition of morbid obesity is a body mass index greater than 37 (Fleming et al., 2002). Responses were examined according to the BMI lying to either side of this value. The subgroup analysis was conducted to find out, first, if an initial body mass could be a predictor for successful treatment with metformin. Second, if hazards of morbid obesity have an influence on clinical and biochemical outcomes of metformin treatment.

Table 13 demonstrates the results of the study for the weight-matched groups.

Endocrine And Metabolic Parameters

Table 13: Endocrine and metabolic parameters in women with PCOS before and after metformin treatment.

Subgroup analysis

	Obesity BMI < 37			**Obesity BMI > 37**		
	n=7			n=12		
Parameter	Pretreatment	16 wk	P	Pretreatment	16 wk	P
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
BMI (kg/m²)	36.8 ± 0.8	34.9 ± 1.4	**0.01**	43.4 ± 3.3	41.9 ± 3.8	**0.01**
LH/FSH	1.57 ± 0.7	1.77 ± 0.4	NS	1.63 ± 1.3	1.76 ± 1.0	NS
Prolactin (ng/ml)	9.7 ± 4.3	9.2 ± 4.3	NS	10.0 ± 5.9	8.75 ± 4.6	NS
total testosterone (ng/ml)	2.1 ± 0.7	2.1 ± 0.6	NS	2.1 ± 1.1	2.0 ± 0.5	NS
free testosterone (pmol/L)	9.2 ± 4.8	10.6 ± 6.7	NS	11.1 ± 6.4	8.4 ± 4.7	NS
SHBG (nmol/L)	23.0 ± 6.8	30.4 ± 13.1	**0.05**	28.9 ± 11.5	36.5 ± 19.1	NS
FAI	9.9 ± 4.2	8.0 ± 3.9	NS	7.0 ± 2.3	7.0 ± 4.5	NS
Fasting insulin (µIU/L)	25.1 ± 10.6	24.3 ± 9.7	NS	19.1 ± 9.3	27.7 ± 13.9	**0.01**
Fasting glucose (mg/dl)	73.3 ± 13.7	72.9 ± 8.7	NS	81.3 ± 16.0	80.2 ± 11.9	NS
AUC, insulin (µIU/L·h)	344.0± 183.2	220.7± 102.2	**0.02**	202.5 ± 82.3	204.6 ± 80.3	NS
AUC, glucose(mmol/L·h)	13.9 ± 2.2	14.4 ± 2.4	NS	14.5 ± 4.1	15.2 ± 3.6	NS
SiM index	0.54 ± 0.38	0.76 ± 0.44	**0.02**	0.7 ± 0.56	0.82 ± 0.6	NS
Leptin (ng/ml)	28.6±9.4	26.8±13.1	NS	45.6 ± 10.5	49.0 ± 20.7	NS
IGF-I (ng/ml)	193.4±124.6	158.2 ±49.6	NS	136.8±22.1	142.4±45.1	NS

	Obesity BMI < 37			**Obesity BMI > 37**		
	n=7			n=12		
Parameter	Pretreatment	16 wk	P	Pretreatment	16 wk	P
	mean ± SD	mean ± SD		mean ± SD	mean ± SD	
Triglycerides (mg/dl)	172.6 ± 74.6	170.3± 70.7	NS	131.6 ± 54.7	132.0 ± 43.8	NS
HbA1c (%)	5.0 ± 2.06	5.5 ± 0.38	NS	6.0 ± 0.6	5.6 ± 0.2	**0.03**

There was no significant difference in pretreatment levels of total testosterone, SHBG and cholesterol among these groups. Leptin levels were significantly higher in more obese patients (45.6 ± 10.5 (women with BMI<37) vs. 28.6±9.4 (women with BMI >37)).

However, it is interesting that women with BMI<37 have higher AUC insulin levels and fasting insulin levels (P<0.04) than patients with BMI>37.

After 16 weeks of metformin treatment AUC insulin and SHBG concentrations changed significantly only in leaner PCOS women. SHBG concentrations increased from 23.0 ± 6.8 to 30.4 ± 13.1 nmol/L , P=0.05. Area Under the Curve (insulin) decreased from 344.0± 183.2 to 220.7± 102.2 µIU/L, P=0.02.

Fasting insulin levels increased significantly after 16 weeks of metformin treatment in more obese women with BMI>37 (19.1 ± 9.3 (pretreatment) vs. 27.7 ± 13.9 (posttreatment) µIU/L, P=0.01). Fasting insulin levels did not change in the metformin-treated patients with BMI<37.

Free androgen index (FAI) showed a tendency to decrease in the leaner subgroup (9.9 ± 4.2 (pretreatment) vs. 8.0 ± 3.9 (posttreatment)) but P value did not achieve statistical significance.

Menstrual Cycle

Before treatment, three of seven women with BMI<37 had amenorrhea, two had oligomenorrhea and two patients had a regular menstrual cycle. In the group of patients with BMI>37, three of twelve women had amenorrhea, six women had chronic oligomenorrhea and three patients had a regular menstrual cycle.

Table 14 demonstrates changes in menstrual history in patients with PCOS after metformin treatment.

Table 14: Menstrual cycle in women with PCOS before and after metformin treatment, subgroup analysis

	BMI<37 n=7		***BMI>37 n=12***	
	0 week	16 weeks	0 week	16 weeks
Amenorrhea	3	1	3	2
Oligomenorrhea	2	3	6	2
Regular cycle	2	3	3	8

3.8. Correlation Analysis

The correlation analysis was undertaken to reveal a relationship between several clinical and metabolic parameters, such as BMI, leptin, insulin, SHBG and lipids.

Leptin

Leptin level correlated strongly with Body Mass Index in women with PCOS (r=0.53, P<0.001). No significant correlation was found between serum leptin concentrations and insulin.

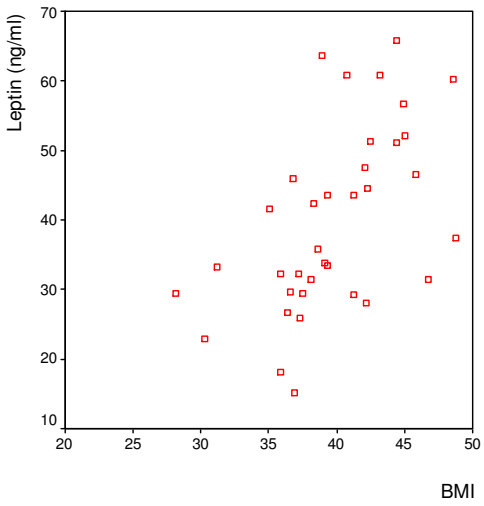

Figure XIX. Correlation between BMI and leptin concentrations in women with PCOS, r=0.53; n=46

Insulin

AUC insulin significantly correlated with free androgen index (r=0.38; P<0.01).

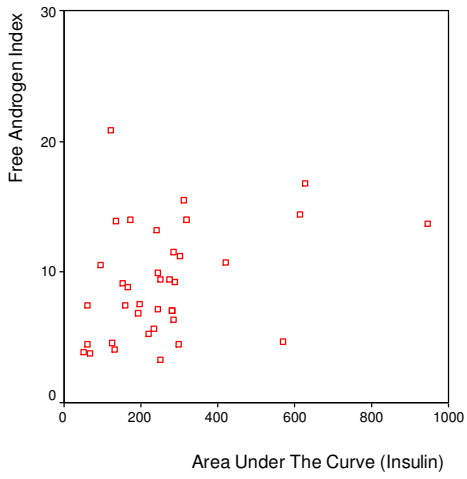

Figure XX. Correlation between Free Androgen Index (FAI) and AUC insulin, n=46

A significant inverse correlation between fasting insulin and HDL cholesterol levels was demonstrated (r=-0.37, P<0.02).

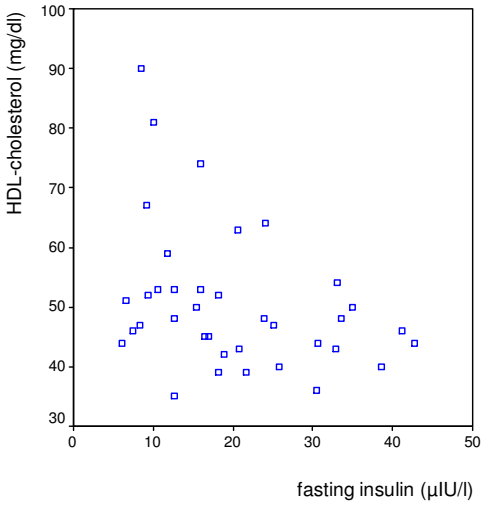

Figure XXI. Correlation between HDL total cholesterol and fasting insulin in women with PCOS, n=46

A significant positive correlation between 2-hours insulin and total cholesterol (r=0.5; P<0.002) was shown.

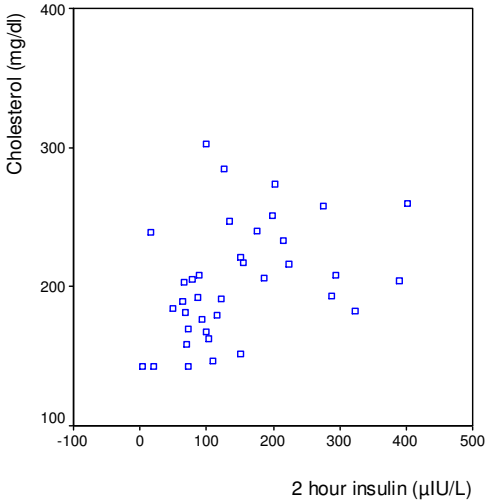

Figure XXII. Correlation between total cholesterol and 2 hour insulin in in women with PCOS, n=46

3.9. Menstrual Cycle

There was a strong correlation between menstrual disfunction and insulin resistance in the whole group. As one can see in the table 13, eight of ten (80%) patients with amenorrhea fulfilled criteria for insulin resistance (Insulin sensitivity index (SiM)<1). Eighteen of twenty (80%) oligomenorrheic women had insulin resistance as well. There were three of seven (42.8%) patients with regular cycle who fulfilled criteria for insulin resistance.

Table 15: Menstrual disfunction and insulin resistance.

	Amenorrhea n=10	***Oligomenorrhea n=20***	***Regular Cycle n=7***
	mean±SD	mean±SD	mean±SD
Insulin Sensitivity Index (SiM)	0.54±0.31	0.57±0.3	1.4±1.1
	8 of 10 (80%)	18 of 20 (80%)	3 of 7 (42.8%)

IGF-I

After 16 weeks of metformin treatment changes in IGF-I level correlated with changes in AUC insulin (r=0.56, P<0.01).

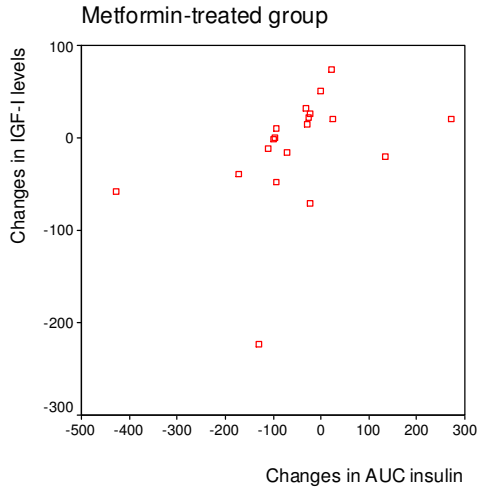

Figure XXIII. Correlation between changes in IGF-I levels and AUC insulin in the metformin-treated group, n=19

Changes in IGF-I level showed a tendency to correlate with changes in HDL-cholesterol concentrations (r=-0.59; P<0.008) in the metformin-treated group.

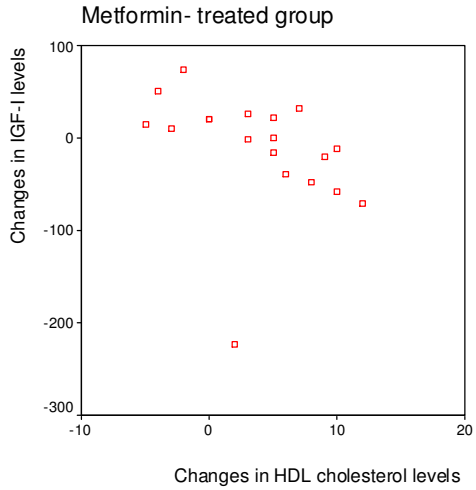

Figure XXIV. Correlation between changes in IGF-I and HDL cholesterol levels in the metformin-treated group, n=19

4. DISCUSSION

In this study on women with PCOS, treatment with metformin resulted in a significant decrease in body mass index, improvement of insulin action with a parallel increase of SHBG concentrations. This study contributed to a few randomized double blind placebo controlled trials and assessed metformin effects on metabolic and endocrine profiles in polycystic ovary syndrome.

4.1. Body Weight And Body Mass Index

In the metformin-treated group, mean body mass index averaged from 40.6 before the trial to 38.9 after trial. The absolute difference in these two values was 1.7, which came to a 4.2% reduction in the mean BMI after metformin therapy. Decrease of BMI in the placebo-taking group was not significant. These findings are consistent with results of almost all observational studies, where a decrease in the BMI ranged from 1% to approximately 4.3%.

Velasquez et al. (1994); Glueck et al. (1999); Kolodziejczyk et al. (2000) additionally found an improvement in menstrual cyclicity during metformin therapy. The metformin-induced weight loss was supposed to be the real cause of improvement rather than direct actions of metformin. It is also well documented in the study by Kiddy and co-workers (1992) that more than 5% decrease in body weight can mitigate reproductive abnormalities. However, the following four double-blind placebo control studies of Moghetti et al. (2000); Morin-Papunen et al. (1998); Kocak et al. (2002) and Fleming et al. (2002) did not find a significant decrease in BMI despite the amelioration in hyperinsulinemia and hyperandrogenism. Furthermore, in the study by Moghetti et al., the metformin group had a significantly lower body mass index than the placebo group, which could explain the nonsignificant result upon completion.

To answer the question if weight loss with metformin is better than weight loss alone, three trials comparing effects of low calorie diet and metformin therapy were conducted. In a randomized control trial (RCT) including 18 obese women with PCOS, all subjects were given a low calorie diet for 1 month before and during 6 months of the study (Pasquali et al., 2000). Ten were treated with metformin and 8 with placebo. In a further trial by Casimiri and colleagues (1997), 24 patients were given a low calorie diet with placebo or metformin for 26 weeks. In both studies, compared with placebo, metformin improved insulin sensitivity, lowered testosterone levels and improved ovulatory and menstrual functions. One placebo-controlled 16-weeks study was not able to demonstrate any additional effect of metformin over-dieting (Crave et al., 1995). The subjects were all hirsute and very obese (mean BMI 35,2 kg/m^2) and only 15 of the 24 women had polycystic ovaries on ultrasound. They may represent a particularly difficult group to treat successfully.

Lean PCOS subjects can experience similar hormonal and metabolic improvements during metformin treatment compared to obese women. Most of the beneficial changes were observed after 3 months of the treatment and at a dosage of 1 g/d. It was hypothesized that lower doses of metformin than those used in obese PCOS women could be sufficient in lean women (Ibanez et al. 2001; Morin-Papunen et al., 2003).

However, while metformin mainly affects central obesity and lipid metabolism, with minimal effects on hepatic clearance, its action seems to be focused mainly on hepatic clearance of insulin and steroid secretion in non-obese patients. This result suggests a particular characteristic of PCOS in non-obese women or the fact that obesity acts as a confounding factor of this disease (Morales et al.,1996).

In this study, significant weight loss was achieved only in the metformin-treated group, although all patients were advised to follow dietary restriction and to do regular physical exercises. Therefore, it was concluded, first, that metformin could contribute to weight loss, and, second, that weight loss can be significantly greater than observed in majority

of RCTs (where metformin was administrated without dietary restriction) if metformin is combined with a hypocaloric diet or/and regular physical activity.

4.2. Body Fat Distribution and Waist-Hip Ratio

The majority of PCOS women are obese or overweight, however, obesity is not the obligatory feature of this syndrome. Of special importance is the amount of body fat and especially the sex specific kind of fat distribution, which can be indicators of the hormonal and reproductive status of woman. Up to now few studies analysed fat distribution patterns in obese and lean PCOS patients (Bringer et al.,1993; Douchi et al., 1997; Lefebvre et al., 1997; Kirchengast et al., 2001). The authors were able to tended to an android (abdominal) fat pattern in PCOS women in comparison with weight-matched control women.

The present study found a significant decrease in waist and hip circumferences in PCOS patients after metformin treatment. These data are in agreement with the results of all other studies that examined metformin effects on obese PCOS women. Pasquali and co-workers measured, by CT scan, additional anthropometric parameters, such as total adipose tissue (TAT), visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Whereas obese PCOS subjects lost significantly more visceral fat in the abdomen without any significant changes of SAT after metformin, the opposite was observed in the control obese group. These findings, therefore, suggested particular effect of metformin on visceral adipose tissue in PCOS.

In this study, metformin-treated women lost significantly more abdominal fat (waist circumference) compared to controls. A loss of hip circumference was obvious in both groups, however, a waist-to-hip ratio remains constant after 16 weeks of metformin or placebo treatment.

Available data seem to support the concept that PCOS and abdominal obesity have a negative impact on insulin sensitivity (Dunaif A., 1997). The study by Pasquali and co-workers (1993) on normal-weight and obese women with and without PCOS and

different patterns of body fat distribution showed that hyperinsulinemia was more correlated with abdominal fat distribution, regardless of PCOS. In addition, Holte et al. (1995) indicated that hyperinsulinemia and insulin resistance might be significantly reversible through reduction or normalisation of abdominal fat depots. Therefore, it is hypothesized that abdominal (visceral) fatness may have a dominant role in determining these abnormalities in most women with PCOS, regardless of other factors, including genetic predisposition (Dunaif A., 1997).

4.3. Reproductive Hormones

Sex Steroids And SHBG

The absence of change in total testosterone concentrations was accompanied by a rise in SHBG levels in this study. This has also been reported by Velasquez and co-workers. The increase in SHBG was not unexpected, due to the experimental data supporting the role of insulin in the regulation of hepatic SHBG production (Nestler et al., 1991). Patients demonstrated a significant increase in SHBG concentrations parallel to the decrease in total insulin response.

A weak inverse correlation between SHBG, fasting insulin levels and insulin levels in response to oGTT was revealed. This correlation was previously shown in both healthy subjects and hyperinsulinemic disease situations such as PCOS and type 2 diabetes (Sharp et al., 1991; Katsuki et al., 1996 and Jayagopal et al., 2003). The strong association prompted suggestions that a low level of SHBG could be used as a marker to identify individuals with insulin resistance.

In this study it was postulated that metformin would alter ovarian steroid production in women with PCOS. This hypothesis is based on finding that metformin treatment ameliorates the hyperinsulinemia and hyperandrogenemia (Velasquez et al., 1997; Moghetti et al., 2000) and that insulin appears to modulate the 17-hydroxylase and

17,20-lyase activities of the ovarian steroid-forming P450c17 alpha (Ehrmann et al., 1995). These enzymes are characteristically abnormally regulated in women with ovarian androgen excess in PCOS.

Contrary to postulates and results reported by most of other studies, except two (Ehrmann et al., 1997; Fleming et al., 2002), no significant changes in serum total and free testosterone levels after treatment with metformin were found.

On the other hand, a recent study by Maciel et al. (2004), that compared effects of metformin in obese and non-obese patients with PCOS, presented similar results: whereas lean women showed significant decrease in serum total testosterone levels after 6 months' metformin therapy (1,5 g daily), obese patients demonstrated only an improvement in SHBG levels but not in serum total testosterone levels.

Hence, it could be suggested that the major effect of metformin in obese women on androgen secretion is mediated by changes in hyperinsulinemia and subsequently in SHBG rather than by direct inhibition of ovarian androgen production.

DHEAS

After 16 weeks of treatment, DHEAS concentrations significantly decreased in the placebo-treated group. DHEAS levels in the metformin-treated women showed a tendency to rise.

Nestler et al. (1994) demonstrated that metformin administration increased DHEAS levels by 80 % in non-obese women. Kolodziejczyk et al., (1999), Kazerooni et al., (2003) have reported the same results in obese women with normal DHEAS levels.

However, when effects of metformin were analysed separately in subgroups, a decrease in DHEAS levels was observed only in individuals with primarily elevated

DHEAS concentrations (>10.3 μmol/l). The authors supposed that hyperinsulinemia inhibits adrenal 17,20-lyase and thus decreases DHEAS production.

Nevertheless, the interactions between DHEAS and insulin are still poorly understood. A negative correlation between these hormones has been reported (Schriock et al., 1988); furthermore, acute infusion of insulin or an increase of insulin induced by the oral glucose tolerance test led to a decrease in DHEAS levels in healthy subjects (Diamond et al., 1991). However, a decline in DHEAS was not observed after insulin infusion in women with hyperandrogenism and hyperinsulinemia (Falcone et al., 1990).

In the present study, the decrease of insulin levels was not accompanied by significant change in DHEAS in the metformin-treated group. It was not possible to conduct a subgroup analysis according to the baseline DHEAS levels since there were only four patients with elevated DHEAS concentrations in the entire treated group. Based on results of previous studies, one can speculate that the adrenal response to declining levels of insulin may be dependent on the baseline adrenal function.

Adrenal function can be modulated by the IGF system. Receptors and binding proteins for IGF have been identified in adrenocortical tissues; furthermore, both IGF-I and IGF-II have been shown to stimulate DHEAS production through type I IGF receptors (Fottner et al., 1998). Thus, it was expected that metformin treatment would result in a decline in DHEAS along with IGF-I. However, DHEAS declined only in the placebo-taking group and this decrease was not associated with any changes in IGF-I levels. This finding underscores the complexity of interactions between adrenal steroidogenesis and the insulin-IGF system; further studies should include determinations of IGF-II and relevant binding proteins.

Gonadotropins

The present study was not able to demonstrate any changes in FSH and LH concentrations after metformin therapy. These results are consistent with most of previous studies (Ehrmann et al., 1997; Morin-Papunen et al., 2003). We have to

emphasize that the blood samples were obtained without regard to the menstrual cycle. The LH/FSH ratio was used to asses the hypothalamic function in our patients.

The prevalence of increased serum LH in PCOS ranges from 30% to 90% (Rebar et al., 1976; Franks, 1989). The reasons for these variations are unclear but probably include the heterogeneity of PCOS, a variable occurrence of associated obesity, different blood sampling frequencies, and the specificity of the gonadotropin assays.

Studies of rat pituitary cells in vitro suggested that hyperinsulinemia may potentiate both LH and FSH release to GnRH. However, these results have not been confirmed by all in vivo studies and only in two studies was a decrease in plasma insulin by the administration of metformin or troglitazone associated with a reduction in mean LH concentrations (Nestler et al., 1996; Dunaif, 1996). Additionally, obese women with PCOS have higher levels of plasma insulin, but the mean LH was unchanged or lower compared with the level in lean women (Morales et al., 1996).

Thus, the effects of insulin and consequently insulin sensitizers on gonadotropin secretion remain unclear and further research needs to be done.

4.4. Insulin And Glucose Metabolism

According to Dunaif et al. (1987), up to 20% of obese PCOS women have impaired glucose tolerance or frank type 2 DM. This is in agreement with the results of our study, where seven (18%) women had increased 2-hours glucose concentrations in response to oGTT and one patient showed increased fasting glucose levels at the beginning of the study.

We observed no changes in glucose levels (both fasting and after glucose load) and glycosylated haemoglobin A1c concentrations in the metformin- or in the placebo-treated group. These data are consistent with results obtained in most studies in PCOS (Ehrmann et al., 1997; Fleming et al., 2002).

Before the study, we found a highly significant correlation between AUC insulin and androgenicity measured by the free androgen index (FAI). Several previous studies have also demonstrated this positive correlation (Burghen et al., 1980; Lobo et al., 1983). Furthermore, the severity of hyperinsulinemia correlates with the degree of clinical expression of PCOS syndrome (Robinson et al., 1993).

Whether hyperandrogenism results from hyperinsulinemia, or vice versa, has been debated since this correlation was demonstrated. The experiments in which hyperandrogenemia was diminished by bilateral oophorectomy (Nagamani et al., 1986) or the administration of GnRH-agonist (Dunaif et al., 1990) did not demonstrate changes in the hyperinsulinemic stage in women with PCOS. Diamanti-Kandarakis et al., (1995) reported that antiandrogen therapy did not alter insulin sensitivity in PCOS. Studies in which insulin levels have been lowered by agents that either decrease insulin secretion such as diazoxide (Nestler et al., 1989) or somatostatin (Prelevic et al., 1990); or improve insulin sensitivity such as metformin (Velasquez et al., 1994) or troglitazone (Dunaif et al., 1996) indicated a decrease of androgen production in PCOS. In summary, these findings demonstrate that disordered insulin action precedes the increase in androgens.

Most of studies demonstrated reduced insulin sensitivity in both obese and non-obese women with PCOS syndrome (Dunaif et al., 1992; Morales et al., 1996).

The effects of metformin therapy on insulin sensitivity in PCOS remain controversial. Some investigators, assessing insulin sensitivity by way of the intravenous insulin tolerance test (Unluhizarci et al., 1999) or the euglycemic clamp technique (Diamanti-Kandarakis et al., 1998; Moghetti et al., 2000), have shown significant improvement of insulin sensitivity after metformin use. Other authors have failed to confirm this (Acbay et al., 1996; Ehramann et al., 1997; Crave et al., 1995).

The possible reason for this discrepancy is that there is no consensus for the most accurate measurement of insulin sensitivity. The hyperinsulinemic euglycemic clamp

technique is still regarded as the gold standard diagnostic test. Its routine use in the clinical setting is not practical for large randomized studies and was used only in three trials (see above). Other investigators used indices such as fasting glucose-to-insulin ratio, homeostasis assessment model (HOMA) and quantative insulin sensitivity check index (QUICKI). The Avignon sensitivity index (SiM) was used in this study to assess insulin sensitivity. It was shown by Chiampelli and co-workers (2005) that SiM has the best correlation with the hyperinsulinemic euglycemic clamp technique.

We did not directly measure insulin sensitivity by mean of clamp technique, however, we calculated the Avignon sensitivity index (SiM) and the measured substitute markers of insulin sensitivity, such as fasting serum insulin and serum insulin excursion after oral glucose ingestion.

There was a significant decrease in integrated insulin response to oGTT (AUC insulin) in the metformin group compared with controls. However, fasting insulin levels increased after both metformin and placebo treatment despite the amelioration of insulin action in response to oral glucose. These findings are partly in agreement with reports by Velasquez et al., (1997); Pasquali et al., (2000); Gambineri et al., (2004) who demonstrated considerable increase of insulin concentrations both fasting and after glucose load.

We analysed baseline predictors of clinical response to metformin according to efficacy of treatment on menstrual disturbances. Responders differed from nonresponders in several characteristics. Responders had significantly lower insulin levels in response to oGTT (AUC insulin) and significantly higher DHEAS levels. Fasting insulin levels showed a tendency to be lower in responders. The degree of insulin resistance (SIM index) tended to be more pronounced in nonresponders. It is important to notice that the majority of patients in both responder and nonresponder groups were insulin-resistant but the degree of insulin resistance was more severe in nonresponders. These data appear to suggest that metformin treatment may be effective in hyperinsulinemic and insulin-resistant patients with a mild degree of insulin resistance.

An unexpected finding of this study was the increase of fasting insulin levels in PCOS women after metformin treatment. Series of studies by Acbay et al., (1996); Ehrmann et al., (1997); Vandermolen et al., (2001); Fleming et al., (2002) revealed no changes in fasting insulin levels after metformin treatment. It is important to note that patients in these studies were manifestly obese (mean BMI ~ 37.6 –39.1) like women in the present study (mean BMI = 39.3).

Ehrmann and co-workers supposed that the ability of metformin to alter insulin secretion in obesity of this magnitude appears to be limited. This hypothesis could explain the results of subgroup analysis which revealed a significant decrease in insulin concentrations in response to oGTT in patients with BMI<37 after 16 weeks of metformin treatment. There were no changes in insulin concentrations in response to oGTT in patients with morbid obesity. At the same time, no changes in insulin fasting levels in women with BMI<37 were observed. Patients with BMI>37 demonstrated a significant increase of fasting insulin levels after 16 weeks of treatment with metformin.

In the present study, less obese women had higher fasting insulin concentrations and in response to oGTT insulin concentrations at baseline compared to more obese patients. This suggests that metformin treatment can be more efficient in hyperinsulinemic PCOS patients irrespective of their obesity.

A recent study of Maciel et al., (2004) comparing effects of metformin in obese and nonobese women with PCOS in a placebo-controlled trial demonstrated for the first time that non-obese women with PCOS respond better than obese women to metformin treatment at a dosage of 1500 mg/day for 6 months.

Thus, an alternative explanation for these findings and those of the present study include the possibilities that metformin was administrated in a dose or duration that was insufficient for very obese women.

4.5. Insulin Like Growth Factor-I

The IGF-I levels remained unchanged during the study. Nevertheless, IGF-I concentrations in the metformin-treated group demonstrated a tendency to decline. This tendency was much stronger in less obese patients who demonstrated a 20% decrease (P not significant), whereas metformin treatment did not modify IGF-I levels in more obese women.

Similar results are reported by Kowalska et al. (2001) and De Leo et al. (2000). In addition, De Leo and colleagues observed a significant increase in IGF-I binding protein concentrations in women with PCOS after 30-32 days of metformin treatment. They also calculated the IGF-I/IGF-I binding protein ratio, which was significantly reduced too. Significant changes were also observed in IGF-I and IGF-I binding protein concentrations after therapy with another insulin-sensitizing drug, rosiglitazone (Belli et al., 2004).

IGF-I may contribute to ovarian hyperandrogenemia in PCOS. The in vitro-study by Nahum et al. (1995) documented that IGF-I stimulates androgen output by increasing DNA synthesis and expression of LH-receptors in theca cells. IGF-I has been shown to cause estrogen production by granulosa cells (Erikson et al., 1990) and to act synergistically with FSH and LH controlling aromatase levels in granulosa cells.

In PCOS, plasma IGF-I levels are within the normal range, whereas serum IGF-I binding protein (IGFBP-I) levels are reported to be significantly lower than in normal women. Insulin has been shown to reduce IGFBP-I concentrations, inhibiting its production in the liver. This leads to an increased bioavailability of IGF-I to the ovaries and subsequently to stimulation of androgen production.

Insulin in high concentrations can also mimic IGF-I effects by acting via IGF-I receptor (Leroith et al., 1995). Some authors suggested that this mechanism could be

responsible for insulin-mediated hyperandrogenism. However, insulin has been shown to bind the IGF-I receptor with an affinity of 50-500 times lower than that of IGF-I. Moreover, Willis & Franks, 1995, using anti-insulin receptor and antitype-I IGF receptor antibodies, not only demonstrated that insulin effects on human granulosa cell steroidogenesis *in vitro* must be mediated via its own receptor, but also excluded both the insulin/type-I IGF hybrid receptor and the type-I IGF receptor as possible insulin action-mediated receptors.

The subgroup analysis revealed a strong positive correlation between changes in IGF-I concentrations and AUC insulin after metformin treatment in less obese women. Hence, based on the report of De Leo, Belli and colleagues, we could imagine that the lowering of insulin levels with metformin would be responsible for the increase in IGFBP-1 synthesis in the ovaries with a lowering of free IGF-1.

4.6. Leptin

Before treatment, a positive correlation between BMI and serum leptin levels was observed, as previously reported (Krotkiewski *et al.,* 2003). The subgroup analysis revealed significant higher leptin concentrations in women with BMI>37 than in women with BMI<37. Consequently, circulating leptin appears to be a predictor for body mass and percentage of body fat.

In spite of significant weight reduction after metformin treatment, leptin levels did not decline. We could not find any explanation for this finding. It was hypothsized that the metformin effect on the leptin secretion may occur during the prolonged therapy. Another hypothesis is that changes in body mass are not accompanied by changes of leptin concentrations immediately and need some time to adapt.

Leptin is the hormone product of the obesity (*ob*) gene and is synthesized exclusively in adipose tissue. This hormone acts on the hypothalamus through a special receptor

resulting in the suppression on food intake and increase of energy consumption. Leptin deficiency and high leptin levels are both associated with infertility (Barash et al., 1996).

A potential contribution of leptin to the pathogenesis of PCOS was suggested in a study by Brzechffa and colleagues (1996) in which a subgroup of women with PCOS appeared to have higher leptin levels than controls. However, the majority of research supported the view that serum leptin concentrations in women with PCOS are not significantly different from a control group with a similar BMI (Chapman et al., 1997; Laughlin et al., 1997; Maliqueo et al., 1999).

Several studies suggest that leptin modulates hypothalamic–pituitary–gonadal axis functions. Leptin may stimulate the release of gonadotropin releasing hormone (GnRH) from the hypothalamus and of gonadotropins from the pituitary. A synchronicity of LH and leptin pulses was demonstrated in the follicular phase of the menstrual cycle of healthy women (Licinio et al., 1998) and in patients with polycystic ovarian syndrome (Sir-Petermann et al., 1999), suggesting that leptin may regulate the episodic secretion of LH.

On the basis of our results, we could not demonstrate any correlation between leptin and LH levels. However, we measured a one-off secretion and not pulsatile hormone concentrations, as described.

Studies of insulin regulation of leptin in human yielded conflicting results. This relationship was investigated in a detailed study by Laughlin et al. (1997). They found that independently of body mass and percentage body fat, only 24-hour mean insulin concentrations contributed significantly to leptin levels. Despite this relationship and the 2-fold higher mean insulin concentrations in patients with PCOS compared with controls, serum leptin was not increased. The authors explained their results by the presence of a PCOS-specific form of insulin resistance in adipocytes, which impairs the stimulatory effect of insulin on leptin secretion. Additionally, it is considered that leptin secretion in women with PCOS is less than expected because of insulin resistance and accumulation of visceral fat that secrets less leptin than subcutaneous fat (Jacobs et al., 1999).

This hypothesis could explain our findings where the improvement of hyperinsulinemia did not alter leptin levels despite the significant weight reduction. The present results are in agreement with the studies by Mantzoros et al, (1997) and Belli et al., (2003), who also found no changes in leptin concentrations after treatment with thiazolidinedione, which is considered to be a more effective insulin-sensitizing agent.

4.7. Lipids

Triglycerides, cholesterol and lipoprotein concentrations approached the upper limit of the normal range and did not significantly change after treatment. Nevertheless, HDL cholesterol levels in the metformin-treated group showed a tendency to rise, but P=0.07 did not achieve a significant value. These findings are in line with the results obtained by Moghetti et al., (2000) who found modest improvement in HDL- cholesterol levels after six months metformin treatment. In most studies on women with PCOS, metformin and other insulin-sensitizing agent, like troglitazone produce no improvements in dyslipidemia in women with PCOS (Velasquez et al., 1997; Morin-Papunen et al., 1998; Legro et al., 2003). Interesting results have been presented in the study of Gambineri and colleagues (2004), who investigated the effects of long-term metformin and flutamide treatment, given alone and in combination.

Whereas flutamide administration showed a significant reduction of total and LDL cholesterol, the combined metformin + flutamide therapy tended additionally to increase HDL levels. The effects on total and LDL cholesterol appear to depend on the antiandrogenic effects of flutamide. Androgens are known to be directly involved in the regulation of cholesterol and lipoprotein metabolism (Anderson et al., 2002). The interaction between metformin and flutamide appear to confirm the role of insulin and androgens on HDL metabolism (Von Eckardsein, 1998).

Multiple studies have also reported that insulin resistance has been associated with decreased HDL-cholesterol and elevated triglyceride levels in PCOS women (Robinson et al., 1996; Rajkhowa et al., 1997; Mather et al., 2000). This finding is in agreement with results of correlation analysis in our study, which revealed a significant inverse

correlation between fasting insulin and HDL cholesterol, and a significant positive correlation between 2-hour insulin and total cholesterol.

To summarize, HDL cholesterol concentrations demonstrated a noticeable trend to rise, and we can suppose that metformin treatment may have an impact on lipids as cardiovascular risk factors.

4.8. Menstrual Abnormalities

After metformin treatment, in two of six patients with severe amenorrhea, the cycle returned to normal, one woman became oligomenorrheic and three patients remained amenorrheic. After placebo treatment, one of four amenorrheic patients became oligomenorrheic and three patients remained amenorrheic. There were no patients in the placebo treated group who returned to regular cycle. These results appear to suggest that metformin administration was associated with improvement of menstrual function in PCOS women with severe menstrual disorders.

Several studies have documented the restoration of regular menstrual cycles and supported that this effect is not limited to a single ethnic group or geographic area (Nestler et al., 1996; Pirwany et al., 1999; Glueck et al., 2001; Kazerooni et al., 2003). Nonetheless, most of these studies were small and uncontrolled. There are only two controlled and randomized trials demonstrating a significant improvement in regular menstrual cycles with metformin (Moghetti et al., 2000; Fleming et al., 2002).

In the present study, 50% of the women given metformin experienced no improvement of their menstrual cycle. The reasons for the differences in clinical response to metformin among the individual PCOS subjects are not easy to explain.

Kolodziejczyk et al. (2000) found that the improvement in the length of the menstrual cycle was significantly greater in women with high DHEAS levels (>400 µg/dL or >10,3 µmol/l) than in those with normal DHEAS.

Moghetti et al. (2000), based on multiple regression analysis, predicted clinical improvement for PCOS patients treated with metformin. These predictors were higher plasma insulin levels, less severe menstrual abnormalities and lower serum androstendione levels. These data strengthen the hypothesis that metformin may be effective only in the insulin-resistant subjects.

By contrast, subgroup analysis in the study by Fleming and co-workers (2002) revealed that body mass index and insulin resistance did not predict the ability to establish normal ovarian function and menstrual cycle. High SHBG concentrations and lower free index predicted the ability to establish normal ovarian function and menstrual cycle.

Pirwany et al. (1999) focused on the effect of metformin alone on menstrual cyclicity and ovulation rates. They also divided women into subgroups to see if various baseline parameters predicted a response to metformin. For example, patients with elevated testosterone levels had significant improvements in ovulation rates: 14. 8% to 38.9% (P = 0.005). Similarly, the subgroup of patients with fasting hyperinsulinemia (FI > 14 mIU/ml) at baseline had a significant increase in ovulation rate from 12.5% to 39.4% (P = 0.012). However, those with normal baseline insulin concentrations did not show an improvement.

We would draw a parallel between our own finding and those obtained by Douchi et al., (2002). The author and his colleagues examined eighty-three obese women (mean BMI = 31.9) who were divided into two groups according to their menstrual status: one with menstrual disorders and the other group with regular menstruation. They compared anthropometric characteristics between the two groups and found that women with irregular menses had higher trunk fat mass and trunk-leg fat ratio than controls. It was

postulated that upper body obesity and not a degree of obesity, are associated with menstrual disorders.

We provided a subgroup analysis in order to test if body weight is an important factor for successful treatment with metformin. There was no difference apparent between women with BMI<37 and morbid obese patients in the menstrual response to treatment. This finding suggests that body weight possibly is not a predictive factor for menstrual response to metformin treatment.

The data of the present study are in agreement with results obtained by Fleming and colleagues (2005). The authors showed no difference between obese and morbid obese groups in the proportions attaining a normal menstrual rhythm.

In the present study, the subgroups contained small numbers of subjects (seven and twelve patients each). Large numbers of patients will be required to show convincing differences in many parameters.

Therefore, we concluded that until now there are not enough data to limit metformin treatment to a specific subgroup of women with PCOS. One of the factors to be determined is the relationship between dose and body mass index.

4.9. Metformin and Pregnancy Rates

There were three conceptions in three patients during the study, and one miscarriage in the first trimester. The distribution of pregnancies was: metformin, 1 of 23 patients; and placebo, 2 of 23 patients. This finding is a surprising observation, taking into account that menstrual cyclicity improved in the metformin-treated group. However, there were some limitations that could explain the results of the study. First, not all patients were tested for tubal factors of infertility. Second, spermiogramm analysis was done only in partners who had been already treated within the scope of *in-vitro* fertilisation. Oligozoospermia or azoospermia were diagnosed in twelve (32.4%) partners (both placebo and metformin) which contributed to low pregnancy rates.

On the other hand, we assume that the16 week period of metformin treatment was too short to influence the reproductive function in our patients more profoundly.

The clinical pregnancy rate reported by trials comparing metformin as a single agent with placebo did not show evidence of benefit (Ng et al., 2001; Fleming et al., 2002). However, the addition of metformin to clomiphene citrate (CC), results in an improved ovulation and pregnancy rate in both unselected and CC-resistant PCOS patients. In the trial by El-Biely and co-workers (2001), metformin in combination with CC was compared to CC alone in 90 women, with both ovulation and pregnancy as an end point. Patients in the CC plus metformin group had a higher ovulation rate (80% vs. 65%), higher pregnancy rate per patient (29% vs. 8%), and a lower rate of ovarian hyperstimulation syndrome (9% vs. 69%).

Taken together, our findings suggest that metformin treatment may restore menstrual abnormalities but the duration of the trial and the dose of metformin were possibly insufficient to influence fertility in obese women with PCOS.

5. SUMMARY

5.1. English Version

The present randomised, placebo-controlled study was designed to compare the antihyperglycemic drug metformin *vs.* placebo combined with lifestyle modification in the treatment of polycystic ovary syndrome in obese women. Special attention was paid to the effects of this medication on insulin and glucose metabolism, endocrine and biochemical parameters, and menstrual function.

Forty six infertile women with PCOS, aged between 22-39 years and mean BMI 38.1; (range 28.1–49.0 kg/m^2) were recruited in the Division of Reproductive Medicine at the University Hospital Charité. Fifteen (32%) patients revealed regular menstrual cyclicity, 24 (52%) patients had less than four cycles in the last six months and seven (16%) women had chronic amenorrhoea. The polycistic ovaries were demonstrated by transvaginal ultrasound in all volunteers.

The trial included five monthly visits. At each visit (at week 0, 4, 8, 12 and 16) all women underwent ultrasound scanning of the ovaries, recording of menstrual pattern, anthropometric and blood pressure measurements and recording of side-effects of metformin. Group therapy with aspects in nutrition and physical activity was conducted monthly. Each woman received individual counselling by a dietician. Therapy with metformin or placebo was initiated in a randomised double-blind trial of 1.5 g daily for 16 weeks.

Changes of endocrinological and metabolic parameters such as FSH, LH, prolactin, estradiol, testosterone, free testosterone, DHEA-S and SHBG, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, insulin, glucose, HbA1c, leptin and IGF-I were assessed at the first, third and fifth visit (at week 0, 8 ,16).

Blood for the endocrine investigations was obtained before the start of the metabolic studies. A 75-g oral glucose dose was administered, and intravenous blood was obtained for glucose and insulin determination, basally, at 60 and 120 min through the catheter. The response of glucose and insulin to the OGGT was analysed by calculating the AUC (area under the curve). Statistical analysis was performed by the SPSS (Statistical Package for the Social Sciences), Version 11.0. Changes during the treatment within groups were evaluated by means of the Wilcoxon matched-pairs test.

In the present study, metformin therapy resulted in a reduction of hyperinsulinemia in obese women with PCOS, with a parallel increase in SHBG concentrations and a tendency for improvement of high density lipoprotein status and amelioration of the menstrual pattern. A significant weight loss was achieved only in the metformin-treated group although all patients were advised to follow dietary restriction and to take regular exercises.

However, contrary to our postulate and results reported by most other studies, we found no significant changes in serum total and free testosterone levels in response to metformin. We postulated that the major effect of metformin in obese women on androgen secretion is mediated by changes in hyperinsulinemia and subsequently in SHBG rather than by direct inhibition of ovarian androgen production.

The subgroup analysis, according to the body mass index, demonstrated significant changes in AUC insulin levels and SHBG concentrations in obese PCOS women with BMI<37 after 16 weeks of metformin treatment. Patients with morbid obesity exhibited a significant increase in fasting insulin levels. There was no difference in insulin concentrations in response to oGTT and SHBG concentrations in morbid obese women. This finding suggests that body weight is an important factor for clinical response to metformin. On the other hand, less obese women had significantly higher fasting insulin concentrations and in respond to oGTT insulin concentrations before the treatment. This could mean that metformin treatment can be more efficient in hyperinsulinemic patients with PCOS irrespective of their obesity.

After metformin treatment, in two of six patients with severe amenorrhea the cycle returned to normal, one woman became oligomenorrheic and three patients remained amenorrheic. These results appear to suggest that metformin administration was associated with improvement in menstrual function in PCOS women with severe menstrual disturbances.

Three women got pregnant during the study; one woman in the metformin-treated group and two women in the placebo-treated group. This finding was unexpectedly low for us, taking into consideration the results of previous studies. The male infertility factor in the present study contributed to the low pregnancy rate. We believe that a higher dose of metformin and/or longer duration of administration may have a distinct effect on thereproductive function in very obese women with PCOS.

Metformin therapy was well tolerated by the majority of patients. Three of twenty three (13%) patients in the metformin-treated group dropped out of the trial. Clinically, this low dropout rate is important and indicates that the usual side effects on this dosage are not severe. Nevertheless, women prescribed metformin should be adequately counselled and actively supported during metformin treatment.

In conclusion, we have demonstrated that metformin treatment has beneficial effects on menstrual function and metabolic factors in obese women with PCOS. This finding support a future therapeutic role of metformin, however, it should be used in combination with general lifestyle improvements, and not as a replacement for physical activity and a diet. Further well-designed, controlled trials are required to determine proper dosages and duration of treatment. The primary end point of subsequent studies should be pregnancies and live-birth rate.

5.2. Deutsche Version

Ziel der vorliegenden randomisierten, placebo-kontrollierten Studie war es, die Auswirkung des antihyperglykämischen Medikaments Metformin in Kombination mit Ernährungsberatung und Sport auf übergewichtige Frauen mit PCOS zu ermitteln. Besondere Aufmerksamkeit wurde der Wirkung des Medikaments hinsichtlich Insulin und Glukose-Stoffwechsel, endokrinologischen und biochemischen Parametern, sowie dem Menstruationszyklus gewidmet.

An der Studie der Abteilung für Reproduktionsmedizin der Charité, Berlin, nahmen 46 infertile Frauen mit PCOS teil. 15 (32%) der Patientinnen wiesen einen regulären Menstruationszyklus auf, 24 (52%) hatten weniger als vier Zyklen in den letzten sechs Monaten und sieben (16%) der Frauen litten an chronischer Amenorrhoe. Die polyzystischen Ovarien wurden mittels transvaginalen Ultraschalls festgestellt.

Die verabreichte Dosis von Metformin bzw. Plazebo betrug 1,5 g täglich über einen Zeitraum von 16 Wochen. Fünf monatliche Visiten wurden durchgeführt. Bei jeder Visite (zur ersten, vierten, achten, zwölften und vierzehnten Woche) wurden die Ovarien mit Ultraschall untersucht, der Menstruationszyklus festgestellt, Gewicht und Blutdruck gemessen sowie Nebenwirkungen überprüft. Monatlich wurde eine Ernährungsberatung angeboten. Jede Teilnehmerin erhielt darüber hinaus individuelle dietätische Beratung.

Die Blutabnahme für die Hormonbestimmung erfolgte jeweils vor dem oralen Glucose-Toleranz-Test. Das Blut für die Glucose- und Insulinbestimmung wurde basal, zur 60. und 120. Minute entnommen. Als Parameter für die Insulinsekretion wurde mit der Trapezmethode die „area-under-the-curve (AUC)“ für Insulin berechnet. Die statistische Auswertung erfolgte mit Hilfe von SPSS (Statistical Package for the Social Sciences), Version 11.0. Veränderungen während der Behandlung wurden mittels des Wilcoxon-Tests für verbundene Stichproben ermittelt.

Das zentrale Ergebnis der vorliegenden Studie ist: Die Metformin-Therapie führte zu einer Reduktion der Hyperinsulinämie bei übergewichtigen Frauen mit PCOS. Daneben wurden eine signifikante Zunahme des SHBG-Spiegels festgestellt. Ein weiterer Befund war eine Tendenz zur Verbesserung der HDL-Werte. Die Menstruationszyklen zeigten eine Tendenz zur Normalisierung. Diese Effekte traten unabhängig von einer Gewichtsreduktion auf. Ein deutlicher Gewichtsverlust wurde nur in der Metformingruppe beobachtet, obwohl allen Patientinnen Diät und Sport empfohlen worden waren.

Entgegen unseren Erwartungen und den Ergebnissen anderer Studien wurde keine signifikante Veränderung beim gesamt- und freien Testosteron festgestellt. Wir vermuten, dass der Haupteffekt der Metformintherapie auf die androgene Sekretion bei adipösen Frauen durch Veränderungen der Insulinsekretion und in der Folge im SHGB-Spiegel zu suchen sind, und nicht in der direkten Verhinderung von ovarieller Androgenproduktion.

Nach 16wöchiger Metformintherapie zeigte die Subgruppen-Analyse mittels des BMI-Indexes eine signifikante Veränderung der Insulinwerte im oGTT und des SHBG-Spiegels bei PCOS-Patientinnen mit BMI<37. Adipöse Frauen mit BMI>37 wiesen eine deutliche Zunahme des nüchternen Insulin-Spiegels sowie keine Veränderungen der Insulinkonzentration im oGTT und SHBG-Spiegel auf. Dieses Ergebnis lässt vermuten, dass Körpergewicht ein wichtiger Faktor für klinischen Erfolg bei Metformin-Therapie darstellt. Andererseits hatten weniger adipöse Patientinnen höhere nüchterne und 2-h Insulinwerte im oGTT-Ausgangsniveau. Dies kann bedeuten, dass Metformintherapie bei hyperinsulinemischen Frauen unabhängig vom Körpergewicht effektiver sein kann.

Nach 16wöchtiger Metformin-Therapie bekamen zwei von sechs Patientinnen mit Amenorrhoe einen regulären Zyklus, eine Frau bekam Oligomenorrhoe und drei Patientinnen blieben amenorrhoisch. Diese Ergebnisse deuten darauf hin, dass Metformin-Einnahme zu einer Verbesserung der Menstruationsfunktion bei PCOS-Patientinnen mit schweren Störungen des Menstruationszyklus führt.

Drei Frauen wurden während der Studie schwanger: eine Frau in der Metformin-therapierten Gruppe und zwei Frauen in der Placebo-Gruppe. Dieses Ergebnis erscheint angesichts früherer Studien überraschend niedrig. Der männliche Infertilitätsfaktor in der vorliegenden Studie dürfte zu dieser niedrigen Schwangerschaftsrate beigetragen haben. Unsere Studienergebnisse sind möglicherweise auch dadurch beeinflusst, dass die Dosierung für adipöse Patientinnen zu niedrig war bzw. die Behandlungsdauer zu kurz war, um die Reproduktionsfunktion zu beeinflussen.

Metformin-Therapie wurde von den meisten Patientinnen gut vertragen. Drei von 23 Patinentinnen (13%) mussten die Studie vorzeitig abbrechen. Aus klinischer Sicht ist diese niedrige Abbruchrate wichtig, weil sie zeigt, dass Nebeneffekte bei dieser Dosierung nicht sehr stark ausgeprägt waren. Dennoch sollten Frauen, denen Metformin verschrieben wird, angemessen beraten und während der Therapie betreut werden.

Zusammenfassung: Wir haben zeigen können, dass Metformin -Therapie günstige Auswirkungen auf den Menstruationszyklus und metabolische Faktoren bei adipösen Frauen mit PCOS hat. Dieses Ergebnis spricht für die Behandlung mit Metformin, die allerdings nicht ohne allgemeine Ernährungsumstellung und begleitende körperliche Aktivitäten geschehen sollte.

Weitere kontrollierte Studien sind nötig, um die optimale Dosierung und Dauer der Behandlung mit Metformin festzulegen. Das Endziel künftiger Studien sollten Schwangerschaftsraten und Lebendgeburten sein.

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7. LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropin Hormone
ALT	Alanine Aminotransferase
AST	Aminotransferase
AUC	Area Under The Curve
BMI	Body Mass Index
CC	Clomiphene Citrate
CT	Computer Tomography
DDT	Chlorophenotanum technicum
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DM	Diabetes mellitus
E2	Estradiol
EM	Endometrial Cancer
FAI	Free Androgen Index
FFA	Free Fatty Acids
FSH	Follicle-Stimulating Hormone
GGT	Gamma-Glutamyltransferase
Hb1Ac	Glycated Haemoglobin
HCG	Human Chorion Gonadotropin
HDL- cholesterol	High-Density Lipoprotein (HDL) cholesterol
HOMA	Homeostasis Assessment Model

IGF	Insulin like Growth Factor
IGFBP	Insulin like growth Factor Binding Protein
IR	Insulin Resistance
LCOS	Laparoscopic Ovarian Drillling
LDL-cholesterol	Low-Density Lipoprotein (LDL) cholesterol
LH	Luteinizing Hormone
NS	Not Significant
OGTT	oral Glucose Tolerance Test
PCO	Polycistic Ovaries
PCOS	Polycystic Ovary Syndrome
QUICKI	Quantative Insulin Sensitivity Check Index
RCT	Randomized Controlled Trial
RIA	Radio Immuno Assay
SAT	Subcutaneous Adipose Tissue
SD	Standard Deviation
SHBG	Sex-Hormone Binding Protein
SPSS	Statistical Package for the Social Sciences
T	Testosterone
TAT	Total Adipose Tissue
TSH	Thyroid Stimulating Hormone
TT	Total Testosterone
VAT	Visceral Adipose Tissue
WHR	Waist-to-Hip Ratio

8. DECLARATION

I hereby declare:

- that I produced the present dissertation entirely on my own and only by using the documents and sources mentioned .
- that neither previously nor simultaneously a second dissertation was done.
- that I am aware of the currently valid rules and regulations for dissertations.

9. ACKNOWLEDGMENTS

The present dissertation was prepared under the auspices of Dr. med. Bettina Pfüller at Humboldt University, Charité, who also initiated the study. My special thanks go to her for consistent support and inspiration, and for allowing me to take part in a magnificent project.

Many colleagues and friends contributed to the endeavour in various ways:

In particular, I would like to thank:

For help in organising and conducting the study:

Prof. Dr. med. Jürgen Scholze

Susanne Klaua

Kati Voigt

Professor Wolfgang Rohde and Dr. med. Andrea Machlitt made valuable comments on earlier drafts of this paper. Professor Rohde and his team also supported hormone analysis in the laboratory of the Institute for Experimental Endocrinology, Charité Hospital.

PD. Dr. med. Manfred Ventz shared his advice and suggestions during conducting the study.

Gerda Siebert of the Institute for Biometrics offered essential support with the data analysis.

Ms. Korlach of the hormone laboratory of Humboldt University, Charité, conducted hormone and other tests.

A team of excellent nurses provided superb care for the study participants:

S. Gerlinde Höhndorf

S. Angela Glaumann

S. Doris Bronk

S. Sibylle Diedrich

Sylvia Schattenfroh of the press department for her help in recruiting participants of the study through an advertisement in the local newspaper, „Berliner Zeitung“.

Finally, I am gratefully indebted to all patients of the study without whose co-operation the present study could not have been performed.

10. PUBLICATIONS

Pfüller B., Ibragimova E, Klaua S, Machlitt A, Voigt K., Rohde W. (2004) Effect of metformin treatment combined with lifestyle modification on reproductive features, endocrine and metabolic profiles in obese infertile women with PCOS. 20th annual meeting of the European Society for Human Reproduction and Embryology in Berlin, Germany June 27 to 30 2004. P-519

Klaua S., Wiesner M., Ibragimova E., Voigt K., Pfüller B., Scholze J. (2004) Charité Adipose Polycystic Ovary Syndrome Study – greater weight reduction in metformin treated less heavier PCOS women. 13th European Congress of Obesity; 2004 26-29 Mai 2004 ; Prague.

Klaua S., Wiesner M., Ibragimova E., Voigt K., Pfüller B., Scholze J (2004) Bioelectrical characteristics in PCOS women – a prospective randomized placebo controlled metformin trial. 13th European Congress of Obesity; 2004 26-29 Mai 2004 ; Prague.

11. ATTACHMENT

11.1. Personal And Family History Sheet

Name, Vorname:

Geburtsdatum:

Anschrift:

Telefon:

Datum:

1. Jetzige oder frühere Beschwerden und Krankheiten

Table 16: Symptome

Beschwerden	**j**	**n**	**Symptome/ Beschwerden**	**j**	**n**
Leistungsschwäche			Unregelmäßiger Herzschlag		
Müdigkeit/ Abgeschlagenheit			Herzschmerzen unter Belastung		
Unruhe/ Nervosität			Herzschmerzen in Ruhe		
Schwitzen			Luftnot unter Belastung		
Hitzewallungen			Luftnot in Ruhe		
Kältegefühl			Nächtliches Wasserlassen/ wie oft?		
Schwindel			Wadenschmerzen unter Belastung		
Sehstörungen			Wadenschmerzen in Ruhe		
Konzentrationsschwächen			Kribbeln in Händen/ Füßen		
Vergesslichkeit			Knochen-, Gelenkschmerzen / wo?		
Schlafstörungen			Gelegentlich Durchfall		

Einschlafen am Tage			Gelegentlich Verstopfung		
Schnarchen/ nächtliche Atemaussetzer			Heißhunger		
Nasenbluten			Libidostörungen		
Herzklopfen			andere		

Table 17: Krankheiten

Krankheit	**ja**	**seit wann?**	**nein**	**unbekannt**
Bluthochdruck				
Erhöhte Blutfette				
Gestörte Glucosetoleranz				
Herzgefäßerkrankung				
Herzinfarkt				
Herzschwäche				
Verengung andere Gefäße				
Schilddrüsenerkrankungen				
Sonstige Hormonstörungen				
Nierenerkrankungen				
Gallensteine				
Thrombose				
Andere				

Frühere Erkrankungen/Operationen (an Organen, Nerven, Knochen, Gelenken, Wirbelsäule, Tumore) .....
.....
.....
.....

Geburtsgewicht (wenn bekannt).....

Geburtsgroße (wenn bekannt).....

2. Familiärer Hintergrund

Besteht oder bestand bei Ihren Verwandten folgende Erkrankungen oder Beschwerden?

Table 18: Familiärer Hintergrund

	Mutter	Vater	Kinder	Geschwister	Großeltern	Onkel/Tanten
Erkrankungen	*ja/nein*	*ja/nein*	*ja/nein*	*ja/nein*	*ja/nein*	*ja/nein*
Übergewicht						
Bluthochdruck						
Schlaganfall						
Herzkranz-gefäßerkrankung						
Herzinfarkt						
Nierener-krankungen						
Altersdemenz						

Zuckerkrankheit						
Gallenstein-Operation						
Schilddrüsener-krankungen						
Sonstige Hormon-störungen						
Tumoren						
Andere						

3. Weitere Angabe zur Person

Große Gewicht

Übergewicht: ja/nein wenn ja, seit wann

Ursachen/Gründe Ihrer Meinung nach:

.....

.....

Bewusste Versuche der Gewichtsabnahme:

Wie oft? .....

Methoden:

.....

Erfolg:

.....

.....

Abnahme/ Zunahme pro Zeitraum(in letzen Jahren):

.....

.....

.....

.

Sportliche Betätigung (was, wie viel pro Woche)

.....

.....

Sind Sie mit bestimmten Aspekten Ihres Lebens unzufrieden - welche, warum?.....

.....

.....

Hobbys:.....

.....

Haben Sie Probleme beim Stuhlgang? ja/nein

oder beim Wasserlassen? ja/nein

Nehmen oder nahmen Sie Abführmittel(Laxantien)? Ja/nein

Nehmen oder nahmen Sie Entwässerungsmittel(Diuretika?) ja/nein

Andere Medikamente (möglichst alle aufzählen, von wann bis wann)

.....

.....

Sind Sie gegen irgendetwas allergisch (Medikamente, Nahrungsmittel, Pollen usw.)?

.....

.....

Rauchen Sie (wenn ja, seit wann und wie oft) ja/nein

.....

Alkohol:

.....

Kaffee:.....

Sind Sie an ihrem Arbeits-/ ausbildungsplatz besonderen Belastungen ausgesetzt (künstliches Licht, Lärm, Staub, Gas , andere)?.....

4. Gynäkologische Angaben

In welchem Alter trat zum ersten mal die Regelblutung auf?.....

Wann war die letzte Regelblutung? .....

Abstand, Dauer(in Tagen) und Stärke (1 schwach, 2 mittel, 3 stark) der Regelblutung:.

...../...../.....

Beginn auffälliger Unregelmäßigkeiten

.....

Zwischenblutungen.....

Dauer des Kinderwunsches.....

Glauben Sie, daß es bisher Unstände gab, die das Eintreten einer Schwangerschaft verhinderten?.....

Arbeitsplatz/-zeiten .....

Abwesenheit/Trennung v. Partner z. B. beruflich, räumlich.....

andere.....

Wie häufig haben Sie durchschnittlich sexuellen Verkehr?

ca..... pro Woche

ca. ..... pro Monat

Pille (welche, in welchem Zeitraum)

.....

Andere hormonelle Medikamente

.....

Geburten(wann).....

Fehlgeburten(wann).....

Schwangerschaftsabbrüche:

.....

Eileiterschwangerschaft.....

Gynäkologische Operationen/Erkrankungen:

.....

.....

IVF-Behandlung (künstliche Befruchtung)

.....

Hirsutismus *Score*

Oberlippe

Kinn

Brust

Bauch

Pubes

Arme

Beine

Rücken

Gesäß

Seborrhöe(ölige Beschaffenheit der Haut und Haare) ja /nein

Akne(wo).....

Haarausfall ja/nein

Haben Sie in den letzen Monaten oder Jahren die morgendliche Temperatur gemessen und/ oder Temperaturkurve geführt? ja/nein

5. Ernährungsanamnese

Wie viele Mahlzeiten nehmen Sie am Tag zu sich?.....

Wo essen Sie? .....

Wann nehmen Sie die Hauptmahlzeiten zu sich?(Uhrzeit)

.....

Was essen Sie am liebsten?

.....

Naschen Sie gern? Wann?
Was?.....

Haben Sie schon Diäten ausprobiert, wenn ja welche?

.....

Haben Sie schon versucht, durch Erbrechen abzunehmen?.....
.

12.2. Patient Consent

Einwilligungserklärung an der Studie

Hiermit willige ich in die Teilnahme zum prospektiven randomisierten placebokontrollierten Parallelgruppenvergleich über 16 Wochen hinsichtlich Schwangerschafts- und Ovulationsraten bei übergewichtigen/adipösen Patientinnen (BMI $\geq$ 27 kg/m^2) mit Polyzystischem Ovarsyndrom unter einer Therapie mit Metformin (500-1500 mg/d) ein.

Ich erkläre die Einwilligung zur Teilnahme an der Studie, meine Teilnahme ist freiwillig und kann jederzeit von mir ohne Nachteil für meine medizinische Behandlung widerrufen werden. Solange ich an der Studie teilnehme, verpflichte ich mich, regelmäßig zu den Visiten und Untersuchungen zu erscheinen und die Studie regulär oder auf Wunsch vorzeitig mit einer Abschlussuntersuchung zu beenden.

Ort, Datum Unterschrift des Patienten

Erklärung des Arztes

Ich habe den Patienten über Wesen, Bedeutung und Tragweite der Studie aufgeklärt. Die Patientenaufklärung wurde mit dem Patienten besprochen. Eine Kopie der Einwilligungserklärung wurde dem Patienten ausgehändigt.

……. ……

Ort, Datum Unterschrift des Arztes

Vertraulichkeit der Daten

Ich erkläre mein Einverständnis zur Überprüfung der richtigen Übertragung meiner Untersuchungsbefunde in anonymisierte Dokumentationsbogen und entbinde nur für diesen Zweck meinen behandelnden Arzt von der ärztlichen Schweigepflicht und der Einhaltung der Bestimmungen des Bundesdatenschutzes.

……. ……

Ort, Datum Unterschrift des Patienten

12.3. Patient Information

Sehr geehrte Patientin,

seit langem wünschen Sie sich mit ihrem Lebenspartner ein Baby. Sie haben schon einige IVF-Versuche ohne Erfolg unternommen. Ihr behandelnder Gynäkologe hat ein Polyzystisches Ovarsyndrom (**PCOS**) diagnostiziert.

Etwa fünf bis sieben Prozent der Frauen leiden unter diesem Syndrom, oftmals ohne es zu wissen: In ihren Eierstöcken (Ovarien) haben sich Zysten (mit Flüssigkeit gefüllte Bläschen) gebildet und häufig reift kein befruchtungsfähiges Ei heran, denn die Produktion der weiblichen Geschlechtshormone (Östrogene) in den Ovarien ist gestört. Es handelt sich hierbei um eine Störung, die sowohl die Funktion der Eierstöcke als auch die der Schleimhaut der Gebärmutter beeinträchtigt. Gleichzeitig kommt es wegen des Mangels an Östrogenen zu einem relativen **Überschuß der männlichen (androgenen) Geschlechtshormone.**

Durch Veränderungen im Hormonhaushalt kommt es zu Regelblutungsstörungen (Amenorrhoe = keine Regelblutung; Oligomenorrhoe = unregelmäßige Regelblutung), Veränderung der Behaarung mit typischem männlichen Erscheinungsbild (Bartwuchs, Haarausfall am Stirnansatz, Behaarung in der Mittellinie des Unterbauches), Neigung zur Akne.

Weniger bekannt sind die Zusammenhänge dieser Hormonstörungen mit dem Zucker- und Fettstoffwechsel. Patientinnen mit einem PCOS sind häufiger als gleichaltrige Frauen ohne diese Störung übergewichtig. Damit verbunden besteht eine relative Unempfindlichkeit der Muskulatur für Insulin (Hormon, welches die Bauchspeicheldrüse nach der Resorption von Zucker, entstanden durch den Abbau der Kohlenhydrate im Darm, in das Blut ausschüttet). Der Zucker wird normalerweise durch Insulin in die Muskelzellen eingeschleust. Durch die relative Unempfindlichkeit der Muskulatur für Insulin wird Zucker bevorzugt in die Fettzelle und die Leber gebracht. Dadurch entsteht die Neigung zum Übergewicht und zur Produktion von Fett aus Zucker.

Reaktiv schüttet die Bauchspeicheldrüse nach Nahrungszufuhr mehr Insulin aus, um das Problem der schlechten Nährstoffversorgung der Muskulatur zu lösen. Sie können sich vorstellen, dass Bewegungsmangel und ungünstige Ernährung diese Situation stark verschlechtern kann. Nach vielen Jahren des Bestehens einer derartigen Störung sind die Blutfettwerte erhöht bzw. kann eine Diabetes mellitus (Zuckerkrankheit) resultieren. Der permanent in diesem Kreislauf steigende Insulinspiegel stört die

normale Produktion von weiblichen Geschlechtshormonen und verstärkt damit das PCOS.

Aus diesem Grund ist es für eine erfolgreiche Therapie zur Behandlung Ihres unerfüllten Kinderwunsches unbedingt notwendig, diesen Kreislauf zu durchbrechen. Dann nämlich normalisieren sich die Hormonspiegel von Östrogenen, Androgenen und Insulin und Sie haben gute Chancen, schwanger zu werden.

In Zusammenarbeit mit der Poliklinik der Charité bieten wir Ihnen eine kombinierte Behandlung zur Gewichtsreduktion und zur Verbesserung der hormonellen Stoffwechsellage an. Ziel der Behandlung, die im Rahmen einer klinischen Studie mit insgesamt sechzig Frauen durchgeführt wird, ist die Normalisierung der Monatszyklen bzw. der Eintritt einer Schwangerschaft.

Hierfür kommen folgende Therapieansätze in Frage:

Medikamentöse Therapie mit Metformin:

Metformin wird bei der Behandlung von Diabetes mellitus Typ II b (infolge von Versagen der Kreislaufes: Insulinunempfindlichkeit am Muskel → erhöhte Insulinausschüttung → absinkender Blutzuckerspiegel → Heißhunger → erhöhte Nahrungszufuhr kombiniert mit Bewegungsmangel, dann absinkender Insulinspiegel mit Erhöhung des Zuckerspiegels) eingesetzt.

Metformin steigert die Aufnahme von Zucker in die Körperzellen, insbesondere in die Leberzellen. Gleichzeitig wird die Neubildung von Zucker in der Leber gebremst. Dadurch wird der Zuckerstoffwechsel günstig beeinflusst und die Bauchspeicheldrüse schüttet weniger Insulin aus.

In zahlreichen internationalen Studien konnte gezeigt werden, dass durch die sinkenden Insulinspiegel die männlichen Hormone vermindert und der Menstruationszyklus normalisiert werden konnte. Es ist zu vermuten, dass sich unter einer Therapie mit Metformin die Vorraussetzungen für die Produktion von Eizellen und auch für die Einnistung eines befruchteten Eis günstiger sind.

Jede medikamentöse Therapie hat Nebenwirkungen, bei einer Therapie mit Metformin sind diese selten: z.B. Durchfälle, vereinzelt Kopfschmerzen.

Steigerung der körperlichen Betätigung/Umstellung der Ernährung:

Durch Steigerung der körperlichen Betätigung insbesondere durch eine Training, welches jeweils zur Hälfte aus Herz-Kreislauf- und Widerstandstraining besteht sowie durch Reduktion der Fettzufuhr, der Saccharose, Glucose und Fructosezufuhr (Einfach- und Zweifachzucker) wird der Zucker-, Fett- und Insulinstoffwechsel positiv beeinflusst.

Einzelne Fälle aus unserer Sprechstunde lassen vermuten, dass damit die Vorraussetzungen für die Produktion von Eizellen und auch für die Einnistung eines befruchteten Eis günstiger sind.

Wir möchten ihnen die Teilnahme an einer Studie wie folgt anbieten:

Gruppe 1:	Gruppe 2:
Metformin	Placebo
Gruppentherapie Ernährung	Gruppentherapie Ernährung
und Bewegung	und Bewegung

Die Behandlung läuft über 4 Monate.

Die Wahl der Therapie erfolgt per Zufallsgenerator.

Bei Teilnahme erklären sie sich mit jedem der aufgeführten Therapieverfahren einverstanden.

Die Untersuchungen und Therapien können sie dem beigefügten Visitenplan entnehmen und mit ihrem Arzt besprechen.

Im Lauf der Studie wird bei Ihnen ein Oraler Glukosetoleranz-Test (zur Bestimmung der Glukosestoffwechselstörungen) durchgeführt.

Sie müssen sich auf diesen Test vorbereiten, d.h.:

mindestens 3 Tage lang übliche Essengewohnheiten.

Mindestens 3 Tage lang Absetzen störender Medikamente(Z.B.: Anthihyperhtensiva, Laxantien, Salycylate, Transquilizer, Corticosteroide), sofern dies ohne Gefahr möglich ist.

Fortsetzung der normalen körperlichen Tätigkeit, Bettlägerigkeit oder übermäßige körperliche Aktivitäten sind auszuschließen.

24 Stunden vorher keinen Alkohol.

12 Stunden vor dem Test nüchtern (d.h., wenn Sie zuletzt am Vorabend etwas gegessen haben, am Morgen des Untersuchungstermins bitte kein Frühstück zu sich nehmen).

Durchführung des Tests:

Nach venöser Blutentnahme zur Bestimmung der Nüchternglucose und des Nüchterninsulins erfolgt die Verabreichung von 75 g Glucose in ca. 200 ml ungesüßter Flüssigkeit (warmer Tee). Nach 30, 60, 90 und 120 min werden weitere venöse Blutentnahmen zur Bestimmung von Glucose und Insulin vorgenommen.

Bio-Impedanz-Analyse (BIA): Die Untersuchung zur Körperzusammensetzung wird mit einem Gerät zur Impedanzanalyse bei verschiedenen Frequenzen tetrapolar durchgeführt. Es dient so der Ermittlung des Gesamtkörperwassers (TBW), der Fettmasse (FM), der Magermasse (LBM), der zellulären und extrazellulären Körperbestandteile (BCM,ECM).

Die weiteren Untersuchungen, die nötig sind, führt der folgende Visitenplan auf.

Dieser Plan zeigt Ihnen, wann welche Untersuchungen geplant sind.

Liebe Patientinnen,

wir freuen uns, dass Sie sich zur Teilnahme an der Studie entschlossen haben. Wir hoffen, dass Sie Ihnen Erfolg bringen wird und wünschen Ihnen Alles Gute.

Wenn Sie Fragen haben, stehen wir Ihnen jederzeit zur Verfügung. Sie erreichen uns telefonisch unter der Nummer 030- 450-56-4287.

Unser Team besteht aus:

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Table 19: Visitenplan (Übersicht)

Rd.-Nr. Name, Vorname, Geburtsdatum Anschrift Tel.-Nr.:						
	Sprechstunde	Woche 0	Woche 4	Woche 8	Woche 12	Woche 16
	Visite 0	Visite 1	Visite 2	Visite 3	Visite 4	Visite 5
Datum						
Prüfung Ein-/Aus-schlusskriterien	X					

Demogr. Daten	X					
Anamnese	X					
Dokumentation Begleiterkrankungen, Medikamente	X	X	X	X	X	X
Dokumentation un-erwünschte Ereignisse		X	X	X	X	X
Körperl. Status	X					X
Messung Körperhöhe, Körpergewicht, Taillenumfang, Hüftumfang, Berechnung BMI, WHR	X	X	X	X	X	X
Messung Blutdruck, Herzfrequenz	X	X	X	X	X	X
Sonogr. Kontrolle	X	X	X	X	X	X
Routinelabor (Na, K, Ca, Krea, HST, ASAT, ALAT, GGT, Urinstik)	X					X

Labor (weib./ männl. Geschl. hormone, Thyroidhormone. Leptin, IGF, G-Chol., HDL-Chol., LDL-Chol., Gluc., HbA1c, oGTT + Insulin).		X		X		X
BIA		X		X		X
Ernährungsanalyse		X				X
Aufklärungsgruppengespräche (Ernährung, Bewegung)		X	X	X	X	X