

## Original Article

## Metformin Therapy in Girls with Polycystic Ovary Syndrome: A Self-Controlled Clinical Trial

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**Background:** Polycystic ovary syndrome is not uncommon in women of reproductive age. We conducted this study to determine the clinical, hormonal, and biochemical effects of metformin therapy on girls with polycystic ovary syndrome.

**Methods:** In a self-controlled clinical trial conducted at the Gynecology Center of Yahyanejad Hospital, affiliated to Babol University of Medical Sciences, Babol, North of Iran, 36 girls with polycystic ovary syndrome were studied. The patients were treated with 500 mg of metformin three times a day for six months. Clinical symptoms, menstrual pattern, and hirsutism, as well as serum concentration of sex steroid (follicular stimulating hormone, luteinizing hormone, dihydroepiandrosterone, testosterone, 17-OH progesterone, estradiol), and lipids (triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein) were assessed pre- and posttreatment.

**Results:** Sixteen girls (53% of those with menstrual disturbances) experienced more regular cycles during the treatment. A significant decrease in weight ( $P = 0.004$ ) and body mass index ( $P = 0.006$ ) was noticed after six months of treatment. The number of patients with hirsutism was also decreased. The mean levels of triglyceride ( $P = 0.03$ ) and low-density lipoprotein ( $P = 0.001$ ) were decreased while high density lipoprotein ( $P = 0.003$ ) was increased significantly. We also found a significant decrease in luteinizing hormone ( $P = 0.006$ ), estradiol ( $P = 0.005$ ), and dihydroepiandrosterone ( $P = 0.026$ ) after treatment. Changes in the follicular stimulating hormone and 17-OH progesterone levels were not significant.

**Conclusion:** Metformin therapy has many useful effects in these patients.

*Archives of Iranian Medicine, Volume 10, Number 2, 2007: 176 – 181.*

**Keywords:** Hirsutism • metformin • polycystic ovary syndrome • obesity

### Introduction

Polycystic ovary syndrome (PCOS), with an incidence of 5 – 10% in women of reproductive age,<sup>1</sup> is characterized by anovulation, infertility, hyperandrogenism, and clinical manifestations of irregular menstrual cycles, hirsutism, obesity, and acne, thus PCOS resembles different metabolic syndromes such as type 2 diabetes mellitus, lipid profile disorders, or atherosclerosis.<sup>2</sup>

Metabolic disturbances such as elevated serum

concentration of luteinizing hormone (LH), testosterone, insulin, and prolactin are common. By the age of 40, up to 40% of patients would have type 2 diabetes mellitus or impaired glucose tolerance.<sup>3</sup> PCOS is therefore an important health-care problem affecting young women nowadays.<sup>4</sup> In these patients, the chronic hyperinsulinemia leads to over-production of ovarian androgens, which cause chronic anovulation, menstrual disturbances, and hirsutism.<sup>5</sup> The associated insulin resistance increases the risk of developing glucose intolerance, type 2 diabetes mellitus, and gestational diabetes mellitus.<sup>6–8</sup>

As a consequence, drugs that reduce insulin levels by improving sensitivity to insulin may provide a new therapeutic option for patients with PCOS. Metformin, an oral biguanide-insulin sensitizing agent, acts by suppressing hepatic

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Accepted for publication: 16 August 2006

gluconeogenesis accompanied by a reducing circulating insulin levels and improving hyperandrogenemia.<sup>9-11</sup>

The most common side-effect of metformin is gastrointestinal disturbance. This can be reduced by taking metformin with meals and increasing the dosage up to the effective dose of 1.5 – 2 g/day slowly. Lactic acidosis is another side-effect, which is extremely rare.<sup>12</sup>

Vrbikova et al reported that after six months of treatment with metformin (1 g/day), 58% of patients showed a significant improvement in their menstrual cycle pattern. Also, a significant reduction in gonadotropin-releasing hormone analogue and stimulated levels of testosterone ( $P < 0.05$ ), LH ( $P < 0.05$ ), and estradiol ( $P < 0.01$ ) were found.<sup>13</sup>

Moggetti et al evaluated the effects of long-term administration of metformin (500 mg three times a day) on obese (with a mean  $\pm$  SD body mass index [BMI] of  $30.0 \pm 1$  kg/m<sup>2</sup>) patients with PCOS who maintained their usual dietary habits. In about 50% of subjects improvement of menstrual abnormalities was noticed. Women receiving metformin showed a reduction in plasma insulin levels and an increase in sensitivity to insulin. Concurrently, ovarian hyperandrogenism was attenuated as indicated by significant reduction in the serum free testosterone level after stimulation test. In subjects with regular menses, 80% of cycles were ovulatory after the treatment but only minor changes were found in BMI.<sup>14</sup>

Patients with PCOS may or may not be insulin-resistant. Insulin resistance may explain the effectiveness of metformin in certain populations such as Venezuelan or Finnish women, but not in American or Turkish ones.<sup>15</sup>

These kinds of studies have been mostly performed on infertile patients (induction of ovulation) and not on unmarried girls; so, we decided to evaluate the effect of metformin on single girls suffering from PCOS.

## Patients and Methods

This self-controlled clinical trial was conducted on 36 girls with PCOS attending to the Gynecology Clinic of Yahyanejad Hospital, affiliated to Babol University of Medical Sciences, Babol, North of Iran, from April 2001 through April 2002. The study protocol was approved by the Ethics Committee of Babol University of Medical

Sciences.

The girls with at least three of the following criteria were included in this study:

- Menstrual disturbances (oligomenorrhea, amenorrhea, and polymenorrhea).
- Obesity, hirsutism, and acne.
- A LH to follicular stimulating hormone (FSH) ratio of  $>3$ .

Polycystic ovary was confirmed by abdominal ultrasonography in all patients. All ultrasonographies were performed by one gynecologist with an Aloka ultrasonography unit (Aloka 500, Japan) with a 5 MHz probe. After obtaining a written consent from each patient, they were treated with metformin for six months.

Hirsutism was evaluated using Ferriman-Galloway criteria. Nine body areas were assessed and classified as: no (0), minimal (1), moderate (2), severe (3), marked, and very severe hirsutism.<sup>4</sup>

The BMI was calculated by dividing the weight in kilograms by squared height in meters. A BMI of  $<25$  was considered normal; BMI of 25 – 29 was considered “overweight”; and a BMI of  $>30$  was considered “obese.”

A menstrual cycle was considered as normal if it lasted from 21 to 35 days with less than seven days of bleeding. An infrequent, irregularly timed episode of bleeding and very light menstrual flow was considered as “oligomenorrhea.” A frequent but regularly-timed episode of bleeding with normal amount of bleeding was considered as “polymenorrhea.” Amenorrhea was defined as the absence of menstruation for three normal cycles or six months in those who had previously normal menstrual cycles.

Biochemical laboratory tests included fasting blood sugar (FBS), 2-hour glucose tolerance test, fasting insulin, triglyceride (TG), total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol.

Hormonal assays were performed before the treatment and at the beginning of the follicular phase. They included FSH, LH, dihydroepiandrosterone (DHEA), testosterone, 17-OH progesterone, and estradiol. These tests were done using radioimmunoassay method. All laboratory tests were repeated six months after the beginning of the treatment with metformin to determine the effect of this drug on hormonal and biochemical parameters and on clinical presentations. All tests were done in the same laboratory using the same kits.

We explained the side-effects of the drug and duration of the treatment for all patients. The treatment was initiated after confirmation of a normal renal and hepatic function.

The patients were treated with Apometformin® (Apotex, Canada) 500 mg three times a day. To prevent the side-effects of the drug, we prescribed one tablet daily in the first week, two tablets in the second, and three tablets in the third week.

The patients who had taken other medications (e.g., oral contraceptive pills) or had changed their lifestyle or nutritional habits (e.g., weight loss) were excluded from the study.

Data were encoded and analyzed by SPSS. Paired sample Student's *t*- and McNemar tests were used to compare before and after treatments. A *P* value of < 0.005 was considered statistically significant.

## Results

Of 36 patients found eligible to enter the study, six were excluded from the study; two for nausea and vomiting and drug intolerance, two for consumption of oral contraceptive pills, and two for lack of compliance. Thirty patients completed the study.

The patients had a age of  $22.5 \pm 4.0$  (mean  $\pm$  SD) (range: 15 – 29) years. The height of them was  $63.4 \pm 3.8$  cm. The weight, waist circumference, and BMI before and after treatment with metformin are shown in Table 1.

Metformin treatment induced normal menstrual cycles in 67% of the patients (Table 2). The number of patients with hirsutism and the severity of the disease was significant reduced after metformin therapy ( $P = 0.026$ ). Before the treatment, 7% of the patients had no hirsutism; 13% had mild, 50% moderate, and 30% severe hirsutism. After the metformin therapy, 20% of the participants had no hirsutism; 60% had mild, and 20% had moderate hirsutism — no one had severe hirsutism.

Metformin treatment also ameliorated acne (20 patients before the treatment vs. 8 after metformin therapy;  $P = 0.003$ ) and ultrasonographic features

**Table 2.** Menstrual abnormalities before and after treatment with metformin.

Menstrual cycle	Number of patients (%)		<i>P</i> value
	Before treatment	After treatment	
Regular	4 (13)	20 (67)	0.0004
Oligomenorrhea	9 (30)	4 (13)	0.063
Amenorrhea	14 (47)	5 (17)	0.004
Polymenorrhea	3 (10)	1 (3)	0.5

of PCOS (30 vs. 14 patients).

The fasting insulin dropped from  $24.7 \pm 12.4$  to  $19.5 \pm 8.0$  mIU/mL ( $P = 0.004$ ), with a reduction of  $5.3 \pm 6.9$  mIU/mL. The FBS also dropped from  $89.7 \pm 17.4$  to  $83.8 \pm 11.0$  mg/dL ( $P = 0.037$ ), with a reduction of  $5.9 \pm 4.3$  mg/dL. The mean 2-hour glucose level decreased from  $114.0 \pm 30.0$  to  $112.3 \pm 29.3$  mg/dL ( $P = 0.52$ ).

The mean levels of TG, total and LDL cholesterol had significant reduction; the mean level of HDL cholesterol was increased significantly (Table 3).

When FBS to fasting insulin ratio is <4.5, resistance to insulin is present. Before the treatment, we had insulin resistance in 19 patients; insulin resistance remained in 16 patients after metformin therapy ( $P = 0.25$ ).

A significant reduction in the serum level of LH, DHEA, and testosterone was observed after the treatment ( $P < 0.05$ ). No significant changes in FSH and 17-OH progesterone levels were observed (Table 4).

## Discussion

PCOS is a common endocrine disorder that affects many women in reproductive ages. Recognition of insulin resistance as an important factor in the pathogenesis of PCOS causes vast use of insulin-lowering agents called insulin-sensitizing agents. Metformin is the most common insulin-lowering agent used in the treatment of PCOS.

In our study, 30 unmarried girls with PCOS were treated with metformin 500 mg three times a day, the same dose administered as in most studies.<sup>16–19</sup> Acbay and Gundogdu,<sup>20</sup> Ehrmann et al,<sup>21</sup> and Diamanti-Kandarakis et al,<sup>22</sup> however, prescribed a higher dose of 850 mg, two or three

**Table 1.** Weight, waist circumference, and BMI before and after metformin therapy.

Parameter	Mean $\pm$ SD			<i>P</i> value
	Before treatment	After treatment	Difference	
Weight (kg)	$73.56 \pm 11.82$	$71.13 \pm 11.22$	$2.47 \pm 16.3$	0.004
Waist circumference (cm)	$85.6 \pm 13.3$	$84 \pm 12.5$	$1.1 \pm 18.2$	0.003
BMI (kg/m <sup>2</sup> )	$27.5 \pm 4.4$	$26.6 \pm 4.1$	$0.9 \pm 1$	0.006

**Table 3.** Changes in the blood lipid levels before and after treatment with metformin.

Parameter (mg/dL)	Mean $\pm$ SD		Mean $\pm$ SD difference	P value
	Before treatment	After treatment		
Cholesterol	203.5 $\pm$ 47.3	189.5 $\pm$ 40.7	14 $\pm$ 17.9	0.008
TG	151.4 $\pm$ 83.9	140.9 $\pm$ 67.52	10.5 $\pm$ 26.6	0.039
LDL	124.4 $\pm$ 34.7	117.3 $\pm$ 32.9	7.1 $\pm$ 14.1	0.01
HDL	37 $\pm$ 10.1	48.7 $\pm$ 13	11.6 $\pm$ 11.6	0.003

times a day.

In our study, the mean weight loss was 2.47 kg and the mean change in BMI was 0.89 kg/m<sup>2</sup>. Crave et al<sup>23</sup> and Kelly and Gordon<sup>24</sup> also reported improved weight loss with metformin. Some researchers<sup>17, 25, 26</sup> mentioned a little reduction in BMI, albeit statistically significant, but others<sup>19, 20, 22</sup> did not report significant changes in BMI.

Pouliot et al showed that a waist circumference of >90 cm in women is predictive of abnormal endocrinology and metabolic function and is associated with increasing risk of cardiovascular diseases.<sup>27</sup> In our study, the mean change in waist circumference was 1.1 cm. Velazquez et al<sup>17</sup> reported a reduction in waist circumference after metformin therapy, but Morin-Papunen et al<sup>19</sup> and Acbay and Gundogdu<sup>20</sup> did not show any changes in waist circumference after treatment with metformin. According to the significant reduction of weight loss, BMI, and specially waist circumference with metformin, this drug can play a role as a preventive agent in cardiovascular disease and metabolic disturbances.

In our study, the mean fasting insulin and FBS reduced significantly. However, there was no significant difference in oral glucose tolerance test before and after the treatment. Most studies agree with the decrease in insulin level after metformin therapy.<sup>17 - 19</sup> But Acbay and Gundogdu, and Ehrmann et al reported no change in the insulin level with this treatment.<sup>20, 21</sup> Kazerooni and Dehghan-Kooshkyhazi reported a significant reduction in FBS.<sup>25</sup> Morin-Papunen et al's study, published in 2003, reported that metformin had no effect on glucose tolerance but it caused significant reduction in fasting insulin,<sup>28</sup> which are in keeping with our findings. Nevertheless, Nestler and

Jakubowicz showed that along with reduction of fasting insulin, patient's glucose tolerance was also reduced markedly.<sup>16</sup>

In one study, metformin administration had no effect on insulin resistance in very obese women with PCOS.<sup>29</sup> In the women with anovulation and hyperinsulinemia, metformin therapy decreased hyperandrogenism, but made no changes in body weight; although, the reduction of waist to hip ratio was associated with reduction of hyperinsulinemia. Studies have shown that obese and nonobese hyperinsulinemic patients respond to metformin treatment.<sup>29</sup>

Hyperinsulinemia increases the risk of cardiovascular disease indirectly by its atherogenic activity and directly by its adverse effects on lipid profile. Women with anovulation, hyperandrogenism, and hyperinsulinemia are at a great risk of developing noninsulin-dependent diabetes mellitus and the onset of the disease is approximately 30 years earlier than the general population. Therefore, control of blood sugar and lipid profile in these patients in premenopausal ages is very important.

Morin-Papunen et al and Acbay and Gundogdu demonstrated no changes in lipid profiles with metformin.<sup>19, 20</sup> Chou et al prescribed metformin to 30 obese, nondiabetic women with PCOS. After 90 days of treatment, significant reduction in total cholesterol level was observed,<sup>30</sup> which is in agreement with our results.

The lipid profile in androgenized women with PCOS is the same as men, i.e., higher levels of TG, total and LDL cholesterol, and lower levels of HDL, which is independent of body weight.

In our study, the LH level was significantly reduced after the treatment ( $P = 0.006$ ); no

**Table 4.** Changes in the hormone levels before and after treatment with metformin.

Parameter	Mean $\pm$ SD		Mean $\pm$ SD difference	P value
	Before treatment	After treatment		
LH (mIU/mL)	11.2 $\pm$ 5	9.6 $\pm$ 4.3	1.6 $\pm$ 2.21	0.006
FSH (mIU/mL)	5.8 $\pm$ 3.9	5.2 $\pm$ 2.9	0.6 $\pm$ 3.2	0.149
DHEA( $\mu$ g/mL)	3.5 $\pm$ 1.5	2.9 $\pm$ 1.29	0.6 $\pm$ 0.8	0.001
Testosterone (ng/mL)	2.1 $\pm$ 1.8	1.5 $\pm$ 1.2	0.6 $\pm$ 0.9	0.001
17-OH progesterone (ng/mL)	1.8 $\pm$ 1.3	1.6 $\pm$ 1.1	0.2 $\pm$ 3.4	0.36
Estradiol (pg/mL)	90.4 $\pm$ 37.6	80.2 $\pm$ 36.6	0.6 $\pm$ 1.1	0.005

significant change was observed in the FSH level ( $P = 0.149$ ). Kazerooni and Dehghan-Kooshkyhazi demonstrated that metformin treatment has no effect on FSH and LH levels,<sup>25</sup> whereas Nestler and Jakubowicz, and Velazquez et al showed a significant reduction in LH level.<sup>16, 17</sup>

We found a significant reduction in the testosterone ( $P = 0.005$ ), DHEA ( $P = 0.0001$ ), and estradiol ( $P = 0.001$ ) levels after metformin therapy, but we did not find any significant differences in the 17-OH progesterone level before and after the treatment ( $P = 0.36$ ) (Table 3).

Kazerooni and Dehghan-Kooshkyhazi reported no change in DHEA level, but Velazquez et al reported a significant reduction in androgen levels, and denoted a significant reduction in circulatory estradiol level in patients taking metformin.<sup>25</sup>

The patients with acne improved remarkably after the treatment ( $P = 0.003$ ). Moreover, 20 of the patients developed regular menses ( $P = 0.008$ ). Casmiri et al, Morin-Papunen et al, and Kazerooni and Dehghan-Kooshkyhazi demonstrated improvements in acne status and menstrual cycles after metformin therapy.<sup>18, 19, 25</sup>

In our study, metformin therapy was very effective on hirsutism. Four patients were completely cured, and after the treatment, no one had very severe hirsutism ( $P = 0.026$ ). In addition to abnormal uterine bleeding, amenorrhea, hirsutism, acne, and unopposed estrogen effect put the patient in a remarkable risk of endometrial and probably breast cancers. The risk of endometrial cancer increases up to three folds in these patients; therefore, it is very important to reduce circulatory androgen levels with a drug.

General goals of treatment in unmarried girls with PCOS include reducing the production and circulatory levels of androgens, protection of endometrium against unopposed estrogen, reducing the risk factors of cardiovascular disease, and avoiding hyperinsulinemic effects on the development of cardiovascular diseases and diabetes mellitus. It seems that metformin can provide all these goals.

With regards to the above-mentioned studies, variable effects of metformin on PCOS were reported. As these studies differ in their inclusion criteria, ethnic background, method of measuring insulin sensitivity, as well as the BMI of the patients and dosage and length of treatment of metformin, it seems difficult to compare them. The results of our study in the northern part of Iran proved that metformin has many useful effects on

these patients. However, for long-term treatment with metformin, more investigations are required. Since the type of patients with PCOS may differ, it is important, in future investigations, to look over different points including lean vs. obese, the degree of hyperandrogenemia, and the way insulin resistance is measured.

## References

- 1 Fleming R, Hopkinson ZE, Wallace AM, Greer IA, Sattar N. Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double-blind placebo-controlled trial. *J Clin Endocrinol Metab.* 2002; **87**: 569 – 574.
- 2 Hahn S, Quadbeck B, Elsenbruch S, Gartner R, Finke R, Mann K, et al. Metformin, an efficacious drug in the treatment of polycystic ovary syndrome [in German]. *Dtsch Med Wochenschr.* 2004; **129**: 1059 – 1064.
- 3 Legro RS. Diabetes prevalence and risk factors in polycystic ovary syndrome. *Obstet Gynecol Clin North Am.* 2001; **28**: 99 – 109.
- 4 Lord JM, Flight IH, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *BMJ.* 2003; **327**: 951 – 953.
- 5 Barbieri RL, Smith S, Ryan KJ. The role of hyperinsulinemia in the pathogenesis of ovarian hyperandrogenism. *Fertil Steril.* 1988; **50**: 197 – 212.
- 6 Dahlgren E, Janson PO, Johansson S, Lapidus L, Oden A. Polycystic ovary syndrome and risk for myocardial infarction. Evaluated from a risk factor model based on a prospective population study of women. *Acta Obstet Gynecol Scand.* 1992; **71**: 599 – 604.
- 7 Franks S, Gilling-Smith C, Watson H, Willis D. Insulin action in the normal and polycystic ovary. *Endocrinol Metab Clin North Am.* 1999; **28**: 361 – 378.
- 8 Legro RS, Kunesman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab.* 1999; **84**: 165 – 169.
- 9 Velazquez E, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. *Obstet Gynecol.* 1997; **90**: 392 – 395.
- 10 Wollen N, Bailey CJ. Metformin potentiates the antigluconeogenic action of insulin. *Diabete Metab.* 1988; **14**: 88 – 91.
- 11 Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. *N Engl J Med.* 1998; **338**: 1876 – 1880.
- 12 Stadtmayer LA, Wong BC, Oehninger S. Should patients with polycystic ovary syndrome be treated with metformin? Benefits of insulin sensitizing drugs in polycystic ovary syndrome--beyond ovulation induction. *Hum Reprod.* 2002; **17**: 3016 – 3026.
- 13 Vrbikova J, Hill M, Starka L, Vondra K. Prediction of the effect of metformin treatment in patients with polycystic ovary syndrome. *Gynecol Obstet Invest.* 2002; **53**: 100 – 104.
- 14 Moghetti P, Castello R, Negri C, Tosi F, Perrone F,

- Caputo M, et al. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J Clin Endocrinol Metab.* 2000; **85**: 139 – 146.
- 15 Homburg R. *Polycystic Ovary Syndrome*. London: Martin Dunitz; Malden, MA: Distributed in the U.S. by Blackwell Science; 2001.
  - 16 Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *N Engl J Med.* 1996; **335**: 617 – 623.
  - 17 Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism.* 1994; **43**: 647 – 654.
  - 18 Casmiri F, Bisscotti M, Gambineri S. Metformin improves insulin, body fat distribution, and androgen in obese women with and without the polycystic ovary syndrome. *Int Obsity.* 1997; **3**: 561.
  - 19 Morin-Papunen L, Koviunen R, Ruokenon A. Metformin therapy improves menstrual pattern of endocrine and metabolic effect in women with polycystic ovary syndrome. *Fertil Steril.* 1998; **69**: 691 – 696.
  - 20 Acbay O, Gundogdu S. Can metformin reduce insulin resistance in polycystic ovary syndrome? *Fertil Steril.* 1996; **65**: 946 – 949.
  - 21 Ehrmann DA, Cavaghan MK, Imperial J, Sturis J, Rosenfield RL, Polonsky KS. Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1997; **82**: 524 – 530.
  - 22 Diamanti-Kandarakis E, Kouli C, Tsianateli T, Bergiele A. Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. *Eur J Endocrinol.* 1998; **138**: 269 – 274.
  - 23 Crave JC, Fimbel S, Lejelane H. Effect of diet and metformin administration on sex hormone binding globulin, androgens, and insulin in hirsute and obese women. *J Clin Endocrinol Metab.* 1995; **80**: 2057 – 2062.
  - 24 Kelly CS, Gordon S. The effect of metformin on hirsutism in polycystic ovary syndrome. *Eur J Endocrinol.* 2002; **147**: 217 – 221.
  - 25 Kazerooni T, Dehghan-Kooshkyhazi M. Effects of metformin therapy on hyperandrogenism in women with polycystic ovarian syndrome. *Gynecol Endocrinol.* 2003; **17**: 51 – 96.
  - 26 Barbieri RL. Metformin for the treatment of polycystic ovary syndrome. *Obstet Gynecol.* 2003; **101**: 785 – 793.
  - 27 Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol.* 1994; **73**: 460 – 468.
  - 28 Morin-Papunen L, Vauhkonen I, Koivunen R, Ruokonen A, Martikainen H, Tapanainen JS. Metformin versus ethinyl estradiol-cyproterone acetate in the treatment of nonobese women with polycystic ovary syndrome. A randomized study. *J Clin Endocrinol Metab.* 2003; **88**: 148 – 156.
  - 29 Kumari AS, Haq A, Jayasundaram R. Metformin monotherapy in lean women with polycystic ovary syndrome. *Reprod Biomed.* 2005; **10**: 100 – 104.
  - 30 Chou KH, von Eye Corleta H, Capp E, Spritzer PM. Clinical, metabolic and endocrine parameters in response to metformin in obese women with polycystic ovary syndrome: a randomized, double-blind, and placebo-controlled trial. *Horm Metab Res.* 2003; **35**: 86 – 91.