

Does adding metformin to clomifene citrate lead to higher pregnancy rates in a subset of women with polycystic ovary syndrome?

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BACKGROUND: An RCT among newly diagnosed, therapy naive women with polycystic ovary syndrome (PCOS) showed no significant differences in ovulation rate, ongoing pregnancy rate or spontaneous abortion rate in favour of clomifene citrate plus metformin compared with clomifene citrate. We wanted to assess whether there are specific subgroups of women with PCOS in whom clomifene citrate plus metformin leads to higher pregnancy rates. **METHODS:** Subgroup analysis based on clinical and biochemical parameters of 111 women randomized to clomifene citrate plus metformin compared with 114 women randomized to clomifene citrate plus placebo. The data for age, BMI, waist–hip ratio (WHR) and plasma testosterone were available in all women, 2 h glucose in 80% of women and homeostatic model assessment for assessing insulin sensitivity (HOMA) in 50% of women. **RESULTS:** Of the women who were allocated to the metformin group, 44 women (40%) reached an ongoing pregnancy. In the placebo group, 52 women (46%) reached an ongoing pregnancy. There was a significantly different chance of an ongoing pregnancy for metformin versus placebo between subgroups based on age and WHR ($P = 0.014$). There was a positive effect of metformin versus placebo on pregnancy rate in older women (≥ 28 years) with a high WHR, a negative effect of metformin versus placebo in young women (< 28 years) regardless of their WHR and no effect in older, not viscerally obese women. No significant differences in effect of treatment were found for groups based on BMI, 2 h glucose, HOMA or plasma testosterone. **CONCLUSIONS:** Metformin may be an effective addition to clomifene citrate in infertile women with PCOS, especially in older and viscerally obese patients.

Keywords: clomifene; metformin; polycystic ovary syndrome; pregnancy; subgroup analysis

Introduction

Polycystic ovary syndrome (PCOS) is characterized by oligo-anovulation, clinical or biochemical hyperandrogenism and/or polycystic ovaries (Franks, 1995; Knochenhauer *et al.*, 1998; Fauser, 2004). Insulin resistance (IR) accompanied by compensatory hyperinsulinaemia constitutes another major biochemical feature of PCOS (Nestler and Jakubowicz, 1996; Dunaif, 1997; Nestler, 1997; Jakubowicz *et al.*, 2001; Fleming *et al.*, 2002). The syndrome affects 5–10% of women during reproductive age (Asuncion *et al.*, 2000).

Because of the link between IR and PCOS, metformin has been put forward as a drug to induce ovulation in women

with PCOS. In fact, metformin either alone or in combination with clomifene citrate is now the most widely used insulin-sensitizer for ovulation induction in women with PCOS (Nestler, 2002; Glueck *et al.*, 2003; ASRM, 2004). The rationale of this treatment was based on only a few small studies with conflicting results (Nestler *et al.*, 1998; El-Biely and Habba, 2001).

Recently, we demonstrated in an RCT among newly diagnosed, therapy naive women with PCOS that there were no significant differences in either ovulation rate, ongoing pregnancy rate or spontaneous abortion rate in women using clomifene citrate plus metformin compared with women using clomifene

citrate (Moll *et al.*, 2006). Since then, our results considering ongoing pregnancy rates have been confirmed in another large RCT (Legro *et al.*, 2007).

However, PCOS is a heterogeneous condition and thus both studies included a heterogeneous group of women. Surprisingly, there is only one small study involving 32 patients with PCOS that has investigated the effect of metformin in specific subgroups. This study showed in a multivariable analysis that higher insulin, lower androstenedione and less severe cycle abnormalities appeared to be independent significant parameters for better response to metformin (Moggetti *et al.*, 2000).

Increasing age and high waist–hip ratio (WHR) are known risk factors in developing IR and, as such, these factors may also affect clinical response to metformin (Despres, 1993; Legro *et al.*, 1999; Pasquali *et al.*, 2000; NCEP Expert Panel, 2001; Laine and Wilson, 2007). Apart from these parameters, some authors suggest that metformin would most likely be beneficial in women with high BMI (Lord, *et al.*, 2003).

Thus, although many investigators claim a beneficial effect of metformin in specific subgroups of women, the evidence is limited.

We therefore wanted to determine whether co-treatment with metformin improves pregnancy rates compared with the standard treatment of clomifene citrate alone in subgroups of women based on clinical and biochemical variables. For this purpose, we reanalysed the data from a previously published randomized trial.

Materials and Methods

The data used in this study were collected in an RCT that has been reported elsewhere (Moll *et al.*, 2006). This double blinded trial took place from June 2001 to May 2004 in 20 Dutch hospitals. All patients with chronic anovulation (a menstrual cycle ≥ 35 days), World Health Organization type II criteria (normogonadotrophic normoestrogenic oligo- or anovulation), polycystic ovaries diagnosed by transvaginal ultrasonography and a desire to conceive were randomly allocated to clomifene citrate (50–150 mg a day for five consecutive days in the beginning of a menstrual cycle, per os) plus metformin (500 mg 4/day) or to clomifene citrate plus placebo. Primary exclusion criteria were other causes of anovulation, age over 40 years and liver, kidney or heart disease/failure (i.e. abnormal results on liver function tests, serum creatinine concentration $>95 \mu\text{mol/l}$ or a history of heart disease/failure) and sperm quality indicating male subfertility (total motile count $<10 \times 10^6$).

Randomization was carried out in the coordinating centre (AMC, Amsterdam), using computer-generated blocks of four. The containers with study medication were prepared by Merck Santé, France. The randomization was stratified per centre, and the centres received blinded, numbered containers with medication. Each included patient received the container with the next number in their own hospital.

Patients continued to take the study medication until a positive pregnancy test (4 weeks after the first day of menstruation), six ovulatory cycles or clomifene citrate resistance occurred, whichever came first. Women had to discontinue their medication as soon as they had a positive pregnancy test as the safety and benefit of using metformin during pregnancy have not yet been proven.

Ongoing pregnancy was defined as a viable pregnancy at 12 weeks of gestation. The study was approved by the Institutional Review

Boards of all hospitals. Written informed consent was obtained from all participants.

At the start of the study, we measured height, weight, WHR and plasma testosterone. Testosterone was measured in several laboratories. Total plasma testosterone concentrations were determined using an in-house radioimmunoassay, without extraction and chromatography and with tritiated testosterone as label (Pratt *et al.*, 1975). Intra-assay and inter-assay coefficients of variation were 4–7% and 5–9%, respectively. An oral glucose tolerance test was also performed. Subgroup analysis was performed on age, BMI, WHR, testosterone, 2 h glucose and HOMA (homeostatic model assessment for assessing insulin sensitivity) as a measure of IR (Ehrmann *et al.*, 1999; Legro *et al.*, 1999, 2004; NCEP Expert Panel, 2001; Fauser, 2004; Samaras *et al.*, 2006). Since not all data were available for all women, in some analyses only a proportion of the women could be included.

The subgroup analysis which we present here was planned in the original protocol. The parameters to be analysed were chosen, after completion of the trial, according to the most recent literature (Despres, 1993; Legro *et al.*, 1999; Moggetti *et al.*, 2000; Pasquali *et al.*, 2000; NCEP Expert Panel, 2001; Lord, *et al.*, 2003; Laine and Wilson, 2007).

Data analysis

For this subgroup analysis, we explored differences in treatment effect on ongoing pregnancy rate in subgroups defined on age, BMI, WHR, plasma testosterone, 2 h glucose level and IR evaluated by HOMA-IR. For this purpose, we performed logistic regression analysis including treatment, the subgroup indicator and the interaction between treatment and subgroup.

We assessed the linearity of the association between the continuous variables age, BMI, WHR, testosterone, 2 h glucose level and HOMA and the ongoing pregnancy rate using spline functions (Harrell *et al.*, 1988). Non-linear associations were accommodated by redefining the corresponding variables.

By testing the interaction in logistic regression, we compared directly the effect sizes of treatment for the subgroups. If the interaction is statistically significant, this is a clear indication that the effect of treatment differs between subgroups (Matthews and Altman, 1996).

Next, to explore potential interesting subgroup features, we combined subgroups for which the interaction with treatment was $P < 0.20$. In all analyses, P -values < 0.05 were considered significant. Data were analysed using the Statistical Package for the Social Sciences 11.5.1.

Results

Baseline characteristics are presented in Table I. Out of the 111 women in the clomifene citrate plus metformin group, 44 (40%) reached an ongoing pregnancy versus 52 (46%) of the 114 women in the clomifene citrate group, a difference which was not significant [relative risk (RR) 0.87; 95% confidence interval (CI) 0.64–1.2]. A significantly larger proportion of patients in the metformin group discontinued treatment because of side effects (18/111 versus 6/114; RR 2.9; 95% CI 1.2–7.1). The total dropout rate in both treatment arms was not statistically different (28/111 versus 21/114; RR 1.3; 95% CI 0.82–2.2).

The data for age, BMI, WHR and testosterone levels were complete. The data for 2 h glucose level were available in 80% of patients, and for HOMA, in 50% of patients.

Table I. Baseline characteristics of patients with PCOS who were treated with clomifene citrate plus metformin or clomifene citrate alone. Figures are mean (SD) unless stated otherwise.

Characteristics	<i>n</i> (metformin versus placebo)	Clomifene citrate and metformin	Clomifene citrate and placebo
Total	111 versus 114		
Age (years)	111 versus 114	27.9 (3.7)	28.4 (4.7)
Parity <i>N</i> (%)			
Nulliparous	89 versus 95	89 (80%)	95 (84%)
Multiparous	22 versus 18	22 (20%)	18 (16%)
Duration of desire to conceive (years)	100 versus 107	1.6 (1.2)	1.3 (1.1)
BMI (kg/m ²)	104 versus 109	28 (7.1)	27 (6.7)
WHR	102 versus 103	0.82 (0.1)	0.83 (0.1)
LH/FSH ratio	106 versus 109	1.9 (1.3)	2.1 (1.4)
Testosterone (nmol/l)	104 versus 108	3.5 (3.7)	3.6 (3.5)
Free androgen index	70 versus 69	12 (18)	12 (12)
Volume ovaries (ml)	71 versus 65	9.1 (6.1)	10.1 (4.9)
Total motile sperm count ($\times 10^6$)	102 versus 105	134 (223)	222 (370)
Glucose, fasting (mmol/l)	101 versus 99	4.9 (0.7)	4.9 (0.6)
Glucose, 2 h (mmol/l)	91 versus 90	5.0 (1.4)	5.1 (1.3)
Insulin, fasting (pmol/l)	56 versus 59	85 (169)	60 (131)
Insulin, 2 h (pmol/l)	58 versus 57	233 (274)	270 (386)
HOMA	56 versus 57	11 (12)	10 (11)

HOMA, homeostatic model assessment for assessing insulin sensitivity; WHR, waist–hip ratio.

Table II. Pregnancy rates for subgroups of women with PCOS treated with clomifene citrate plus metformin or clomifene citrate alone.

	<i>N</i>	Metformin <i>n/N</i> (%)	Placebo <i>n/N</i> (%)	RR (95% CI)	<i>P</i> -value of interaction
Overall	225	44/111	52/114	0.87 (0.64–1.2)	—
Age (years) < 28 years	91	13/48 (27)	19/43 (44)	0.61 (0.35–1.1)	0.11
Age (years) \geq 28 years	134	31/63 (49)	33/71 (47)	1.1 (0.74–1.5)	
BMI (kg/m ²) < 28	118	27/56 (48)	32/62 (52)	0.93 (0.65–1.3)	0.23
BMI (kg/m ²) \geq 28	95	16/48 (30)	19/47 (40)	0.82 (0.49–1.4)	
WHR < 0.85	134	33/70 (47)	37/64 (58)	0.82 (0.59–1.1)	0.012
WHR \geq 0.85	71	10/32 (31)	11/39 (28)	1.1 (0.54–2.3)	
Testosterone (nmol/l) \leq 3.5	143	30/71 (42)	34/72 (47)	0.89 (0.62–1.3)	0.87
Testosterone (nmol/l) > 3.5	69	13/33 (39)	15/36 (42)	0.95 (0.53–1.7)	
Glucose 2 hours (mmol/l) \leq 5	101	22/48 (46)	28/53 (53)	0.87 (0.58–1.3)	0.22
Glucose 2 hours (mmol/l) > 5	80	16/43 (37)	12/37 (32)	1.1 (0.63–2.1)	
HOMA < 3.8	42	10/23 (44)	6/19 (32)	1.4 (0.61–3.1)	0.88
HOMA \geq 3.8	71	13/33 (39)	14/38 (37)	1.1 (0.59–1.9)	

RR, relative risk; CI, confidence interval.

In women \geq 28 years of age, the effect of metformin versus placebo showed a small trend towards a different effect compared with the effect of metformin versus placebo in women < 28 years (*P*-value of interaction 0.11) (Table II). In women with a WHR \geq 0.85, the effect of metformin versus placebo was significantly different from the effect of metformin versus placebo in women with a WHR < 0.85 (*P*-value of interaction 0.012) (Table II).

We did not find significant differences in effect of treatment for groups based on BMI, plasma testosterone, 2 h glucose values or HOMA.

On the basis of these findings, we combined age and WHR and created four subgroups. We found a significantly different chance of reaching an ongoing pregnancy of metformin versus placebo between these subgroups (*P*-value of interaction 0.014) (Table III). A positive effect of metformin versus placebo on pregnancy rate was found in older women (\geq 28 years) with a WHR \geq 0.85, a negative effect of metformin versus placebo in young women regardless of their WHR and no effect in the older, not viscerally obese women (Fig. 1).

Discussion

In the present analysis, there was a significantly different chance of an ongoing pregnancy of metformin versus placebo between subgroups, based on age and WHR. A positive effect of metformin versus placebo on pregnancy rate was found in older women with a high WHR, a negative effect of metformin versus placebo in young women regardless of their WHR and no effect in older, not viscerally obese women.

We have included women with PCOS, who had never used clomifene citrate and were seeking treatment for their fertility problems for the first time. We evaluated eligibility irrespective of their BMI. By using these criteria, our study group reflects the largest group of women with PCOS a fertility clinic will see and treat.

Our results contradict those from the only other subgroup analysis, where no differential effect of metformin was shown in subgroups of women based on age and WHR (Moggetti *et al.*, 2000): this study did show that in multivariable analysis higher insulin, lower androstenedione and less

Table III. Pregnancy rates for combined subgroups in women with PCOS treated with clomifene citrate plus metformin or clomifene citrate alone.

	<i>N</i>	Metformin <i>n/N</i> (%)	Placebo <i>n/N</i> (%)	RR (95% CI)	<i>P</i> -value
Age (years) < 28, WHR < 0.85	54	10/32 (31)	13/22 (59)	0.53 (0.28–0.98)	0.014
Age (years) ≥ 28, WHR < 0.85	80	23/38 (61)	24/42 (57)	1.1 (0.73–1.5)	
Age (years) < 28, WHR ≥ 0.85	29	3/14 (21)	5/15 (33)	0.64 (0.19–2.2)	
Age (years) ≥ 28, WHR ≥ 0.85	42	7/18 (39)	6/24 (25)	1.6 (0.98–3.8)	

severe cycle abnormalities appeared to be significant parameters for better response to metformin, variables we did not assess in our study. However, our data may be more valid, because we analysed over 200 patients, 10 times more than in the Moghetti *et al.* (2000) subanalysis and because our study was based on a double-blinded RCT as opposed to the open protocol used in the previous subanalysis.

The most likely reason why metformin has a beneficial effect on pregnancy rates in older and viscerally obese women is that IR is common in these women (Cowie *et al.*, 2006; Lord *et al.*, 2006). However, we did not find a significant interaction between pregnancy rates and the outcome of other surrogate markers of IR, including BMI, plasma testosterone, 2 h glucose value and HOMA. This is probably due to the fact that WHR is the most powerful predictor of IR of all surrogate markers (Rexrode *et al.*, 1998; Lord *et al.*, 2003; Alberti *et al.*, 2006; Lord *et al.*, 2006). We did not measure IR directly by means of the gold standard, the glucose clamp technique, since this is considered to be too invasive and burdensome to be part of a large clinical trial in outpatients (Legro *et al.*, 2004; Mohlig *et al.*, 2006; Piche *et al.*, 2007).

The lack of effect of metformin in patients with high BMI, increased plasma testosterone, higher 2 h glucose levels and HOMA may be real, if confirmed by further studies. However, another possibility is that the present study did not have enough power to show a difference. This might especially hold true for HOMA, since we only had data on half of the patients.

A limitation of the study is that we did not collect all parameters in all patients. In particular, fasting insulin is missing in 49% of the patients. When comparing baseline characteristics of the patients for whom data on insulin were available with those for whom data were not available, we found no

significant differences (data not shown). The lack of data appears, therefore, to be due to a random effect of not executing the complete study protocol rather than a deliberate omission of patients with certain clinical or biochemical characteristics.

An additional limitation of the study relates to the testosterone analysis. After our study protocol was written (1999) and our study performed (2001–2004), it became clear that testosterone assays show a wide inter-laboratory variability (Kane *et al.*, 2007; Rosner *et al.*, 2007). When we had finished the trial, it was impossible to adjust for this. The calculations performed in this trial were therefore performed without correcting for this inter-laboratory imprecision, as is the case in all previous literature. It is possible that the variation is sufficiently high to render the result not significant. This relatively new understanding of the variability of testosterone assays may have great impact, not only on existing but also on future literature on PCOS, especially in multicentre studies.

Our RCT was not powered for these subgroup analyses; therefore, the data need to be interpreted with great caution. Nonetheless, we feel that our data argue for performing a trial comparing metformin and clomifene citrate with clomifene citrate alone in older visceral obese women with PCOS.

Conclusion

Although our initial randomized trial showed no benefit for metformin, our current findings suggest that metformin may be an effective addition to clomifene citrate in infertile, older and visceral obese women with PCOS. Randomized studies should be performed specifically for this subgroup, or data from other existing trials should be re-analysed before we can consider implementing these findings in clinical practice.

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References

- Alberti KG, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus statement from the International Diabetes Federation. *Diabet Med* 2006;**23**:469–480.
- ASRM The Practice Committee of the American Society for Reproductive Medicine. Use of insulin sensitizing agents in the treatment of polycystic ovary syndrome. *Fertil Steril* 2004;**82**:181–183.
- Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J Clin Endocrinol Metab* 2000;**85**:2434–2438.
- Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, Saydah SH, Williams DE, Geiss LS, Gregg EW. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health and Nutrition Examination Survey 1999–2002. *Diabetes Care* 2006;**29**:1263–1268.

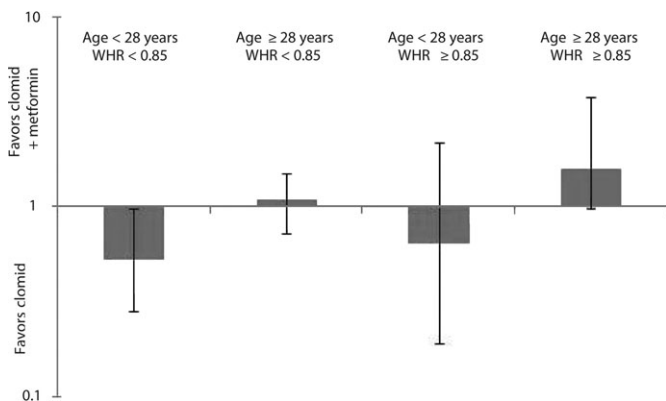


Figure 1: Relative Risks and 95% confidence intervals for combined subgroups of women treated with clomifene citrate (clomid) plus metformin or clomifene citrate alone with respect to pregnancy rates. WHR, waist hip ratio.

- Despres JP. Abdominal obesity as important component of insulin-resistance syndrome. *Nutrition* 1993;**9**:452–459.
- Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* 1997;**18**:774–800.
- Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care* 1999;**22**:141–146.
- El-Biely MM, Habba M. The use of metformin to augment the induction of ovulation in obese infertile patients with polycystic ovary syndrome. *Middle East Fertil Soc J* 2001;**6**:43–49.
- Fauser BC. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;**19**:41–47.
- 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.
- Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995;**333**:853–861.
- Glueck CJ, Papanna R, Wang P, Goldenberg N, Sieve-Smith L. Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. *Metabolism* 2003;**52**:908–915.
- Harrell FE, Jr, Lee KL, Pollock BG. Regression models in clinical studies: determining relationships between predictors and response. *J Natl Cancer Inst* 1988;**80**:1198–1202.
- Jakubowicz DJ, Seppala M, Jakubowicz S, Rodriguez-Armas O, Rivas-Santiago A, Koistinen H, Koistinen R, Nestler JE. Insulin reduction with metformin increases luteal phase serum glycodelin and insulin-like growth factor-binding protein 1 concentrations and enhances uterine vascularity and blood flow in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2001;**86**:1126–1133.
- Kane J, Middle J, Cawood M. Measurement of serum testosterone in women: what should we do? *Ann Clin Biochem* 2007;**44**:5–15.
- Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *J Clin Endocrinol Metab* 1998;**83**:3078–3082.
- Laine C, Wilson JF. Type 2 diabetes. *Ann Intern Med* 2007;**146**: ITC1–ITC15.
- Legro RS, Kunselman 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.
- Legro RS, Castracane VD, Kauffman RP. Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. *Obstet Gynecol Surv* 2004;**59**:141–154.
- Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG, Cataldo NA *et al.* Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med* 2007;**356**:551–566.
- Lord J, Thomas R, Fox B, Acharya U, Wilkin T. The central issue? Visceral fat mass is a good marker of insulin resistance and metabolic disturbance in women with polycystic ovary syndrome. *BJOG* 2006;**113**:1203–1209.
- Lord JM, Flight IH, Norman RJ. Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. *Cochrane Database Syst Rev* 2003; CD003053.
- Matthews JN, Altman DG. Statistics notes. Interaction 2: compare effect sizes not *P*-values. *BMJ* 1996;**313**:808.
- Moggetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. 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.
- Mohlig M, Spranger J, Ristow M, Pfeiffer AF, Schill T, Schlosser HW, Moltz L, Brabant G, Schoff C. Predictors of abnormal glucose metabolism in women with polycystic ovary syndrome. *Eur J Endocrinol* 2006;**154**: 295–301.
- Moll E, Bossuyt PM, Korevaar JC, Lambalk CB, Van der Veen F. Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double blind clinical trial. *BMJ* 2006;**332**: 1485–1489.
- NCEP Expert Panel. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486–2497.
- Nestler JE. Insulin regulation of human ovarian androgens. *Hum Reprod* 1997;**12** (Suppl 1):53–62.
- Nestler JE. Should patients with polycystic ovarian syndrome be treated with metformin?: an enthusiastic endorsement. *Hum Reprod* 2002;**17**: 1950–1953.
- 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.
- 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.
- Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, Fiorini S, Cognigni GE, Filicori MAM. Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2000;**85**:2767–2774.
- Piche ME, Lemieux S, Corneau L, Nadeau A, Bergeron J, Weisnagel SJ. Measuring insulin sensitivity in postmenopausal women covering a range of glucose tolerance: comparison of indices derived from the oral glucose tolerance test with the euglycemic-hyperinsulinemic clamp. *Metabolism* 2007;**56**:1159–1166.
- Pratt JJ, Wiegman T, Lappöhn RE, Woldring MG. Estimation of plasma testosterone without extraction and chromatography. *Clin Chim Acta* 1975;**59**:337–346.
- Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ, Willett WC, Manson JE. Abdominal adiposity and coronary heart disease in women. *JAMA* 1998;**280**:1843–1848.
- Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Position statement: utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *J Clin Endocrinol Metab* 2007;**92**:405–413.
- Samaras K, McElduff A, Twigg SM, Proietto J, Prins JB, Welborn TA, Zimmet P, Chisholm DJ, Campbell LV. Insulin levels in insulin resistance: phantom of the metabolic opera? *Med J Aust* 2006;**185**:159–161.

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