Clinical Endocrinology (2009) **70**, 233–237 doi: 10.1111/j.1365-2265.2008.03309.x

ORIGINAL ARTICLE

Effect of metformin, orlistat and pioglitazone treatment on mean insulin resistance and its biological variability in polycystic ovary syndrome

L. W. Cho*, E. S. Kilpatrick†, B. G. Keevil‡, A. M. Coady§ and S. L. Atkin*

**Department of Medicine University of Hull, Hull, UK, †Department of Clinical Biochemistry, Hull Royal Infirmary, Hull, UK, ‡Department of Clinical Biochemistry, Wythenshawe Hospital, Manchester, UK, §Department of Radiology, Hull Royal Infirmary, Hull, UK*

Summary

Context Mean insulin resistance (IR) is greater and it is also more variable in overweight women with polycystic ovarian syndrome (PCOS) compared to weight matched controls. Whilst treatment will reduce the mean IR, it is not known if the IR variability is also reduced.

Objective To compare the change in IR and its variability before and after treatment with insulin sensitization through metformin and pioglitazone, compared to that induced by weight loss with orlistat.

Design Randomized, open labelled parallel study.

Setting Endocrinology outpatient clinic at a referral centre.

Patients Thirty obese PCOS patients [BMI 36·0 ± 1·2 kg/m^2 (mean ± SEM)] participated in the study.

Intervention The change in biological variability (BV) was assessed by measuring IR (homeostasis model assessment method) at 4-day intervals on 10 consecutive occasions before and 12 weeks after randomization to metformin, pioglitazone or orlistat.

Outcome measured The primary end point of the study was a change in BV of IR.

Results Treatment with pioglitazone, orlistat and metformin reduced the overall IR by 41·0 ± 4·1%, 19·7 ± 6·4% and 16·1 ± 6·8% ($P = 0{\cdot}005$, $P = 0{\cdot}013$, $P = 0{\cdot}17$, respectively) and IR variability by 28·5 ± 18·0%, 41·8 ± 11·6% and 23·7 ± 17·0 ($P = 0{\cdot}20$, $P = 0{\cdot}015$ and $P = 0{\cdot}28$, respectively). Free androgen index reduced significantly with all treatments.

Conclusion Only orlistat reduced both IR and its variability significantly, though all three drugs were effective in reducing hyperandrogenism within the 12-week period of the study.

(Received 18 February 2008; returned for revision 14 March 2008; finally revised 31 March 2008; accepted 19 May 2008)

Correspondence: Li Wei Cho, Centre for Diabetes and Endocrinology, 220-236 Anlaby Road, Hull, HU3 2RW, UK. Tel.: +44 0 1482 675385; Fax: +44 0 1482 675395. E-mail: l.cho@hull.ac.uk

Introduction

Increased insulin resistance (IR) is an important feature in polycystic ovary syndrome (PCOS) that contributes to the increased risk of developing type 2 diabetes and is a feature of the adverse cardiovascular risk profile seen in the metabolic syndrome.[1,2] Jayagopal *et al*. have shown that the absolute and intra-individual variation in IR are much greater in overweight women with PCOS compared to weight matched women without PCOS,[3] but it is unknown if this innate variation of IR is modified following treatment and whether treatments such as insulin sensitization or weight loss have the largest impact. Therefore, this study has compared the effects of insulin sensitization with metformin, pioglitazone, to that of weight loss (with orlistat) on mean IR and its biological variability (BV) in women with PCOS.

Research design and methods

This was a randomized, open labelled, parallel study comparing treatment with metformin (500 mg three times a day), orlistat (120 mg three times a day) and pioglitazone (45 mg once daily). Thirty obese hyperandrogenic, anovulatory Caucasian women with PCOS [BMI 36·0 ± 1·2 kg/m^2 and age of 26·4 ± 1·5 year (mean ± SEM)] were recruited from the Hull Royal Infirmary endocrinology clinic, where they were referred by their primary care physicians for investigation of menstrual abnormalities, with or without hirsutism. PCOS was diagnosed by the ESRM Rotterdam criteria;[4] all patients had oligomenorrhea or amenorrhoea, hyperandrogenaemia and polycystic ovaries on transvaginal ultrasound. None were on any medications that would alter their IR at the time or for the preceding 3 months of entering the trial. Diabetes was excluded by a 75-g oral glucose tolerance test. All subjects gave their informed written consent that had been approved by the local research ethics committee. The clinical trial registration number for this study is ISRCTN58369615 and the study is funded by University of Hull.

After initial screening, all women began with an 8-week run-in period where they were given dietary advice and compliance checked. At the end of the run-in phase, they were randomized using a computer program. The number of patients to be recruited was based on a previous study.[5] Using NQuery version 4 and powered for IR, a sample size of 10 patients in each treatment arm was

determined for study completion. The dose of metformin was in accord with a previous study showing the beneficial effect of metformin in PCOS[6] and increased step-wise, from 500 mg once daily for the first week to 500 mg twice daily for the next week, and to 500 mg three times daily for the remainder of the study period. The dose of orlistat was 120 mg three times daily before each meal. Pioglitazone was started at 30 mg daily for the first 2 weeks and further increased to 45 mg for the remainder of the study period. After randomization, the subjects were advised not to modify their eating habits throughout the study. Clinical and biochemical assessments were performed at each visit on 10 consecutive occasions at 4-day intervals before and 12-weeks after treatment. The primary end points of the study were the change in IR and its biological variability.

Study measurements

After an overnight fast, weight and blood pressure were measured and blood samples were taken at screening, randomization, and on completion of the study period. Compliance was monitored based on counting returned medication. Fasting venous blood was collected into serum gel tubes (Becton Dickinson) and one fluoride oxalate tube at the same time each day (08·00–09·00) on 10 consecutive occasions at 4-day intervals before and 12-week after treatment. Samples were separated by centrifugation at 2000 ***g*** for 15 min at 4 °C, and two aliquots of the serum were stored at –20 °C within 1 h of collection. Plasma glucose was analysed within 4 hours of collection. The serum samples were split before assay.

Reagents

Serum insulin was assayed using a competitive chemiluminescent immunoassay, supplied by Euro/DPC, Llanberis, UK. The assay was performed on a DPC Immulite 2000 analyser (Euro/DPC, Llanberis, UK), using the manufacturer's recommended protocol. There was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyser (Beckman-Coulter, High Wycombe, UK), using the manufacturer's recommended protocol. The coefficient of variation for this assay was 1·2% at a mean glucose value of 94·6 mg/dl (5·3 mmol/l). The IR was calculated using the homeostasis model assessment (HOMA) method (HOMA-IR = (insulin × glucose)/22·5).[7] Serum testosterone was measured by isotope dilution liquid chromatography-tandem mass spectromentry (Waters Corporation, Manchester, UK) and SHBG was measured by immunometric assay with fluorescence detection on the DPC Immulite 2000 analyser using the manufacturer's recommended protocol. The free androgen index was obtained as the quotient 100 T/SHBG. Before analysis, all the serum samples were thawed and thoroughly mixed. The duplicate samples (i.e. two per visit) were randomized and then analysed in a single continuous batch using a single batch of reagents.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 14·0. Comparisons of percentage changes from baseline in the metformin, orlistat and pioglitazone groups were carried out using the paired *t*-test in data showing a Gaussian distribution. In data where distribution between individuals violated the assumptions of normality when tested using the Kolmogorov–Smirnov test, differences were calculated by paired Wilcoxon method. Biovariability data was analysed by calculating analytical, within subject, and between subject variances (SD_A^2, SD_I^2, SD_G^2, respectively) according to the methods of Fraser and Co-workers.[8,9] Using this technique, analytical variance (SD_A^2) was calculated from the difference between duplicate results for each specimen ($SD_A^2 = \sum d^2/2N$ where d is the difference between duplicates, and N is the number of paired results). The variance of the first set of duplicate results for each subject on the 10 assessment days was used to calculate the average biological intra-individual variance (SD_I^2) by subtraction of SD_A^2 from the observed dispersion (equal to $SD_I^2 + SD_A^2$). Subtracting $SD_I^2 + SD_A^2$ from the overall variance of the set of first results determined the inter-individual variance (SD_G^2). The intra-individual (SD_I) and inter-individual (SD_G) variations were estimated as square roots of the respective variance component estimates. For all analysis, a two-tailed $P < 0·05$ was considered to indicate statistical significance.

Results

All subjects completed the study and the results are shown in Table 1. Baseline differences between the three groups were nonsignificant (ANOVA test).

Insulin resistance (IR)

HOMA-IR reduced significantly in both the orlistat ($P = 0·013$) and pioglitazone ($P = 0·005$) treated groups. Women treated with metformin did not show significant reduction in HOMA-IR ($P = 0·17$) for the period studied. Comparison between the three treatments showed a significant reduction in HOMA-IR favouring the pioglitazone group ($P = 0·04$). The differences between the groups for percentage change in HOMA-IR remained significant after adjustment for percentage change in BMI ($P < 0·0001$).

Biological variability (BV) of HOMA-IR

Figures 1–3 show the changes in BV of HOMA-IR after 12 weeks of treatment for all the three groups. BV reduced significantly only in the orlistat treated group compared to baseline ($P = 0·015$). Women in the metformin and pioglitazone treated groups showed a trend to reduction of BV of IR at but did not reach statistical significance for the period studied. A discrepant HOMA-IR of 19·6 in one patient on orlistat was included in the analysis that caused a greater pretreatment variance, but had no influence on our overall findings.

BV HOMA-IR was significantly correlated to HOMA-IR, the Pearson correlation coefficient was 0·72 ($P = 0·001$; two-tailed). The change in BV HOMA-IR significantly correlated with changes in HOMA-IR (correlation coefficient 0·39, $P = 0·035$) and changes in FAI (correlation coefficient 0·57, $P = 0·001$). There were no significant correlations between changes in BV HOMA-IR with changes in BMI or SHBG.

Table 1. Percentage change for each parameter after 3 months of treatment

	Metformin		Orlistat		Pioglitazone	
	Baseline *vs.* 12 weeks	% change	Baseline *vs.* 12 weeks	% change	Baseline *vs.* 12 weeks	% change
HOMA-IR	3·6 ± 0·5 *vs.* 3·1 ± 0·6	–16·1 ± 6·8	5·0 ± 0·8 *vs.* 3·7 ± 0·5	–19·7 ± 6·4*	4·5 ± 0·8 *vs.* 2·5 ± 0·4	–41·0 ± 4·1*
BV HOMA	1·1 ± 0·5 *vs.* 0·7 ± 0·4	–23·7 ± 17·0	3·9 ± 2·0 *vs.* 1·0 ± 0·5	–41·8 ± 11*	1·2 ± 0·5 *vs.* 0·5 ± 0·2	–28·5 ± 18·0
Insulin (μU/ml)	16·8 ± 2·3 *vs.* 15·1 ± 2·9	–12·8 ± 7·7	23·6 ± 3·9 *vs.* 17·7 ± 2·3	–18·4 ± 5·6*	20·0 ± 2·9 *vs.* 12·0 ± 1·5	–37·6 ± 4·2*
SHBG (nmol/l) [range 35–100]	22·1 ± 2·5 *vs.* 25·3 ± 3·2	13·3 ± 3·1*	25·1 ± 3·5 *vs.* 29·5 ± 5·1	14·3 ± 5·0*	22·3 ± 1·6 *vs.* 32 ± 3·2	43·7 ± 10·2*
BMI (kg/m^3)	34·3 ± 1·8 *vs.* 33·2 ± 1·9	–3·4 ± 1·0*	37·4 ± 2·7 *vs.* 35·2 ± 2·4	–5·7 ± 0·8*	36·2 ± 1·8 *vs.* 37·3 ± 1·8	3·1 ± 1·4
FAI (%) [range 0–3·0]	7·9 ± 0·9 *vs.* 6·1 ± 0·9	–22·9 ± 7·4*	8·7 ± 1·9 *vs.* 6·9 ± 1·6	–20·8 ± 5·8*	6·1 ± 1·2 *vs.* 4·7 ± 0·6	–16·1 ± 5·1*

Data are presented as mean ± SEM and local laboratory reference range given in brackets. All serum results are obtained from fasting variables. BV HOMA denotes biological variation of HOMA. Baseline differences between groups were nonsignificant (ANOVA).
*$P < 0·05$ for changes compared to baseline.
To convert values for insulin to picomoles per litre, multiply by 6. To convert values for SHBG to micrograms per decilitre, divide by 34·7.

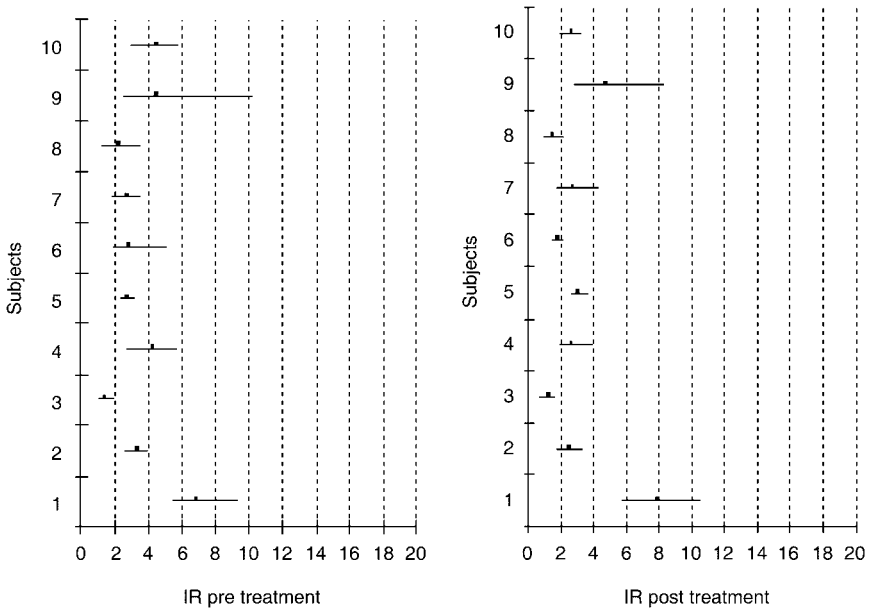

Fig. 1 Changes in biological variability of insulin resistance in women with PCOS pre- and post-treatment with metformin.

BMI

BMI reduced significantly with both metformin ($P = 0·013$) and orlistat ($P = 0·005$), with a trend to an increase with pioglitazone after 12 weeks of treatment. There was no difference between the decrease in BMI with metformin to that of orlistat, $P = 0·07$.

Biochemical hyperandrogenaemia

FAI reduced significantly in all the three groups ($P = 0·017$ in metformin, $P = 0·007$ in orlistat and $P = 0·012$ in pioglitazone). There was no difference between the three treatments for the change in FAI, $P = 0·85$ (Kruskal–Wallis test).

Discussion

Metformin therapy has been shown to have beneficial short-term effects on IR in nondiabetic women with PCOS,[6,10–16] and its use in women with PCOS is now regarded as acceptable practice. Pioglitazone[17–20] and orlistat[5] have also been shown to improve the metabolic and hormonal consequence of PCOS. This study aimed to contrast and compare the metabolic changes and BV of IR that followed treatment with these three medications in women with PCOS.

We have shown that weight reduction with orlistat was comparable to insulin sensitization with pioglitazone in leading to a significant reduction of IR over the time course of the study while treatment with metformin showed a trend to reduction. The apparent lack of effect by metformin may have been due to its reduced efficacy in subjects with a high BMI.[21] The percentage reduction of IR was largest with pioglitazone compared to metformin and orlistat, but with respect to the reduction in hyperandrogenism, the effect of pioglitazone was comparable to the other two treatments.

IR is much more variable in women with PCOS compared to weight matched controls[3] and this study has shown that only weight reduction through orlistat reduced IR variability of the HOMA-IR

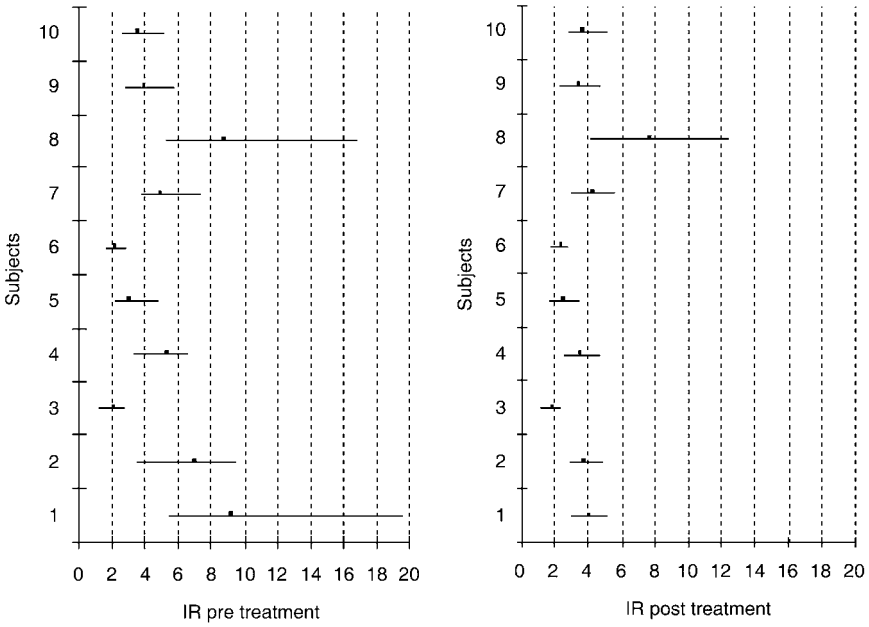

Fig. 2 Changes in biological variability of insulin resistance in women with PCOS pre- and post-treatment with orlistat.

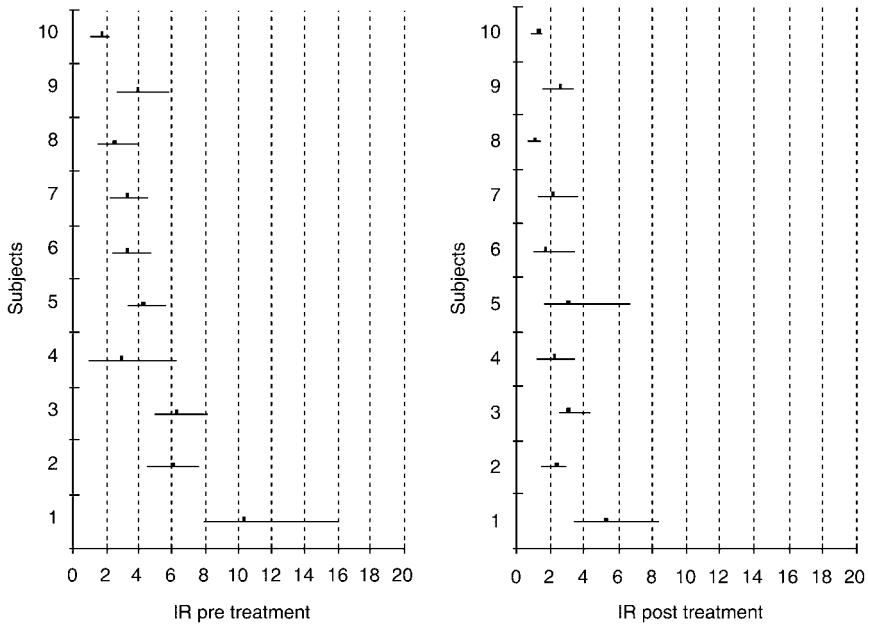

Fig. 3 Changes in biological variability of insulin resistance in women with PCOS pre- and post-treatment with pioglitazone.

measurements, whilst insulin sensitization had no effect. This suggests that physiological reversal of both mean and variability of IR occurs with weight loss, whilst only mean IR is reduced with insulin sensitization. The reduction in variability within the same individual by weight loss also indicates that the large variability in HOMA-IR is likely to be a true reflection of fluctuant IR in these patients, rather than an artefact. The HOMA model itself is a validated technique of assessing IR[7] and studies comparing it to other measures of IR such as clamp studies have shown it to be a good measure of IR[22,23] and in practical terms the only method for such frequent sampling to determine BV.

Why should weight loss reduce the BV of HOMA when insulin sensitization does not? It has to be accepted that the cause for such a large degree of variability in IR amongst PCOS patients is currently unknown. However, speculatively, insulin sensitization by metformin and pioglitazone is unidimensional, in that both reduce IR largely through a single mechanism which, in turn, would seem to predominantly influence only mean IR rather than its variability. In comparison, weight loss (in this case with orlistat) is more likely to have a multidimensional effect by modulating several factors associated with IR and hence the reducing both mean IR and its BV.

While variability in IR has not been proven to add to the risk already present in patients with high mean IR values, glucose variability has certainly been found to be associated with increased free radical damage independently of mean glucose in type 2 diabetes (T2DM).[24] However, it is not known if this relationship is actually a reflection of the inherent variability of IR found in T2DM and PCOS.

In summary, only weight reduction with orlistat resulted in a significant reduction in insulin resistance and its variability whist insulin sensitization with metformin and pioglitazone had no effect on insulin resistance variability despite a significant reduction in mean insulin resistance seen with pioglitazone.

References

1 Glueck, C.J., Papanna, R., Wang, P. *et al.* (2003) Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. *Metabolism*, **52**, 908–915.

2 Ehrmann, D.A., Sturis, J., Byrne, M.M. *et al.* (1995) Insulin secretory defects in polycystic ovary syndrome. Relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. *Journal of Clinical Investigation*, **96**, 520–527.

3 Jayagopal, V., Kilpatrick, E.S., Holding, S. *et al.* (2002) The biological variation of insulin resistance in polycystic ovarian syndrome. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1560–1562.

4 ESHRE/ASRM-Sponsored_PCOS_Consensus_Workshop_Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility and Sterility*, **81**, 19–25.

5 Jayagopal, V., Kilpatrick, E.S., Holding, S. *et al.* (2005) Orlistat is as beneficial as metformin in the treatment of polycystic ovarian syndrome. *Journal of Clinical Endocrinology and Metabolism*, **90**, 729–733.

6 Moghetti, P., Castello, R., Negri, C. *et al.* (2000) 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. *Journal of Clinical Endocrinology and Metabolism*, **85**, 139–146.

7 Matthews, D.R., Hosker, J.P., Rudenski, A.S. *et al.* (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28**, 412–419.

8 Fraser, C.G. & Harris, E.K. (1989) Generation and application of data on biological variation in clinical chemistry. *Critical Reviews in Clinical Laboratory Science*, **27**, 409–437.

9 Gowans, E.M. & Fraser, C.G. (1988) Biological variation of serum and urine creatinine and creatinine clearance: ramifications for interpretation of results and patient care. *Annals of Clinical Biochemistry*, **25**, 259–263.

10 Kocak, M., Caliskan, E., Simsir, C. *et al.* (2002) Metformin therapy improves ovulatory rates, cervical scores, and pregnancy rates in clomiphene citrate-resistant women with polycystic ovary syndrome. *Fertility and Sterility*, **77**, 101–106.

11 Fleming, R., Hopkinson, Z.E., Wallace, A.M. *et al.* (2002) Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism*, **87**, 569–574.

12 Ng, E.H., Wat, N.M. & Ho, P.C. (2001) Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. *Human Reproduction*, **16**, 1625–1631.

13 Pasquali, R., Gambineri, A., Biscotti, D. *et al.* (2000) 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. *Journal of Clinical Endocrinology and Metabolism*, **85**, 2767–2774.

14 Morin-Papunen, L.C., Vauhkonen, I., Koivunen, R.M. *et al.* (2000) Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: a randomized study. *Journal of Clinical Endocrinology and Metabolism*, **85**, 3161–3168.

15 Nestler, J.E. & Jakubowicz, D.J. (1996) Decreases in ovarian cytochrome P450c17α activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *New England Journal of Medicine*, **335**, 617–623.

16 Velazquez, E.M., Mendoza, S., Hamer, T. *et al.* (1994) Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism*, **43**, 647–654.

17 Ortega-Gonzalez, C., Luna, S., Hernandez, L. *et al.* (2005) Responses of serum androgen and insulin resistance to metformin and pioglitazone in obese, insulin-resistant women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **90**, 1360–1365.

18 Glueck, C.J., Moreira, A., Goldenberg, N. *et al.* (2003) Pioglitazone and metformin in obese women with polycystic ovary syndrome not optimally responsive to metformin. *Human Reproduction*, **18**, 1618–1625.

19 Romualdi, D., Guido, M., Ciampelli, M. *et al.* (2003) Selective effects of pioglitazone on insulin and androgen abnormalities in normo- and hyperinsulinaemic obese patients with polycystic ovary syndrome. *Human Reproduction*, **18**, 1210–1218.

20 Brettenthaler, N., De Geyter, C., Huber, P.R. *et al.* (2004) Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **89**, 3835–3840.

21 Maciel, G.A., Soares Junior, J.M., Alves da Motta, E.L. *et al.* (2004) Nonobese women with polycystic ovary syndrome respond better than obese women to treatment with metformin. *Fertility and Sterility*, **81**, 355–360.

22 Bonora, E., Targher, G., Alberiche, M. *et al.* (2000) Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*, **23**, 57–63.

23 Mather, K.J., Hunt, A.E., Steinberg, H.O. *et al.* (2001) Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *Journal of Clinical Endocrinology and Metabolism*, **86**, 5457–5464.

24 Monnier, L., Mas, E., Ginet, C. *et al.* (2006) Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *Journal of the American Medical Association*, **295**, 1681–1687.

