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REVIEW ARTICLE

The effects of metformin on endogenous androgens and SHBG in women: a systematic review and meta-analysis

Maddalena Barba*, Holger J. Schünemann*†‡, Francesca Sperati*, Elie A. Akl†, Felice Musicco§, Gordon Guyatt‡ and Paola Muti*

*Department of Epidemiology, Italian National Cancer Institute Regina Elena, Rome, Italy, †Department of Medicine, State University of New York at Buffalo, Buffalo, New York, USA, ‡Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada and §Pharmacological Service, Italian National Cancer Institute Regina Elena, Rome, Italy

Summary

Objectives Elevated circulating androgens are risk factors for several chronic, metabolic and reproductive disorders. Metformin is an insulin-sensitizing agent that may lower androgen levels. To evaluate the effects of metformin on endogenous androgens and SHBG levels in women, we conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) comparing metformin with placebo or no treatment.

Data source We used OVID to search MEDLINE, EMBASE and CENTRAL until March 2007.

Review methods Two reviewers independently extracted data on methodological quality, participants, interventions and outcomes of interest. Our a priori primary outcome was post-treatment measurements. In a secondary analysis, we evaluated the difference between the pre- and post-treatment levels. We computed the weighted mean difference (WMD) as a measure of effect for each outcome using the DerSimonian–Laird random effects method. We used the I^2 statistic to assess heterogeneity and explored its causes in subgroup analyses of features related to participants' characteristics and study design. Based on a regression model, we conducted sensitivity analyses by investigating the use of placebo as a predictor of effect size.

Results Twenty RCTs fulfilled the inclusion criteria. Pooled WMDs in post-treatment levels between the metformin and control group were –0·31 nmol/l (95% CI –0·65 to 0·03) for total testosterone (TT), 0·10 pmol/l (95% CI –0·89 to 1·10) for free testosterone (FT), 0·14 µmol/l (95% CI –0·34 to 0·62) for dehydroepiandrosteronesulfate (DHEAS), –0·60 nmol/l (95% CI –1·67 to 0·46) for androstenedione (AND) and 5·88 nmol/l (95% CI 2·01–9·75) for SHBG. Pooled WMDs of the pre- to post-treatment differences (i.e. with adjustment for baseline hormone levels) were –0·38 (95% CI –0·51 to –0·25) for TT, –2·71 (95% CI –10·35 to 4·93) for FT, –0·50 (95% CI –0·83 to –0·16) for DHEAS, –1·39 (95% CI –2·30 to –0·49) for AND and 6·63 (95% CI 2·32–10·94) for SHBG. In subgroup analyses, features related to the administered treatment (i.e. metformin as a single agent or as part of combined regimens) partly explained the heterogeneity. Sensitivity analyses of studies using placebo showed similar results to those not using placebo.

Conclusions Our systematic review and meta-analysis provides evidence of metformin-induced changes in circulating androgens and SHBG levels in women but the quality of evidence is not high. However, there are no data from RCTs regarding these effects in postmenopausal women or healthy premenopausal women. High-quality RCTs are required to evaluate whether metformin has effects on surrogate markers and patient-important outcomes in these patient groups.

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Introduction

Metformin is an insulin-sensitizing agent with several mechanisms of action. It decreases hepatic glucose production and insulin secretion, enhances peripheral glucose uptake by muscles, and increases glucose oxidation by adipose tissue.[1,2] These mechanisms combined improve insulin resistance and blood glucose control.[1,3,4] The clinical indications for metformin therapy include several disorders, such as type 2 diabetes mellitus[5–7] and polycystic ovary syndrome (PCOS), for which insulin resistance represents a key pathological mechanism.[5–10]

PCOS is a common disorder of premenopausal women characterized by hyperandrogenism and substantial peripheral insulin resistance.[8,9] Hyperandrogenism results from increased androgen biosynthesis and decreased SHBG synthesis, both associated with hyperinsulinaemia and increased androgen bioavailability.[2,8,11,12] Hyperandrogenism may itself contribute to the development and maintenance of the

Correspondence: Holger Schünemann, Department of Epidemiology, Italian National Cancer Institute Regina Elena, Rome, Italy. Tel.: +39 0652665102; Fax: +39 0652662732; E-mail: hjs@buffalo.edu

insulin resistance state, which precedes and accompanies hyperinsulinaemia and represents the probable link between the metabolic syndrome, cardiovascular diseases and type 2 diabetes.[2,13]

Several reviews report large numbers of trials investigating the effects of metformin administration in women diagnosed with PCOS.[14–20] The most recent review, published in 2003, included trials mostly uncontrolled and with small numbers of participants.[14–20] These focused on outcomes such as fertility, weight, blood pressure, serum concentration of cholesterol and triglycerides, glycaemia and circulating insulin.

Although some of these latter outcomes have led to metformin use for the treatment of endocrinological diseases, the mechanisms of action and the impact on endocrine hormones are not completely understood. To our knowledge, no systematic review has evaluated the extent to which metformin affects endogenous hormones other than insulin. We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) evaluating the effect of metformin on endogenous hormone levels.

Methods

Literature search and selection

We used the OVID platform to search MEDLINE (January 1966 onwards), EMBASE (January 1980 onwards) and the Cochrane Central Register of Controlled Trials Register (CENTRAL) (The Cochrane Library, latest issue) until March 2007. The search strategy combined terms for metformin with a search filter for RCTs (available from the authors upon request). We also used the 'Related Articles' feature in PubMed to identify additional articles and screened the reference lists of included studies without language restriction.

Included studies fulfilled the following criteria: RCTs investigating metformin effects in women, and metformin given as a single agent or as part of combined regimens including drugs other than metformin and/or lifestyle modifications, as long as the administrated co-interventions were the same in all groups within each trial compared with placebo or no treatment. We included RCTs reporting at least one post-treatment measure of blood and/or urinary and/or salivary concentrations of at least one of the following primary outcomes: total testosterone (TT), free testosterone (FT), dehydroepiandrosteronesulfate (DHEAS), androstenedione (ANDS) and SHBG. Secondary outcomes were fasting glycaemia and insulinaemia. We excluded studies in pregnant or lactating women and those with a loss to follow-up of more than 20%.

For trials with a cross-over design, we only included the first post-intervention measurement (i.e. prior to cross-over). For multi-arm RCTs, we included all pairwise comparisons for which the two arms differed by metformin use only. For RCTs including more than one population differing by indication for metformin treatment, we considered the different populations separately.

Data extraction and quality assessment

Two reviewers independently screened the titles and abstracts of the identified articles for potential eligibility, applying sensitive criteria to the first evaluation. Because of poor agreement, a third investigator evaluated all titles and abstracts that only a single reviewer had judged as eligible. Two reviewers independently screened the full text articles judged potentially eligible and then used a piloted form for data extraction and methodological quality assessment. They resolved disagreements by discussion with a third reviewer. The data collected related to participants, intervention and outcomes of interest. Methodological quality criteria included: concealment of allocation, blinding, intention-to-treat (ITT) analyses, and percentage of follow-up. If data were incomplete or unclear, we made at least two attempts to contact the study investigators. We included abstracts only if information related to methodological aspects and study results were available.

Data analysis

We used the kappa statistic (κ) to evaluate the degree of agreement between the two reviewers for titles and abstracts screening. We then assessed raw agreement between the two reviewers for full text eligibility and data extraction.[21]

A priori, we defined the unadjusted analysis of post-treatment measurements as primary analysis and the analysis adjusted for baseline values as secondary analysis. For each of the outcomes we calculated effect estimates using SI units (corresponding forest plot figures available from the authors upon request).

We calculated the I^2 statistic[22,23] to assess heterogeneity across trial results, applying the following interpretation for I^2 (J. Higgins, personal communication): 0–50 = low; 50–80 = moderate and worthy of investigation; 80–100 = severe and worthy of understanding; 95–100 = aggregate with major caution. We explored heterogeneity using preplanned subgroup analyses. The subgroups were defined based on two different features, namely required evidence of clinical and/or biochemical hyperandrogenism and metformin administration as a single agent or as part of combined regimens.

We conducted regression analyses to evaluate the effect on the results of the use (*vs.* no use) of placebos and adjusted for baseline values. We assessed publication bias by visual inspection of funnel plots (available from the authors upon request) that graphically display the magnitude of each study effect estimates against the inverse of the variance.[24] We used Revman 4·2·7 and Stata version 8·2 (Stata Corp., College Station, TX, USA) for statistical analyses, considering the weighted mean difference (WMD) as a measure of effect for each outcome using the DerSimonian–Laird random effects method.

Results

Systematic review flow

Figure 1 shows the trial flow. Twenty RCTs met the eligibility criteria,[25–44] accounting for 848 women. The degree of agreement between the two reviewers was 0·435 (κ) for potential eligibility (based on highly sensitive titles and abstracts screening) and 97% (raw agreement) for full text eligibility and data extraction.

All the included trials reported measuring exclusively blood concentration of the variables of interest. None of the included

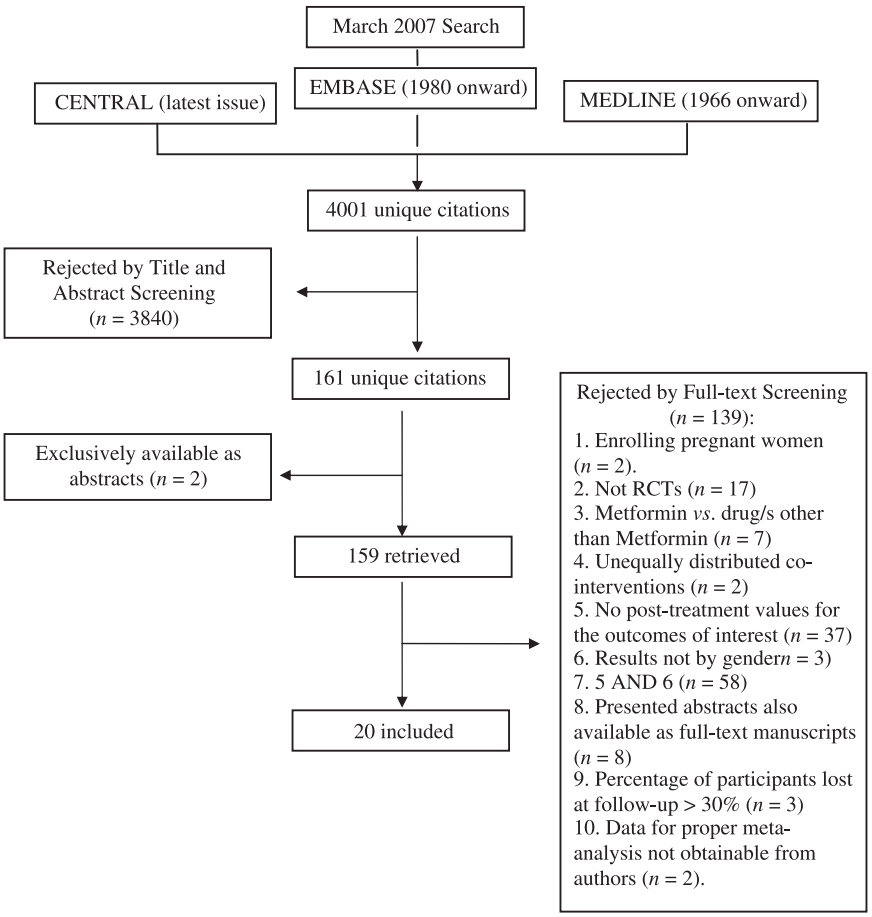


Fig. 1 Flow diagram of the trial selection process.

studies reported on outcomes measured on saliva or urine. Table 1 shows the characteristics of the included studies.

Methodological quality of included RCTs

The methodological quality varied among the included studies. All but five of them reported on the randomization method.[29,36,37,39,40] Three RCTs provided no details regarding blinding,[34,36,39] seven reported blinding patients,[29,30,37,38,40,42,44] two reported blinding patients and investigators,[25,26] two reported blinding patients and caregivers,[27,43] one reported blinding patients and outcome assessors,[28] one reported blinding investigators, caregivers, patients, outcome assessors and manuscript writer,[35] one reported blinding investigators, caregivers, patients and outcome assessors,[41] and one reported blinding investigators.[33] The remaining RCTs had an open-label design.[31,32] Only eight trials reported conducting ITT analyses.[28,31–34,37,39,43] The overall methodological quality was judged as acceptable.

Quantitative data synthesis

Pooling the WMDs for the post-treatment measurements from 11 studies,[27–34,36–38] we found that metformin increased the circulating levels of SHBG (WMD: 5·88 nmol/l, 95% CI 2·01–9·75, I^2: 60·0%) (Fig. 2). The effects of metformin on the other variables were not statistically significant. Heterogeneity decreased (WMD: 9·04 nmol/l, 95% CI 1·05–17·03, I^2: 28·3%) in subgroup analyses including RCTs administering metformin as a single agent.[25,26,31–33,37,43,44]

Pooling the WMDs of the pre- to post-treatment changes[25–44], metformin decreased the circulating levels of TT (–0·38 nmol/l, 95% CI –0·51 to –0·25, I^2: 9·4%), DHEAS (–0·50 μmol/l, 95% CI –0·83 to –0·16, I^2: 0%) and ANDS (–1·39 nmol/l, 95% CI –2·30 to –0·49, I^2: 38·6%), and increased the circulating levels of SHBG (6·63 nmol/l, 95% CI 2·32–10·94, I^2: 43·6%). The results were not statistically significant for FT. Heterogeneity was statistically significant only for SHBG (I^2: 43·6, $P = 0·04$). Subgroup analyses by administered treatment and by required evidence of hyperandrogenism at the study entrance reduced heterogeneity for SHBG (WMD: 12·30, 95% CI 6·30–8·30, I^2: 0, $P = 0·0001$ in subgroup analyses by administered treatment). We also found evidence of a very slight decrease in fasting glycaemia (–0·02 mmol/l, 95% CI –0·03 to –0·01, I^2: 0).

Sensitivity analyses of studies using placebo showed similar results to those not using placebo. We produced funnel plots of the RCTs for each of the investigated outcomes.

Discussion

In this systematic review, we found that, in both the primary and secondary meta-analyses, metformin administration increased

Table 1. Characteristics of the included RCTs

Study	Sample size	Total metformin dosage (mg/day)	Control arm*	Duration of treatment (days)	Participants' characteristics
Baillargeon *et al.* (2004)[25]	128	1700	Placebo	180	Age range 17–40 PCOS[7] BMI ≤ 27 kg/m^2 Normal glucose tolerance Not currently on OC or medications affecting insulin sensitivity
Chou *et al.* (2003)[26]	32	1500	Placebo	90	Age range 16–42 PCOS[8] BMI ≤ 30 kg/m^2 Normal glucose tolerance Non-smokers Not on medications within the previous 3 months
Cibula *et al.* (2005)[27]	30	1500	OC[1]	180	Age range 18–28 PCOS[20] No secondary endocrine disorder or contraindications to OC use
Elter *et al.* (2002)[28]	40	1500	OC[1] and diet	120	Age range 16–36 PCOS[9] BMI ≤ 26 kg/m^2 Normal glucose tolerance Not on medications affecting carbohydrates or lipid metabolism within the previous 6 months
Gambineri *et al.* (2004)[29]	40	1700	Flutamide[2] and diet	180	Age range 21–33 PCOS[9] BMI ≤ 28 kg/m^2 Normal glucose tolerance Not on medications within the previous 3 months Not on diet in previous 3 months No significant body weight modifications in the previous 3 months
Gambineri *et al.* (2006)[30]	80	1700	Flutamide[2] and diet Placebo and diet	360	Age range 21–31 PCOS[17] BMI ≤ 28 kg/m^2 Reproductive age range 18–45 Waist circumference > 88 cm[19]
Ibanez *et al.* (2004)[31]	24	850	No treatment	360	Age range 10–14 Low birthweight[10] BMI ≤ 26 kg/m^2 Precocious pubarche[11] Hyperinsulinaemia on a standard test[12] Normal glucose tolerance No personal or familial history of diabetes mellitus Subclinical ovarian hyperandrogenism[13] Not currently on OC or any medication affecting gonadal function or carbohydrate metabolism
Ibanez *et al.* (2004)[33]	33	425	No treatment	180	Age range 7–8 Low birthweight[10] BMI ≤ 21 kg/m^2 Precocious pubarche[11] Hyperinsulinaemia on a standard test[12] Subclinical ovarian hyperandrogenism[13] No personal or familial history of diabetes mellitus Normal glucose tolerance Not currently on OC or any medication affecting gonadal function or carbohydrate metabolism
Ibanez *et al.* (2006)[32]	38	425	No treatment	720	Age (mean) 8 Low birthweight[10] BMI ≤ 22 kg/m^2 Precocious pubarche[17]
Khorram *et al.* (2006)[34]	31	1500	CC[3]	21	Age range 26–28 PCOS[9] BMI > 29 kg/m^2 Normal glucose tolerance Desire for fertility No previous assumption of CC
Kocak *et al.* (2002)[35]	56	1700	Placebo and CC[4]	60	Age range 22–30 PCOS[9] Normal glucose tolerance Documented history of resistance to CC[14] Not on OC or any medication affecting gonadal function or carbohydrate metabolism within the previous 3 months
Lv *et al.* (2005)[36]	50	500	CA[5]	180	Age range 16–36 PCOS[9] BMI ≤ 25 kg/m^2 Normal glucose tolerance Not on CC or any medications within the previous 6 months
Nestler *et al.* (1998)[37]	61	1500	Placebo	35	Age range 27–30 PCOS[9] BMI > 28 kg/m^2 Normal glucose tolerance Not on CC or any medications within the previous 2 months

Table 1. *Continued*

Study	Sample size	Total metformin dosage (mg/day)	Control arm*	Duration of treatment (days)	Participants' characteristics
Pasquali *et al.* (2000)[38]	40†	1700	Placebo and diet	180	Age range 23–38 PCOS[9] BMI > 28 kg/m^2 Not on CC or any medications within the previous 3 months
Refaie *et al.* (2005)[39]	55	1500	CC[6]	180	Age range 22–33 PCOS[9]
Sturrock *et al.* (2002)[40]	26	1500	Placebo and CC[6]	180	Age range 18–40 PCOS[9] Documented history of resistance to CC[14]
Tang *et al.* (2006)[41]	143	1700	Placebo, physical exercise and diet	180	Age range: 18–39 PCOS[9] BMI > 30 kg/m^2 Normal glucose tolerance Desire for fertility No previous ovulation induction therapy No previous ovulation induction therapy Not on hormone therapy currently/within the previous 6 weeks
Vandermolen *et al.* (2001)[42]	27	1500	Placebo and CC[6]	49	Age range 18–35 PCOS[9] Documented history of resistance to CC[14]
van Santbrink *et al.* (2005)[43]	20	1700	Placebo	35	Age range 18–37 Desire for fertility Normal serum oestriol and FSH concentrations Severe oligomenorrhoea or amenorrhoea Insulin resistance[15] Documented history of resistance to CC[14]
Yarali *et al.* (2002)[44]	32	1700	Placebo	42	Age range 23–35 PCOS[9] Documented history of resistance to CC[14] No previous exogenous gonadotrophin treatment Normal glucose tolerance Normal semen analysis No previous genital surgery Normal hysterosalpingography and/or laparoscopy within the previous 6 months

*In each of the included RCTs, the control arm/s and the intervention arm/s differs/differ exclusively by metformin use.
†Recruited participants include 20 women diagnosed with PCOS, whose characteristics are described in this table and 20 controls comparable for age and weight, with regular menses and no evidence of hyperandrogenism.
[1]Oral contraceptive: ethinyl oestradiol (EE), 35 ng, and cyproterone acetate (CA), 2 mg for 21 days per month; [2]flutamide at 500 mg/day; [3]clomiphene citrate (CC), 100 mg/day on cycle days 5–9 only; [4]CC 100 mg/day on cycle days 3–7 only; [5]CP, 1 tablet/day for 21 days/month from the first day of menstruation or progestin-induced bleeding; [6]CC, 50 mg/day on cycle days 2–6 only; [7]PCOS as defined by oligomenorrhoea and hyperandrogenaemia; [8]PCOS as defined by oligomenorrhoea and hyperandrogenism; [9]PCOS as defined by (i) ultrasound examination; (ii) oligomenorrhoea; (iii) manifestations of hyperandrogenism and/or hyperandrogenaemia; [10]birthweight < –1.5 SD (corresponding to 2.7 kg at term in Catalonian girls); [11]defined as having pubic hair at < 8 years of age; [12]defined on a standard 2-h oral glucose tolerance test; [13]defined as excessive response in terms of 17-hydroxyprogesterone to leuprolide acetate administration; [14]documented history of resistance to CC ranging from 50 to 150 mg/day for 5 days; [15]defined as fasting glucose–insulin ratio < 4.5 mg/10^{-4} U; [16]PCOS as defined by oligomenorrhoea or amenorrhoea and also at least one of the criteria of hyperandrogenism including a hirsutism score of > 7 (according to Ferriman and Gallway) and/or an elevated serum concentration of free testosterone (> 4 ng/dl); [17]attributed to exaggerated adrenarche; [18]the diagnosis of PCOS included: chronic anovulation or severe oligomenorrhoea/amenorrhoea, hirsutism or total testosterone levels > 0.72 ng/ml; and polycystic ovarian morphology at ultrasound; [19]consistent with an abdominal fat distribution phenotype; [20]PCOS as defined by oligomenorrhoea, increased concentration of at least one androgen above the upper reference limit and clinical manifestation of hyperandrogenism.

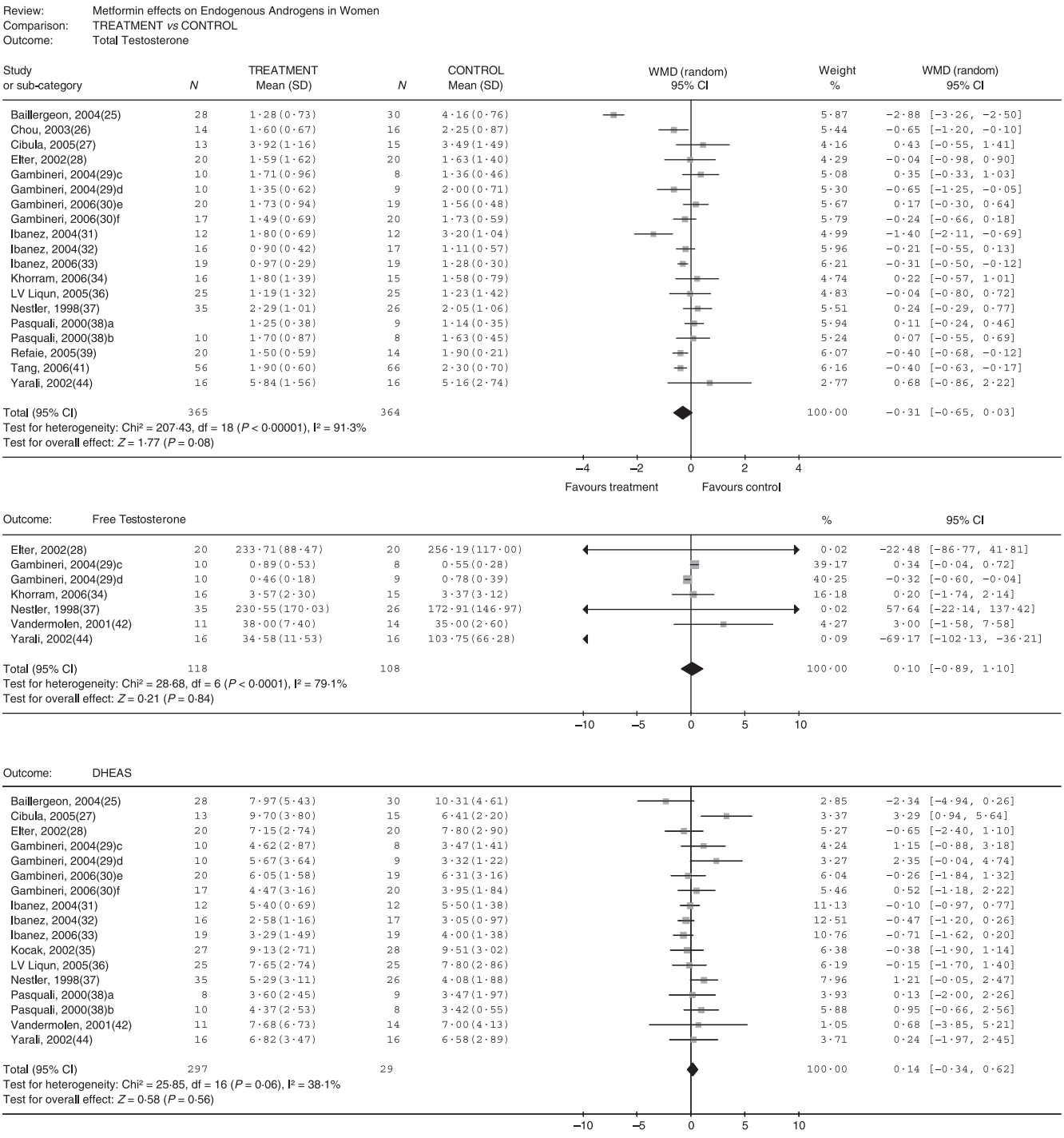
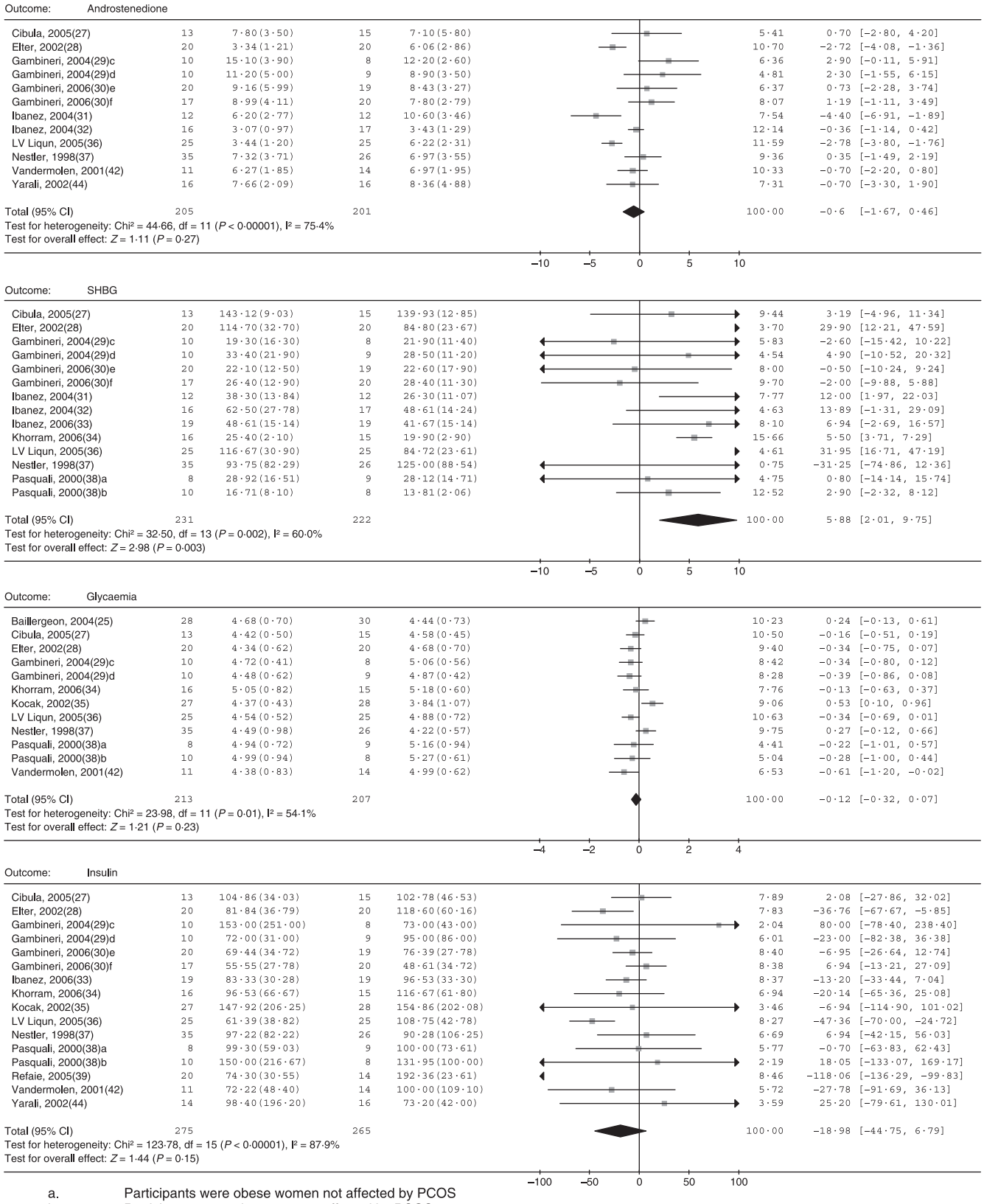


Fig. 2 Meta-analysis of weighted mean differences in post-treatment circulating androgens and SHBG.

SHBG circulating levels. In the secondary analysis, metformin administration also lowered the circulating levels of TT, DHEAS and ANDS.

This systematic review has the following strengths. We followed the Cochrane Collaboration methods for conducting systematic reviews and meta-analyses, including an extensive and systematic search to identify all relevant trials without language restrictions. However, we were unable to include two eligible trials in the meta-analyses[45,46] because relevant data were incompletely reported or not provided by the authors.

There are some limitations to this review. All included participants were premenopausal women, who were either at high risk of developing, or had been diagnosed with, disorders affecting the sexual steroid axis. Thus, our results limit inference regarding metformin effects on circulating androgens and SHBG concentrations in healthy women.



a. Participants were obese women not affected by PCOS
b. Participants were obese women affected by PCOS
c. Participants randomly allocated to Metformin and diet OR Placebo and diet
d. Participants randomly allocated to Metformin, Flutamide and diet OR Flutamide and diet
e. Participants randomly allocated to Metformin and diet OR Placebo and diet
f. Participants randomly allocated to Metformin, Flutamide and diet OR Flutamide and diet

Fig. 2 *Continued.*

We observed low to moderate heterogeneity. The factors we specified a priori as potential effect modifiers (required evidence of clinical and/or biochemical hyperandrogenism, metformin administration as a single agent or as part of combined regimens, and use *vs.* no use of placebos) explained some of the observed heterogeneity. The analysis of the characteristics of included studies revealed additional factors that varied across studies and could potentially explain heterogeneity. These factors include both characteristics of the populations (i.e. diagnosis of PCOS, body mass index (BMI) at inclusion) and of study design (i.e. methodology for measuring circulating androgens, metformin therapy dosage and duration). In fact, overweight and obese PCOS women are more likely to exhibit severe hyperandrogenism and lower SHBG levels when compared to their normal weight counterpart.[47] Direct radioimmunoassay (RIA) methods tend to show higher TT levels when compared to studies using extraction and chromatography in conjunction with RIA.[29,41,48–50] There is also evidence that analogue-based free testosterone RIA is highly unreliable. We conducted post-hoc subgroup analyses based on these additional factors but none of them reduced the heterogeneity.

Considerable experimental and epidemiological evidence supports the association between circulating androgens and SHBG levels and several life-threatening conditions in women. Elevated serum levels of androgens are positively associated with breast cancer risk, while SHBG levels are inversely associated with risk.[51,52] Thus, metformin could, by decreasing androgens levels and increasing SHBG levels, have a potential role in the chemoprevention of breast cancer. However, no clinical evidence is currently available to support this hypothesis.

Androgens and SHBG have been also linked to adverse cardiovascular risk factors in women, with increased testosterone levels and decreased SHBG levels strongly associated with central adiposity, increased triglycerides, and decreased high density lipoprotein (HDL) cholesterol levels. In fact, metformin has been shown to decrease those cardiovascular risk factors such as blood pressure and low density lipoprotein (LDL) cholesterol in PCOS.[14] Although we could not locate studies in non-diabetic patients, a systematic review in patients with diabetes showed that metformin may prevent some vascular complications, and mortality.[53]

Low levels of SHBG have also been associated with higher rates of diabetes.[54] This suggests a potential role of metformin in preventing diabetes. Indeed, a systematic review has found evidence that metformin may reduce the occurrence of type 2 diabetes.[55]

In summary, our systematic review and meta-analysis provides evidence of metformin-induced changes in circulating androgens and SHBG levels in women. The information is helpful for explaining mechanisms related to metformin. The review indicates that a fairly large amount of data from RCTs administering metformin in women affected by PCOS or at risk of developing PCOS is currently available, although the overall methodological quality is moderate. Conversely, there are no data from RCTs regarding the effects of metformin in healthy women. We thus suggest the use of metformin in future RCTs focusing on patient-important outcomes, such those related to the role of androgens as breast cancer promoters and potential mediators of cardiovascular risk in women. We would further add the need for high-quality studies, designed primarily to address the latter outcomes.

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