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Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis

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BACKGROUND: Oxidative stress might be associated with polycystic ovary syndrome (PCOS), but relatively small studies published to date do not permit reaching a definitive conclusion. We aimed at conducting a systematic review and meta-analysis of studies evaluating circulating markers of oxidative stress in patients with PCOS.

METHODS: We conducted a systematic review of studies reporting circulating markers of oxidative stress in women with PCOS and controls published up to June 2012, using Entrez PubMed and EMBASE online facilities. Meta-analysis calculated standardized mean differences (SMDs) and 95% confidence intervals (95CI).

RESULTS: From 1633 potential studies identified electronically, 68 studies, including 4933 PCOS patients and 3671 controls, were selected. For each of nine circulating markers of oxidative stress, an individual meta-analysis was conducted. Compared with control women, patients with PCOS presented higher circulating concentrations of homocysteine (23% increase, SMD 0.6, 95CI, 0.4–0.8), malondialdehyde (47% increase, SMD 1.9, 95CI 1.2–2.6) and asymmetric dimethylarginine (36% increase, SMD 1.1, 95CI 0.6–1.6), and increased superoxide dismutase activity (34% increase, SMD 1.0, 95CI 0.5–1.4) and decreased glutathione levels (50% decrease, SMD −3.7, 95CI −6.2 to −1.2) and paraoxonase-1 activity (32% decrease, SMD −0.9, 95CI −1.3 to −0.4). Similar results were found when restricting the analyses to studies in which patients and controls were matched for age and body mass index.

CONCLUSIONS: Circulating markers of oxidative stress are abnormal in women with PCOS independent of weight excess. This finding suggests that oxidative stress may participate in the pathophysiology of this common disorder.

Key words: oxidative stress / polycystic ovary syndrome / hyperandrogenism / insulin resistance / obesity

Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine and metabolic disorders, affecting 6–14% of women of childbearing age (Knochenhauer *et al.*, 1998; Diamanti-Kandarakis *et al.*, 1999; Michelmore *et al.*, 1999; Asuncion *et al.*, 2000; March *et al.*, 2010; Sanchon *et al.*, 2012).

PCOS has been associated with metabolic disorders, including insulin resistance, obesity and diabetes. The prevalence of obesity in clinical series of patients with PCOS ranges from 30 to 75% (Ehrmann, 2005) and in as many as 28% of overweight and obese women PCOS is also present (Alvarez-Blasco *et al.*, 2006). Even in the absence of obesity, patients with PCOS frequently have excessive body fat and central adiposity (Kirchengast *et al.*, 2001) and have demonstrable insulin resistance (Goodarzi *et al.*, 2011).

PCOS and its metabolic comorbidities may be explained by the existence of a vicious circle of effects. A chronic androgen excess of ovarian and/or adrenal origin, starting prenatally or early in life, results in abdominal adiposity and android obesity in affected women (Escobar-Morreale and San Millan, 2007). Abdominal adiposity favours hypoadiponectinemia and adipose tissue dysfunction (Kadowaki and Yamauchi, 2005; Escobar-Morreale *et al.*, 2006), local and systemic cytokine excess (Gonzalez *et al.*, 1996; Fernandez-Real *et al.*, 2003) and oxidative stress (Fenkci *et al.*, 2003; Gonzalez *et al.*, 2006), among other mechanisms of disease. Abdominal adiposity thus promotes further androgen excess by a direct response of the ovaries and adrenals to inflammatory mediators or indirectly by the development of insulin resistance and compensatory hyperinsulinemia since insulin facilitates androgen secretion by these glands. Either way the vicious circle which began with abdominal adiposity is closed. Of note, the clinical heterogeneity of PCOS with respect to its metabolic complications may be explained by the individual variability in the relative contributions of androgen excess and abdominal adiposity to this vicious circle.

Aside from low-grade chronic inflammation, oxidative stress might also contribute to PCOS and its metabolic associations (Escobar-Morreale *et al.*, 2005). Oxidative stress is defined as an imbalance derived from excessive formation of oxidants in the presence of limited antioxidant defences (Turrens, 2003).

Oxidants are chemical elements that tend to gain electrons losing positive charge. They include products of normal cellular metabolism such as reactive oxygen species (ROS) and reactive nitrogen species that derive from nitric oxide (RNS). ROS derive from molecular oxygen, and include oxygen ions, free radicals (chemical species with unpaired electrons) and peroxides.

ROS and RNS are well recognized for playing a dual role, since they can be either harmful or beneficial to living systems (Valko *et al.*, 2006). At low-to-moderate concentrations, ROS and RNS are involved in physiological roles including defence against infectious agents and a number of cellular signalling systems. But when present in excess, oxidants may damage DNA, cellular lipids and proteins, interfering with their normal function (Valko *et al.*, 2007). Hence, oxidative stress participates in the pathophysiology of many human pathologic conditions as well as in the physiologic process of ageing.

Protective physiological mechanisms against oxidant species involve preventative and repair mechanisms, physical defences and antioxidant defences (Valko *et al.*, 2007). Antioxidants are compounds that are capable of disposing, scavenging or suppressing the formation of oxidants. They include enzymes [such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and paraoxonase], non-enzymatic macromolecules (such as albumin and ferritin) and small molecules (such as ascorbic acid, glutathione and vitamin E).

Mounting data suggest that obesity induces oxidative stress in humans. Several studies have reported increased levels of reactive species or oxidant products in obesity (Van Gaal *et al.*, 1998; Skrha *et al.*, 1999; Dandona *et al.*, 2001; Block *et al.*, 2002; Olusi, 2002; Ozata *et al.*, 2002; Konukoglu *et al.*, 2003; Urakawa *et al.*, 2003). These levels decrease in response to weight reduction, caloric restriction and diets rich in antioxidants (Kisakol *et al.*, 2002; Uzun *et al.*, 2004; Vincent and Taylor, 2005; Murri *et al.*, 2010). Possible contributors to oxidative stress in obesity include hyperglycemia, hyperlipemia, augmented muscle activity to carry excessive weight, hyperleptinemia, chronic inflammation and inadequate antioxidant defences (Vincent and Taylor, 2005).

Furthermore, reactive species production and oxidative stress have been linked to insulin resistance. Oxidative stress impairs glucose uptake in muscle and adipose tissue, and reduces insulin secretion from pancreatic β cells (Matsuoka *et al.*, 1997; Rudich *et al.*, 1998; Takeda *et al.*, 2005). Recent studies have demonstrated that activation of stress-sensitive intracellular signalling pathways results in insulin resistance and impaired insulin secretion, both *in vitro* (Aguirre *et al.*, 2000; Nguyen *et al.*, 2005; Gao *et al.*, 2010) and *in vivo* (Tuncman *et al.*, 2006; Masharani *et al.*, 2011). In addition, antioxidant treatments may improve insulin sensitivity in patients with insulin resistance or type 2 diabetes (Evans and Goldfine, 2000; Evans *et al.*, 2003).

The pathogenesis of PCOS is complex and its underlying basis remains unclear. Several characteristics and associations of PCOS, including androgen excess, abdominal adiposity, insulin resistance and obesity, may contribute to the development of local and systemic oxidative stress (Vincent and Taylor, 2005; Liu *et al.*, 2010) which may reciprocally worsen these metabolic abnormalities (Fig. 1). However, previous studies addressing circulating markers of oxidative stress in women with PCOS yielded controversial results, partly because the relatively small sample sizes of most of these studies and the variability in the markers and assays used in them.

In order to overcome these limitations, we have conducted a systematic review and meta-analysis of high-quality studies addressing differences in circulating markers of oxidative stress between patients with PCOS and control women.

Methods

Literature search

A systematic review of the literature was conducted using Entrez PubMed and EMBASE online facilities. Studies reporting circulating markers of oxidative stress in women with PCOS and controls and published up to June 2012 were identified and analysed. In addition, a hand search of the references of the retrieved articles and relevant reviews was performed to identify other potentially eligible studies.

First, we conducted a generic search of oxidative stress and PCOS. Then, we searched for the different oxidative stress markers in blood cells, plasma and serum. The search terms were 'PCOS, polycystic ovary syndrome, oxidative stress, antioxidant, oxidant, reactive oxygen species, reactive nitrogen species, protein carbonyl, lipid peroxide,

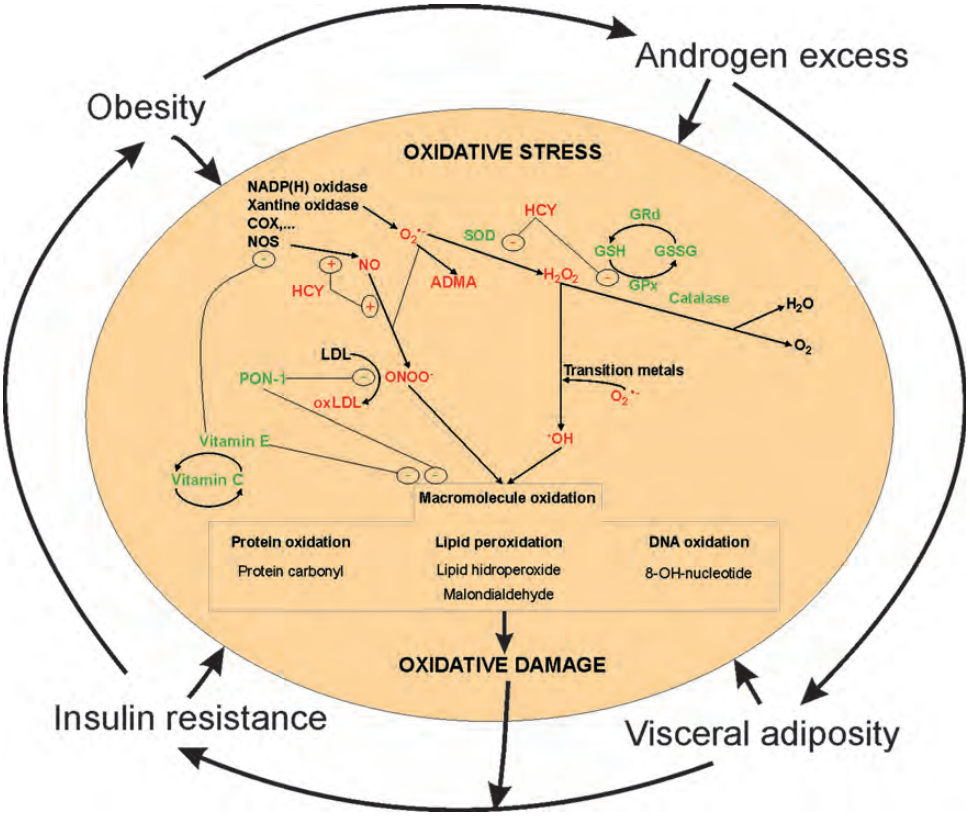

Figure 1 Schematic representation of mechanisms and pathways that may contribute to oxidative stress in polycystic ovary syndrome (PCOS). Several characteristics and associations of PCOS, including androgen excess, abdominal adiposity, insulin resistance and obesity, may contribute to the development of local and systemic oxidative stress which may reciprocally worsen these metabolic abnormalities. Superoxide anion can be produced by NAD(P)H oxidases, xanthine oxidase and cyclooxygenase, among others. Superoxide anion is dismutated by the SOD to hydrogen peroxide. Hydrogen peroxide is scavenged by the enzyme glutathione peroxidase which requires GSH as the electron donor, or by catalase. The oxidized glutathione is reduced back to GSH by glutathione reductase. Some transition metals can breakdown hydrogen peroxide to the reactive hydroxyl radical. The hydroxyl radical can substract an electron from polyunsaturated fatty acid, DNA or proteins. Lipid hydroperoxides can be reduced by the reduced form of Vitamin E, which can be regenerated by Vitamin C, or by GPx using GSH as the electron donor. Nitric oxide, which is produced by nitric oxide synthase, and the superoxide anion may react together to produce peroxynitrite anion, which is a potent oxidizing agent that can cause protein, DNA and lipid oxidation. Paraoxonase 1 prevents the formation of oxidized LDL. Homocysteine might result in oxidative stress by decreasing the transcription, translation and catalytic activity of glutathione peroxidase and SOD, and by inducing inducible nitric oxide synthase. ADMA, asymmetric dimethylarginine; COX, cyclooxigenase; H_2O_2, hydrogen peroxide; HCY, homocysteine; GPx, glutathione peroxidase; GRd, glutathione reductase; GSH, reducted glutathione; GSSG, oxidized glutathione; LDL, low-density lipoprotein; NO, nitric oxide; NOS, nitric oxide synthase; $O_2^{\cdot-}$ superoxide anion; $^{\cdot}OH$, hydroxyl radical; ONOO$^-$, peroxynitrite; oxLDL, oxidized low-density lipoprotein; PON-1, paraoxonase 1; SOD, superoxide dismutase.

malondialdehyde, 8-hydroxydeoxyguanosine, thiobarbituric acid reactive substances, nitric oxide, nitrotyrosine, sulfhydryl group, asymmetric dimethylarginine, oxidized LDL lipoprotein, xanthine oxidase, total oxidant status, total antioxidant capacity, superoxide dismutase, glutathione, glutathione peroxidase, glutathione reductase, glutathione transferase, catalase, homocystein, paraoxonase-1, vitamin A, vitamin C, vitamin E', including their acronyms and synonyms.

Inclusion and exclusion criteria

Inclusion criteria included: (i) studies where reproductive-age women with PCOS were compared with non-hyperandrogenic controls for circulating markers of oxidative stress; (ii) strict diagnosis of PCOS using National Institute of Child Health and Human Development (NICHD) conference definition (Zawadzki and Dunaif, 1992), European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) definition (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), or Androgen Excess & PCOS Society (AE-PCOS) definition (Azziz *et al.*, 2009) and (iii) articles written in English. Meta-analysis was conducted for markers studied in five or more studies. To avoid over-representation of cases, when several studies on the same series of cases and controls have been published, only the report with the larger sample size was included in the meta-analysis.

Exclusion criteria included: (i) studies in which the diagnosis of PCOS was not strict; (ii) studies addressing markers of oxidative stress in samples other than blood and its derivatives; (iii) studies reporting less than 10 cases and/or controls and (iv) articles not written in English.

Study selection was performed independently by three investigators (M.M., M.I. and M.O.-O.). Disagreements were discussed and resolved by consensus.

Data extraction

The following data were extracted from the retrieved studies: author's names, study design, publication date, country, sample size, diagnostic criteria for PCOS, mean age and BMI, mean and standard deviation of circulating markers of oxidative stress and analytical method.

Quality assessment

The studies included for the meta-analysis were appraised for their methodological quality according to a modification of the Newcastle-Ottawa Quality Assessment Scale for case–control studies (Wells *et al.*, 2000), as presented in Table I. The following items were evaluated in order to score the studies to maximum of eight points:

Selection

(1) Is the case definition adequate? (a) Yes, with independent validation [one point]; (b) yes, e.g. record linkage or based on self-reports; (c) no description.
(2) Representativeness of the cases: (a) consecutive or obviously representative series of cases (cases with outcome of interest over a defined period of time, all cases in a defined catchment area, all cases in a defined hospital or clinic, group of hospitals, health maintenance organization, or an appropriate sample of those cases) [one point]; (b) potential for selection biases or not stated.
(3) Selection of controls: (a) community controls [one point]; (b) hospital controls; (c) no description.
(4) Definition of controls: (a) no history of disease (end-point) [one point]; (b) no description of source.

Comparability

(1) Comparability of cases and controls on the basis of design or analysis: (a) study controls for age [one point]; (b) study controls for any additional factor [one point]. Statements of no differences between groups or that the differences were not statistically significant are sufficient for establishing comparability.

Exposure

(1) Ascertainment of exposure: (a) secure record (PCOS diagnosis were clinically assessed) [one point]; (b) written self-report or medical record only; (c) no description.
(2) Same method of ascertainment for cases and controls: (a) yes [one point]; (b) no.

Statistical analysis

Meta-analyses were performed using the MIX program version 1.7 (Bax *et al.*, 2006). We used Hedge's g standardized mean differences (SMDs) as a measure of effect size because the outcomes of the studies were frequently measured by different assays and techniques. SMD becomes dimensionless and the scales become uniform across the different studies. Results are given as SMD and 95% confidence intervals (95CI). In order to help readers judge the practical importance of the effect found (namely, having PCOS versus being a control), we translated the effect size into Cohen's U3–50% index. This index is the percentile place difference between the two distributions (it will be 0 if there is no difference) and may be interpreted as the increase or decrease in patients compared with controls, as a percentage.

A separate random-effects model was constructed for each oxidative stress marker using the DerSimonian–Laird weighting method, which incorporates between-study variability into the calculations. This method was selected because of the considerable likelihood that there was heterogeneity and inconsistency across studies resulting from the variability in the ethnicity and race of the subjects, differences among studies in the criteria used to define PCOS, and inconsistent control of confounding factors such as obesity. We tested heterogeneity using Cochran's Q and Higgins's I^2 statistics.

A subsequent subgroup analysis was restricted to studies in which the PCOS and control groups were matched for BMI and age. If a study mismatched for these variables included a subgroup of patients and controls that were matched for age and BMI, these subjects were included in subanalysis. We asked the corresponding authors of the original articles for the mean and SD of the groups as needed (i.e. when the data were reported as medians or geometric means).

Evidence dissemination bias was estimated by funnel plot asymmetry and Egger's regression test (Egger *et al.*, 1997) when 10 or more studies were analysed. All P values are two-sided, and $\alpha = 0.05$ was set as a level of statistical significance.

Results

Meta-analysis of circulating markers of oxidative stress

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow charts, from identification of studies to meta-analysis, are presented in Fig. 2 and the characteristics of the eligible studies for meta-analysis are summarized in Table II. The methodological quality assessment of the included studies showed that 82% of the articles were of high quality and the remaining were of medium quality (Table I).

Meta-analysis of homocysteine

The search yielded 487 manuscripts, of which 347 were duplicate articles, and 1 additional article was identified from the reference lists of those publications (Mohan and Vishnu, 2009). Then 59 manuscripts were immediately excluded (31 reviews, 11 congress abstracts/articles, 5 editorials, 6 letters, and 6 non-English articles). The remaining 82 studies were retrieved for a more detailed evaluation. Of these 82 studies, 33 were excluded for the following reasons: (i) not relevant for the research question (5 manuscripts); (ii) non-strict diagnosis of PCOS (Loverro *et al.*, 2002; Schachter *et al.*, 2003; Yilmaz *et al.*, 2005b; Badawy *et al.*, 2007); (iii) absence of a control group (Randeva *et al.*, 2002; Vrbikova *et al.*, 2002; Kilic-Okman and Kucuk, 2004; Kilicdag *et al.*, 2005a, b; Cagnacci *et al.*, 2006; Carlsen *et al.*, 2007; Schachter *et al.*, 2007; Gul *et al.*, 2008; Kazerooni *et al.*, 2008; Haydardedeoglu *et al.*, 2009; Makedos *et al.*, 2010; Mancini *et al.*, 2010; Palomba *et al.*, 2010; Altug Sen *et al.*, 2011; Gharakhani *et al.*, 2011; Kilic *et al.*, 2011; Rajagopal *et al.*, 2012); (iv) studies not showing the homocysteine concentrations of the control group (Luque-Ramirez *et al.*, 2009) or of the controls and patients (Glueck *et al.*, 2003); (v) homocystein was measured in follicular fluid (Berker *et al.*, 2009) and (vi) no response to requests for data clarification (Pamuk *et al.*, 2010; Moini *et al.*, 2011, 2012). Moreover, 14 studies were excluded due to over-representation of cases also included in larger series by the same authors (Kucuk and Kilic-Okman, 2005; Yilmaz *et al.*, 2005a; Palep-Singh *et al.*, 2007, 2008; Yilmaz *et al.*, 2008; Erdogan *et al.*, 2008a; Mancini *et al.*, 2009; Kaya *et al.*, 2009a, b, c, 2010b; Arikan *et al.*, 2010; Karadeniz *et al.*, 2010, 2011).

The remaining 35 studies containing usable information were used to perform the meta-analysis of circulating homocysteine concentrations (Yarali *et al.*, 2001; Morgante *et al.*, 2002; Orio *et al.*, 2003;

Table I Quality assessment of studies included in meta-analyses using a modified Newcastle–Ottawa Quality Assessment Scale for case–control studies.

Author (year)	Selection	Comparability	Exposure	Total points	Quality assessment rating[a]
Arikan *et al.* (2009)	4	2	2	8	High
Atamer *et al.* (2008)	3	2	2	7	High
Baskol *et al.* (2012)	2	2	2	6	High
Battaglia *et al.* (2008)	2	2	2	6	High
Bausenwein *et al.* (2010)	2	1	1	4	Medium
Bayrak *et al.* (2012)	4	2	2	8	High
Bayraktar *et al.* (2004)	3	2	2	7	High
Bayram *et al.* (2012)	2	2	2	6	High
Bickerton *et al.* (2005)	3	2	1	6	High
Boulman *et al.* (2004)	2	1	1	4	Medium
Caglar *et al.* (2011)	3	2	1	6	High
Cakir *et al.* (2011)	3	1	1	5	Medium
Carmina *et al.* (2005)	4	2	2	8	High
Cetinkalp *et al.* (2009)	2	2	1	5	Medium
Charitidou *et al.* (2008)	4	1	1	6	High
Demirel *et al.* (2007)	3	1	2	6	High
Dincer *et al.* (2005)	2	2	2	6	High
Dursun *et al.* (2006)	4	2	2	8	High
Dursun *et al.* (2011)	3	2	2	7	High
Espinos-Gomez *et al.* (2012)	4	1	2	7	High
Fan *et al.* (2012)	3	0	2	5	Medium
Fenkci *et al.* (2003)	2	2	2	6	High
Fenkci *et al.* (2007)	4	2	2	8	High
Fulghesu *et al.* (2010)	4	2	2	8	High
Guzelmeric *et al.* (2007)	3	2	2	7	High
Harmanci *et al.* (2013)	3	2	2	7	High
Hemati *et al.* (2011)	3	2	1	6	High
Heutling *et al.* (2008)	4	2	2	8	High
Karadeniz *et al.* (2011)	4	2	2	8	High
Karaer *et al.* (2010)	3	2	1	6	High
Kaya *et al.* (2009b)	3	2	2	7	High
Kaya *et al.* (2010c)	3	2	2	7	High
Kilic-Okman *et al.* (2004)	1	2	1	4	Medium
Kurdoglu *et al.* (2012)	4	2	2	8	High
Kuscu and Var, (2009)	3	2	2	7	High
Macut *et al.* (2006)	4	2	2	8	High
Macut *et al.* (2011)	2	2	2	6	High
Markou *et al.* (2010)	3	2	2	7	High
Mohamadin *et al.* (2010a)	3	2	1	6	High
Mohamadin *et al.* (2010b)	3	2	1	6	High
Mohan and Vishnu, (2009)	3	1	2	6	High
Moran *et al.* (2009)	3	2	1	6	High
Morgante *et al.* (2002)	2	1	2	5	Medium
Nacul *et al.* (2007)	3	2	2	7	High
Nafiye *et al.* (2010)	2	2	2	6	High
Ngo *et al.* (2011)	3	2	1	6	High

Continued

Table I *Continued*

Author (year)	Selection	Comparability	Exposure	Total points	Quality assessment rating[a]
Oktem *et al.* (2009)	3	2	2	7	High
Orio *et al.* (2003)	2	2	2	6	High
Ozgurtas *et al.* (2008)	4	2	2	8	High
Palacio *et al.* (2006)	2	2	1	5	Medium
Rajendran *et al.* (2009)	2	1	1	4	Medium
Sabuncu *et al.* (2001)	3	2	2	7	High
Sahin *et al.* (2007)	4	2	1	7	High
Salehpour *et al.* (2011)	4	2	2	8	High
San Millan *et al.* (2006)	4	1	2	7	High
Soares *et al.* (2009)	4	2	2	8	High
Soyman *et al.* (2011)	3	2	2	7	High
Temel *et al.* (2010)	2	2	2	6	High
Topcu *et al.* (2006)	3	2	1	6	High
Torun *et al.* (2011)	1	2	1	4	Medium
Turkcuoglu *et al.* (2011)	4	1	1	6	High
Verit and Erel, (2008)	3	2	2	7	High
Victor *et al.* (2011)	3	2	2	7	High
Vrbikova *et al.* (2003)	4	0	2	6	High
Wijeyaratne *et al.* (2004)	2	1	1	4	Medium
Yarali *et al.* (2001)	2	1	2	5	Medium
Yildizhan *et al.* (2011)	4	1	1	6	High
Yilmaz *et al.* (2005c)	2	2	2	6	High

[a]Studies scoring 0 to 2 points were considered of low quality, from 3 to 5 points, studies were considered of medium quality, and from 6 to 8 points studies were considered of high quality.

Vrbikova *et al.*, 2003; Bayraktar *et al.*, 2004; Boulman *et al.*, 2004; Kilic-Okman *et al.*, 2004; Wijeyaratne *et al.*, 2004; Bickerton *et al.*, 2005; Carmina *et al.*, 2005; Yilmaz *et al.*, 2005c; Topcu *et al.*, 2006; Guzelmeric *et al.*, 2007; Sahin *et al.*, 2007; Atamer *et al.*, 2008; Battaglia *et al.*, 2008; Heutling *et al.*, 2008; Arikan *et al.*, 2009; Cetinkalp *et al.*, 2009; Mohan and Vishnu, 2009; Oktem *et al.*, 2009; Soares *et al.*, 2009; Fulghesu *et al.*, 2010; Karaer *et al.*, 2010; Markou *et al.*, 2010; Nafiye *et al.*, 2010; Temel *et al.*, 2010; Mohamadin *et al.*, 2010b; Kaya *et al.*, 2010c; Caglar *et al.*, 2011; Hemati *et al.*, 2011; Salehpour *et al.*, 2011; Bayrak *et al.*, 2012; Espinos-Gomez *et al.*, 2012; Harmanci *et al.*, 2013).

This meta-analysis included 3511 women (2090 women with PCOS and 1421 controls). Mean homocysteine concentrations were increased by 23% in women with PCOS compared with controls (SMD 0.6, 95CI 0.4–0.8, z = 6.5, $P < 0.01$, Fig. 3). As suspected, heterogeneity was present (Q 207, $P < 0.01$; I^2 84%, 95CI 78–88%), but dissemination bias was not present (Egger's regression intercept 2.3, 95CI −0.2 to 4.9, $P = 0.07$, Fig. 3).

To rule out any influence of obesity and age in these results, we restricted the meta-analysis to studies in which patients with PCOS and controls were matched for age and BMI. We also included subgroups of patients and controls that were matched for age and BMI within studies that, overall, were not matched for age and BMI. The meta-analysis excluding six mismatched studies (Morgante *et al.*, 2002; Vrbikova *et al.*, 2003; Boulman *et al.*, 2004; Wijeyaratne *et al.*, 2004; Mohan and Vishnu, 2009; Hemati *et al.*, 2011) and maintaining only the subgroup of patients and controls presenting with similar BMI and age of another mismatched study (Espinos-Gomez *et al.*, 2012) yielded similar results (24% increase in PCOS, SMD 0.7, 95CI 0.4–0.9, $z = 6.2$, $P < 0.01$; Q 192, $P < 0.01$; I^2 85%, 95CI 79–89%; Egger's regression intercept 2.4, 95CI −0.6 to 5.4, $P = 0.11$).

Meta-analysis of malondialdehyde

The initial search returned 200 manuscripts, of which 158 were duplicate articles. One additional article was identified from the reference lists of those publications (Mohan and Vishnu, 2009). Then 9 manuscripts were immediately excluded (4 congress abstracts/articles and 5 non-English articles). The remaining 34 studies were retrieved for detailed evaluation. Of these 34 studies, 18 were excluded for the following reasons: (i) not relevant for the research question (12 manuscripts); (ii) non-strict diagnosis of PCOS (Dincer *et al.*, 2001); (iii) absence of a control group (Kaya *et al.*, 2010d); (iv) malondialdehyde (MDA) was measured in follicular fluid (Yildirim *et al.*, 2007; Berker *et al.*, 2009) and (v) no response to requests for data clarification (Kaya *et al.*, 2010b; Kulkarni *et al.*, 2011). Moreover, two studies were excluded due to over-representation of cases also included in larger series by the same authors (Karadeniz *et al.*, 2008; Erdogan *et al.*, 2008b).

The remaining 14 studies containing usable information were used to perform the meta-analysis of circulating MDA concentrations

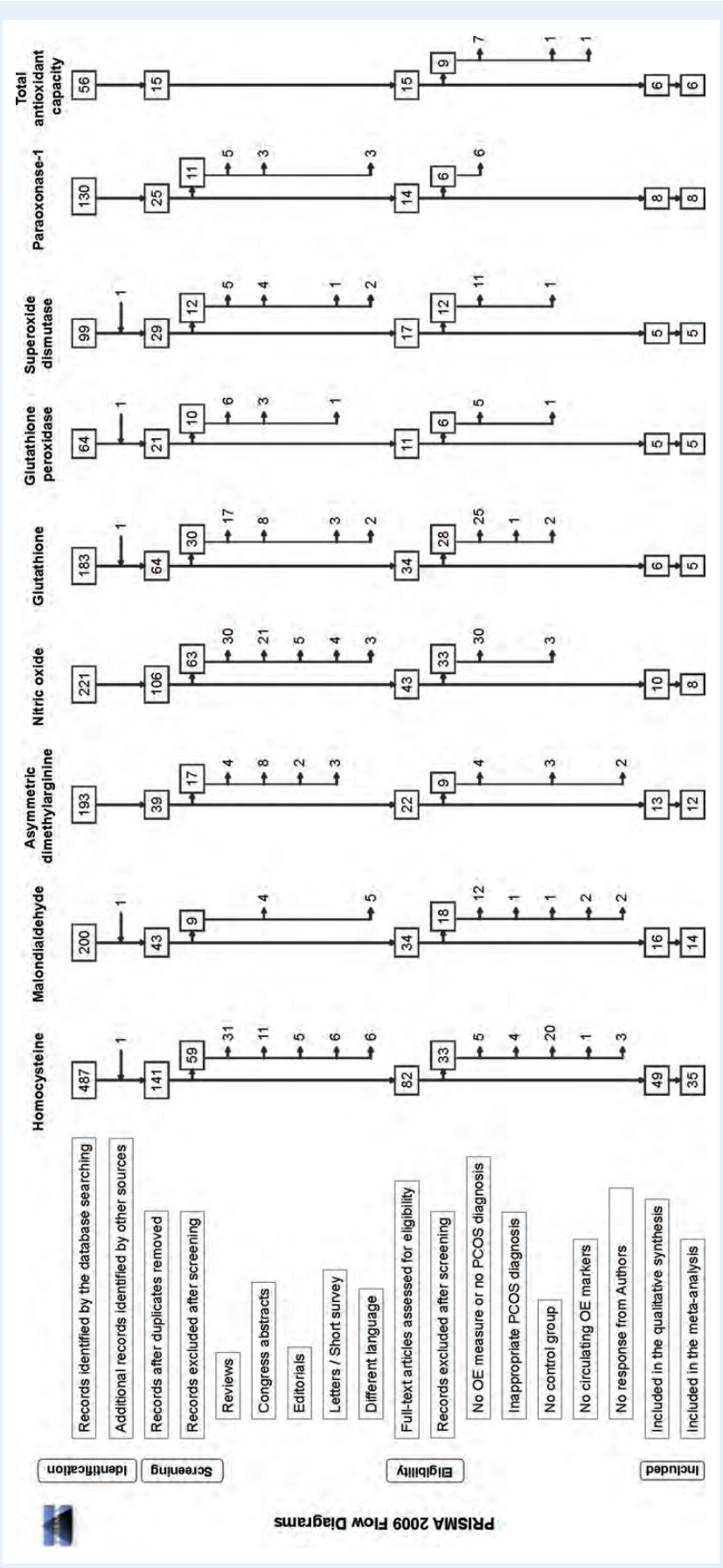

Figure 2 Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flowcharts of the circulating markers of oxidative stress submitted to meta-analysis. OE, oxidative stress; PCOS, polycystic ovary syndrome.

Table II Characteristics of the studies included in meta-analyses and in the descriptive review.

Author (year)	Country	Simple size (*n*)		Mean age (years)		Mean BMI (kg/m^2)		Participant selection	Definition of PCOS
		PCOS	Controls	PCOS	Controls	PCOS	Controls		
Arikan *et al.* (2009)	Turkey	39	30	22.8	24.6	21.5	20.9	Age and BMI matched	ESHRE/ASRM
Atamer *et al.* (2008)	Turkey	35	30	27.3	27.9	28.0	27.6	Age and BMI matched	ESHRE/ASRM & NICHD & AE-PCOS
Baskol *et al.* (2012)	Turkey	30	20	28.2	29.5	26.1	25.1	Age and BMI matched	ESHRE/ASRM
Battaglia *et al.* (2008)	Italy	28	15	25.2	24.9	25.2	25.9	Age and BMI matched	NICHD
Bausenwein *et al.* (2010)	Germany	62	22	29.6	31.9	27.6	25.0	BMI matched	ESHRE/ASRM & NICHD & AE-PCOS
Bayrak *et al.* (2012)	Turkey	77	25	21.8	22.5	23.9	22.1	Age and BMI matched	ESHRE/ASRM
Bayraktar *et al.* (2004)	Turkey	50	25	21.4	21.6	23.9	22.1	Age and BMI matched	NICHD
Bayram *et al.* (2012)	Turkey	45	17	24.0	25.7	23.8	21.4	Age matched, no difference in BMI	ESHRE/ASRM
Bickerton *et al.* (2005)	United Kingdom	11	12	33.5	30.7	35.3	31.0	No differences in age or in BMI	NICHD
Boulman *et al.* (2004)	Israel	33	16	NA	NA	NA	NA	No differences in BMI	NICHD
Caglar *et al.* (2011)	Turkey	61	21	22.6	23.4	22.7	21.1	No differences in age or in BMI	ESHRE/ASRM
Cakir *et al.* (2011)	Turkey	52	36	23.6	25.5	24.2	22.6	Age matched, no difference in BMI	ESHRE/ASRM
Carmina *et al.* (2005)	Italy	254	127	23.7	24.1	26.4	24.3	Age matched	ESHRE/ASRM
Cetinkalp *et al.* (2009)	Turkey	129	91	24.6	25.5	24.5	24.2	Age and BMI matched	ESHRE/ASRM
Charitidou *et al.* (2008)	Greece	106	30	21.2	29.1	24.0	25.5	BMI matched	ESHRE/ASRM
Demirel *et al.* (2007)	Turkey	44	31	15.3	15.5	25.8	21.1	Age matched, only one subgroup of patients was matched for BMI	NICHD
Dincer *et al.* (2005)	Turkey	35	24	23.0	22.0	25.6	24.9	Age and BMI matched	NICHD
Dursun *et al.* (2006)	Turkey	23	23	24.4	25.1	23.0	22.2	Age and BMI matched	ESHRE/ASRM
Dursun *et al.* (2011)	Turkey	25	27	22.7	24.2	22.4	20.6	Age and BMI matched	ESHRE/ASRM & NICHD & AE-PCOS
Espinos-Gomez *et al.* (2012)	Spain	196	21	26.2	28.5	29.8	21.6	No difference in age, only one subgroup of patients showed no differences in BMI with the controls	NICHD
Fan *et al.* (2012)	China	291	281	25.0	28.4	22.9	20.9	Not matched	ESHRE/ASRM
Fenkci *et al.* (2003)	Turkey	30	31	25.8	26.1	24.3	25.2	Age matched, no difference in BMI	NICHD
Fenkci *et al.* (2007)	Turkey	31	33	24.0	25.4	24.5	23.4	Age and BMI matched	ESHRE/ASRM & NICHD & AE-PCOS
Fulghesu *et al.* (2010)	Italy	71	94	18.6	18.1	24.0	22.6	Age and BMI matched	ESHRE/ASRM
Glintborg *et al.* (2008)	Denmark	30	14	NA	NA	33.1	33.2	Age and BMI matched	NICHD
Gonzalez *et al.* (2006)	USA	16	15	27.5	31.5	29.5	31.5	Weight matched, no difference in age	ESHRE/ASRM
Guzelmeric *et al.* (2007)	Turkey	44	26	23.5	25.9	26.5	24.1	Age and BMI matched	NICHD
Hamurcu *et al.* (2010)	Turkey	36	29	21.8	23.4	26.0	23.6	Age and BMI matched	ESHRE/ASRM
Harmanci *et al.* (2013)	Turkey	23	23	22.0	21.7	21.9	21.8	Age and BMI matched	ESHRE/ASRM & NICHD & AE-PCOS
Hemati *et al.* (2011)	Iran	64	50	31.1	29.1	22.7	22.1	Age and physical activity matched	ESHRE/ASRM

Continued

Table II *Continued*

Author (year)	Country	Simple size (*n*)		Mean age (years)		Mean BMI (kg/m^2)		Participant selection	Definition of PCOS
		PCOS	Controls	PCOS	Controls	PCOS	Controls		
Heutling *et al.* (2008)	United Kingdom	83	39	27.8	27.8	30.4	29.1	Age and BMI matched	ESHRE/ASRM
Karadeniz *et al.* (2011)	Turkey	98	93	24.4	25.6	24.8	24.3	Age and BMI matched	ESHRE/ASRM
Karaer *et al.* (2010)	Turkey	31	31	27.9	27.9	28.9	28.9	Age and BMI matched	ESHRE/ASRM
Kaya *et al.* (2009b)	Turkey	35	30	NA	NA	NA	NA	Age and BMI matched	ESHRE/ASRM
Kaya *et al.* (2010c)	Turkey	68	68	24.2	24.2	23.4	24.1	Age and BMI matched	ESHRE/ASRM
Kilic-Okman *et al.* (2004)	Turkey	29	25	23.9	25.2	25.3	23.3	No difference in age or BMI	NICHD
Kurdoglu *et al.* (2012)	Turkey	31	29	24.3	26.8	21.9	22.7	Age matched, no difference in BMI	ESHRE/ASRM
Kuscu and Var, (2009)	Turkey	31	23	23.8	22.5	21.8	20.5	Age and BMI matched	ESHRE/ASRM
Macut *et al.* (2006)	Serbia	179	56	24.3	24.4	25.3	23.5	Age and BMI matched	ESHRE/ASRM & NICHD & AE-PCOS
Macut *et al.* (2011)	Serbia	34	23	23.8	25.3	23.0	21.1	Age and BMI matched	ESHRE/ASRM
Markou *et al.* (2010)	Greece	17	17	25.1	25.9	20.9	21.1	Age and BMI matched	ESHRE/ASRM
Mohamadin *et al.* (2010a)	Saudi Arabia	50	40	30.2	29.3	29.3	25.7	No difference in age or BMI	ESHRE/ASRM
Mohamadin *et al.* (2010b)	Saudi Arabia	35	30	29.2	28.5	28.1	24.9	No difference in age or BMI	ESHRE/ASRM
Mohan *et al.* (2009)	India	56	56	NA	NA	24.8	21.1	Age matched	ESHRE/ASRM & NICHD & AE-PCOS
Moran *et al.* (2009)	Australia	80	27	34.1	33.8	36.0	37.4	Age and BMI matched	ESHRE/ASRM & NICHD & AE-PCOS
Morgante *et al.* (2002)	Italy	20	12	24.0	23.0	25.1	21.6	No difference in age	NICHD
Nacul *et al.* (2007)	Brazil	31	21	22.4	26.7	NA	NA	Age matched	NICHD
Nafiye *et al.* (2010)	Turkey	36	61	29.6	29.2	26.3	24.7	No difference in age or BMI	ESHRE/ASRM
Ngo *et al.* (2011)	Australia	27	20	31.5	33.1	26.9	26.3	Age and weight matched	ESHRE/ASRM
Oktem *et al.* (2009)	Turkey	31	31	25.6	25.6	29.7	29.3	Age and BMI matched	ESHRE/ASRM
Orio *et al.* (2003)	Italy	70	70	22.5	21.9	24.1	23.8	Age and BMI matched	NICHD
Ozgurtas *et al.* (2008)	Turkey	44	22	NA	NA	21.8	21.4	Age and BMI matched	ESHRE/ASRM
Palacio *et al.* (2006)	Spain	10	21	28.6	31.1	22.1	24.6	No difference in age or BMI	NICHD
Rajendran *et al.* (2009)	Australia	24	12	32.1	30.5	27.5	21.2	Age matched, only one subgroup of patients showed no differences in BMI with the controls	ESHRE/ASRM
Sabuncu *et al.* (2001)	Turkey	27	18	26.7	28.8	31.4	30.0	Age and BMI matched	NICHD
Sahin *et al.* (2007)	Turkey	20	20	21.6	22.9	22.4	23.2	Age and BMI matched	ESHRE/ASRM
Salehpour *et al.* (2011)	Iran	85	83	29.0	27.3	26.7	25.8	BMI matched	NICHD
San Millan *et al.* (2006)	Spain	107	58	25.0	31.0	29.6	29.5	BMI matched	NICHD
Soares *et al.* (2009)	Brazil	40	50	24.5	24.5	22.7	23.1	Age and BMI matched	ESHRE/ASRM
Sova *et al.* (2010)	Finland	50	20	27.0	30.6	27.6	26.8	Age and BMI matched	ESHRE/ASRM
Soyman *et al.* (2011)	Turkey	30	30	24.3	23.3	23.5	22.6	Age and BMI matched	ESHRE/ASRM
Temel *et al.* (2010)	Turkey	30	30	23.1	23.3	22.3	20.8	Age and BMI matched	ESHRE/ASRM
Topcu *et al.* (2006)	Turkey	28	26	27.1	28.8	26.6	24.7	Age matched	ESHRE/ASRM & NICHD & AE-PCOS
Torun *et al.* (2011)	Turkey	30	20	23.5	25.9	26.8	26.4	Age and BMI matched	ESHRE/ASRM

Turkcuoglu *et al.* (2011)	Turkey	40	35	25.7	27.9	24.9	23.4	No difference in BMI	ESHRE/ASRM
Verit and Erel, (2008)	Turkey	63	58	24.4	24.8	21.2	21.8	Age and BMI matched	ESHRE/ASRM
Victor *et al.* (2011)	Spain	39	43	24.2	26.0	22.2	21.8	Age and BMI matched	ESHRE/ASRM
Vrbikova *et al.* (2003)	Czech Republic	40	11	25.8	33.0	27.8	22.3	Not matched	NICHD
Wijeyaratne *et al.* (2004)	United Kingdom	161	78	27.6	32.7	29.0	23.7	Not matched	NICHD
Yarali *et al.* (2001)	Turkey	30	30	27.9	31.4	27.3	25.0	BMI matched	NICHD
Yildizhan *et al.* (2011)	Turkey	57	27	26.4	25.4	26.9	23.9	Age matched, only one subgroup of patients showed no differences in BMI with the controls	ESHRE/ASRM
Yilmaz *et al.* (2005c)	Turkey	50	35	24.1	24.4	21.9	22.5	Age and weight matched	ESHRE/ASRM

AE-PCOS, Androgen Excess & Polycystic Ovary Syndrome Society; BMI, body mass index; ESHRE/ASRM, European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine; NA, not available; NICHD, National Institute of Child Health and Human Development; PCOS, polycystic ovary syndrome.

(Sabuncu *et al.*, 2001; Yilmaz *et al.*, 2005c; Dursun *et al.*, 2006; Palacio *et al.*, 2006; Kuscu and Var, 2009; Mohan and Vishnu, 2009; Rajendran *et al.*, 2009; Kaya *et al.*, 2009b; Mohamadin *et al.*, 2010a; Karadeniz *et al.*, 2011; Macut *et al.*, 2011; Bayram *et al.*, 2012; Fan *et al.*, 2012; Kurdoglu *et al.*, 2012). The meta-analysis included 1481 women (790 women with PCOS and 691 controls). Mean MDA levels were increased by 47% in women with PCOS compared with controls (SMD 1.9, 95CI 1.2–2.6, $z = 5.2$, $P < 0.01$, Fig. 4), yet there was evidence of dissemination bias for this marker (Egger's regression intercept 6.7, 95CI 2.4–11.1, $P < 0.01$, Fig. 4). In addition, high heterogeneity was observed (Q 392, $P < 0.01$; I^2 97%, 95CI 96–98%).

The meta-analysis excluding two studies mismatched for age or BMI (Mohan and Vishnu, 2009; Fan *et al.*, 2012) and maintaining only the subgroup of patients and controls presenting with similar BMI and age of another mismatched study (Rajendran *et al.*, 2009) yielded similar results (43% relative increase in PCOS, SMD 1.5, 95CI 0.8–2.2, $z = 4.0$, $P < 0.01$; Q 210, $P < 0.01$; I^2 95%, 95CI 92–96%; Egger's regression intercept 9.7, 95CI 4.5–14.9, $P < 0.01$).

Meta-analysis of asymmetric dimethylarginine (ADMA)

The initial search yielded 193 manuscripts, of which 154 were duplicate articles, and 17 manuscripts were immediately excluded (4 reviews, 8 congress abstracts/articles, 3 letters and 2 editorials). The remaining 22 studies were retrieved for a more detailed evaluation. Of these 22 studies, 9 were excluded for the following reasons: (i) not relevant for the research question (4 studies); (ii) studies not showing the ADMA concentrations of the control group (Kebapcilar *et al.*, 2009; Teede *et al.*, 2010; Kilic *et al.*, 2011) and (iii) no response to requests for data clarification (Pamuk *et al.*, 2010; Lakhani *et al.*, 2011). Furthermore, one study was excluded due to over-representation of cases (Moran *et al.*, 2011).

The remaining 12 studies (Demirel *et al.*, 2007; Charitidou *et al.*, 2008; Heutling *et al.*, 2008; Ozgurtas *et al.*, 2008; Moran *et al.*, 2009; Rajendran *et al.*, 2009; Mohamadin *et al.*, 2010b; Ngo *et al.*, 2011; Soyman *et al.*, 2011; Turkcuoglu *et al.*, 2011; Yildizhan *et al.*, 2011; Bayrak *et al.*, 2012) containing usable information were used to perform the ADMA meta-analysis. This meta-analysis included 1000 women (662 women with PCOS and 338 controls). Mean ADMA levels were 36% higher in women with PCOS compared with controls (SMD 1.1, 95CI 0.6–1.6, z = 4.2, $P < 0.01$, Fig. 4), yet there was evidence of dissemination bias for this meta-analysis (Egger's regression intercept 10.6, 95CI 1.5–19.7, $P = 0.03$, Fig. 4). Heterogeneity was also observed (Q 132, $P < 0.01$; I^2 92%, 95CI 87–95%).

The meta-analysis excluding one mismatched study (Turkcuoglu *et al.*, 2011) and maintaining only the subgroup of patients and controls presenting with similar BMI and age of three mismatched studies (Demirel *et al.*, 2007; Rajendran *et al.*, 2009; Yildizhan *et al.*, 2011) yielded similar results but without statistically significant evidence of dissemination bias (37% increase in PCOS, SMD 1.1, 95CI 0.6–1.7, $z = 3.9$, $P < 0.01$; Q 128, $P < 0.01$; I^2 92%, 95CI 88–95%; Egger's regression intercept 8.6, 95CI −0.3 to 17.5, $P = 0.06$).

Meta-analysis of nitric oxide (NO)

The initial search yielded 221 manuscripts, of which 115 were duplicate articles. Then 63 manuscripts were immediately excluded (30 reviews, 21 congress abstracts/articles, 5 editorials, 4 letters

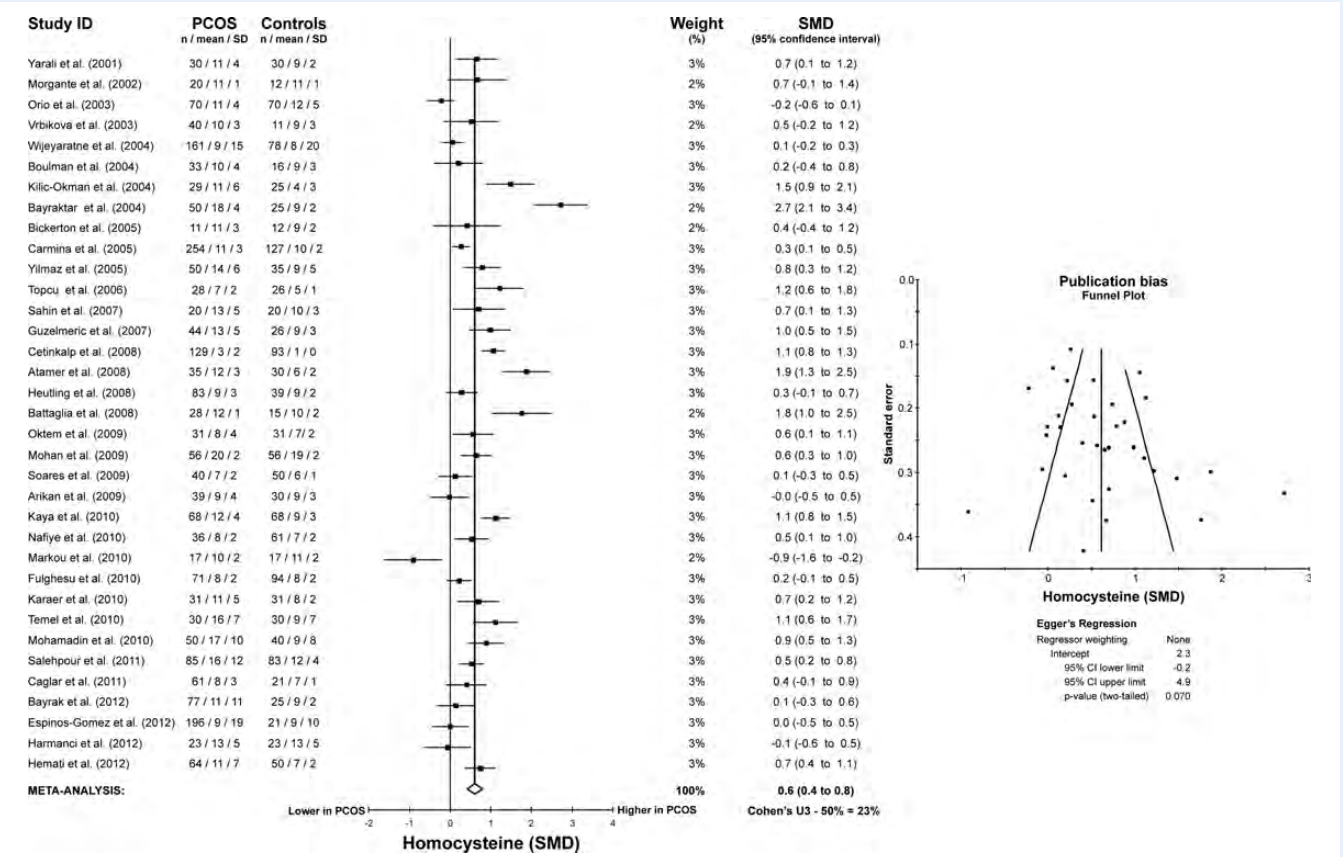

Figure 3 Meta-analysis of studies addressing circulating homocysteine levels (in μmol/l) in patients with PCOS and control women. Results are shown as Hedges' g standardized mean differences (SMDs) and Cohen's U3−50% index. Evidence of dissemination bias was assessed using funnel plot asymmetry and Egger's regression.

and 3 non-English articles). The remaining 43 studies were retrieved for a more detailed evaluation. Of the 43 studies, 33 were excluded for the following reasons: (i) not relevant for the research question (30 studies) and (ii) studies not showing nitric oxide concentrations of the control group (Battaglia *et al.*, 2010; Kaya *et al.*, 2010a; Kilic *et al.*, 2011). Moreover, two studies were excluded as over-representation of cases (Karadeniz *et al.*, 2008; Erdogan *et al.*, 2008b)

The remaining eight studies (Nacul *et al.*, 2007; Kuscu and Var, 2009; Mohamadin *et al.*, 2010b; Dursun *et al.*, 2011; Karadeniz *et al.*, 2011; Turkcuoglu *et al.*, 2011; Baskol *et al.*, 2012; Bayram *et al.*, 2012) containing usable information were used to perform a meta-analysis that included 626 women (350 women with PCOS and 276 controls). There was no statistically significant difference in NO levels in women with PCOS compared with controls (SMD −0.3, 95CI −0.8 to 0.3, $z = 1.0$, $P = 0.32$, Fig. 4). Heterogeneity was observed (Q 74, $P < 0.01$; $I^2 = 91\%$, 95CI 84–94%).

The meta-analysis excluding one mismatched study (Turkcuoglu *et al.*, 2011) yielded similar results (SMD −0.4, 95CI −1.0 to 0.2, $z = 1.3$, $P = 0.21$; Q = 68, $P < 0.01$; $I^2 = 91\%$, 95CI 84–95%).

Meta-analysis of glutathione

The initial search yielded 183 manuscripts, of which 120 were duplicate articles. In the reference list of those publications, one additional article was identified for the current review (Mohan and Vishnu, 2009). Then 30 manuscripts were immediately excluded (17 reviews, 8 congress abstracts/articles, 2 letters, 1 short survey and 2 non-English articles). The remaining 34 studies were retrieved for a more detailed evaluation. Of these 34 studies, 28 were excluded for the following reasons: (i) not relevant for the research question (25 studies); (ii) non-strict diagnosis of PCOS (Dincer *et al.*, 2001); (iii) studies not showing the glutathione concentrations of the control group (Taskin *et al.*, 1999; Dona *et al.*, 2012). Moreover, one study was excluded to avoid over-representation of cases (Victor *et al.*, 2009).

The remaining five studies containing usable information were used to perform the glutathione meta-analysis (Sabuncu *et al.*, 2001; Dincer *et al.*, 2005; Mohan and Vishnu, 2009; Victor *et al.*, 2011; Kurdoglu *et al.*, 2012), including 358 women (188 women with PCOS and 170 controls). The mean glutathione levels were 50% lower in women with PCOS compared with controls (SMD −3.7, 95CI −6.2 to −1.2, $z = 2.9$, $P < 0.01$, Fig. 5). Heterogeneity was observed (Q = 231, $P < 0.01$; $I^2 = 98\%$, 95CI 97–99%).

The meta-analysis excluding one mismatched study (Mohan and Vishnu, 2009) yielded similar results (49% decrease in PCOS, SMD −2.4, 95CI −4.3 to −0.5, $z = 2.4$, $P < 0.01$; Q 98.8, $P < 0.01$; $I^2 = 97\%$, 95CI 95–98%).

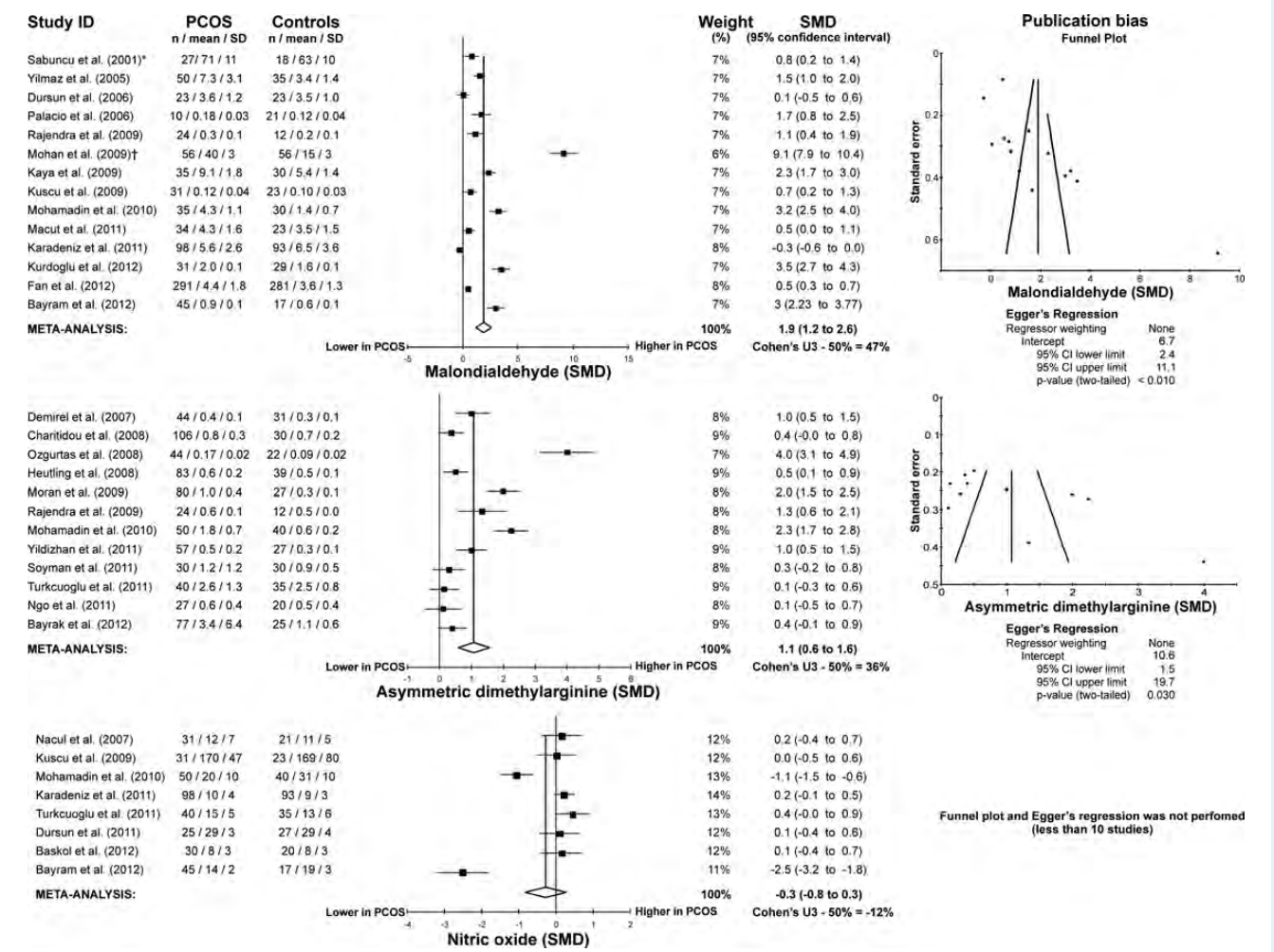

Figure 4 Meta-analysis of studies addressing circulating malondialdehyde, asymmetric dimethylarginine and nitric oxide levels in patients with PCOS and control women. All units are μmol/l except *μmol/mol Hb and †nmol/g Hb. Results are shown as Hedges' g SMDs and Cohen's U3−50% index. Evidence of dissemination bias was assessed by funnel plot asymmetry and Egger's regression only for malondialdehyde and asymmetric dimethylarginine, because less than 10 studies were included in the nitric oxide meta-analysis.

Meta-analysis of GPx activity

The initial search yielded 64 manuscripts, of which 44 were duplicate articles. One additional article (Mohan and Vishnu, 2009) was identified from the reference list of those publications. Then 10 manuscripts were immediately excluded (6 reviews, 3 congress abstracts/articles and 1 letter). Of the remaining 11 studies, 6 were excluded for the following reasons: (i) not relevant for the research question (5 studies) and (ii) one study where the GPx activity of the control group was not shown and where GPx activity was measured in peritoneal tissues (Taskin *et al.*, 1999).

The remaining five studies (Sabuncu *et al.*, 2001; Mohan and Vishnu, 2009; Bausenwein *et al.*, 2010; Macut *et al.*, 2011; Baskol *et al.*, 2012) containing usable information were used to perform the GPx meta-analysis that included 348 women (169 women with PCOS and 179 controls). There was no statistically significant difference in GPx activity in women with PCOS compared with controls (SMD 0.5, 95CI −0.4 to 1.4, $z = 1.2$, $P = 0.24$, Fig. 5). Heterogeneity was observed ($Q = 55$, $P < 0.01$; $I^2 = 93\%$, 95CI 86−96%). The meta-analysis excluding two mismatched studies (Mohan and Vishnu, 2009; Bausenwein *et al.*, 2010) yielded similar results with no heterogeneity (SMD = 0.2, 95CI −0.2 to 0.5, $z = 1.0$, $P = 0.34$; $Q = 1.94$, $P = 0.38$; $I^2 = 0\%$, 95CI 0−90%).

Meta-analysis of SOD activity

The initial search yielded 99 manuscripts, of which 71 were duplicate articles. In the reference list of those publications, one additional article was identified for the current review (Mohan and Vishnu, 2009). Then 12 manuscripts were immediately excluded (5 reviews, 4 congress abstracts/articles, 1 letter and 2 non-English articles). The remaining 17 studies were retrieved for a more detailed evaluation. Of these 17 studies, 12 were excluded for the following reasons: (i) not relevant for the research question (eleven studies) and (ii) one study not showing SOD activity of the control group and where SOD activity was measured in peritoneal tissues (Taskin *et al.*, 1999).

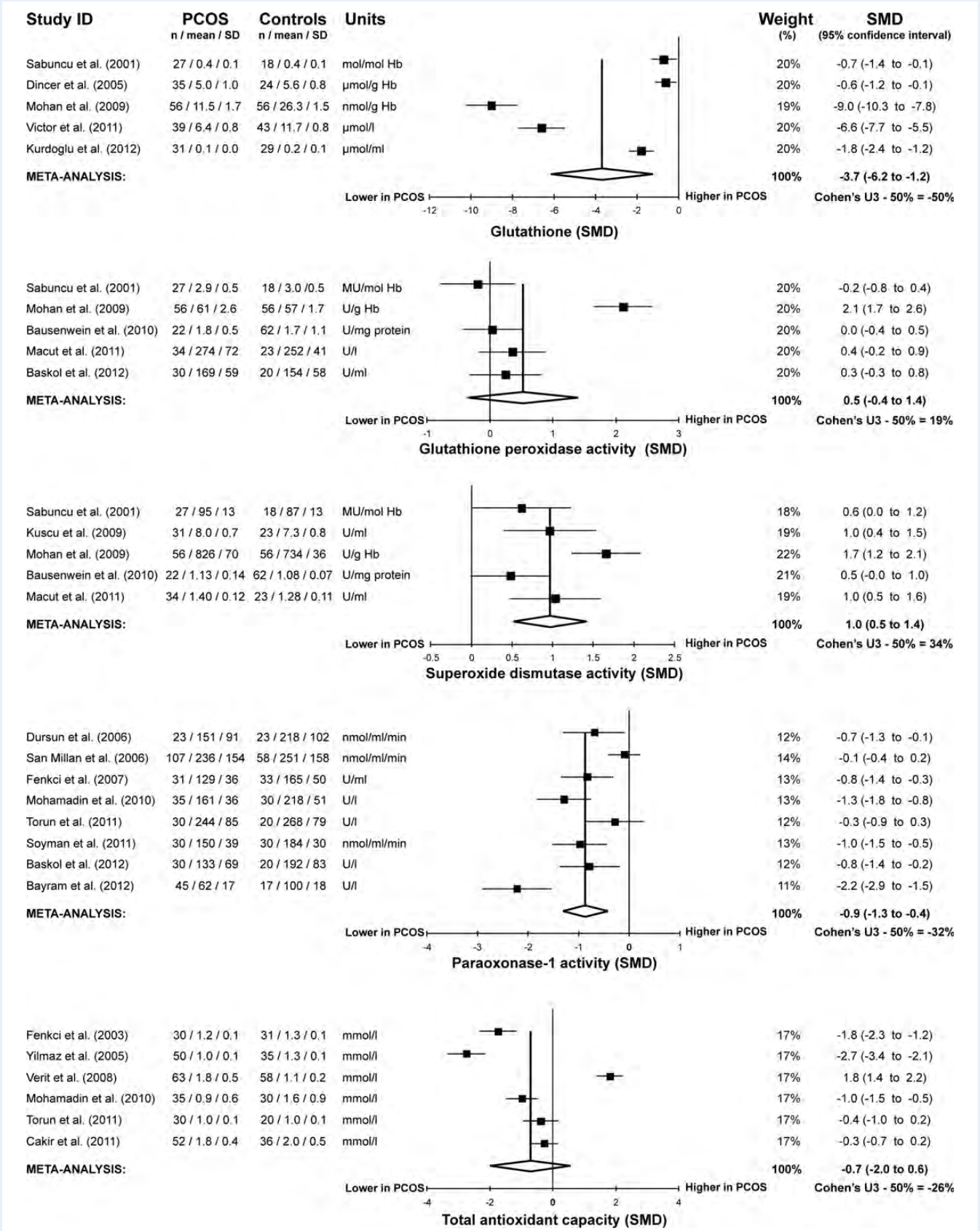

Figure 5 Meta-analysis of studies addressing circulating antioxidant molecules, including glutathione levels, glutathione peroxidase, SOD and paraoxonase-1 activities, and TAC, in patients with PCOS and control women. Units are given in the figure. Results are shown as Hedges' g SMDs and Cohen's U3−50% index. SOD, superoxide dismutase activity; TAC, total antioxidant capacity.

The remaining five studies (Sabuncu *et al.*, 2001; Kuscu and Var, 2009; Mohan and Vishnu, 2009; Bausenwein *et al.*, 2010; Macut *et al.*, 2011) containing usable information were used to perform the SOD meta-analysis that included 352 women (170 women with PCOS and 182 controls). Mean SOD activity was 34% higher in women with PCOS compared with controls (SMD = 1.0, 95CI 0.5–1.4, $z = 4.2$, $P < 0.01$, Fig. 5). Heterogeneity was observed (Q = 15, $P < 0.01$; $I^2 = 73\%$, 95CI 32–89%).

The meta-analysis excluding two mismatched studies (Bausenwein *et al.*, 2010; Mohan and Vishnu, 2009) yielded similar results but without heterogeneity (31% increase in PCOS, SMD = 0.9, 95CI 0.6–1.2, $z = 5.2$, $P < 0.01$; Q = 1.1, $P = 0.58$; $I^2 = 0\%$, 95CI 0–90%).

Meta-analysis of paraoxonase 1 (PON-1) activity

The initial search yielded 130 manuscripts, of which 105 were duplicate articles. Then 11 manuscripts were immediately excluded (5 reviews, 3 congress abstracts/articles and 3 non-English articles). The remaining 14 studies were retrieved for a more detailed evaluation. Of these, 6 studies were excluded as they were not relevant for the research question.

The remaining eight studies (Dursun *et al.*, 2006; San Millan *et al.*, 2006; Fenkci *et al.*, 2007; Mohamadin *et al.*, 2010a; Soyman *et al.*, 2011; Torun *et al.*, 2011; Baskol *et al.*, 2012; Bayram *et al.*, 2012) which contained usable information were included in the PON-1 meta-analysis that included 562 women (331 women with PCOS and 231 controls).

Mean PON-1 activity was 32% lower in women with PCOS compared with controls (SMD −0.9, 95CI −1.3 to −0.4, $z = 3.8$, $P < 0.01$, Fig. 5). Heterogeneity was observed (Q = 41, $P < 0.01$; $I^2 = 83\%$, 95CI 68–91%).

The meta-analysis excluding one mismatched study (San Millan *et al.*, 2006) yielded similar results with moderate heterogeneity (34% decrease in PCOS, SMD −1.0, 95CI −1.4 to −0.6, $z = 4.8$, $P < 0.01$; Q = 21, $P < 0.01$; $I^2 = 72\%$, 95CI 39–87%).

Meta-analysis of total antioxidant capacity

The initial search yielded 56 manuscripts, of which 41 were duplicate articles. The remaining 15 studies were retrieved for a more detailed evaluation. Of these 15 studies, 9 were excluded for the following reasons: (i) not relevant for the research question (7 articles); (ii) studies not showing the total antioxidant capacity (TAC) concentrations of the control group (Verit *et al.*, 2007) and (iii) TAC was measured in follicular fluid (Chattopadhayay *et al.*, 2010).

The remaining six studies containing usable information were used to perform the TAC meta-analysis (Fenkci *et al.*, 2003; Yilmaz *et al.*, 2005c; Verit and Erel, 2008; Mohamadin *et al.*, 2010a; Cakir *et al.*, 2011; Torun *et al.*, 2011). This meta-analysis included 470 women (260 women with PCOS and 210 controls). There was no significant difference in TAC in women with PCOS compared with controls (SMD −0.7, 95CI −2.0 to 0.6, $z = 1.1$, $P < 0.28$, Fig. 5), and heterogeneity was observed (Q = 188, $P < 0.01$; $I^2 = 97\%$, 95CI 96–98%).

Systematic review of other oxidative stress markers

Several other circulating markers that have been studied in less than five studies with usable information are reviewed below.

Increased total oxidant status in patients with PCOS compared with controls has been reported in two studies (Verit and Erel, 2008; Torun *et al.*, 2011), yet no differences were found in another (Cakir *et al.*, 2011). Also, polymorphonuclear cells from women with PCOS produced more ROS than those of controls (Victor *et al.*, 2011).

By-products of oxidative damage would be expected to be increased in patients with PCOS if oxidative stress plays a significant role in the pathogenesis of this disorder. However, the studies conducted to date have yielded relatively conflicting results.

Plasma nitrotyrosine levels, products of nitrosative damage formed most notable by the reaction of superoxide and nitric oxide radicals with protein tyrosine residues, were significantly higher in patients with PCOS compared with healthy controls (Macut *et al.*, 2011). Serum protein carbonyl levels, a marker of oxidative modification of proteins, were increased in patients with PCOS compared with controls in two studies (Fenkci *et al.*, 2003; Kurdoglu *et al.*, 2012). Similarly, patients with PCOS presented with increased circulating concentrations of oxidized low-density lipoprotein (which promote atherogenesis through foam cell formation and inflammatory responses) in three studies (Macut *et al.*, 2006; Glintborg *et al.*, 2008; Bausenwein *et al.*, 2010), yet no differences were found between patients and controls in another study (Demirel *et al.*, 2007).

Lipid peroxides are the products of chemical damage done by oxygen free radicals to the polyunsaturated fatty acids of cell membranes. Serum lipid peroxide concentrations were increased in patients with PCOS in one study (Torun *et al.*, 2011), yet plasma thiobarbituric acid-reactive substances, a byproduct of lipid peroxidation, were similar in patients with PCOS and controls in another (Gonzalez *et al.*, 2006). However, circulating 8-hydroxydeoxyguanosine concentrations, which are a commonly used marker of oxidative stress-derived DNA damage, have been found to be similar (Hamurcu *et al.*, 2010) or even decreased (Sova *et al.*, 2010) in patients with PCOS compared with controls. Similarly, the reduction–oxidation system represented by thiol levels (also known as sulphhydryl groups) have been reported as either decreased (Torun *et al.*, 2011), similar (Baskol *et al.*, 2012) or even increased (Erdogan *et al.*, 2008b) in patients with PCOS compared with controls.

The results of studies addressing enzymes that participate in the generation or scavenging of oxidants are inconclusive. Serum xanthine oxidase, an enzyme that plays an important role in the catabolism of purines in humans and generates ROS, was increased in PCOS in one study (Baskol *et al.*, 2012). The circulating levels of glutathione-*S*-transferase, an enzyme that inactivates toxic endogenous compounds formed as secondary metabolites during oxidative stress, has been found to be either decreased (Kurdoglu *et al.*, 2012) or increased (Mohan and Vishnu, 2009) in patients with PCOS compared with controls. Similar conflicting results have been found for catalase, an enzyme involved in the detoxification of hydrogen peroxide, as its activity has been reported to be similar (Kurdoglu *et al.*, 2012), increased (Bausenwein *et al.*, 2010) or decreased (Mohan and Vishnu, 2009) in patients with PCOS compared with controls.

Finally, several vitamins with antioxidant properties derived from their scavenging of oxidant molecules have been studied in PCOS. Serum (Kurdoglu *et al.*, 2012) and erythrocyte (Mohan and Vishnu, 2009) vitamin C concentrations are lower in patients with PCOS compared with controls, and the same applies to circulating vitamin E concentrations (Mohan and Vishnu, 2009; Kurdoglu *et al.*, 2012). Vitamin

A and β-carotene, on the contrary, were not different in patients with PCOS and controls (Kurdoglu et al., 2012).

Discussion

Many studies that have addressed oxidative stress and PCOS to date have not given a definitive conclusion about their possible association (Lee et al., 2010). With a few notable exceptions (Wijeyaratne et al., 2004; Carmina et al., 2005; San Millan et al., 2006; Charitidou et al., 2008; Espinos-Gomez et al., 2012; Fan et al., 2012), these studies have suffered from small sample sizes and included less than 100 patients with PCOS. Moreover, many different circulating markers used to estimate oxidative stress and the frequent finding of conflicting results across studies addressing the same markers may explain why the question of whether or not PCOS is associated with oxidative stress still remains open.

Our present systematic review, allowing the meta-analysis of data of nine different circulating markers of oxidative stress obtained from 68 individual studies that included a total of 4933 patients with PCOS and 3671 control women, may shed some light into this unresolved issue.

Meta-analyses of these studies showed that the concentrations of several promoters and by-products of oxidative stress were significantly increased in patients with PCOS compared with control women. Homocysteine induces oxidative stress by promoting ROS production by increasing NADPH oxidase and decreasing thioredoxin (Tyagi et al., 2005), and its concentrations were increased in PCOS patients compared with control women according to our meta-analysis. A similar increase in ADMA concentrations, which also enhances ROS production (Sydow and Munzel, 2003), and in malondialdehyde, which is an end-product of lipid peroxidation and a good marker of oxidant-mediated damage and oxidative stress (Del Rio et al., 2005), were found in PCOS.

On the other hand, our meta-analyses indicated that some circulating antioxidant markers were decreased in PCOS. Glutathione concentrations were decreased in patients with PCOS compared with controls. Glutathione plays a main protective role against oxidative stress, being a cofactor of several antioxidant enzymes, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of GPx, but being able to regenerate the most important antioxidants, vitamins C and E, back to their active forms. A similar decrease in the activity of PON-1 in patients with PCOS may contribute to oxidative stress because PON-1 is an antioxidant enzyme that prevents oxidation of lipoproteins and hydrolyzes atherogenic products of oxidative lipid modification such as phospholipid peroxides and cholesterol ester hydroperoxides. Furthermore, even the increase found in patients with PCOS in the activity of SOD, a potent protective enzyme scavenging superoxide anion radical by catalyzing its dismutation to H_2O_2 and O_2 (Pollack and Leeuwenburgh, 1999), may be interpreted as a compensatory mechanism in response to the increased production of oxidant molecules mentioned above.

Although meta-analyses did not result in significant differences among patients with PCOS and control women in nitric oxide levels, GPx activity and TAC, overall, the decrease in glutathione and paraoxonase-1 levels in parallel with an increase of oxidant molecules such as homocysteine, ADMA and MDA suggests an imbalance in the oxidative status of patients with PCOS.

However, because obesity enhances ROS production promoting oxidative stress, and PCOS and obesity are frequently associated, the possibility exists that the association of PCOS with markers of oxidative stress could be spurious, actually deriving from the association of PCOS with obesity. The fact that the abnormalities in circulating markers of oxidative stress persisted when we restricted the meta-analyses to studies and subgroups of women matched for age and BMI indicates that oxidative stress may be present in PCOS irrespective of its well-known association with weight excess, obesity and ageing. Moreover, the systematic review identified individual studies showing differences between patients with PCOS and controls in other oxidants, by-products of oxidative stress and antioxidant vitamins and compounds, further supporting the participation of oxidative stress in PCOS.

Because PCOS is a life-time heterogenous endocrinopathy, long-term management of this frequent disorder must consider all the consequences of the syndrome, including the metabolic comorbidities in which oxidative stress may play a role. For this purpose, the finding of a clinically helpful individual marker of oxidative stress in patients with PCOS would be of importance. Our present review suggests that glutathione, paraoxonase-1, homocysteine, ADMA and MDA levels might be appropriate to estimate the oxidative status of women with PCOS. However, the magnitude of the changes in markers of oxidative stress associated with PCOS was relatively small and of uncertain clinical consequences and, therefore, routine clinical measurements of these markers cannot be recommended with the evidence gathered to date.

Regarding therapeutic strategies, correction of oxidative stress by improving antioxidant defences through body fat mass reduction, pharmacological agents, exercise and/or dietary modification might have beneficial effects in PCOS, as has been demonstrated for insulin-resistant disorders (Abdel-Wahab et al., 2002; Vincent et al., 2007; Wright and Sutherland, 2008). Although experimental data suggest that strategies targeting oxidative stress might prove useful for PCOS (Masharani et al., 2010; Rzepczynska et al., 2011), the paucity of studies addressing antioxidant therapy in patients with PCOS precludes making any recommendation for routine clinical use of antioxidants in this disorder.

The strengths of our current systematic review and meta-analysis include the wide range of markers of oxidative stress evaluated, the large number of subjects included in the analyses, the exclusion of duplicate reports and the medium- to high-quality requirement for inclusion of studies in the meta-analysis as assessed by a modified Newcastle-Ottawa Quality Assessment Scale for case–control studies (Wells et al., 2000).

However, our study is not free of limitations. First, the magnitude of the changes in circulating markers of oxidative stress associated with PCOS was modest (50% or less) and therefore the results must be interpreted with caution. Processes of oxidative stress participate in the pathogenesis of disease mostly at the tissue level and may not translate entirely into its circulating markers, explaining the modest changes observed here. Secondly, two of the largest meta-analyses showed evidence for dissemination (publication) bias, suggesting that studies with positive results (namely, those showing statistically significant differences between patients and controls) were more likely to be published than studies with negative results, another reason for a careful interpretation of the results. Thirdly, most if not all the

studies conducted to date compared patients with PCOS with control women selected as to have no evidence of androgen excess and, in some cases, other metabolic disorders. This use of 'hypernormal' controls may lead to false-positive results that could have been avoided by the more appropriate recruitment of unselected control women from the general population (Hattersley and McCarthy, 2005). Fourthly, there was considerable heterogeneity in the studies included in the meta-analyses. Factors contributing to this heterogeneity include the differences in the criteria used for the definition of PCOS, differences in race and ethnicity of the subjects and the use of different assays and different samples (plasma, serum, leukocytes and erythrocytes). Furthermore, blood sampling after an adequate fasting period is a critical issue when analysing oxidative stress as fasting may modify the results of assays (Pollack and Leeuwenburgh, 1999); however, this key issue for the assessment of markers of oxidative stress was not specified with detail in most studies.

In order to overcome the impact that the heterogeneity resulting from clinical and/or methodological issues might have had on the results of the meta-analyses we chose Hedges's g SMD as a measure of the effect size (Durlak, 2009) and implemented random-effects models using the DerSimonian–Laird weighting method (DerSimonian and Laird, 1986). Random-effects models assume that the estimated effects in the different studies are variable but there is some type of specific distribution, using the observed effect from individual studies to estimate this distribution. Specifically, the DerSimonian–Laird weighting method estimates the magnitude of heterogeneity and assigns a greater variability (confidence interval) to the effect size to compensate for this heterogeneity. Of note, clinical factors were possibly responsible for the heterogeneity observed in some of the meta-analyses (i.e. GPx and SOD activities) as the subanalysis of studies and subgroups matched for age and BMI were no longer heterogeneous. In any case, the overall results of the meta-analyses were similar including or excluding mismatched groups and studies, indicating that heterogeneity was correctly compensated by the statistical approach used here.

Table III Summary of the meta-analysis of circulating markers of oxidative stress in polycystic ovary syndrome (PCOS).

Marker	Action	Changes in PCOS
Promoters and byproducts of oxidative stress		
Homocysteine	Promotes reactive species	↑
ADMA	Promotes reactive species	↑
Malondialdehyde	End-product of lipid peroxidation	↑
Nitric oxide	Promotes reactive nitrogen species	↔
Antioxidants		
Glutathione	Detoxifies hydrogen peroxide and lipid peroxides, prevents protein from oxidation	↓
Paraoxonase-1	Prevents oxidation of lipoproteins by reactive species	↓
SOD	Converts superoxide anions to hydrogen peroxide and molecular oxygen	↑
Glutathione peroxidase	Detoxifies hydrogen peroxide, peroxynitrites and lipid peroxides	↔
TAC	Prevents oxidation and detoxifies oxidants	↔

↓, decreased; ↑, increased; ↔, unchanged; ADMA, asymmetric dimethylarginine; SOD, superoxide dismutase activity TAC, total antioxidant capacity.

In conclusion, as summarized in Table III, our systematic review and meta-analysis indicates that several circulating markers of oxidative stress are abnormal in women with PCOS independent of weight excess. Although modest in magnitude, these abnormalities suggest that oxidative stress may participate in the pathophysiology of this common disorder. However, in light of the evidence available at present, neither the routine measurement of markers of oxidative stress nor the use of antioxidant therapies can be recommended as yet for the clinical management of PCOS.

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Authors' roles

M.M. contributed to the study conception and design and data collection and analysis, and wrote the draft of the article. M.L-R. analysed the data and revised the article critically for important intellectual content. M.I. and M.O.-O. contributed to data collection and analysis. H.F.E-M. contributed to the study conception and design, analysed the data, revised the article critically for important intellectual content and wrote the final version of the manuscript. All authors approved the final version of the article and take full responsibility for the accuracy of its content.

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Conflict of interest

None declared.

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