

Carotid artery intima-media thickness in polycystic ovary syndrome: a systematic review and meta-analysis

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BACKGROUND: Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder associated with cardiovascular disease (CVD) risk factors and metabolic disturbances. This systematic review and meta-analysis was conducted to determine whether carotid intima-media thickness (CIMT), a marker of subclinical atherosclerosis, is higher in women with PCOS compared with women without PCOS.

METHODS: Primary articles reporting the mean CIMT in women with PCOS and controls were identified using Ovid MEDLINE, EMBASE and PUBMED. We performed a random-effects meta-analysis and created forest plots of the mean difference in CIMT and conducted tests for heterogeneity and publication bias. Studies were grouped by quality, defined by reporting reproducibility of CIMT and averaging both common carotid arteries versus one side for CIMT.

RESULTS: From the 36 eligible full-text studies, 8 studies were included in the systematic review and 19 studies were included in the meta-analysis (total $n = 1123$ women with PCOS, $n = 923$ controls). The summary mean difference in CIMT among women with PCOS compared with controls was 0.072 mm [95% confidence interval (CI) 0.040, 0.105, $P < 0.0001$] for highest quality studies, 0.084 mm (95% CI 0.042,

0.126, $P = 0.0001$) for good quality studies, 0.041 (95% CI -0.038 , 0.120, $P = 0.310$) for fair-quality studies and 0.045 (95% CI -0.020 , 0.111, $P = 0.173$) for lower quality studies.

CONCLUSIONS: Larger studies with a well-defined PCOS population using rigorous methodology may be required to draw a more robust conclusion. However, these results suggest women with PCOS are at a greater risk of premature atherosclerosis, which emphasizes the importance of screening and monitoring CVD risk factors in women with PCOS.

Key words: polycystic ovary syndrome / carotid-intima media thickness / subclinical cardiovascular disease / systematic review

Introduction

Polycystic ovary syndrome (PCOS) is a reproductive endocrine disorder that affects ~7 million women or ~6–10% of women in the USA (Azziz *et al.*, 2004; Nestler, 2008). Women with PCOS experience acne, excessive hair, weight gain and irregular periods. In addition, these women also have an increase in cardiovascular disease (CVD) risk factors with more insulin resistance (IR) (Glueck *et al.*, 2009), dyslipidemia (Legro *et al.*, 2001), abdominal obesity (Casella *et al.*, 2008), type 2 diabetes (Moran *et al.*, 2010) and inflammation (Boulman *et al.*, 2004) than those without this disorder. These risk factors and metabolic disturbances may be associated with functional and structural impairments of the vascular system resulting in acceleration of atherosclerosis as these women age.

The extent to which there is an increased risk of subclinical atherosclerosis and CVD events among women with PCOS remains controversial. Studies of CVD events in women with PCOS are limited but a recent meta-analysis showed women with PCOS had two times the relative risk of coronary heart disease or stroke than controls (de Groot *et al.*, 2011). Some studies have found that women with PCOS had more severe subclinical atherosclerosis as measured by coronary calcification scores (CAC) (Christian *et al.*, 2003; Talbott *et al.*, 2004, 2008; Shroff *et al.*, 2007), carotid artery intima-media thickness (CIMT) (Talbott *et al.*, 2000; Lakhani *et al.*, 2004; Orio *et al.*, 2004; Vryonidou *et al.*, 2005; Vural *et al.*, 2005; Carmina *et al.*, 2006; Heutling *et al.*, 2008) and endothelial dysfunction measured by flow-mediated dilation (FMD) (Tarkun *et al.*, 2004; Kravariti *et al.*, 2005; Lowenstein *et al.*, 2007; Casella *et al.*, 2008) compared with controls. However, these studies have not shown consistent results (Meyer *et al.*, 2005; Costa *et al.*, 2008; Arikani *et al.*, 2009; Soares *et al.*, 2009). Most of these investigations for subclinical atherosclerosis were limited by small sample sizes and evaluated young women of reproductive age.

Several excellent reviews have discussed the association of PCOS with CVD risk factors and the risk of CVD (Legro, 2003; Loverro, 2004; Cussons *et al.*, 2006; Dokras, 2008; Mak and Dokras, 2009; Wild *et al.*, 2010) but a systematic review has yet to be conducted of studies on subclinical atherosclerosis in women with PCOS. Thus, the aim of this study was to review the literature regarding CVD risk assessment by CIMT in women with PCOS compared with controls. CIMT is a non-invasive ultrasound measure of the thickness of the intima-media of the common carotid arteries. CIMT is a widely used structural marker of subclinical atherosclerosis that is associated with CVD risk factors (van der Meer *et al.*, 2003; Hurst *et al.*, 2007) and CVD events (Poredos, 2004; Espeland *et al.*, 2005; Lorenz *et al.*, 2007a, b). The protocol for this report

follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati *et al.*, 2009; Moher *et al.*, 2009).

Methods

Eligibility criteria

Primary articles investigating CIMT among women with PCOS and without PCOS (controls) were included if they: (i) were a peer-reviewed primary article, (ii) had a study population of women with PCOS [diagnostic criteria for PCOS specified by the National Institutes of Health (NIH)] (Zawadzki and Dunaif, 1992), the European Society of Human Reproduction and Embryology/American Society of Reproductive Medicine (ESHRE/ASRM, 2004) and/or the Androgen Excess Society (AES) criteria (Azziz *et al.*, 2006), and were compared with controls without PCOS, (iii) reported a measure of CIMT (unadjusted or adjusted) and (iv) were published in the English language. Studies without a control group were excluded.

Search strategy and study selection

Articles assessing CIMT in women with PCOS were identified using Ovid MEDLINE, EMBASE and PUBMED. The primary search was conducted in Ovid MEDLINE through 19 November 2010 (M.L.M.). The search terms for Ovid MEDLINE included carotid artery diseases, tunica media, carotid artery, common/tunica intima, arteriosclerosis, intima-media thickness and PCOS: physiopathology, pathology, complications, etiology, mortality, ultrasonography, epidemiology, prevention and control (Fig. 1). The search terms for EMBASE were intima-media thickness, ovary polycystic disease limited to humans and limited to the publication years to 1980–2010. A search from 1980 to 2010 through PUBMED did not identify any new references. Two independent investigators reviewed reference lists from the primary search (M.L.M. and E.O.T.). Review papers were assessed to find possible references not identified in the Medline and EMBASE journal databases. Before finalizing this meta-analysis, a PUBMED search in February 2011 identified an additional study published in 2011.

Data extraction

The data from the studies were extracted into a table and re-verified (M.L.M.). The information from each study included: first author, journal, study design, whether the study controlled for age and BMI or weight, PCOS diagnostic criteria used, source of the control population, number of participants, mean age and BMI of the participants, measure of CIMT (both unadjusted and adjusted measures were extracted if reported), P -value for the difference between cases and controls and the methodology for the CIMT measurement (carotid segments used, the calculation of CIMT and reproducibility information).

Two investigators met to discuss the eligibility of studies to be included in the meta-analysis (M.L.M. and E.O.T.). In cases of disagreement, a third

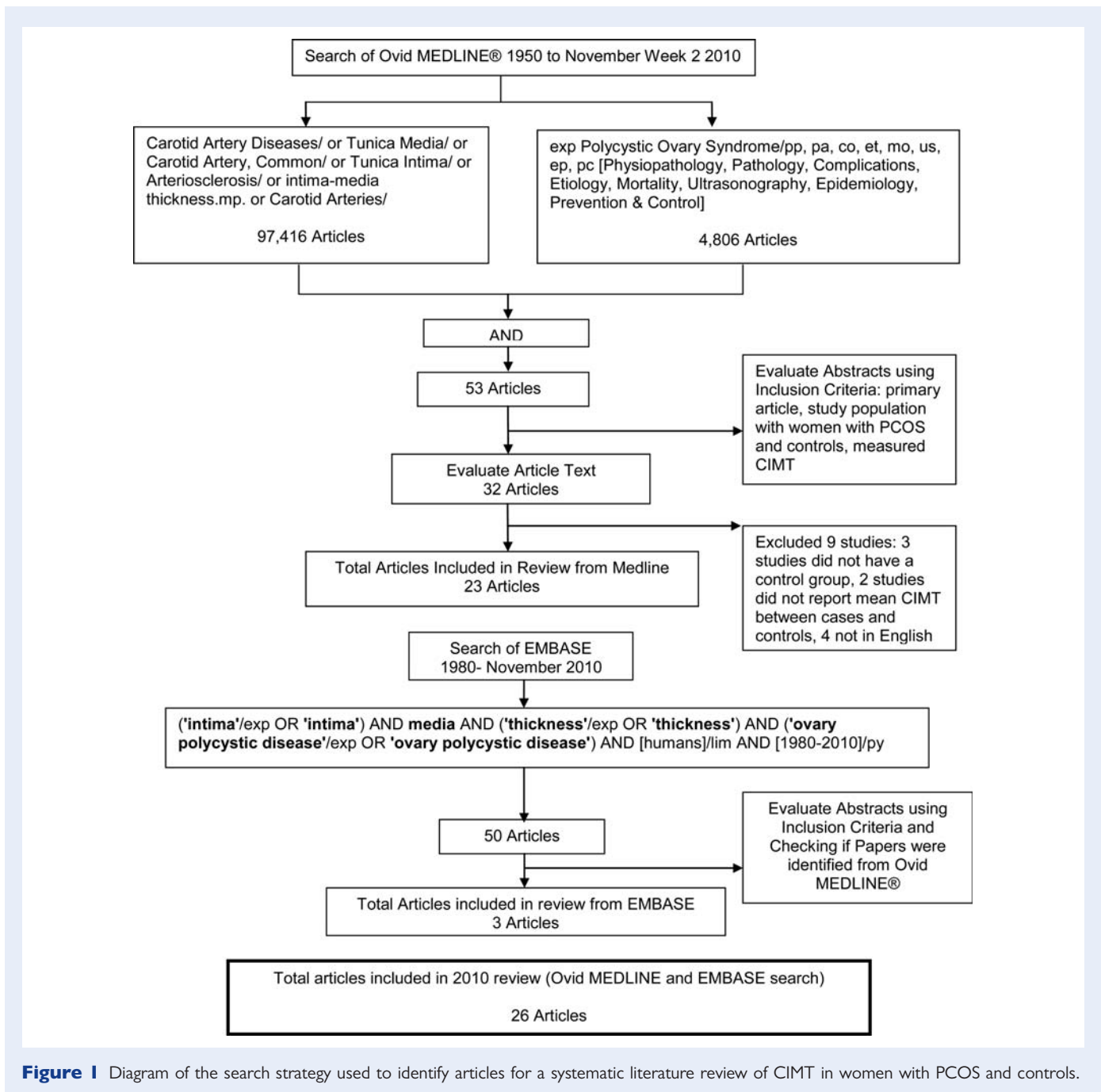


Figure 1 Diagram of the search strategy used to identify articles for a systematic literature review of CIMT in women with PCOS and controls.

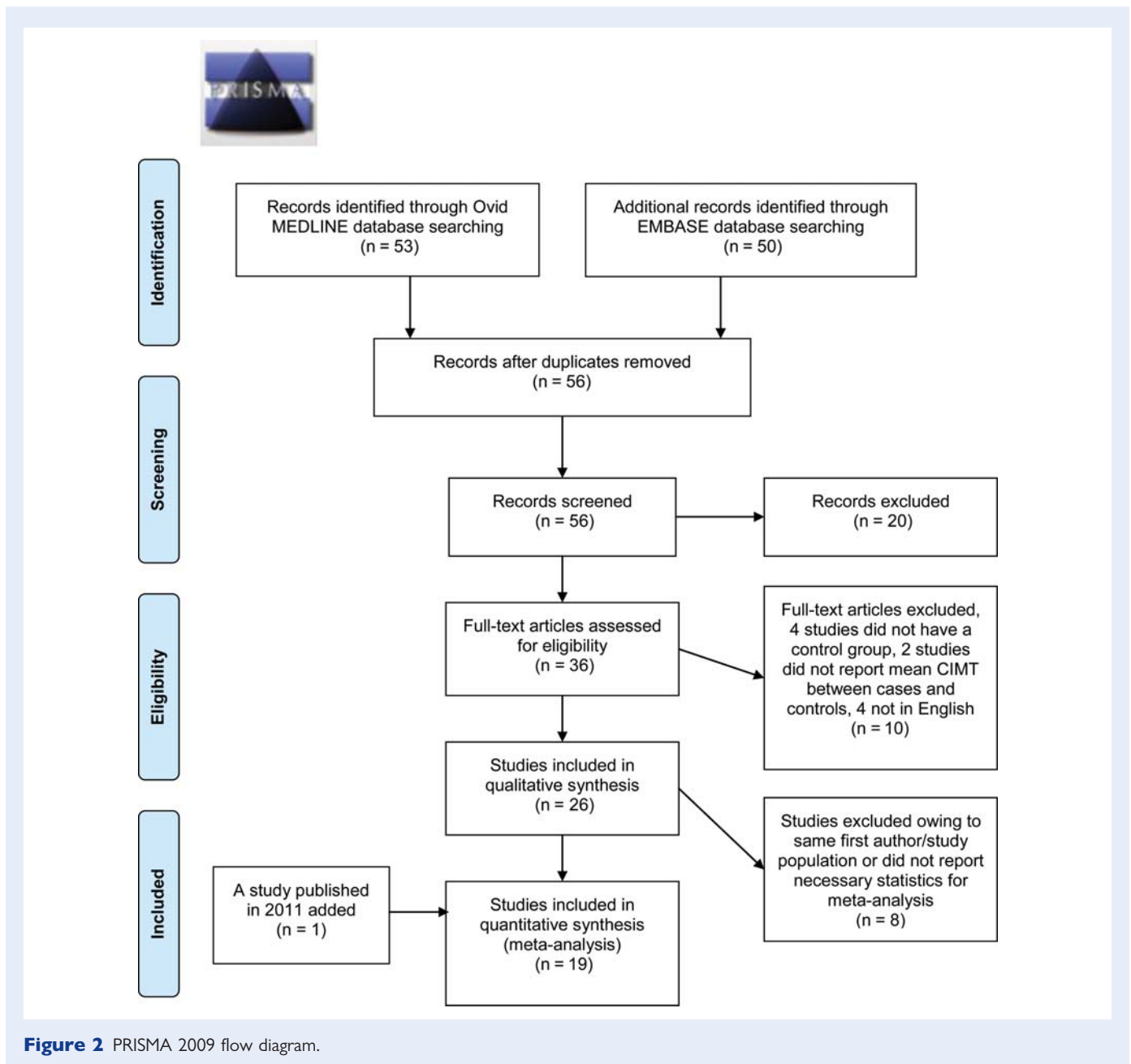
arbitrator was consulted (R.A.W.). Two of the three investigators were required to be in agreement. After reviewing articles, 26 studies met the inclusion criteria and 19 studies were suitable for the meta-analysis (Fig. 2). One article published in 2011, as mentioned previously, was added to the meta-analysis (Pepene et al., 2011). For the statistical analysis, three studies were excluded that did not report necessary information for the meta-analysis (Meyer et al., 2005; Vryonidou et al., 2005; Pamuk et al., 2010), one paper for reporting unusual CIMT values (Adali et al., 2010) and four papers that were from the same author or study population (Guzick et al., 1996; Carmina et al., 2006; Cascella et al., 2006; Erdogan et al., 2008). In instances of duplicate papers from the same first author, the most recent study containing the larger sample size was included in the meta-analysis.

Assessment of risk of bias

Two investigators independently assessed limitations and possible biases within each study (M.L.M. and E.O.T.). This information was used to determine whether studies were adequate for the meta-analysis and to determine possible sources of heterogeneity. A priori, it was hypothesized that the studies might differ according to the protocol and reproducibility of CIMT and the PCOS diagnostic criteria that was used. Publication bias across studies was assessed using a funnel plot and Egger's test.

Data analysis

The primary outcome of interest was the mean difference in CIMT between women with PCOS and women without PCOS. The



meta-analysis was performed using a random-effects model to compute the mean difference in CIMT and the 95% confidence intervals (CIs) for each study and an overall summary estimate. The mean CIMT, the SD and the sample size were available for most of the studies. Four studies were included that reported means and exact *P*-values (Talbot *et al.*, 2000; Alexandraki *et al.*, 2006; Pepene *et al.*, 2011), and three studies were included that used an adjusted mean CIMT (Talbot *et al.*, 2000; Lakhani *et al.*, 2004; Orio *et al.*, 2004). Two studies were included two times because the case–control groups were stratified by age (Talbot *et al.*, 2000) and obesity (Ketel *et al.*, 2010). The numbers in the manuscript reflect those studies being included as one, instead of two.

Three studies were ineligible to be included for the meta-analysis because they did not report the necessary information (Meyer *et al.*, 2005; Vryonidou *et al.*, 2005; Pamuk *et al.*, 2010). One study was excluded

because the reported CIMT was unusually low in both women with PCOS and controls (Adali *et al.*, 2010). One study stratified cases by levels of the homeostasis model assessment-IR (HOMA) but not the control group (Karadeniz *et al.*, 2008). For this study, the CIMT from the case group that was most similar to controls was used to give a conservative estimate. The results for right CIMT were used for three studies that reported the left and right CIMT separately (Karadeniz *et al.*, 2008; Trakakis *et al.*, 2008; Erdogan *et al.*, 2009).

Forest plots were created with the random-effect model to obtain an estimate of the overall mean difference in CIMT across the studies. The random-effects model was used to incorporate greater variability or heterogeneity between the studies. The a priori hypothesis was that the heterogeneity may be related to differences in PCOS diagnostic criteria, the age and BMI of the study populations, the protocol for CIMT and the observer variation of the technician(s) performing the ultrasound

assessments. Beyond visual assessment for heterogeneity, homogeneity was tested using the χ^2 test Cochran's Q -statistic. A $P < 0.10$ was used to suggest heterogeneity. The I^2 statistic was computed to measure the proportion of inconsistency that could not be explained by chance in each of the individual studies (Higgins et al., 2003). I^2 ranges between 0 and 100% with lower values representing less heterogeneity. The recommended guidelines for low, moderate and high I^2 values are <25 , 50 and $>75\%$, respectively (Higgins et al., 2003). However, the power to detect bias is under 0.80 with a meta-analysis of less than 20 studies and including studies with less than 80 participants (Hardy and Thompson, 1998; Gava-ghan et al., 2000; Huedo-Medina et al., 2006).

To examine possible sources of heterogeneity between studies, the meta-analysis was conducted by grouping the studies by the quality of the CIMT measurement. The quality of the studies was determined by evaluating if the study reported reproducibility of CIMT and if the study used an average of the left and right common carotid artery (CCA) for CIMT versus just one side. This criterion was used because the average of measures from the left and right CCA would be more stable than the average of one side (Thompson et al., 2001). Finally, to assess possible publication bias, a funnel plot was created to assess for symmetry and the Egger regression test was performed to test for asymmetry of the funnel plot. The Egger test evaluates the association between the standardized effect estimate (estimate divided by SE) and the precision ($1/SE$) through the use of linear regression for Y-intercept = 0 (Egger et al., 1997). All analyses were performed using Comprehensive Meta-Analysis software [Borenstein M, Hedges L, Higgins J, Rothstein H. Comprehensive Meta-analysis Version 2, Biostat, Englewood NJ (2005)].

Results

Description of studies

A total of 56 articles were identified for the review through a database search of Ovid MEDLINE and EMBASE after adjusting for duplicates (Fig. 2). The investigator screened the title and abstracts and excluded 20 articles because they were reviews, did not have a control group or did not report a CIMT measurement. There were 36 full-text studies that met the eligibility criteria for this systematic review and meta-analysis. Of these, 10 studies were excluded because they were not in English, did not have a control group or did not report a CIMT value. Eight studies were excluded from the meta-analysis because they had the same first author or study population, the necessary statistics were not reported or the CIMT values were abnormal compared with the other studies, as previously mentioned. In summary, the search identified 8 studies for the systematic review and 18 studies for the meta-analysis. An additional article published in 2011 was identified and subsequently added to the meta-analysis, for a total of 19 studies to be included in the meta-analysis.

The 8 studies in the qualitative systematic review and the 19 studies in the meta-analysis were cross-sectional studies with the exception of one RCT (Heutling et al., 2008). Eight studies were included in the qualitative review (Table I). As previously mentioned, these studies had either a duplicate study population as a study included in the meta-analysis or incomplete statistics for the meta-analysis. This analysis focused on the 19 studies that were included in the meta-analysis (Table II). The 19 studies in the meta-analysis involved a total of 1123 women with PCOS and 923 non-PCOS controls. The sample sizes ranged from 18 to 200 women with PCOS and from 12 to 142

controls. Women with PCOS were diagnosed using the NIH criteria, the AES criteria or the Rotterdam criteria. The studies enrolled patients (Karadeniz et al., 2008; Soares et al., 2009), community controls (Guzick et al., 1996; Talbott et al., 2000; Heutling et al., 2008) and doctors, medical students and nurses as controls (Lakhani et al., 2004; Vural et al., 2005; Alexandraki et al., 2006; Saha et al., 2008; Trakakis et al., 2008; Arikani et al., 2009). Some studies did not specify where the healthy controls came from or how they were recruited (Orio et al., 2004; Carmina et al., 2006; Cascella et al., 2006; Luque-Ramirez et al., 2007; Costa et al., 2008; Erdogan et al., 2008; Ciccone et al., 2009; Pepene et al., 2011).

The studies enrolled women with a mean age range from 22 to 40 years and a mean BMI range from 21 to 30 kg/m². The women were premenopausal with the exception of one study (Talbott et al., 2000). All but four studies matched or adjusted for age and BMI or weight between women with PCOS and controls for the CIMT estimate (Vural et al., 2005; Saha et al., 2008; Ciccone et al., 2009; Pepene et al., 2011). CIMT was assessed using B-mode ultrasound of the CCA and calculated as a mean of measurements of the far wall of the left and right CCA. One study used the maximum CIMT (Pepene et al., 2011). Most studies averaged the right and left CCA together, whereas a few reported them separately. The mean CIMT ranged from 0.41 to 0.75 mm in women with PCOS and from 0.33 to 0.74 mm in controls.

Quality control measures for CIMT were reported in nine studies. The most common reported reproducibility statistic was the intra-observer coefficient of variation (CV). The CV shows the variability between measures, where low CV values indicate less variability in the measures. The intra-observer CV for seven studies were $<11\%$ (Lakhani et al., 2004; Orio et al., 2004; Luque-Ramirez et al., 2007; Cascella et al., 2008; Heutling et al., 2008; Carmina et al., 2009b; Pepene et al., 2011), and the inter-observer CV for one study was 12% (Orio et al., 2004). One study reported an intra-class correlation coefficient of 0.86 (Talbott et al., 2000), where higher values indicate more measurement variability related to differences between patients rather than other sources of error. One study reported an intra-observer error of <0.03 mm (Vural et al., 2005). The other 10 studies did not mention the quality control measures for CIMT (Karadeniz et al., 2008; Saha et al., 2008; Trakakis et al., 2008; Erdogan et al., 2009; Soares et al., 2009; Ketel et al., 2010). But, five of these studies indicated that there was one technician reading the CIMT images (Alexandraki et al., 2006; Costa et al., 2008; Arikani et al., 2009; Ciccone et al., 2009).

From this information, the studies were ranked by the quality of the CIMT assessment. There were seven studies considered to be of the highest quality because they reported a reproducibility statistic and used the left and right CCA for CIMT (Talbott et al., 2000; Orio et al., 2004; Vural et al., 2005; Cascella et al., 2008; Heutling et al., 2008; Carmina et al., 2009b; Pepene et al., 2011). Two studies were considered of good quality because they reported a reproducibility statistic and used one CCA for CIMT (Lakhani et al., 2004; Luque-Ramirez et al., 2007). Five studies were considered to be of fair quality because they did not report a reproducibility statistic and used the left and right CCA for CIMT (Alexandraki et al., 2006; Costa et al., 2008; Saha et al., 2008; Arikani et al., 2009; Ciccone et al., 2009). The remaining five studies were considered lower quality because they did not report a reproducibility statistic and

Table 1 Summary of eight studies of CIMT in women with PCOS and controls for the qualitative review.

| Study | Control population | Controlled for age and/or BMI | n | Age ± SD | BMI ± SD | Mean CIMT (mm) ± SD | P-value | Segment(s) used to measure CIMT | CIMT protocol | Rational for excluding from meta-analysis |
|--------------------------------|---|-------------------------------|---|--|--|---|----------------------|---------------------------------|---|---|
| Guzick <i>et al.</i> (1996) | Community | Age | 16 cases ^a 16 controls | 44.4 ± 0.9 ^c 43.9 ± 1.3 ^c | 32.7 ± 2.3 ^c 25.3 ± 1.2 ^c | 0.680 ± 0.019 ^c 0.630 ± 0.012 ^c | P = 0.035 | CCA, Bif, ICA | Mean, left and right, near and far wall CCA, far wall bulb and ICA | Same population as Talbott <i>et al.</i> (2000) |
| Meyer <i>et al.</i> (2005) | Community | NA | 100 cases ^a 20 controls | 32.7 ± 1.8 ^c 33.2 ± 2.3 ^c | 37.3 ± 2.43 ^c 36.7 ± 1.28 ^c | 0.55 ± 0.01 ^c 0.54 ± 0.01 ^c | P > 0.05 | CCA | Mean, right, far wall | Did not report necessary statistics |
| Vryonidou <i>et al.</i> (2005) | Patients seeking treatment for thyroid function and obesity | Age and BMI | 75 cases ^a 55 controls | 23.9 ± 5.4 24.7 ± 5.3 | 27.3 ± 7.0 26.3 ± 7.7 | 0.58 (0.42–0.80) ^d 0.47 (0.38–0.63) ^d | P < 0.001 | CCA | Mean and max, left and right, far wall | Did not report necessary statistics |
| Carmina <i>et al.</i> (2006) | NA | Age and weight | 50 cases ^a 50 controls | 25.2 ± 1 ^c 25.1 ± 0.7 ^c | 28.7 ± 0.8 ^c 28.5 ± 0.5 ^c | 0.50 ± 0.01 ^c 0.41 ± 0.01 ^c | P < 0.01 | CCA | Mean, left and right, near and far walls | Included Carmina <i>et al.</i> (2009a,b) |
| Cascella <i>et al.</i> (2006) | NA | Age and BMI | 50 cases ^b 50 controls | 21.9 ± 2.7 22.2 ± 2.8 | 24.6 ± 2.5 24.4 ± 2.8 | 0.50 ± 0.07 0.40 ± 0.05 | P < 0.001 | CCA | Mean, left and right, far wall | Included Cascella <i>et al.</i> (2008) |
| Erdogan <i>et al.</i> (2008) | NA | NA | 68 cases ^b 26 controls | 24.3 ± 5.4 26.4 ± 5.7 | 24.4 ± 5.4 23.4 ± 5.0 | Right CIMT 0.42 ± 0.5 Left CIMT 0.43 ± 0.56 Right CIMT 0.44 ± 0.56 Left CIMT 0.44 ± 0.77 | P > 0.05 P > 0.05 | NA | NA | Included Erdogan <i>et al.</i> (2009) |
| Adali <i>et al.</i> (2010) | Outpatient clinic | Age | 24 overweight or obese cases ^b 26 non-obese cases ^b 25 controls | 26.7 ± 2.2 24.7 ± 2.9 25.0 ± 2.3 | 29.7 ± 3.6 24.4 ± 4.2 23.9 ± 4.0 | 0.04 ± 0.01 0.03 ± 0.01 0.03 ± 0.01 | P > 0.05 | CCA | Mean of left and right, average of five measurements | Unusual CIMT values |
| Pamuk <i>et al.</i> (2010) | Healthy hospital staff | Age and BMI | 35 cases ^b 31 controls | 26 (18–35) ^d 27 (18–33) ^d | 29.7 (23.9–34.4) ^d 28.4 (23.1–33.8) ^d | 0.52 (0.45–0.72) ^d 0.49 (0.40–0.71) ^d | P = 0.51 | CCA | Right and left, five measurements for each side averaged | Did not report necessary statistics |

NA, not addressed; CCA; common carotid artery; Bif, carotid bifurcation; ICA, internal carotid artery; P values are cases versus controls.

^aNIH PCOS Criteria.

^bRotterdam PCOS Criteria.

^cData expressed as SE.

^dData expressed as median (minimum–maximum).

Table II Summary of 19 studies of CIMT in women with PCOS and controls for the meta-analysis.

| Study | Control population | Controlled for age and/ or BMI | n | Age \pm SD | BMI \pm SD | Mean CIMT (mm) \pm SD | P-value | Segment(s) used to measure CIMT | CIMT protocol | |
|------------------------------------|--|--------------------------------|--|--|--|--|--|--------------------------------------|--|--|
| Talbot <i>et al.</i> (2000) | Community | Age and BMI | 125 cases ^a | 37.5 \pm 6.2 ^c | 30.1 \pm 0.7 ^c | Age-BMI adjusted (CI) Overall 0.70 (0.68–0.73) 30–44 years 0.65 (0.62–0.69) \geq 45 years 0.77 (0.74–0.81) (n = 125) (n = 78) (n = 47) | P = 0.299 P = 0.565 P = 0.005 | CCA, Bif, ICA | Mean, left and right, near and far walls, intra-class correlation coefficient 0.86 | |
| | | | 142 controls | 39.0 \pm 6.2 ^c | 26.5 \pm 0.5 ^c | Overall 0.67 (0.65–0.69) 30–44 years 0.64 (0.61–0.67) \geq 45 years 0.71 (0.68–0.75) (n = 142) (n = 82) (n = 60) | | | | |
| Lakhani <i>et al.</i> (2004) | Staff members | Age | 19 PCOS ^b | 29.2 \pm 4.0 | 31.3 \pm 8.2 | 0.54 \pm 0.11 | 0.53 \pm 0.09 ^d CI (0.47–0.98) | P = 0.006, P = 0.034 ^d | CCA, Bif | Mean, right side, CCA and Bif reported separately, intra-observer CV 8% |
| | | | 12 PCO | 27.7 \pm 4.0 | 22.5 \pm 3.8 | 0.51 \pm 0.18 | 0.50 \pm 0.15 ^d CI (0.81–0.7) | P = 0.038, P = 0.841 ^d | | |
| Orio <i>et al.</i> (2004) | NA | Age and BMI | 12 controls | 27.5 \pm 4.0 | 24.2 \pm 3.4 | 0.40 \pm 0.09 | 0.44 \pm 0.09 ^d | P < 0.05 | CCA | Mean, right and left, far wall, intra-observer CV 7% and inter-observer CV 12% |
| | | | 30 cases ^b 30 controls | 22.2 \pm 2.5 22.6 \pm 2.3 | 22.4 \pm 2.1 22.1 \pm 1.8 | 0.53 \pm 0.09 ^e 0.39 \pm 0.08 ^e | | | | |
| Vural <i>et al.</i> (2005) | Medical students and nurses | Age | 43 cases ^b 43 controls | 21.4 \pm 1.8 20.8 \pm 2.2 | 23.4 \pm 4.7 21.5 \pm 3.0 | 0.75 \pm 0.11 0.61 \pm 0.11 | P < 0.001 | CCA | Left and right, near and far walls, intra-observer error <0.03 mm | |
| Alexandraki <i>et al.</i> (2006) | Doctors and medical students | Age and BMI | 27 cases ^a 27 controls | 25.4 \pm 0.8 ^c 27.3 \pm 0.8 ^c | 27.42 \pm 1.1 ^c 25.05 \pm 1.2 ^c | 0.49 \pm 0.01 ^c 0.51 \pm 0.02 ^c | P = 0.19 | CCA, Bif, ICA | Mean, left and right, far wall one reader | |
| Luque-Ramirez <i>et al.</i> (2007) | 12 patients for obesity problems, 8 healthy controls | | 40 cases ^a 20 controls | 24.5 \pm 5.8 27.2 \pm 6.8 | 29.4 \pm 6.3 28.2 \pm 6.9 | 0.41 \pm 0.11 0.33 \pm 0.08 | P = 0.005 | CCA | Left, far wall, intra-observer CV 10.8% | |
| Cascella <i>et al.</i> (2008) | NA | Age and BMI | 200 cases ^b 100 controls | 24.6 \pm 3.2 24.0 \pm 2.8 | 28.5 \pm 2.8 28.8 \pm 2.7 | 0.46 \pm 0.16 0.38 \pm 0.09 | P < 0.001 | CCA | Mean, left and right, far wall, intra-observer CV 7.0% | |
| Costa <i>et al.</i> (2008) | NA | | 57 cases ^b 37 controls | 25.5 \pm 5.3 26.6 \pm 5.4 | 27.6 \pm 5.8 26.7 \pm 4.9 | 0.52 \pm 0.08 0.53 \pm 0.08 | P = 0.35 | CCA | Mean, left and right, far wall, one reader | |
| Heutling <i>et al.</i> (2008)* | Public advertising | | 83 cases ^b 39 controls | 24.8 \pm 4.7 27.8 \pm 5.6 | 30.4 \pm 5.9 29.1 \pm 4.8 | 0.48 \pm 0.07 0.42 \pm 0.05 | P < 0.001 | CCA | Mean, left and right, far wall, intra-observer CV 6.8% | |

| | | | | | | | | | |
|----------------------------------|-----------------------------|----------------|--|--|--|--|--------------------------|---------------|---|
| Karadeniz <i>et al.</i> (2008) | Patients | Age | Cases ^b HOMA-IR > 1.75 (n = 37) | 23.8 ± 5.5 | 25.6 ± 5.6 | Right: 0.41 ± 0.05 Left: 0.43 ± 0.06 | P > 0.05 P > 0.05 | CCA | NA |
| | | | Cases ^b HOMA-IR < 1.75 (n = 21) | 24.9 ± 5.1 | 22.2 ± 4.1 | Right: 0.43 ± 0.05 Left: 0.45 ± 0.06 | P > 0.05 P > 0.05 | | |
| | | | 25 controls | 27.2 ± 4.2 | 23.4 ± 5.2 | Right: 0.44 ± 0.05 Left: 0.44 ± 0.05 | | | |
| Saha <i>et al.</i> (2008) | Staff members | | 30 cases ^a 30 controls | 26.1 ± 4.2 28.7 ± 7.1 | 25.8 ± 4.6 22.0 ± 3.0 | 0.63 ± 0.19 0.44 ± 0.05 | P < 0.001 | CCA, Bif, ICA | Mean, left and right, NS |
| Trakakis <i>et al.</i> (2008) | Nurses and medical students | | 53 cases ^b | 26.1 ± 5.5 | 28.7 ± 7.1 | Right: 0.67 ± 0.15 Left: 0.68 ± 0.13 | P < 0.0001 P < 0.0001 | CCA, ICA | Mean CIMT, left and right, near and far wall |
| | | | 53 controls | 25.4 ± 4.7 | 28.7 ± 7.1 | Right: 0.46 ± 0.16 Left: 0.42 ± 0.16 | | | |
| Arikan <i>et al.</i> (2009) | Staff and medical students | Age and BMI | 39 cases ^b 30 controls | 22.8 ± 5.5 24.6 ± 4.2 | 21.5 ± 6.5 20.9 ± 6.0 | 0.45 ± 0.82 0.44 ± 0.11 | P > 0.05 | CCA | Mean, left and right, far wall, one technician |
| Carmina <i>et al.</i> (2009a, b) | Family members of staff | Age and weight | 95 cases ^f 90 controls | 24.2 ± 3.0 23.9 ± 3.0 | 27.6 ± 5.8 27.5 ± 3.0 | 0.61 ± 0.18 0.53 ± 0.15 | P < 0.01 | CCA | Mean average of 10 measurements of left and right far wall, intra-observer CV < 7.0% |
| Ciccone <i>et al.</i> (2009) | NA | Age | 29 cases ^b 26 controls | 22.0 ± 3.8 22.0 ± 3.8 | 26.3 ± 4.5 20.5 ± 1.6 | 0.651 ± 0.59 0.637 ± 0.133 | P > 0.05 | CCA, ICA, Bif | Mean of average of three measures from right and left, plaque free segments, one technician |
| Soares <i>et al.</i> (2009) | Basic health clinic | Age and BMI | 40 cases ^b 50 controls | 24.5 ± 3.8 24.5 ± 5.1 | 22.7 ± 3.3 23.1 ± 3.2 | 0.44 ± 0.10 0.42 ± 0.09 | P = 0.41 | CCA | Mean of average of four measures of right CCA |
| Erdogan <i>et al.</i> (2009) | Outpatient clinic | Age and BMI | 88 cases ^b | 24.1 ± 1.3 | 24.4 ± 4.1 | Right: 0.74 ± 0.59 Left: 0.73 ± 0.80 | P > 0.05 | CCA | NA |
| | | | 119 controls | 25.0 ± 2.1 | 23.5 ± 4.1 | Right: 0.74 ± 0.61 Left: 0.74 ± 0.60 | | | |
| Ketel <i>et al.</i> (2010) | Clinic, local newspaper ads | Age and weight | 22 lean cases ^b | 28.6 ± 4.5 | 22.0 ± 2.2 | 0.53 ± 0.08 | P > 0.05 | CCA | Right side, mean of three measurements |
| | | | 18 obese cases ^b | 30.3 ± 4.2 | 36.2 ± 5.9 | 0.56 ± 0.17 | | | |
| | | | 17 lean controls | 27.7 ± 5.3 | 22.2 ± 1.7 | 0.48 ± 0.07 | | | |
| | | | 13 obese controls | 28.6 ± 5.3 | 40.5 ± 7.0 | 0.60 ± 0.11 | | | |
| Pepene <i>et al.</i> (2011) | NA | Age | 64 cases ^f 20 controls | 28.6 ± 5.4 ^c 28.6 ± 5.5 ^c | 29.9 ± 0.8 ^c 26.3 ± 1.3 ^c | 0.572 ± 0.017 ^c 0.635 ± 0.062 ^c | P = 0.323 | CCA, Bif, ICA | Single max CIMT, left and right, one tech, CV 5% |

RCT; randomized control trial; NA, not addressed.

^aNIH PCOS criteria.

^bRotterdam PCOS criteria.

^cData expressed as SE.

^dCIMT adjusted for age, systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, cholesterol and insulin.

^eAdjusted for age, BMI, waist to hip ratio, smoking status, glucose and insulin levels, free androgen index, lipid profile, homocysteine concentrations and SBP and DBP;

^fAndrogen Excess Society (AES) PCOS criteria.

*P values are cases versus controls.

used one CCA for CIMT (Karadeniz et al., 2008; Trakakis et al., 2008; Erdogan et al., 2009; Soares et al., 2009; Ketel et al., 2010). (Two studies Talbott et al., 2000; Ketel et al., 2010 were included two times because the case–control groups were stratified by age or obesity. The numbers in the manuscript reflect those studies being included as one, instead of two.)

Risk of bias

The funnel plot of the seven high-quality studies suggested no evidence of publication bias because the studies were symmetrical around the mean and the Egger's regression test was not significant at $P = 0.94$ (data not shown). Publication bias was not assessed among the two good quality studies as this requires more than three studies to run publication bias procedures. The funnel plots of the fair- and low-quality studies were also symmetrical around the mean (data not shown) and the Egger's test P -value was 0.48 for the five studies without reproducibility and used the right and left CCA, and the P -value was 0.61 for the five studies without reproducibility and used one CCA.

Mean difference in CIMT

The forest plots showed that the mean difference in CIMT between women with PCOS and controls varies across the groups of studies (Figs 3 and 4, Table III). The forest plot of the seven high-quality studies suggested that the cases had a greater CIMT than controls as most of the estimated difference in means, except one, were to the right of zero (Fig. 3a). The widths of the 95% CIs were similar, which indicated that the studies had similar precision in the estimates. The summary random-effect mean difference in CIMT showed that women with PCOS had a significantly greater CIMT than controls (0.072, 95% CI 0.040, 0.105, $P < 0.0001$). The Q -statistic for heterogeneity was significant ($\chi^2 = 36.82$, $P < 0.0001$, $I^2 = 80.99$).

Like the previous studies, the forest plot of the two good quality studies showed that the estimated difference in means and 95% CIs were similar and were located to the right of zero (Fig. 3b). The summary random-effect mean difference in CIMT showed that women with PCOS had a significantly greater CIMT than controls (0.084, 95% CI 0.042, 0.126, $P < 0.0001$). The Q -statistic for heterogeneity was not significant ($\chi^2 = 0.05$, $P = 0.82$, $I^2 = 0.00$). However, as noted earlier, heterogeneity estimates should be interpreted with caution because they are not reliable with a small number of studies and small numbers of participants within some studies.

The forest plot of the five fair-quality studies showed that most of the estimates and 95% CIs cross zero, except for one study (Fig. 4a). Two studies had a wide CI compared with the rest, which indicated less precision in the estimate. In contrast to the high-quality studies, the estimates of the difference in CIMT across studies did not show a consistent pattern. The summary random-effect mean difference in CIMT was not significant between women with PCOS and controls (0.041, 95% CI -0.038 , 0.120, $P = 0.310$), and the Q -statistic for heterogeneity was significant ($\chi^2 = 30.11$, $P < 0.0001$, $I^2 = 86.72$).

Similar to the fair-quality studies, the forest plot of the five low-quality studies showed that most of the estimates were around zero and the 95% CIs crossed zero, except for one study (Fig. 4b). Similar to the fair-quality studies, there was not a consistent pattern

in the estimated difference in means and CIs across studies. The summary random-effect mean difference in CIMT was not significant between women with PCOS and controls (0.045, 95% CI -0.020 , 0.111, $P = 0.173$), and the Q -statistic for heterogeneity was significant ($\chi^2 = 43.58$, $P < 0.0001$, $I^2 = 88.53$).

Discussion

Summary of evidence

This meta-analysis demonstrates that women with PCOS have a higher mean CIMT compared with non-PCOS controls. The summary estimate of the mean difference in CIMT was 0.072 mm for women with PCOS compared with controls (95% CI 0.040–0.105, $P < 0.0001$) for the high quality and was similar to the good quality studies (0.084 mm, 95% CI 0.042, 0.126, $P = 0.0001$). The summary estimate of the difference in CIMT for the fair- and low-quality studies was higher among women with PCOS but was not significantly different, $P > 0.05$. The average change in CIMT for women is estimated to be around 0.009 (Chambless et al., 2002) and 0.015 mm per year (Johnson et al., 2007), thus the summary mean difference corresponds to about a 7 year progression in CIMT. This difference in CIMT was detected despite including small studies of young women.

These results should be viewed in light of the significant heterogeneity across studies. As previously mentioned, these tests may have had low power. Nonetheless, heterogeneity could be related to between the study differences in PCOS phenotypes, age, BMI, CVD risk factors and technical factors related to assessment of CIMT. Larger studies with a well-defined PCOS population using rigorous methodology may be required to draw a more robust conclusion. However, the evidence to date suggests women with PCOS are at risk for premature atherosclerosis. This emphasizes the importance of screening for and addressing CVD risk factors as a way of reducing progression of CVD in this high-risk subgroup.

Strengths and limitations of the review

The limits of the search and the inclusion of studies only in the English language may have introduced possible publication bias in the meta-analysis. However, only three non-English studies were excluded and we did not detect evidence of publication bias from the funnel plots or Egger's test. Another limitation was the heterogeneity that suggested the populations and CIMT measurements were not the same across studies. The heterogeneity was addressed by using the random-effects model and grouping the studies according to quality of the CIMT measurement.

CIMT is a reproducible measure but has within and between study variability owing to random error and error from study participants and technicians. Larger variability of CIMT would decrease the reproducibility and require larger sample sizes to maintain adequate power. The subgroup analysis showed that the consistency of the estimates across studies increased as the quality of the CIMT measurement increased. The high-quality studies had consistent CIMT estimates across studies and a more robust summary estimate. This is in contrast to the fair- and low-quality studies in which the estimates had more variation across studies. There were a few estimates with a wide CI that suggested lower precision in the estimate, and the summary estimate was much weaker and not significant at $P < 0.05$.

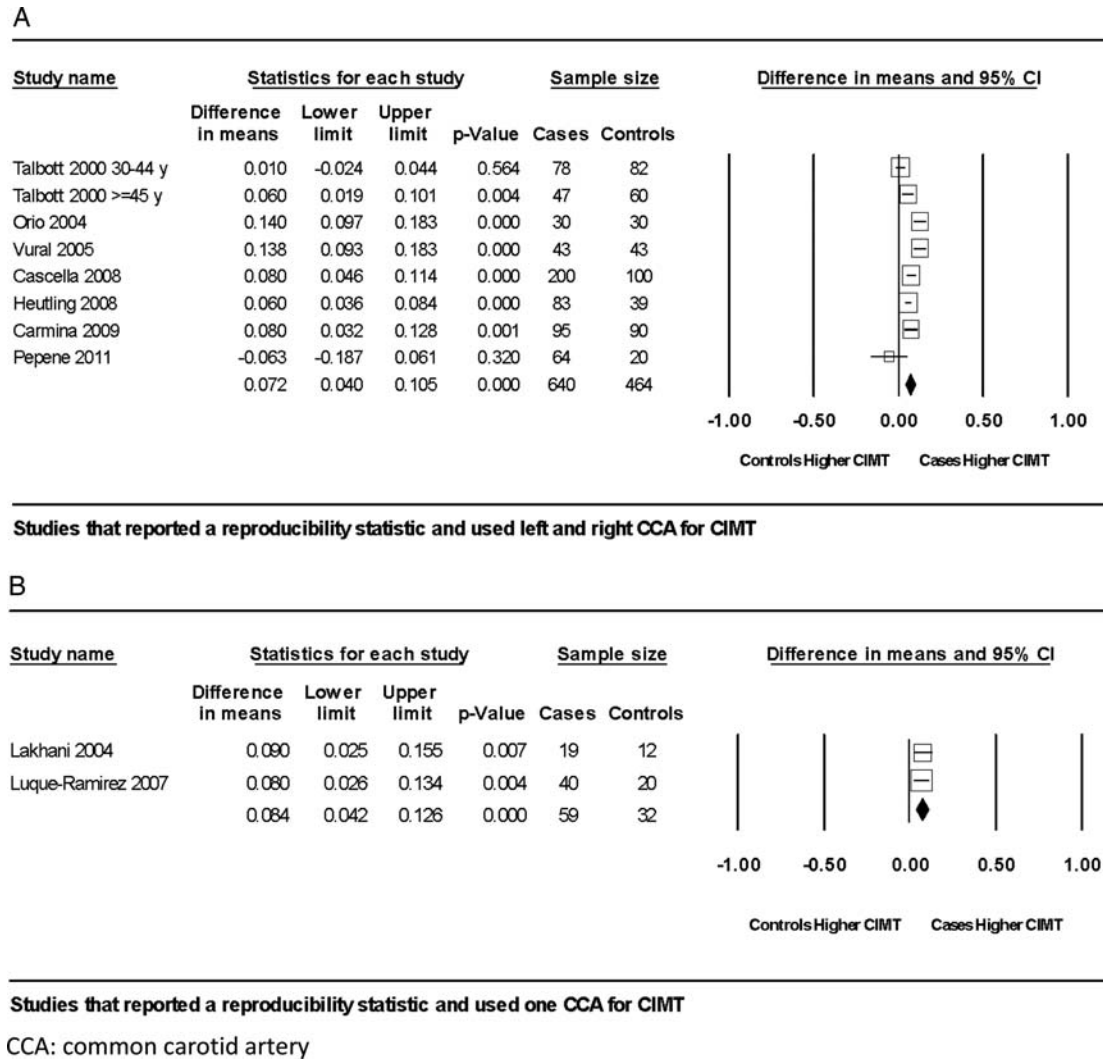


Figure 3 (A) Boxes represent the estimated difference in mean CIMT and lines indicate the 95% CI. (B) Boxes represent the estimated difference in mean CIMT and lines indicate the 95% CI.

These observations demonstrate the importance of reporting quality control measures and describing the protocol and reproducibility of CIMT. A large portion of the studies did not describe quality control measures for the CIMT measurement (Alexandraki et al., 2006; Costa et al., 2008; Karadeniz et al., 2008; Saha et al., 2008; Tra-kakis et al., 2008; Arikan et al., 2009; Ciccione et al., 2009; Erdogan et al., 2009; Soares et al., 2009; Ketel et al., 2010).

Heterogeneity between studies could also be caused by differences in the prevalence of CVD risk factors and PCOS phenotypes. The Rotterdam criteria add an additional, less severe, phenotype to the diagnosis, which could increase heterogeneity and may lower the power of a study to detect a difference between participants with and without PCOS. There is evidence that the prevalence of CVD risk factors varies by PCOS phenotype (Jovanovic et al., 2010). Women with the classical definition of PCOS had a higher prevalence of one or more CVD risk factors, which include C-reactive protein (CRP),

lipids and homocysteine, than ovulatory women with PCOS (Carmina et al., 2005). Women with classical PCOS also had more abdominal obesity than ovulatory women with PCOS with similar BMI ($P < 0.05$) (Carmina et al., 2009a). On the other hand, non-hyperandrogenic women with PCOS that are included in the Rotterdam criteria and not the NIH criteria had normal androgen levels and lower prevalence of IR and metabolic abnormalities than women with classical or ovulatory PCOS (Chae et al., 2008).

This meta-analysis is also vulnerable to limitations within each study that include their cross-sectional study designs and small sample sizes. Smaller studies may have lacked sufficient power to detect a difference in CIMT between cases and controls as the sample sizes of the studies ranged from 18 to 200 women with PCOS and 12–142 controls. Another limitation was the potential selection bias within the studies. The response rate of recruitment was not reported in any of the studies, which may lead to potential selection bias. In addition,

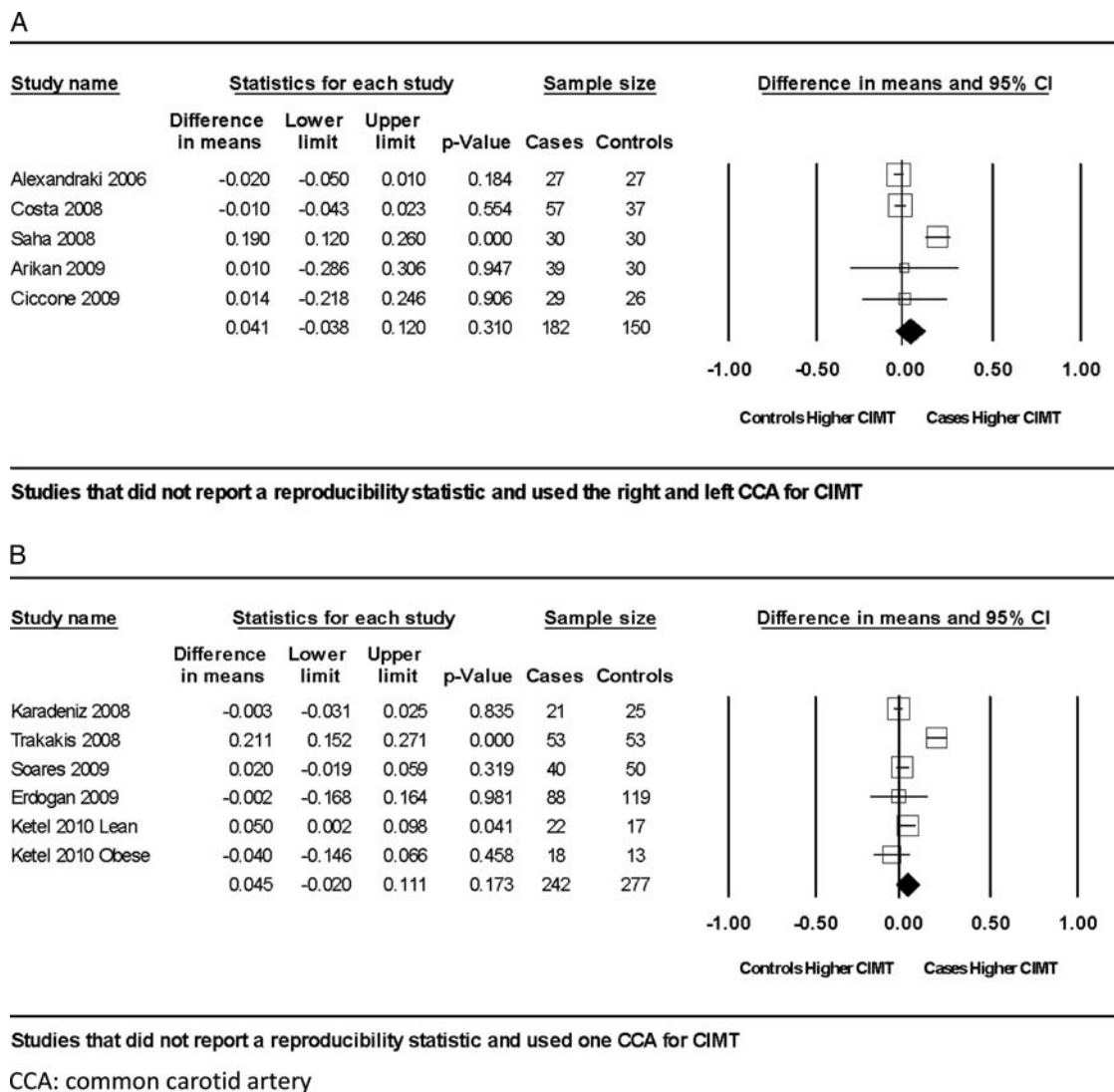


Figure 4 (A) Boxes represent the estimated difference in mean CIMT and lines indicate the 95% CI. (B) Boxes represent the estimated difference in mean CIMT and lines indicate the 95% CI.

there were differences in the average age and BMI between women with PCOS and controls in some studies, but this was controlled for in all of the studies except three (Vural et al., 2005; Saha et al., 2008; Cicccone et al., 2009). Exclusion of these three studies from the analysis did not change the results.

The strength of this paper is that the meta-analysis was able to summarize results from the conflicting body of literature and increase statistical power by estimating a summary effect for the studies limited by small sample size. Also, grouping studies by the quality of the CIMT measurement identified potential sources of heterogeneity and demonstrated the robustness of the results. This is the first meta-analysis to investigate differences in CIMT between women with PCOS and controls, and showed the presence of more pronounced subclinical atherosclerosis in women with PCOS.

Comparison with previous research

Overall, the results indicated that women with PCOS had a 0.072–0.084 mm higher CIMT compared with controls. This is similar to studies of subclinical atherosclerosis as measured by CAC and FMD among women with PCOS. Women with PCOS had a higher prevalence of CAC defined by none versus any (Christian et al., 2003; Talbott et al., 2004; Shroff et al., 2007), and more CAC defined by an Agatston score of <10 versus ≥ 10 (Talbott et al., 2008) and significantly lower FMD (Kravariti et al., 2005; Meyer et al., 2005; Carmina et al., 2006; Cascella et al., 2008) when compared with controls.

There are several mechanisms that may explain the increase in CIMT among women with PCOS. Higher levels of circulating insulin (Carmina et al., 2009b), total cholesterol and low-density lipoprotein

Table III PCOS and CIMT meta-analysis results for random-effects models by quality of CIMT measurement.

| Random-effects model | Number of studies | Point estimate | SE | 95% CI | P-value | Heterogeneity | |
|---|-------------------|----------------|-------|---------------|---------|---------------------------------|--------|
| | | | | | | χ^2 (P-value) ^a | I^2 |
| 1 Studies reporting reproducibility and using right and left CCA for CIMT | 8 ^b | 0.072 | 0.017 | 0.040, 0.105 | <0.0001 | 36.818 (<0.0001) | 80.988 |
| 2 Studies reporting reproducibility and using one CCA for CIMT | 2 | 0.084 | 0.021 | 0.042, 0.126 | <0.0001 | 0.054 (0.817) | 0 |
| 3 Studies not reporting reproducibility and using right and left CCA for CIMT | 5 | 0.041 | 0.040 | -0.038, 0.120 | 0.3098 | 30.113 (<0.0001) | 86.717 |
| 4 Studies not reporting reproducibility and using one CCA for CIMT | 6 ^c | 0.045 | 0.033 | -0.020, 0.111 | 0.1734 | 43.575 (<0.0001) | 88.526 |
| All studies | 21 | 0.059 | 0.014 | 0.031, 0.088 | <0.0001 | 144.804 (<0.0001) | 86.188 |

CI, confidence interval.

^a χ^2 from Q-value based on fixed effects model.

^bTalbott *et al.* (2000) included two subgroups.

^cKetel *et al.* (2010) included two subgroups.

cholesterol (Alexandraki *et al.*, 2006; Saha *et al.*, 2008), triglycerides (Saha *et al.*, 2008), CRP (Casella *et al.*, 2008), serum interleukin-18 (Kaya *et al.*, 2010), lower levels of high-density lipoprotein cholesterol (Vryonidou *et al.*, 2005) and abdominal obesity (Casella *et al.*, 2008; Carmina *et al.*, 2009b) were each associated with CIMT in women with PCOS. This analysis showed women with PCOS have greater CIMT than controls, but we could not evaluate the influence of CVD risk factors that are strongly associated with PCOS and CIMT, given the design of this meta-analysis. Women with PCOS had higher insulin levels or IR compared with controls in all studies included in the meta-analysis except four (Lakhani *et al.*, 2004; Alexandraki *et al.*, 2006; Arikan *et al.*, 2009; Soares *et al.*, 2009). Further classification of PCOS phenotypes and CVD risk factors in PCOS is needed to understand the complexity of PCOS and the risk of CVD.

Clinical implications

This meta-analysis suggests that women with PCOS have more early adverse structural changes in the vascular system than normal menstruating women, having about a 0.08 mm greater CIMT, indicating an increased risk of a cardiovascular event. To put the results in perspective, every 0.10 mm increase in CIMT has been estimated to increase the risk of a myocardial infarction (MI) by 15% and the risk of stroke by 18% (Lorenz *et al.*, 2007b). Other reports show for every 0.16 mm increase in CIMT, the risk of an MI, stroke or death increases by 24% (Staub *et al.*, 2006) and by 19% after adjusting for age and sex (Lorenz *et al.*, 2006).

The increase in subclinical CVD suggests that primary CVD prevention would be beneficial for women with PCOS. CIMT could be used for risk stratification to identify women who are at the highest risk for CVD and who would benefit from an intervention or advanced therapy. CIMT can be reduced and progression can be slowed with diet alone (Markus *et al.*, 1997), or combined with lifestyle interventions (Wildman *et al.*, 2004), or with the use of metformin (Heutling *et al.*, 2008) or cholesterol-lowering medications (Probstfield *et al.*, 1995).

It is important to monitor women with PCOS as they age and transition through menopause. Post-menopausal women have higher CIMT (Sutton-Tyrrell *et al.*, 1998) and greater CIMT progression (Wildman *et al.*, 2004) compared with premenopausal women. All studies except one included in this analysis enrolled premenopausal women. Our results show that women with PCOS have more subclinical CVD before menopause that is likely to progress and develop to more advanced CVD with age compared with women without PCOS.

Conclusions

The findings from this meta-analysis on subclinical atherosclerosis in women with PCOS demonstrated higher CIMT in women with PCOS than that in controls. Heterogeneity was observed across studies, which may be related to that fact that PCOS is a complex heterogeneous syndrome associated with CVD risk factors. The results showed greater variation in the CIMT estimates across studies as the quality of the CIMT measurement decreased, which may partially explain inconsistencies in the literature. This can be improved by using standardized ultrasound protocols and reporting detailed methods for CIMT.

Identifying PCOS as a risk factor for CVD is difficult given the young age of onset of PCOS with CVD events that tend to occur with aging. To date, most studies have been conducted in young women but the risk of CVD may not be evident until later in life. Large prospective studies with detailed PCOS phenotypic data and change in subclinical atherosclerosis are needed to provide a better estimate of the risk of CVD in women with PCOS.

In the absence of these studies, PCOS is accompanied by CVD risk factors that put these women at an increased risk of atherosclerosis. These findings support recommendations for screening and monitoring CVD risk factors in women with PCOS, as endorsed by the Androgen Excess and PCOS society (Wild *et al.*, 2010). This is of important public health significance as it will allow for the early identification of

hypertension, type 2 diabetes and premature atherosclerosis in this high-risk population.

Authors' roles

M.L.M., R.A.W. and E.O.T. contributed to the concept and design of the study; M.T.K. contributed to the design of study. M.L.M., A.M.M., R.A.W. and E.O.T. contributed to the acquisition of the data. M.L.M., A.M.M., R.A.W., E.O.T. and M.T.K. contributed to the analysis and interpretation of the data. M.L.M., A.M.M., R.A.W., M.T.K. and E.O.T. contributed to the drafting and editing of the manuscript.

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Conflict of interest

None declared.

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