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

Michelle L. Meyer [1,*], Angela M. Malek [2], Robert A. Wild [3], Mary T. Korytkowski [4], and Evelyn O. Talbott [2]

[1]Department of Epidemiology, University of North Carolina at Chapel Hill, Bank of America Center, 137 E. Franklin St., Suite 306, Chapel Hill, NC 27514, USA [2]Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA [3]Departments of Obstetrics and Gynecology and Epidemiology and Biostatistics and Medicine, Oklahoma University Health Sciences Centre, Oklahoma City, OK, USA [4]Division of Endocrinology and Metabolism, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

*Correspondence address. Tel: +1-919-966-4596; Fax: +1-919-966-9800; E-mail: mlmeyer@unc.edu

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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 (Cascella *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; Cascella *et al.*, 2008) compared with controls. However, these studies have not shown consistent results (Meyer *et al.*, 2005; Costa *et al.*, 2008; Arikan *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
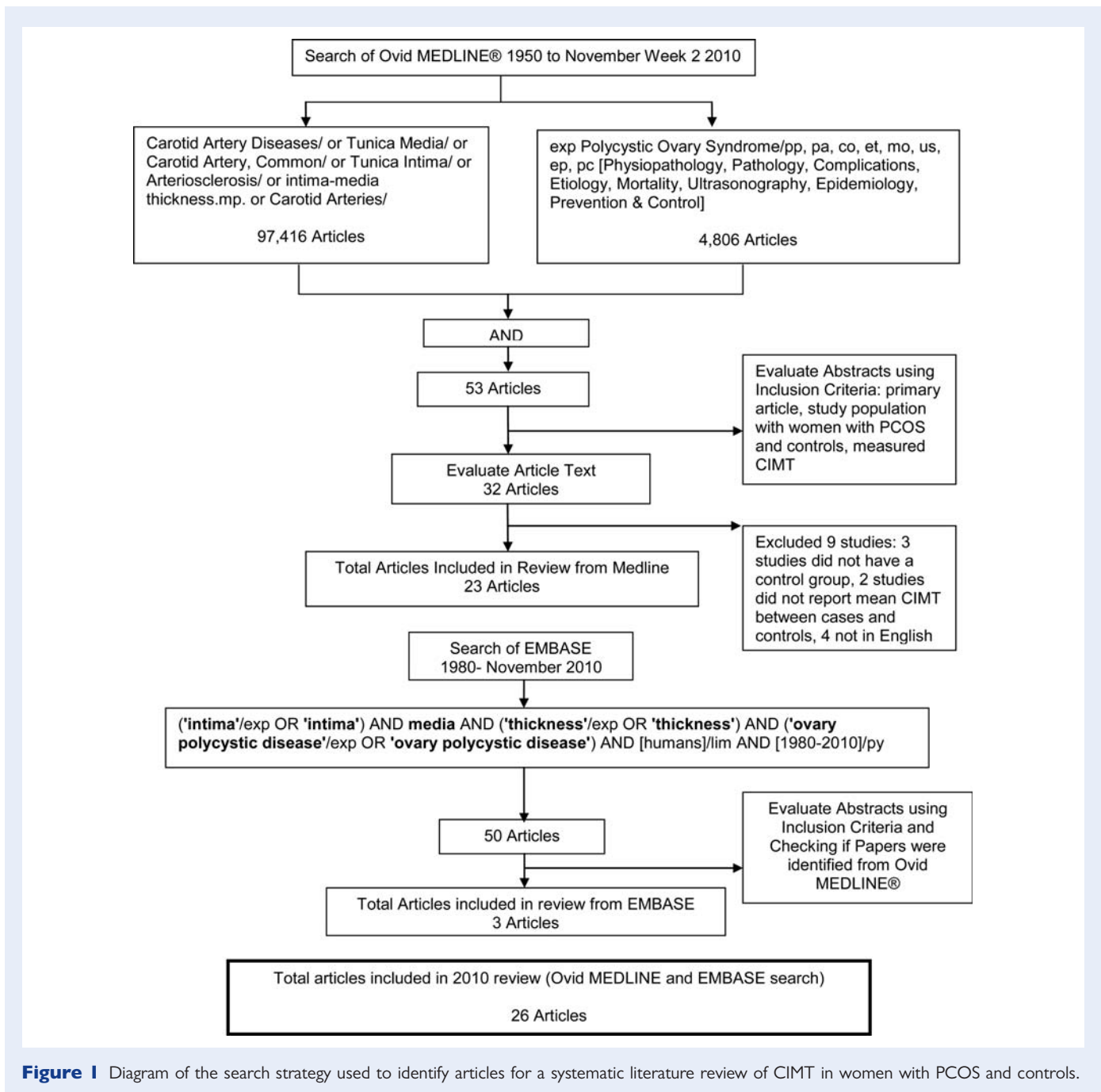
Figure 1 Diagram of the search strategy used to identify articles for a systematic literature review of CIMT in women with PCOS and controls.

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
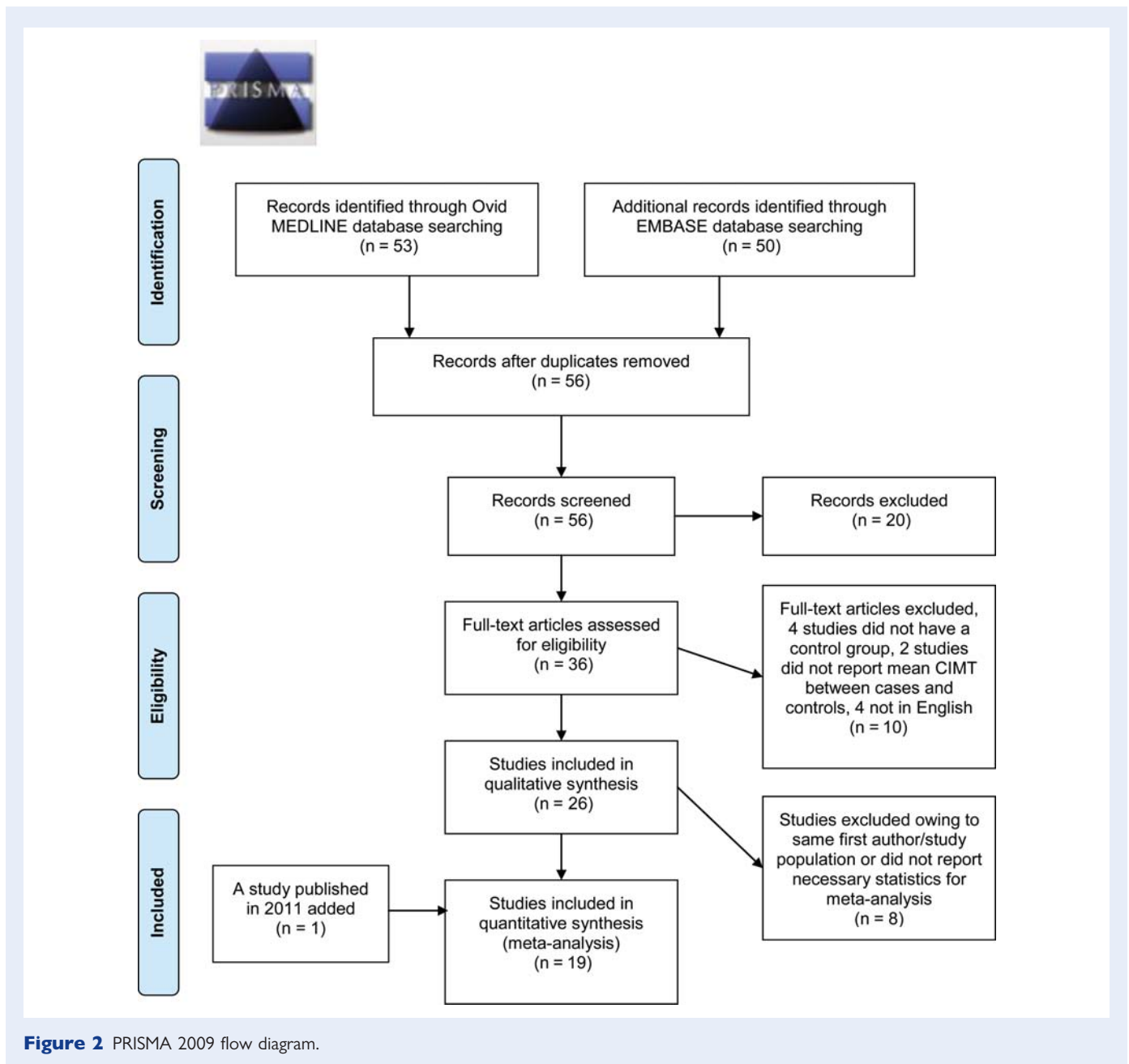
Figure 2 PRISMA 2009 flow diagram.

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 (Talbott et al., 2000; Alexandraki et al., 2006; Pepene et al., 2011), and three studies were included that used an adjusted mean CIMT (Talbott 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 (Talbott 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%, respectfully (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; Gavaghan *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; Arikan *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/m^2. 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; Arikan *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; Arikan *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 I 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 et al. (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 et al. (2000)
Meyer et al. (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 et al. (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 et al. (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 et al. (2009a,b)
Cascella et al. (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 et al. (2008)
Erdogan et al. (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 et al. (2009)
Adali et al. (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 et al. (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.
[a]NIH PCOS Criteria.
[b]Rotterdam PCOS Criteria.
[c]Data expressed as SE.
[d]Data 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 ± SD	BMI ± SD	Mean CIMT (mm) ± SD	P-value	Segment(s) used to measure CIMT	CIMT protocol
Talbott et al. (2000)	Community	Age and BMI	125 cases[a]	37.5 ± 6.2[c]	30.1 ± 0.7[c]	Age-BMI adjusted (CI) Overall (n = 125) 0.70 (0.68–0.73); 30–44 years (n = 78) 0.65 (0.62–0.69); ≥45 years (n = 47) 0.77 (0.74–0.81)	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 ± 6.2[c]	26.5 ± 0.5[c]	Overall (n = 142) 0.67 (0.65–0.69); 30–44 years (n = 82) 0.64 (0.61–0.67); ≥45 years (n = 60) 0.71 (0.68–0.75)			
Lakhani et al. (2004)	Staff members	Age	19 PCOS[b]	29.2 ± 4.0	31.3 ± 8.2	0.54 ± 0.11; 0.53 ± 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 ± 4.0	22.5 ± 3.8	0.51 ± 0.18; 0.50 ± 0.15[d] CI (0.81–0.7)	P = 0.038, P = 0.841[d]		
			12 controls	27.5 ± 4.0	24.2 ± 3.4	0.40 ± 0.09; 0.44 ± 0.09[d]			
Orio et al. (2004)	NA	Age and BMI	30 cases[b]	22.2 ± 2.5	22.4 ± 2.1	0.53 ± 0.09[e]	P < 0.05	CCA	Mean, right and left, far wall, intra-observer CV 7% and inter-observer CV 12%
			30 controls	22.6 ± 2.3	22.1 ± 1.8	0.39 ± 0.08[e]			
Vural et al. (2005)	Medical students and nurses	Age	43 cases[b]	21.4 ± 1.8	23.4 ± 4.7	0.75 ± 0.11	P < 0.001	CCA	Left and right, near and far walls, intra-observer error <0.03 mm
			43 controls	20.8 ± 2.2	21.5 ± 3.0	0.61 ± 0.11			
Alexandraki et al. (2006)	Doctors and medical students	Age and BMI	27 cases[a]	25.4 ± 0.8[c]	27.42 ± 1.1[c]	0.49 ± 0.01[c]	P = 0.19	CCA, Bif, ICA	Mean, left and right, far wall one reader
			27 controls	27.3 ± 0.8[c]	25.05 ± 1.2[c]	0.51 ± 0.02[c]			
Luque-Ramirez et al. (2007)	12 patients for obesity problems, 8 healthy controls		40 cases[a]	24.5 ± 5.8	29.4 ± 6.3	0.41 ± 0.11	P = 0.005	CCA	Left, far wall, intra-observer CV 10.8%
			20 controls	27.2 ± 6.8	28.2 ± 6.9	0.33 ± 0.08			
Cascella et al. (2008)	NA	Age and BMI	200 cases[b]	24.6 ± 3.2	28.5 ± 2.8	0.46 ± 0.16	P < 0.001	CCA	Mean, left and right, far wall, intra-observer CV 7.0%
			100 controls	24.0 ± 2.8	28.8 ± 2.7	0.38 ± 0.09			
Costa et al. (2008)	NA		57 cases[b]	25.5 ± 5.3	27.6 ± 5.8	0.52 ± 0.08	P = 0.35	CCA	Mean, left and right, far wall, one reader
			37 controls	26.6 ± 5.4	26.7 ± 4.9	0.53 ± 0.08			
Heutling et al. (2008)*	Public advertising		83 cases[b]	24.8 ± 4.7	30.4 ± 5.9	0.48 ± 0.07	P < 0.001	CCA	Mean, left and right, far wall, intra-observer CV 6.8%
			39 controls	27.8 ± 5.6	29.1 ± 4.8	0.42 ± 0.05			

Study	Setting	Matched for	Group	Age	BMI	CIMT	P value	Site	Measurement
Karadeniz et al. (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 et al. (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 et al. (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 et al. (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 et al. (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 et al. (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 et al. (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 et al. (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 et al. (2010)	Clinic, local newspaper ads	Age and weight	22 lean cases[b] 18 obese cases[b] 17 lean controls 13 obese controls	28.6 ± 4.5 30.3 ± 4.2 27.7 ± 5.3 28.6 ± 5.3	22.0 ± 2.2 36.2 ± 5.9 22.2 ± 1.7 40.5 ± 7.0	0.53 ± 0.08 0.56 ± 0.17 0.48 ± 0.07 0.60 ± 0.11	P > 0.05	CCA	Right side, mean of three measurements
Pepene et al. (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 trail; NA, not addressed.
[a]NIH PCOS criteria.
[b]Rotterdam PCOS criteria.
[c]Data expressed as SE.
[d]CIMT adjusted for age, systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, cholesterol and insulin.
[e]Adjusted for age, BMI, waist to hip ratio, smoking status, glucose and insulin levels, free androgen index, lipid profile, homocysteine concentrations and SBP and DBP;
[f]Androgen 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 $P < 0.05$.
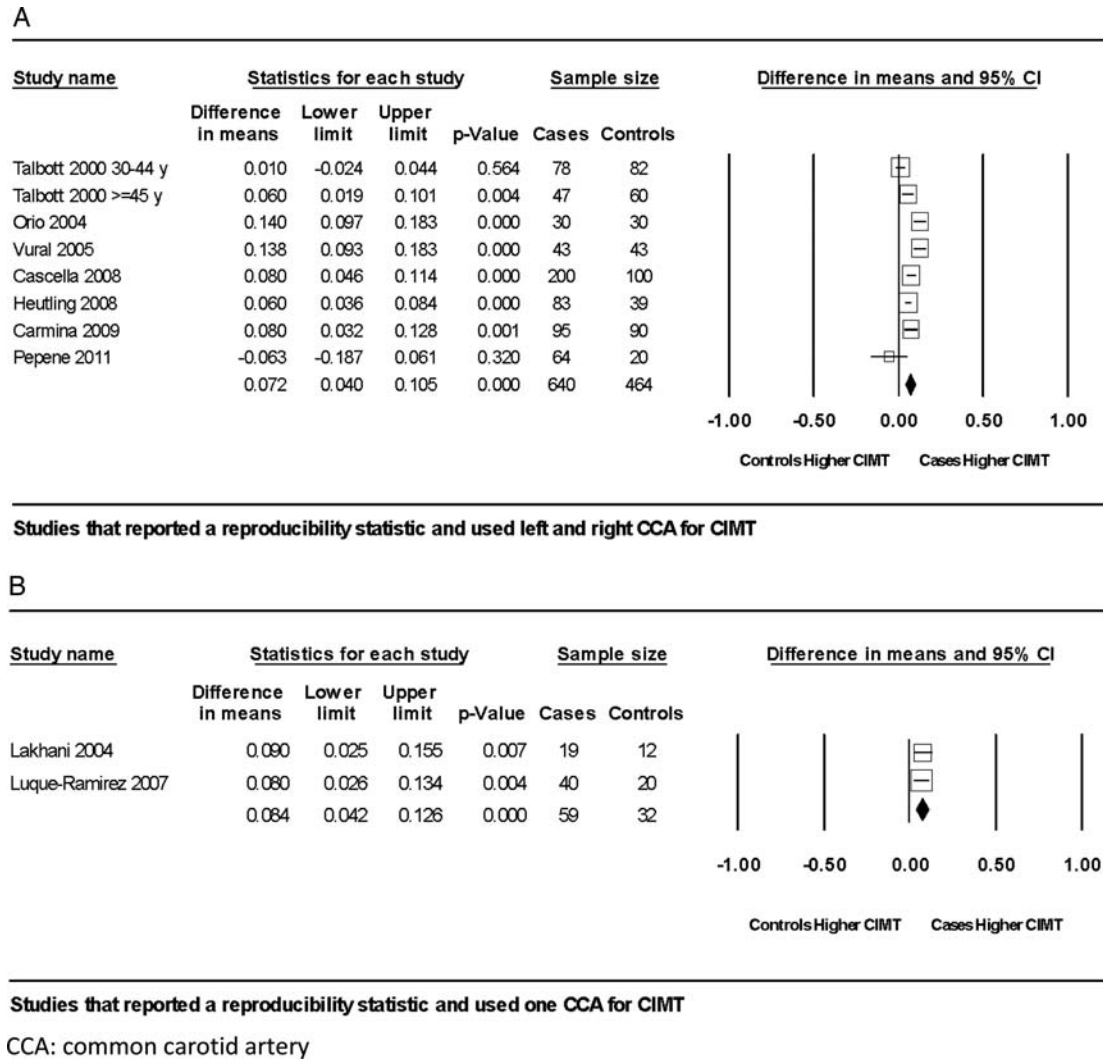
A

Study name	Statistics for each study				Sample size		Difference in means and 95% CI
	Difference in means	Lower limit	Upper limit	p-Value	Cases	Controls	
Talbott 2000 30-44 y	0.010	-0.024	0.044	0.564	78	82	
Talbott 2000 >=45 y	0.060	0.019	0.101	0.004	47	60	
Orio 2004	0.140	0.097	0.183	0.000	30	30	
Vural 2005	0.138	0.093	0.183	0.000	43	43	
Cascella 2008	0.080	0.046	0.114	0.000	200	100	
Heutling 2008	0.060	0.036	0.084	0.000	83	39	
Carmina 2009	0.080	0.032	0.128	0.001	95	90	
Pepene 2011	-0.063	-0.187	0.061	0.320	64	20	
	0.072	0.040	0.105	0.000	640	464	

-1.00   -0.50   0.00   0.50   1.00

Controls Higher CIMT   Cases Higher CIMT

Studies that reported a reproducibility statistic and used left and right CCA for CIMT

B

Study name	Statistics for each study				Sample size		Difference in means and 95% CI
	Difference in means	Lower limit	Upper limit	p-Value	Cases	Controls	
Lakhani 2004	0.090	0.025	0.155	0.007	19	12	
Luque-Ramirez 2007	0.080	0.026	0.134	0.004	40	20	
	0.084	0.042	0.126	0.000	59	32	

-1.00   -0.50   0.00   0.50   1.00

Controls Higher CIMT   Cases Higher CIMT

Studies that reported a reproducibility statistic and used one CCA for CIMT

CCA: common carotid artery

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; Trakakis et al., 2008; Arikan et al., 2009; Ciccone 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,
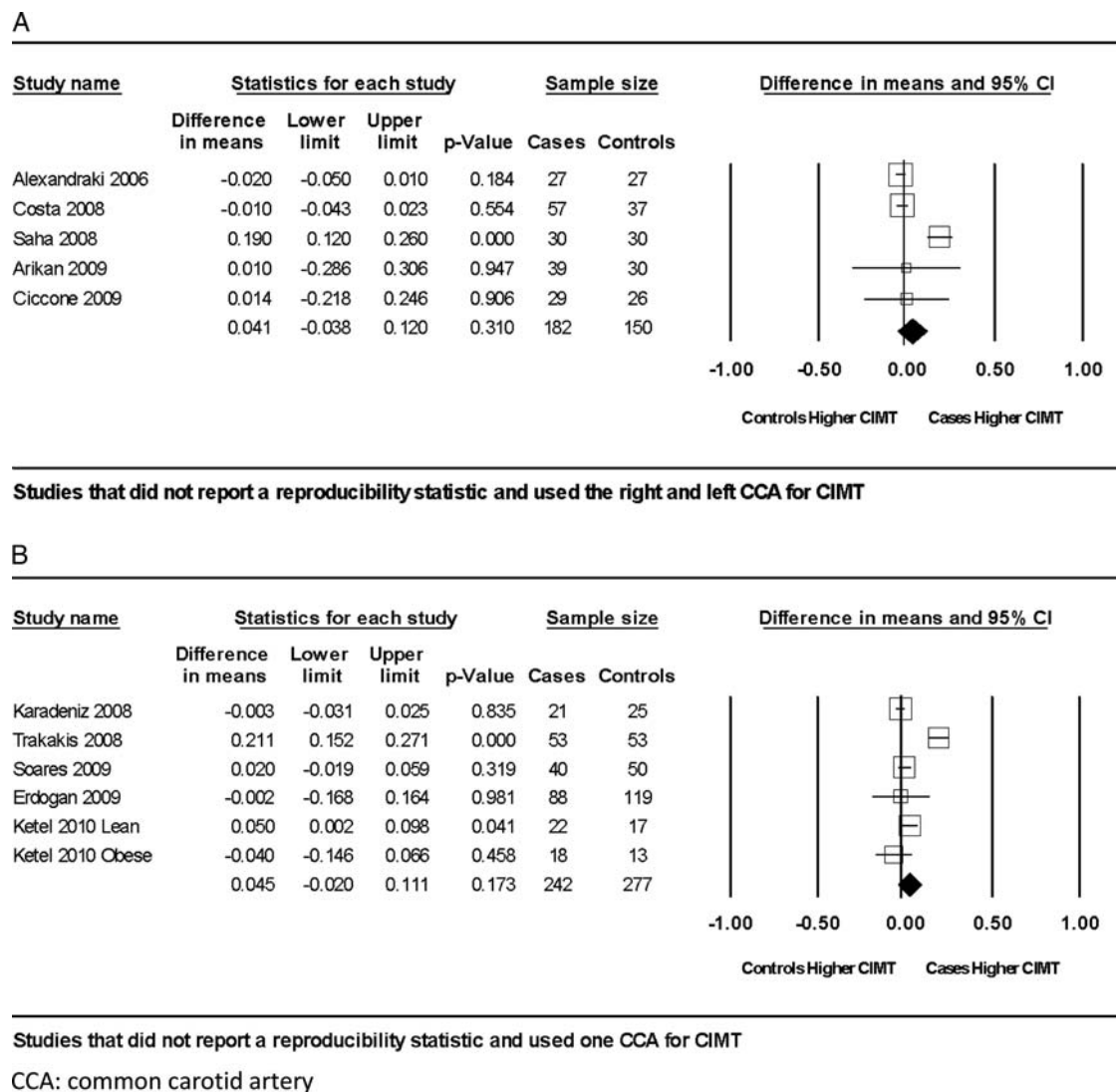
A

Study name	Statistics for each study				Sample size		Difference in means and 95% CI
	Difference in means	Lower limit	Upper limit	p-Value	Cases	Controls	
Alexandraki 2006	-0.020	-0.050	0.010	0.184	27	27	
Costa 2008	-0.010	-0.043	0.023	0.554	57	37	
Saha 2008	0.190	0.120	0.260	0.000	30	30	
Arikan 2009	0.010	-0.286	0.306	0.947	39	30	
Ciccone 2009	0.014	-0.218	0.246	0.906	29	26	
	0.041	-0.038	0.120	0.310	182	150	

Studies that did not report a reproducibility statistic and used the right and left CCA for CIMT

B

Study name	Statistics for each study				Sample size		Difference in means and 95% CI
	Difference in means	Lower limit	Upper limit	p-Value	Cases	Controls	
Karadeniz 2008	-0.003	-0.031	0.025	0.835	21	25	
Trakakis 2008	0.211	0.152	0.271	0.000	53	53	
Soares 2009	0.020	-0.019	0.059	0.319	40	50	
Erdogan 2009	-0.002	-0.168	0.164	0.981	88	119	
Ketel 2010 Lean	0.050	0.002	0.098	0.041	22	17	
Ketel 2010 Obese	-0.040	-0.146	0.066	0.458	18	13	
	0.045	-0.020	0.111	0.173	242	277	

Studies that did not report a reproducibility statistic and used one CCA for CIMT

CCA: common carotid artery

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; Ciccone 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.
[b]Talbott et al. (2000) included two subgroups.
[c]Ketel et al. (2010) included two subgroups.

cholesterol (Alexandraki et al., 2006; Saha et al., 2008), triglycerides (Saha et al., 2008), CRP (Cascella et al., 2008), serum interleukin-18 (Kaya et al., 2010), lower levels of high-density lipoprotein cholesterol (Vryonidou et al., 2005) and abdominal obesity (Cascella 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 by18% (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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