Endothelium-dependent But Not Endothelium-independent Flow-mediated Dilation Is Significantly Reduced in Patients with Systemic Lupus Erythematosus without Vascular Events: A Metaanalysis and Metaregression

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ABSTRACT. ***Objective.*** To assess whether endothelium-dependent and endothelium-independent flow-mediated dilation (FMD) are impaired in patients with systemic lupus erythematosus (SLE) with no history of vascular event; and to determine factors moderating impaired FMD in SLE.

Methods. Electronic databases were searched for case-control studies that compared endothelium-dependent and/or endothelium-independent FMD at the brachial artery between SLE patients who were naive for vascular events and matched healthy controls. Effect size as standardized mean difference (SMD) and 95% confidence intervals of FMD between SLE patients and controls was pooled using the inverse variance method. Mixed-model metaregression was performed to identify potential demographic and clinical factors associated with the effect size.

Results. Thirteen relevant studies involving 580 patients and 381 matched healthy controls were included. Endothelium-dependent FMD was significantly lower in SLE patients than in controls (SMD –0.832, 95% CI –1.172 to –0.492, $p < 0.001$). Endothelium-independent FMD, however, did not differ between the 2 groups (SMD –0.179, 95% CI –0.433 to 0.075, $p = 0.167$). Metaregression revealed that increasing age ($r = 0.047$, $p = 0.037$) and duration of SLE ($r = 0.008$, $p = 0.024$) at the time of FMD measurement significantly narrowed the difference of endothelium-dependent FMD between patients and controls; whereas sex, smoking, menopause, diabetes mellitus, body mass index, blood pressure, fasting lipid profile, C-reactive protein, and prednisolone use did not.

Conclusion. Endothelium-dependent, but not endothelium-independent FMD is significantly impaired in lupus patients who are naive for vascular events. Increasing age and longer disease duration may limit the potential of endothelial reactivity as an indicator of early atherosclerosis in SLE.

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Key Indexing Terms:
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Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease that can affect any organ at any time. While survival of lupus patients has improved over the past 30 years, the burden resulting from organ damage accrual remains a major issue[1]. From a variety of damage observed, the occurrence of cardiovascular (CV) disease has been consistently increasing over the past decades and has become a major cause of death in patients with SLE[2,3]. Hence, detection and intervention during the very early stage of atherogenesis may potentially prevent clinical vascular events and improve the survival of patients with SLE.

From the moment patients present with clinical consequences of atherosclerosis, the involvement is essentially advanced and treatment is largely palliative or secondary prevention for future vascular events. While subclinical features of atherosclerosis such as carotid intima-media thickness measured by ultrasonography and coronary artery calcifications detected by computed tomography are increased in lupus patients compared with healthy subjects, these changes are currently considered to occur late in the course of atherosclerosis[4,5]. Recently, the concept of "vascular failure" has been introduced, encompassing a comprehensive syndrome of failed vascular function extending from risk factor exposure and endothelial dysfunction to atheroscle-

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rotic diseases[4]. It is now strongly believed that endothelial dysfunction occurs as an initial step in the pathogenesis of atherosclerosis, and is a predictor for CV events even in patients with normal coronary angiogram[6]. Endothelial dysfunction may be reversible if traditional CV risk factors are corrected. Hence, assessment of endothelial function may be a potential biophysical marker of atherosclerosis in its very early stage, which opens up an avenue for possible earlier intervention to prevent future vascular events in patients with lupus.

Observational studies investigating endothelial reactivity at the brachial artery assessed by flow-mediated dilation (FMD) suggested that endothelial dysfunction is present in SLE patients who are naive for vascular events[7,8,9,10,11,12,13,14,15,16,17,18,19]. However, the results of these studies are discordant. Also, the relative applicability of endothelium-dependent and endothelial-independent FMD in detecting endothelial dysfunction in lupus has not been formally addressed.

Thus, it is imperative to systematically combine these studies using metaanalysis and generate an effect size to address (1) whether FMD is impaired in SLE patients with no history of clinical vascular event; (2) whether endothelium-dependent or endothelium-independent FMD is a better method to detect endothelial dysfunction in lupus patients; and (3) whether there is any limitation of FMD measurement in identifying endothelial dysfunction in lupus patients by investigating if potential demographic and disease-related confounders such as age, sex, disease duration, the presence of comorbidities, inflammatory status, and use of antimalarials and glucocorticoids are crucial predictors for the difference of FMD between SLE patients and healthy controls using metaregression. These factors need to be addressed before the utility of this method as a diagnostic and monitoring tool for early atherosclerosis in patients with SLE can be determined.

MATERIALS AND METHODS

Search strategy. We performed an extensive literature search using the relevant keywords "endothelial," "flow," "dilation," "dilatation," "brachial," "lupus," and "SLE" in various combinations to identify case-control studies published in English from different computerized databases: PubMed (1966 to September 2010), Embase (1980 to September 2010), and the Cochrane Central Register of Controlled Trials (3rd quarter of 2010). Abstracts presented in major international conferences (Annual European Congress of Rheumatology, International Congress on SLE, and American College of Rheumatology meetings) over the past 10 years were manually searched. We also scanned the articles from the bibliographies of the retrieved trials and review articles. The authors of correspondence were contacted for information that was lacking in their published articles.

Criteria for selecting articles. All observational case-control studies addressing the difference in brachial artery FMD between SLE patients and matched healthy controls were included. Studies were included if they met the following criteria: (1) SLE patients and matched healthy controls were compared for FMD at the brachial artery; (2) subjects in both SLE and control groups have had no history of any CV and cerebrovascular diseases; and (3) the procedure of brachial artery FMD measurement was based on a similar published protocol[4,5]. Three investigators (AM, YL, and RCMH) independently assessed the reports generated for relevancy and reports with the following criteria were excluded: (1) not written in English; (2) did not compare SLE patients and matched healthy controls; (3) patients in SLE and control groups had history of cerebrovascular and CV diseases; and (4) measurement of brachial artery FMD deviated substantially from predefined protocol[4,5]. In brief, subjects are asked to rest in supine position for at least 10 minutes before FMD measurement at the same position. FMD at the brachial artery is measured using a high-resolution ultrasound system, in which the ultrasound probe is steadied by a stereotactic holding device that also allows fine positional adjustment. Reactive hyperemia is induced by rapid inflation of a pneumatic cuff placed around the proximal forearm to a pressure between 30 and 50 mm Hg above the systolic blood pressure for around 5 minutes, followed by rapid deflation. Change of vessel diameter at maximum dilatation and percentage of FMD change can hence be detected by the ultrasound probe and calculated by a computer program, with the peak reactive hyperemic blood flow at 45 to 60 seconds after cuff deflation. All FMD studies are preferably performed after abstention from food and exercise for 8 to 12 hours, coffee and tea for 24 hours, and alcohol for 48 hours. Vasoactive drugs such as calcium channel blockers and angiotensin-converting enzyme inhibitors should be withheld for at least 4 to 5 half-lives before FMD measurement. Another established way to assess endothelial reactivity is to measure endothelium-independent FMD of the brachial artery before and after administration of nitroglycerin, which is a direct smooth-muscle relaxant without the need for nitric oxide production and release by endothelium. After 10 to 15 minutes of rest following completion of endothelium-dependent FMD measurement, 0.4 mg nitroglycerin, in the form of sublingual spray or tablet, is given to participants. Peak vasodilation occurs between 3 and 5 minutes after nitroglycerin administration and endothelium-independent FMD can be measured, using the same method as for endothelium-dependent FMD, except no forearm occlusion is required[4,5].

Assessment of quality of studies. The quality of the studies identified was assessed by the Newcastle-Ottawa quality assessment scale for case-control studies[20]. This was jointly developed by the Universities of Newcastle, Australia, and Ottawa, Canada, for assessing the quality of case-control studies for systematic reviews and metaanalyses. A "star system" has been developed in which a study is evaluated based on 3 perspectives; namely, selection of cases and controls, comparability of the selected groups, and ascertainment of either the exposure or outcome of interest. A study can be awarded a maximum of 1 star in each category for each item within the selection and exposure categories, while a maximum of 2 stars can be awarded for comparability[20]. The total score ranges from 0 to 9. Although there is no validated cutoff score to distinguish between good and poor quality studies, we arbitrarily defined a score ≥ 7 to be of high quality.

Data were independently extracted into a standard electronic form. Any discrepancies were resolved by consensus. If consensus could not be reached, the principal investigators (AM and RCMH) would make the final decision for trial eligibility and data extraction.

Outcome measures. The primary and secondary outcomes were the difference between patients with SLE and matched healthy controls with respect to endothelium-dependent FMD and endothelium-independent FMD, respectively, at the brachial artery.

Statistical analysis. Effect size of both primary and secondary outcomes was pooled as the standardized mean difference (SMD) and the corresponding 95% confidence interval. Heterogeneity was assessed by the Cochran Q-test. Because the number of studies of this metaanalysis may be limited, the Cochran Q-test for heterogeneity may yield a low statistical power[21]. A value of significance at 10% ($p \leq 0.1$) was therefore considered statistically significant for heterogeneity[22]. Further, we assessed heterogeneity by I^2, which describes the percentage of total variation across studies caused by heterogeneity rather than chance. High values of I^2 suggest increased heterogeneity. If considerable heterogeneity (arbitrarily if $I^2 > 40$) was encountered, we applied the random effects model with the method sug-

gested by DerSimonian and Laird[23]. For models with considerable heterogeneity and statistically significant effect size, metaregression was performed to identify demographic and disease-related factors that might contribute to the heterogeneity[24]. Since the covariates chosen were not expected to explain all the heterogeneity of the studies, mixed-model metaregression was used in considering the presence of "residual heterogeneity"[24]. The regression coefficients and the associated standard error (SE), the z score, degree of freedom (df), and p values were reported for the metaregression analysis. Publication bias was assessed by Egger's regression analysis.

Sensitivity analyses. We performed 2 sensitivity analyses by (1) excluding studies with poor quality based on our arbitrary cutoff score of 7 of the Newcastle-Ottawa assessment scale; and (2), excluding studies missing data that were essential for this metaanalysis. The statistical method of effect size calculation in the sensitivity analyses was the same as that involved in the analyses of the primary and secondary outcomes.

All statistical analyses in this metaanalysis were performed using the Comprehensive Meta-analysis Programme, Version 2 (Biostat, Englewood, NJ, USA). As metaanalytically essential missing data such as standard deviation might be encountered, it was planned that such missing data were to be estimated by multiple imputation with 1000 imputations, an acceptable method for handling missing data in metaanalysis[25]. However, demographic and study-related missing values such as age, comorbidities, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and FMD were not imputed as estimation of these values was deemed inappropriate. Multiple imputations were performed using PASW Statistics version 18.0 (SPSS Inc., Chicago, IL, USA). To ascertain the quality of this metaanalysis, both the MOOSE (meta-analysis of observational studies in epidemiology) and QUOROM (quality of reporting of meta-analysis) guidelines were followed where applicable.

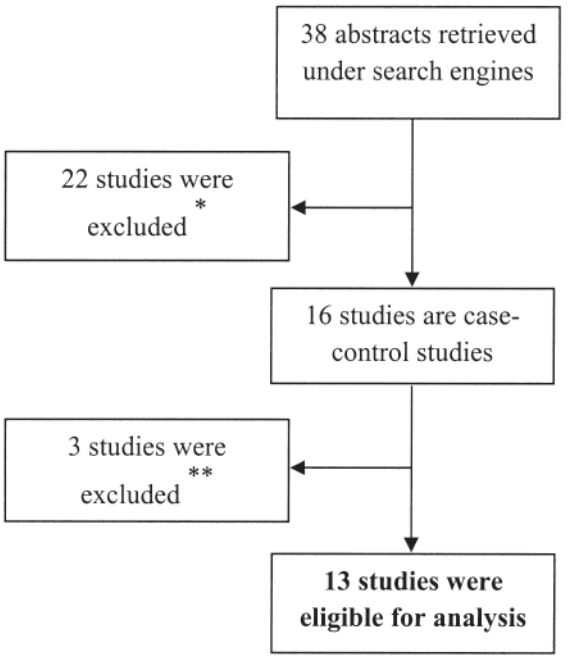

Figure 1. Summary of the literature search. *Three did not study SLE, 1 studied autoimmune disease, 3 were reviews, 1 did not study FMD, 3 were non-English publications, and 11 were not case-control studies. **Patients with history of cardiovascular or cerebrovascular disease were enrolled.

RESULTS

Thirty-eight abstracts were initially identified through database searches. Twenty-two were not eligible because they investigated diseases other than SLE (n = 4), were review articles (n = 3), did not study FMD (n = 1), were non-English abstracts (n = 3), or were not case-control studies (n = 11) (Figure 1). Sixteen studies were then further scrutinized for eligibility. Out of these 16, 3 were excluded because patients with history of cerebrovascular and CV events were included. Hence, 13 studies were finally eligible for this metaanalysis (Table 1). Since missing data that was crucial to this metaanalysis were noted in 11 of these 13 eligible studies, e-mails were sent to the corresponding authors. Six of the authors responded and kindly furnished the missing data that had not been published in their articles.

Agreement between investigators. The interrater reliability agreement of the 2 investigators (AM and RCMH) in terms of inclusion and exclusion of studies was 0.90 and 0.92, respectively, calculated based on the Fleiss statistic[25]. Such level of agreement is considered to be almost perfect[26].

Primary outcomes. Data of 13 studies comprising 580 patients with SLE and 381 age and sex-matched healthy controls were involved in pooling effect size for the primary outcome. Endothelium-dependent FMD was significantly lower in patients with SLE than healthy controls (SMD = –0.832, 95% CI –1.172 to –0.492, p < 0.001; Figure 2). Since Cochran's Q and I^2 statistics revealed a substantial degree of heterogeneity in these studies (I^2 = 81.540, p < 0.001), the random effects model was therefore used.

Secondary outcomes. Two studies were not used in synthesizing the effect size of the secondary outcome because they did not report endothelium-independent FMD[8,11] (Table 1). While the SMD was lower in SLE patients when endothelium-independent FMD was collectively analyzed in 11 studies, the difference did not reach statistical significance (SMD –0.179, 95% CI –0.433 to 0.075, p = 0.167; Figure 2). The random effects model was used because a moderate degree of heterogeneity was revealed in these studies (I^2 = 63.782, p = 0.002).

Metaregression. Mixed-model metaregression revealed that increasing age and disease duration of SLE were significant moderators for a less discrepant endothelium-independent FMD between SLE patients and healthy controls (Table 2). Hydroxychloroquine (HCQ) use was associated with a more discrepant FMD between the 2 groups (Table 2). Other predefined moderators such as percentages of females in the studies, hypertension, diabetes mellitus, menopause, and smoking, as well as body mass index (BMI), blood pressure, fasting lipid profiles, CRP, baseline brachial artery diameter, mean prednisolone dose, and prednisolone use were not significant predictors for the primary outcome (Table 2). ESR and SLE Disease Activity Index (SLEDAI), percentages of patients with high antiphospholipid antibodies and antiphospholipid antibody syndrome, and proportion of aspirin users

Table 1. Characteristics and quality of studies comparing brachial artery flow-mediated dilation in patients with systemic lupus erythematosus (SLE) and matched healthy controls.

	Patients with SLE						Controls					Newcastle-Ottawa Scale			
Study	Mean Age, yrs	N	Female, %	Disease Duration, mo	EDD ± SD, %	EID ± SD, %	Mean Age, yrs	N	Female, %	EDD ± SD, %	EID ± SD, %	Selection	Compara-bility	Exposure	Total
Ahmadi[19]	29.6	84	100	68.4	8.50 ± 7.52*	7.94 ± 12.42*	26.5	18	100	15.84 ± 6.88	24.70 ± 9.42	3	2	2	7
Cypiene[8]	37.33	30	100	96.12	9.25 ± 5.15	NR	37.45	66	100	9.69 ± 3.29	NR	3	1	2	6
Ghosh[9]	31	60	90	60	9.97 ± 5.51	23.64 ± 7.25	34	38	86.8	18.97 ± 6.42	22.53 ± 7.19	3	2	2	7
Johnson[10]	47.1	5	100	198.8	9.62 ± 5.54	14.76 ± 6.04	42.4	5	100	11.08 ± 2.63	12.14 ± 6.05	2	1	2	5
Karadag[11]	40	25	100	90	7.10 ± 2.10	NR	38	22	100	11.40 ± 1.20	NR	3	1	2	6
Kiss[17]	41.15	33	85.2	122.4	8.81 ± 5.28	17.75 ± 8.60	48.54	26	84.6	9.86 ± 3.87	18.23 ± 8.10	1	2	2	5
Lima[15]	29	69	100	NR	5.00 ± 5.00	14 ± 6	29	35	100	12.00 ± 6.00	16.00 ± 6.00	3	2	2	7
Piper[7]	40.6**	36	100	120	5.60** ± 3.13*	21.50** ± 10.55*	46.0**	22	100	8.00** ± 1.38*	21.50** ± 4.57*	2	2	2	6
Rajagopalan[18]	37	43	100	NR	3.70 ± 3.50	20.60 ± 9.0	35	43	50	6.50 ± 3.50	19.60 ± 6.00	2	2	2	6
Svenungsson[14]	52.2	26	100	240	6.40 ± 4.20	18.20 ± 9.40	52.3	26	100	5.10 ± 5.00	19.10 ± 9.10	4	2	2	8
Valdivielso[13]	34	26	96.2	NR	12.49 ± 4.47	23.21 ± 6.55	35	21	95.2	16.91 ± 5.58	25.34 ± 5.78	3	2	2	7
Wright[16]	45	32	88	180	2.40** ± 3.53*	16.20** ± 9.49*	40	19	79	5.80** ± 3.92*	15.40** ± 3.08*	3	2	2	7
Zhang[12]	34.4	111	100	112.8	10.87 ± 5.42	27.02 ± 8.84	34.5	40	100	14.23 ± 4.11	29.13 ± 6.53	3	1	2	6

EDD: endothelium-dependent dilation at brachial artery; EID: endothelium-independent dilation at brachial artery; NR: not reported. * Estimated by multiple imputation with 1000 imputations; ** median.

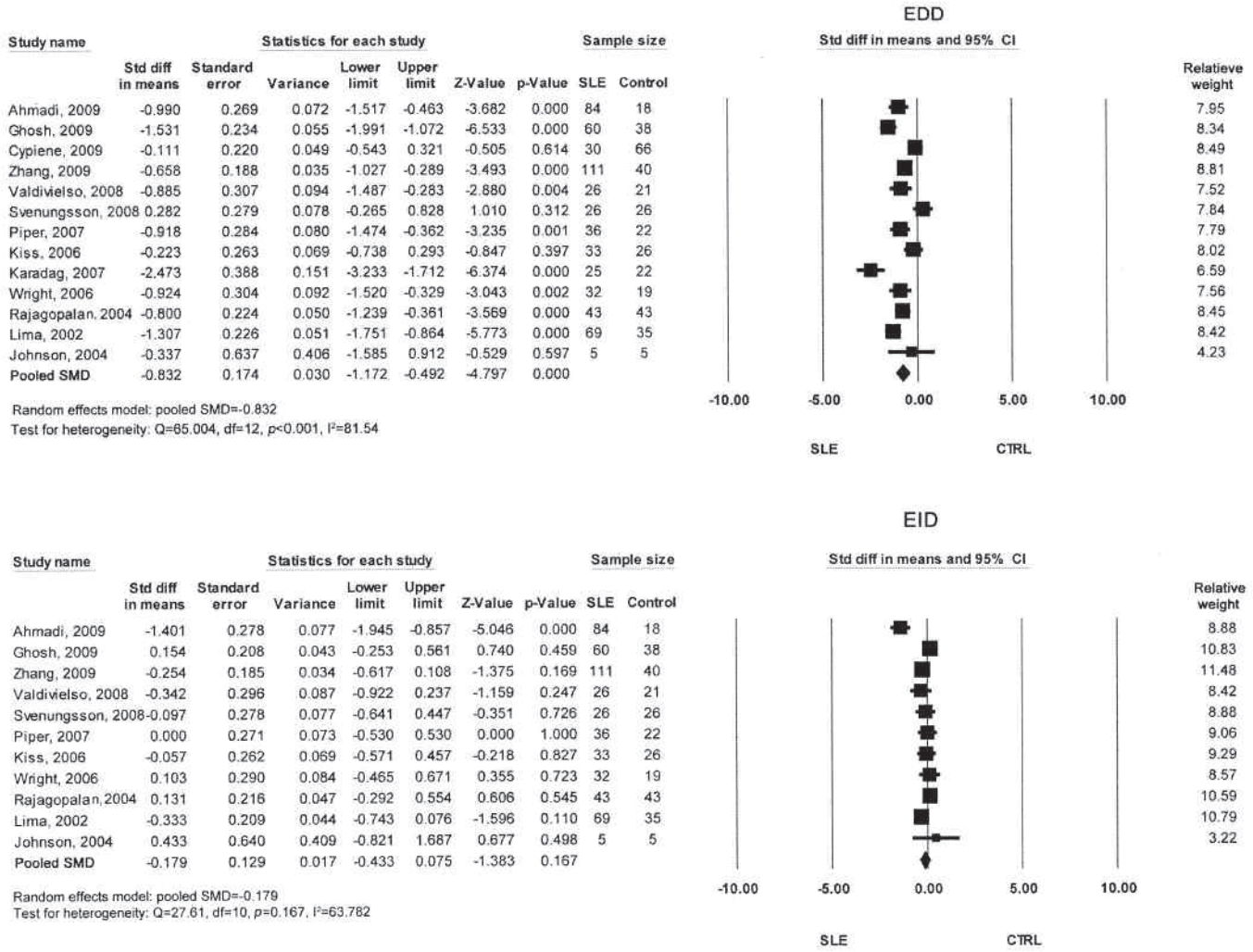

Figure 2. Forest plots of the primary and secondary outcomes: endothelium-dependent flow-mediated dilation (EDD) and endothelium-independent flow-mediated dilation (EID).

Table 2. Metaregression analysis of potential moderators of difference of endothelium-dependent flow-mediated dilation.

Factor	Regression Coefficient (SE)	Z Score	Tau^2	DF	p
Age, yrs	0.04716 (0.02261)	2.08579	0.21018	12	0.037
Female, %	–0.13693 (3.34813)	–0.04090	0.32448	12	0.967
Diabetes mellitus, %	1.26221 (1.09262)	0.11552	0.37872	10	0.908
Hypertension, %	0.24785 (1.17795)	0.21041	0.36537	11	0.834
Smoking, %	1.29497 (1.52302)	0.85026	0.36985	9	0.395
Menopause, %	1.21313 (0.86167)	1.40788	0.31034	8	0.159
Body mass index, kg/m^2	0.09065 (0.12067)	0.75123	0.40031	9	0.452
Systolic blood pressure, mm Hg	0.02016 (0.03157)	0.63849	0.39058	8	0.523
Diastolic blood pressure, mm Hg	–0.01463 (0.05198)	–0.28146	0.45404	7	0.778
Total cholesterol, mg/dl	0.01847 (0.01107)	1.66834	0.31260	9	0.095
HDL, mg/dl	0.02358 (0.01893)	1.24576	0.33741	9	0.213
LDL, mg/dl	0.01432 (0.01220)	1.17414	0.36586	9	0.240
TG, mg/dl	0.00114 (0.00792)	0.14364	0.53103	7	0.886
CRP, U/l	0.03293 (0.09250)	0.35594	0.47379	7	0.722
Prednisolone use, %	0.27961 (1.06867)	0.26164	0.43398	8	0.794
Mean prednisolone dose, mg/day	–0.01615 (0.03806)	–0.42429	0.33187	7	0.671
Hydroxychloroquine use, %	–2.40527 (0.76028)	–3.16360	0.17219	8	0.002
Baseline brachial artery diameter, mm	0.24629 (0.91395)	0.26948	0.29085	5	0.788
Disease durations, mo	0.00775 (0.00342)	2.26409	0.25260	9	0.024

HDL: high density lipoprotein; LDL: low density lipoprotein; TG: total triglyceride; CRP: C-reactive protein.

were not involved in the metaregression because studies were too few to result in meaningful and unbiased metaregression analyses.

Sensitivity analysis. Data of 3 out of the 13 studies needed to be imputed because the standard deviations of the FMD were missing (Table 1). After exclusion of these 3 studies, there is no change in the significance of effect sizes of both the endothelium-dependent and endothelium-independent FMD (Table 3). Similarly, exclusion of studies of poor quality (Newcastle-Ottawa score < 7) did not alter the significance of the primary and secondary outcomes (Table 3).

Publication bias. Using the Egger regression method, no significant publication bias was detected when all 13 studies were pooled for SMD of endothelium-dependent FMD (intercept = –1.392, SE = 2.784, df = 11, 2 tailed p = 0.627). Similarly, no significant bias was noted in pooling the 11 studies for effect size of endothelium-independent FMD (intercept = 0.058, SE = 2.158, df = 9, 2-tailed p = 0.979). Representation of publication bias is shown as funnel plots (Figure 3).

DISCUSSION

The reason for endothelial dysfunction is believed to be a complex interplay of inflammatory, metabolic, immunological, and therapeutic factors[27], in addition to conventional CV risk factors[7,8]. Intuitively, in the context of inflammation and higher prevalence of conventional risk factors in SLE, lupus patients are expected to have poorer endothelial function compared with their age and sex-matched healthy counterparts even before they develop clinical atherosclerosis.

Table 3. Results of sensitivity analyses based on quality of studies and exclusion of studies with missing data.

	No. Studies Involved		Pooled SMD (95% CI)	
	EDD	EID	EDD	EID
Quality of studies				
All studies	13	11	–0.832 (–1.172, –0.492)†	–0.179 (–0.433, 0.075)
High-quality studies*	6	6	–0.904 (–1.411, –0.397)†	–0.312 (–0.748, 0.125)
Missing data				
All studies	13	11	–0.832 (–1.172, –0.492)†	–0.179 (–0.433, 0.075)
Studies without missing data**	10	8	–0.801 (–1.237, –0.364)†	–0.099 (–0.266, 0.068)

* Newcastle-Ottawa quality assessment scale total score ≥ 7. ** Exclusion of Ahmadi, *et al*[19], Piper, *et al*[7], and Wright, *et al*[16]. † p < 0.001. SMD: standardized mean difference; EDD: endothelium-dependent dilation at brachial artery; EID: endothelium-independent dilation at brachial artery.

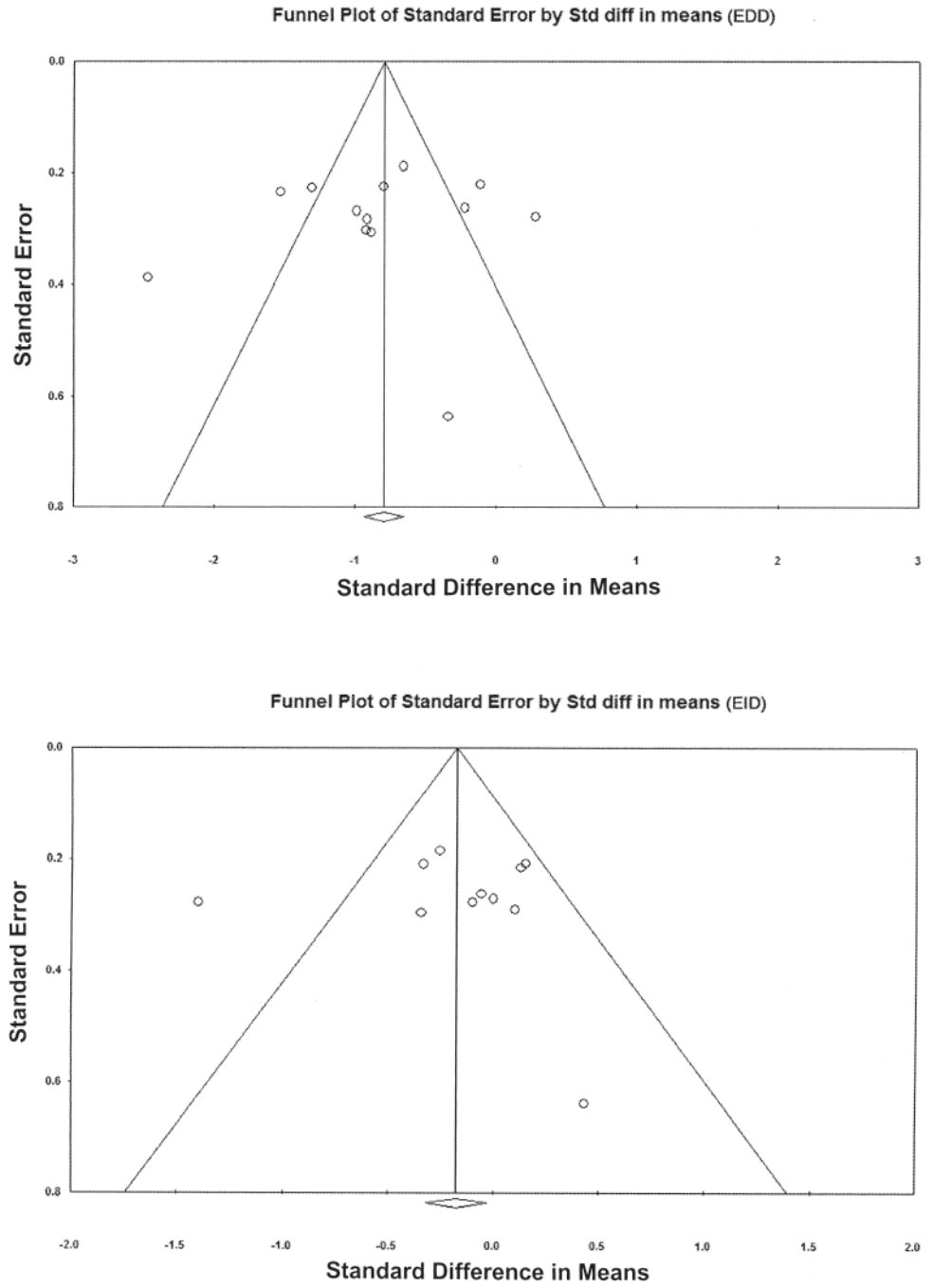

Figure 3. Funnel plots of publication bias in both primary and secondary outcomes (standard error against SMD).

While the literature addressing this assumption has not been in total agreement, this metaanalysis confirms that endothelial function is indeed impaired in lupus patients before the development of clinical atherosclerosis.

Interestingly, endothelial-independent FMD was comparable between lupus patients and healthy controls. Because endothelium-independent FMD depends on relaxation of vascular musculature from exogenous source of nitric oxide generated from nitroglycerin, the endothelium is spared. This implies that the endothelium is preferentially involved during the early process of atherosclerosis rather than vascular smooth-muscle dysfunction or changes in the vascular structures in SLE. Therefore, employing endothelium-independent FMD to detect very early vascular involvement in lupus patients may not be an optimal method.

Our metaregression revealed that the gap of endotheli-

um-dependent FMD between lupus patients and healthy controls converged as age and duration of SLE in patients increased (Table 2). This phenomenon is likely due to 2 reasons. First, endothelial function deteriorates naturally as one gets older even without SLE. Second, for patients with longer disease duration, more endothelial and vascular damage is expected to accrue, although our metaregression could neither delineate the differential effects of either age or duration of SLE on FMD nor address if a relationship between age and duration of SLE existed in the studies we analyzed. Nevertheless, using FMD to identify and monitor endothelial damage as an indicator of very early atherosclerosis may not be applicable in lupus patients with advanced age and long disease duration.

Surprisingly, HCQ use was significantly associated with a narrower difference in FMD between SLE patients and controls. Cautious interpretation of this result is required, because in 3 of the studies that demonstrated the lowest SMD, one reported that all patients used HCQ and 88% used aspirin[9], one reported the presence of antiphospholipid antibody syndrome in 14% of patients[11], and the third reported that over 30% of patients were positive for antiphospholipid antibodies[15]. Hence, confounding by indication might operate and drive the whole metaregression of HCQ use on SMD toward statistical significance. Further, we failed to identify significant correlations between a number of CV risk factors such as hypertension, diabetes mellitus, smoking, menopausal state, lipid level, blood pressure, BMI, and glucocorticoid use and the primary outcome. The reason for this is unclear; the relatively small sample sizes of individual studies and the limited number of studies in the metaanalysis may partly explain this phenomenon.

Despite our precautions, this study has several limitations, which stem mainly from factors intrinsic to metaanalysis. First, while statistical significance of the effect size was achieved, the metaanalysis and metaregression were still based on a small number of studies and the results are subject to random errors and potential bias. Second, although no publication bias was noted statistically, there is no single perfect method to detect publication bias. Third, the results were subject to aggregation bias because the analysis was not based on subjects' individual data[28]. Fourth, the hypothesis that the endothelium but not the vascular musculature is preferentially affected in SLE during the early stage of atherogenesis is based purely on statistical grounds in this study. Physiological studies are undoubtedly required to confirm this hypothesis. Finally, while we demonstrated that patients with SLE had impaired endothelial function, the results cannot be extrapolated to conclude that they have higher CV risk because studies involving patients with clinical atherosclerosis were excluded. Further prospective studies involving serial FMD measurements in patients naive for vascular events and observation of development of CV events among these patients are required.

Endothelium-dependent FMD, but not endothelium-independent FMD, is impaired in patients with SLE. Increasing age and disease duration might reduce the potential advantage of FMD as a biophysical marker of endothelial dysfunction. Further studies are needed to address whether prospective measurement of FMD with modification of risk factors or even treatment at the stage of endothelial dysfunction is potentially applicable in lupus patients for retarding the development of clinical cardiovascular events.

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