A meta-analysis of published studies of endothelial dysfunction does not support its routine clinical use

A. Cardona,[1,2]* S. R. Kondapally Seshasai,[1]* J. Davey,[1] A. L. Arrebola-Moreno,[1,3] G. Ambrosio,[2] J. C. Kaski,[1]† K. K. Ray[1]†

Linked Comment: Ferguson and Sattar. *Int J Clin Pract* 2015; 69: 624–5.

SUMMARY

Background: Endothelial dysfunction is a marker of future cardiovascular disease (CVD) risk, yet epidemiological studies have yielded inconsistent results. We therefore studied the association between endothelial dysfunction and CVD under diverse circumstances. **Methods and results:** Literature-based meta-analysis of prospective observational studies with ≥ 12 months of follow-up published in Medline and having information on endothelial function and CVD outcomes. Tabular data on participant characteristics, endothelial function assessments and incident CVD outcomes were abstracted from individual studies. Random-effects meta-analysis was used to quantify pooled associations, and I^2 statistic to evaluate between-study heterogeneity. Potential sources of heterogeneity were explored by subgroup analyses and meta-regression. Thirty five studies involving 17,206 participants met the inclusion criteria. During more than 80,000 person-years of observation, up to 2755 CVD events were accrued, yielding a pooled relative risk (RR) of 1.25 (95% confidence interval 1.15–1.35) for CVD comparing top (i.e. more severe) vs. bottom (less severe) third of endothelial dysfunction. There was significant between-study heterogeneity and evidence of publication bias. RRs varied importantly according to the method used to ascertain endothelial function, and were higher among older individuals and among participants with risk factors for CVD or established CVD at baseline. **Conclusions:** Although endothelial dysfunction is an important determinant of cardiovascular outcomes in people with pre-existing CVD, current evidence base does not support its use as a potentially useful measurement for risk stratification in people at lower risk of CVD.

Review criteria

Endothelial dysfunction has been proposed as a marker of incident cardiovascular disease (CVD) outcomes, and endothelial function assessments have been considered to represent a useful tool in the evaluation of cardiovascular risk among various individuals. However, epidemiological studies evaluating this association have yielded inconsistent results to date.

Message for the clinic

By combining published information on over 17,000 individuals from 35 prospective studies, we found that people within the highest category of endothelial dysfunction had just 25% excess risk of CVD outcomes, as compared with those within the lowest category. Therefore, although endothelial dysfunction may be an important determinant of vascular disease outcomes in people with pre-existing CVD, current evidence does not support its use as a potentially useful measurement for risk stratification in people at lower risk of CVD.

[1]Cardiovascular and Cell Sciences Research Institute, St. George's, University of London, London, UK
[2]Division of Cardiology, University of Perugia School of Medicine, Perugia, Italy
[3]Division of Cardiology, University Hospital Virgen de Las Nieve, Granada, Spain

Correspondence to:
Juan Carlos Kaski,
Cardiovascular and Cell Sciences Research Institute, St. George's, University of London, London, UK
Tel.: + 442087252628
Fax: + 442087253416
Email: jkaski@sgul.ac.uk

*Joint first authors.

†Senior authors.

Disclosure
None.

Introduction

Endothelial dysfunction represents an early step in the development of atherosclerosis and has been proposed as a marker of cardiovascular disease (CVD) outcomes (1). However, its association with CVD has not been reliably quantified under diverse circumstances (2–4). As reviewed recently, the clinical usefulness of endothelial function assessment is not yet firmly established, nor is any specific method for measuring endothelial dysfunction recommended in guidelines for primary or secondary prevention (4). In this respect, interpretation of the findings from individual studies can be problematic because of differences in: baseline prevalence of CVD and associated risk factors; the extent and severity of underlying ischaemic heart disease and diagnostic techniques used to assess endothelial (dys)function.

A previous meta-analysis of 23 cohort studies reported a significant association between endothelial dysfunction assessed using brachial artery flow-mediated dilatation (FMD) and CVD outcomes (5). However, it was importantly limited because: several contributing studies had combined all-cause mortality along with CVD in their primary end-point resulting in potentially misleading estimates of association; results of the meta-analysis were based solely on FMD measurements precluding any comparisons with other techniques; and, the associations were calculated separately for studies reporting continuous vs. categorical risk estimates, i.e. without any attempts to standardise the risk ratios (RRs). We therefore sought to quantify more precisely than previously possible, the association between endothelial dysfunction and the composite end-point of all CVD outcomes under diverse circumstances, by analysing

data from 35 prospective studies including over 17,000 participants with up to 2755 CVD events (2,3,6–40). Studies included in our meta-analysis were based on five distinct population subtypes: essentially general populations (involving participants not selected on the basis of pre-existing CVD risk factors or baseline CVD); individuals at moderate-to-high risk of CVD; individuals with suspected coronary heart disease (CHD), defined as those undergoing diagnostic cardiac catheterisation for suspected coronary artery disease (CAD) or those with a positive exercise stress test; participants with established CHD (defined as a previous diagnosis of any CAD according to standard criteria (41,42)); and, patients with known congestive heart failure (CHF).

Methods

We searched MEDLINE for prospective studies published before 1 May 2013 that had reported on the association between endothelial function and incident CVD outcomes using keywords and MeSH terms related to endothelium and CHD/CAD/CVD, without restriction to any language. Electronic searches were supplemented by scanning reference lists of published reports. For our meta-analysis, endothelial dysfunction was defined according to study-specific criteria, and studies were included if they had at least 12 months of follow-up and had reported on incident CVD outcomes. CVD was defined as a composite of CHD (fatal or non-fatal myocardial infarction and/or unstable angina); coronary, peripheral or carotid revascularisation; heart failure; stroke; transient ischaemic attack and cardiovascular death. With the exception of heart failure definition which varied importantly across studies, other CVD outcomes were generally defined according to standard criteria, and therefore we conducted further sensitivity analyses by excluding heart failure from the composite CVD end-point, where possible.

Data were extracted independently by two authors (AC, AAM) using a standardised data extraction protocol (Table S1), and potential discrepancies resolved by two additional authors (KKR, SRKS). Where published data were insufficient, investigators were contacted for additional information (9,10,13,14,18,19,21,24,25,37). Studies that enrolled participants following heart transplantation (43), those that used blood-based biomarkers alone to assess endothelial function (44,45), or those that did not record any CVD outcomes and/or failed to provide any risk estimates for CVD were excluded from our meta-analysis (46–48).

The following details were abstracted from individual studies: geographical location, year of publication, population characteristics, sample size, year of baseline enrolment, mean age of participants at baseline, percentage men, mean duration of follow-up and number of CVD events on follow-up. Further details were abstracted regarding the method used to ascertain endothelial dysfunction, including information regarding technique, approach (invasive vs. non-invasive) and vascular bed examined (coronary vs. peripheral). In case of multiple publications, the most up-to-date or comprehensive report was used for data abstraction. Where a single study reported separately on multiple subgroups of the same population, data from the largest group of participants were used for the main analysis with smaller subgroups contributing to stratum-specific estimates of association. Where investigators published data on multiple methods of endothelial function assessment separately, we used information from the report involving the largest number of participants in our main analysis, using data on other methods in subsidiary analyses. As the estimates of association with CVD were reported differently across studies (e.g. RRs comparing the top vs. bottom third of endothelial dysfunction in some studies as compared with RRs per unit decrease in endothelial function in others), we calculated standardised effect estimates for CVD across studies by comparing top vs. bottom third of endothelial dysfunction (i.e. more vs. less severe derangement in endothelial function).

Our primary analysis involved quantifying the association between endothelial dysfunction and CVD by combining study-specific RRs using random-effects meta-analysis (fixed-effect models were used for comparison). Where study-specific RRs were unavailable, we derived the corresponding risk estimates from available data (such as p-values) and by using *riskconv* programme in Stata, or by contacting individual study investigators for further information. For this meta-analysis we assumed that RRs, hazard ratios and odds ratios approximated the same relative risk. As the degree of multivariable adjustments varied between studies, we used RRs from maximally adjusted models for our meta-analysis, where possible. The extent to which the effect estimates varied across studies beyond that attributable to chance (heterogeneity) was quantified using the I^2 statistic. Potential sources of heterogeneity were investigated by subgroup analyses, and by using more stringent p-value thresholds (< 0.01) for statistical significance. Potential publication bias was assessed both visually (funnel plots) and quantitatively (Egger test). All analyses were performed using STATA release 11 software (Stata Corp., College Station, Texas, USA).

Our study was not supported by any bespoke funding, and no separate ethical approval was sought

as it involved re-analysis of previously published data. JCK and KKR take full responsibility for the accuracy of the study results, and the decision to submit it for publication.

Results

The initial Medline search yielded 1927 titles of relevance, and a further 51 titles were identified by screening the reference lists of these reports. After excluding duplicate publications, we studied 1799 abstracts and 79 full text articles for eligibility, yielding 35 studies (from 37 published reports) and involving 17,206 individuals for final quantitative data synthesis (Figure 1). Thirty-three of these were prospective cohort studies (2,3,7,9–19,21–40) and three were case-cohort studies (6,8,20). Four studies (N = 7706) included general populations (3,25,30,39); 12 (N = 2035) included individuals with suspected CHD (2,12,17,18,21,23,26,28,29,31,33,34); six studies (N = 4223) involved participants having cardiovascular risk factors (7,9,22,24,32,38); eight studies (N = 1367) included people with established CHD at baseline (8,10,13,14,16,19,20,37); four studies (N = 922) recruited individuals with CHF (6,11,15,35); two studies (27,36) included participants

having either suspected or established CHD (N = 370) and 1 study (40) involved participants having either cardiovascular risk factors or established CHD. Mean (SD) age at study entry for all participants combined was 60 (6) years, and 64% were male. Average follow-up was 48 (27) months for all participants combined (> 80,000 person-years in total) during which 2755 CVD events were accrued (Table 1). FMD was the most common technique used to assess endothelial function (25 studies), followed by quantitative coronary angiography (QCA, five studies), and venous occlusion plethysmography (VOP, four studies). Positron emission tomography (PET), intravascular Doppler (IDOP) and peripheral arterial tonometry (PAT) were used in one study each.

The overall unstandardised RR for the association between endothelial dysfunction and all CVD events combined was 1.13 (95% CI: 1.09–1.18), with statistically significant heterogeneity between studies (I^2 = 71.1%, p < 0.001) (Figure S1). By comparison, the pooled RR in 27 studies (13,122 individuals, 1719 CVD events) in which risk estimates could be standardised was 1.25 (1.15–1.35; I^2 = 71.5%; p < 0.001) for individuals within the top vs. bottom third of the endothelial dysfunction category (i.e. more vs. less severe disease) (Figures 2 and S2). The
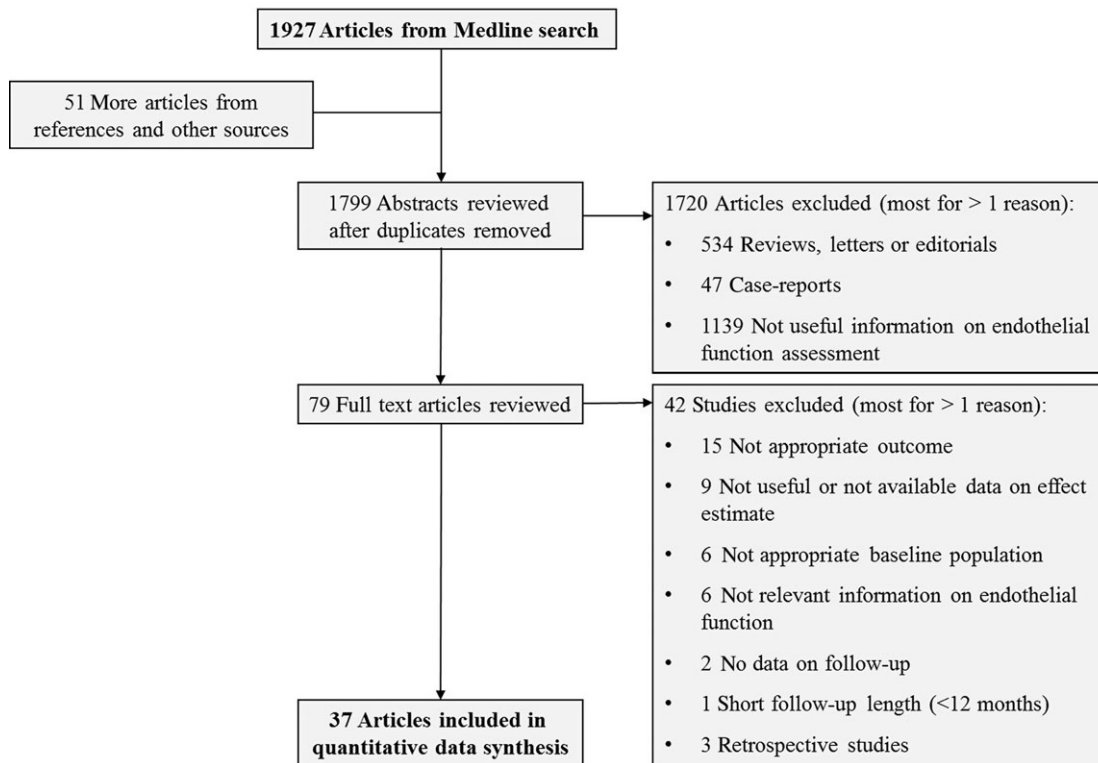

Details of literature review

Figure 1 Flow diagram of study selection

Table 1 Details of studies included in this meta-analysis

Author, year of publication	Location	Population	No. of participants	No. of CVD events	Mean age (years)	Male (%)	Follow-up (months)	Technique	Approach	Site	Stimulus
Akiyama et al. (2012)	Japan	CHF	321	59	72	50	20	PAT	Non-invasive	Peripheral	CI
Anderson et al. (2011)	Canada	Gen Pop	1574	75	49.4	100	86.4	FMD	Non-invasive	Peripheral	CI
Asselbergs et al. (2004)	The Netherlands	Suspected CHD	277	24	57.3	61.0	47.0	QCA	Invasive	Coronary	ACh
Brevetti et al. (2003)*	Italy	CVD risk	131	39	63.5	90.0	22.7	FMD	Non-invasive	Peripheral	CI
Careri et al. (2013)	Italy	Estd CHD	60	14	62	73	32 (median)	FMD	Non-invasive	Peripheral	CI
Corrado et al. (2008)	Italy	CVD risk	84	24	61.9	77.0	24.0	FMD	Non-invasive	Peripheral	CI
Fichtlscherer et al. (2004)	Germany	Estd CHD	198	31	55.3	80.0	47.7	VOP	Invasive	Peripheral	ACh
Fischer et al. (2005)*	Germany	CHF	67	24	60.7	82.0	45.7	FMD	Non-invasive	Peripheral	CI
Frick et al. (2005)	Austria	Suspected CHD	398	41	54	100	39.0	FMD	Non-invasive	Peripheral	CI
Gokce et al. (2003)	USA	Estd CHD	199	35	65.9	76.8	14.4	FMD	Non-invasive	Peripheral	CI
Guazzi et al. (2009)	Italy	Estd CHD	179	44	64.8	61.4	13.7	FMD	Non-invasive	Peripheral	CI
Heitzer et al. (2005)*	Germany	CHF	289	79	60.6	61.0	58.0	VOP	Invasive	Peripheral	ACh
Heitzer et al. (2001)*	Germany	Estd CHD	281	120	60	80.0	53.0	VOP	Invasive	Peripheral	ACh
Hu et al. (2008)*	China	Suspected CHD	279	36	62	58.0	16 (median)	FMD	Non-invasive	Peripheral	CI
Huang et al. (2007)*	Taiwan	Suspected CHD	205	29	63	69.0	24.0	FMD	Non-invasive	Peripheral	CI
Karatzis et al. (2006)	Greece	Estd CHD	98	45	63.1	100	24.8	FMD	Non-invasive	Peripheral	CI
Kitta et al. (2009)	Japan	Estd CHD	251	42	66.8	58.1	31.0	FMD	Non-invasive	Peripheral	CI
von Mering et al. (2004)	USA	Suspected CHD	163	58	55.6	0.0	48.0	QCA	Invasive	Coronary	ACh
Muiesan et al. (2008)	Italy	CVD risk	172	20	55.7	59.3	95.0	FMD	Non-invasive	Peripheral	CI
Neunteufl et al. (2000)*	Austria	Suspected CHD	74	38	51.2	52.0	60.0	FMD	Non-invasive	Peripheral	CI
Perticone et al. (2001)	Italy	CVD risk	225	29	46.4	47.1	31.5	VOP	Invasive	Peripheral	ACh
Rossi et al. (2008)	Italy	Gen Pop	2264	90	55	0.0	45.0	FMD	Non-invasive	Peripheral	CI
Sanchez et al. (2009)	Spain	Suspected CHD	147	33	58	42.0	84.0	QCA	Invasive	Coronary	ACh
Schachinger et al. (2000)	Germany	Suspected or estd CHD	147	28	54	69	80.4	QCA	Invasive	Coronary	ACh/CPT
Schindler et al. (2003)	Switzerland	Suspected CHD	130	37	59	70.0	45.0	QCA	Invasive	Coronary	CPT
Schindler et al. (2005)	Switzerland	Suspected CHD	72	25	58	61.0	66.0	PET	Non-invasive	Coronary	CPT
Shimbo et al. (2007)	USA	Gen Pop	842	30	66.6	42.4	36.0	FMD	Non-invasive	Peripheral	CI
Suessenbacher et al. (2013)	Austria	Suspected CHD	396	216	54	100	141	FMD	Non-invasive	Peripheral	CI
Suzuki et al. (2008)	USA	CVD risk	819	84	66.5	43	81	FMD	Non-invasive	Peripheral	CI
Takase et al. (2008)	Japan	Suspected CHD	103	15	62	76.6	50.0	FMD	Non-invasive	Peripheral	CI
Takase et al. (2006)	Japan	Suspected CHD	70	11	60	67.1	53.0	IDOP	Invasive	Peripheral-coronary	ACh

Table 1 Continued

Author, year of publication	Location	Population	No. of participants	No. of CVD events	Mean age (years)	Male (%)	Follow-up (months)	Technique	Approach	Site	Stimulus
Takishima et al. (2012)*	Japan	CHF	245	33	66	68.5	33	FMD	Non-invasive	Peripheral	CI
Ulriksen et al. (2009)	Denmark	Suspected or estd CHD	223	90	54	76	50	FMD	Non-invasive	Peripheral	CI
Wang et al. (2009)	China	Estd CHD	101	29	62.4	66.3	12.1	FMD	Non-invasive	Peripheral	CI
Yeboah et al. (2007)*	USA	CVD risk	2792	674	78.6	41.4	60.0	FMD	Non-invasive	Peripheral	CI
Yeboah et al. (2009)	USA	Gen Pop	3026	365	61.2	49.8	60.0	FMD	Non-invasive	Peripheral	CI
Yilmaz et al. (2011)	Turkey	CVD risk or estd CHD	304	89	46	52	41	FMD	Non-invasive	Peripheral	CI

*Studies where it was not possible to standardise the effect estimate provided. Abbreviations as in figure legends (Estd, established). CVD was a composite of fatal or non-fatal myocardial infarction, unstable angina, coronary, peripheral or carotid revascularisation, heart failure, stroke, transient ischaemic attack and cardiovascular death.

association was more conservative in fixed-effect meta-analysis (RR 1.06, 95% CI 1.04–1.08) (Figure S3). In analyses restricted to studies that did not include heart failure in their composite CVD end-point (14 studies (2,8,10,19,24,25,27,28,31–33,36,39,40), 8270 participants, 1157 CVD events), the pooled RR [1.22 (95% CI 1.09–1.37)] was very similar to the overall risk estimate, with statistically significant heterogeneity between studies (I^2 = 64.8%, $p < 0.001$) (Figure S4).

Relative risks for total CVD varied importantly across studies based on the method used to ascertain endothelial function, the pooled RR being 1.49 (1.24–1.79) for assessments made by FMD, 1.05 (1.01–1.09) for QCA, 1.85 (1.33–2.56) for VOP and 1.25 (0.83–1.88) for other techniques (including IDOP, PAT and PET) (Figure 2). The associations also varied importantly according to whether invasive (1.09, 1.01–1.17) vs. non-invasive (1.42, 1.21–1.67) methods were used to ascertain endothelial dysfunction, and according to whether peripheral (mainly brachial) or central (typically coronary) arteries were studied (RRs 1.49, 1.28–1.74 vs. 1.05, 0.99–1.12; $p < 0.001$). Further sensitivity analyses that involved exclusion of two outlier studies (one that contributed unduly to the random-effects weights (29) and another having unstable effect estimates (34)) only confirmed the differential association between invasive vs. non-invasive methods for endothelial function assessment and CVD. These analyses additionally showed a borderline association between endothelial dysfunction assessed using invasive methods and incident CVD (Figure S5).

Our meta-analysis also demonstrated a stronger association for CVD in studies that accrued fewer than 50 events on follow-up (1.38, 1.21–1.57) as compared with those that recorded more than 50 events (1.24, 1.04–1.48); this difference was, however, not statistically significant (p = 0.42; Figure 3). The associations were less disparate with regard to the overall sample size of studies. Length of follow-up was found to be an important determinant of the associations, such that studies with a follow-up of approximately less than 4 years (median for this meta-analysis) had a summary RR of 1.75 (1.37–2.24), whereas those with a follow-up beyond this period showed weaker association with CVD (1.08, 1.03–1.14; $p < 0.001$; Figure 3). The association between endothelial dysfunction and CVD also varied importantly according to key demographic and participant characteristics. RRs for CVD were generally lower among studies involving participants at a low risk for future CVD, e.g. general populations (1.23, 1.03–1.46) and people suspected (but not proven) to have CHD at study entry (1.11, 1.01–1.22).
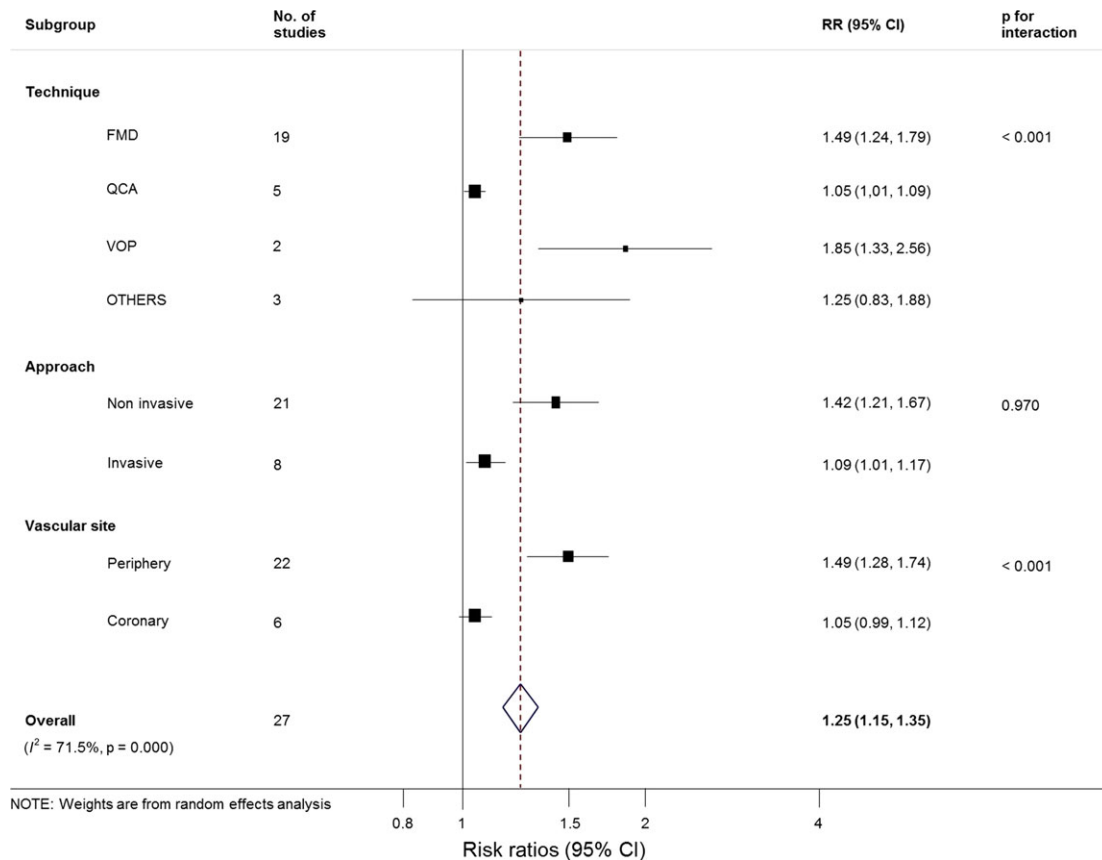
Figure 2 Overall and subgroup-specific associations between endothelial dysfunction and CVD in the standardised dataset*. FMD, flow-mediated dilatation; QCA, quantitative coronary angiography; VOP, venous occlusion plethysmography; PET, positron emission tomography; IDOP, intravascular Doppler. *See text for details

By comparison, the pooled RRs were generally higher within studies that recruited participants with multiple CVD risk factors (2.25, 1.52–3.33), established CHD at baseline (1.87, 1.25–2.78) and CHF (1.35, 1.07–1.71). The study of Schachinger et al. (27) had to be excluded from subgroup analyses as separate estimates for CVD were not available according to whether its participants had suspected or established CVD at baseline (this study did however contribute to the overall analyses). RRs for CVD were also generally higher among studies that involved participants with a higher mean age at entry, and among those that recruited participants after 1999 (p < 0.001). RRs for CVD were importantly different across major geographical regions and according to the stimulus used. There was evidence of publication bias in the associations studied in this meta-analysis (Egger test p < 0.001; Figure S6).

Discussion

By combining evidence across 35 cohort studies involving > 17,000 participants, the current meta-

analysis provides the most robust confirmation yet of the prospective and thereby potentially unbiased association between endothelial dysfunction and CVD. Moreover, by examining this association under diverse circumstances, we were able to provide novel information regarding the clinical relevance of endothelial dysfunction as a marker of incident CVD.

Our systematic approach helped uncover important, yet hitherto unsuspected, limitations of endothelial dysfunction as a marker of incident CVD outcomes, thereby precluding any firm conclusions regarding its role in risk stratification of CVD. First, the methods used to ascertain endothelial dysfunction varied importantly across studies. For instance, our subgroup analyses demonstrate that the association with CVD was stronger for studies that used noninvasive and more peripheral circulation-based tests (as compared with invasive, coronary-based tests). Although differences in population characteristics may explain the underlying heterogeneity to some extent it is possible, however, that the observed associations were in fact genuine. From a technical perspective it is worth emphasising that FMD-based
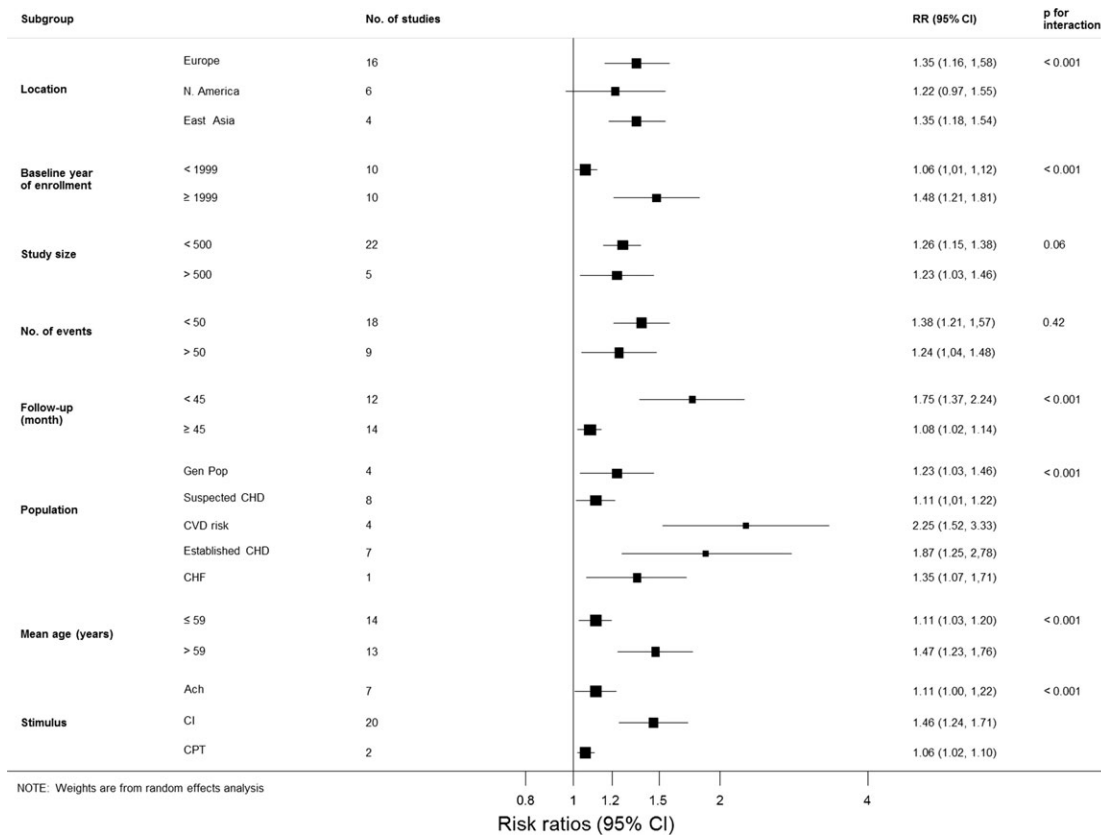
Figure 3 Association between endothelial dysfunction and CVD under diverse circumstances. Risk ratios are shown for the association between top vs. bottom third of endothelial dysfunction and CVD. Gen Pop, general populations; CVD risk, subjects with moderate-to-high levels of CVD risk factors; CI, cuff inflation; Ach, Acetylcholine; CPT, cold pressor test

methods, the most widely used techniques in the last decade, are potentially limited by issues related to reproducibility and variability (49). Hence, further studies are needed not only to clarify the role of individual measures of endothelial dysfunction in relation to CVD outcomes but also to identify methods of endothelial function assessment that are best suited to identify groups of at-risk individuals.

Second, our data also suggest significant publication bias and thereby likely overestimation of the observed associations, with important practical implications for the use of endothelial function assessments in asymptomatic individuals. In fact, our findings of just modest associations with CVD in the subgroup of otherwise healthy participants tend to discourage the widespread use of endothelial function assessments until further evidence becomes available regarding their role in low-risk populations. Third, in addition to this exaggerated association with CVD, we also observed some spurious associations in this meta-analysis. For instance, we found important differences between the baseline year of enrolment and CVD risk, which is unlikely to be a true calendar

effect given that methods used for endothelial function assessments changed considerably with time (FMD-based methods became more widespread after 1999). Fourth, we did not have access to individual-participant data to enable consistent adjustments for confounding factors across studies, nor did we have information regarding serial measurements of endothelial function to enable adjustments for time-varying effects of endothelial function. Finally, although our meta-analysis is the largest to date, information from certain key studies was unavailable from published literature, and could not be provided by their investigators upon request (50–52).

In addition to the abovementioned data-dependent limitations, one of the major conceptual limitations of using endothelial function as a marker of CVD risk relates to the fact that it is uncertain to what extent physiological changes in peripheral arteries reliably reflect abnormalities in more central arteries (coronary or cerebral), as the latter are more directly relevant to clinical CVD events (53). To date, only small-scale investigations have studied this relationship, yielding inconsistent findings (54,55).

Moreover, as simultaneous measurements of endothelial function using multiple techniques in a common subset of participants were generally unavailable, any observed differences in RRs by technique may be attributable to between-study differences rather than true differences in association.

Despite the suggestions by several advocates that endothelial dysfunction is here for prime time use, the totality of the data we present suggests that publication bias and incomplete adjustment of confounders (especially in small studies) may have over inflated its potential clinical utility (4). Furthermore, the modest association observed with CVD among low risk groups suggests that endothelial function assessment is unlikely to alter clinical decision making among them. On the other hand, in people with established CVD, where a stronger association between endothelial dysfunction and CVD was observed, patients are already more likely to be on secondary prevention strategies and therefore, on balance, it appears that there may be more value in considering other emerging modalities for CVD risk prediction. For instance, the Rotterdam study showed that coronary artery calcium (CAC) score outperformed measurements of carotid intima-media thickness (CIMT) and blood-based biomarkers as a predictor of CHD (net reclassification index 19.3% vs. 7.6%, respectively, for CAC vs. biomarkers) (56). In the Framingham Offspring Study, when added to the Framingham risk score, CAC score improved NRI by 46% vs. 6% for CIMT and 2.3% for FMD. Furthermore, current meta-analyses of CAC score suggest improvement in reclassification by over 20% vs. < 1% for CIMT (57,58). To date, large scale data on arterial stiffness and CVD events are not well-established and given the consistent association between CAC score and CVD and its reproducibility compared with other tests, it is unlikely that any other measure of CVD risk currently available will outperform CAC score (59).

Notwithstanding these limitations, the demonstration of a graded association between endothelial dysfunction and CVD risk among people having vastly different vascular disease risk profiles at baseline and the influence of subjects' age on the strength of association with CVD are consistent with the notion of a continuum in the atherosclerosis process (60). In conclusion, although endothelial dysfunction is an important determinant of cardiovascular outcomes in people with pre-existing CVD, current evidence base does not support its use as a potentially useful measurement for risk stratification in people at lower risk for CVD.

Author contributions

Kaski, Ambrosio, Ray: study design; Cardona, Arrebola Moreno, Seshasai: data abstraction; Davey: statistical analyses; Cardona, Seshasai, Ambrosio, Kaski, Ray: manuscript preparation and revisions. All authors were involved in the interpretation of results. Professors Kaski and Ray take full responsibility for the overall content of this manuscript.

References

1 Quyyumi AA. Prognostic value of endothelial function. *Am J Cardiol* 2003; **91**: 19H–24H.

2 Asselbergs FW, Monnink SH, Jessurun GA et al. Assessing the prognostic value of coronary endothelial function in patients referred for a first coronary angiogram. *Am J Cardiol* 2004; **94**: 1063–7.

3 Anderson TJ, Charbonneau F, Title LM et al. Microvascular function predicts cardiovascular events in primary prevention: long-term results from the firefighters and their endothelium (fate) study. *Circulation* 2011; **123**: 163–9.

4 Flammer AJ, Anderson T, Celermajer DS et al. The assessment of endothelial function: from research into clinical practice. *Circulation* 2012; **126**: 753–67.

5 Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *Int J Cardiol* 2013; **168**: 344–51.

6 Akiyama E, Sugiyama S, Matsuzawa Y et al. Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction. *J Am Coll Cardiol* 2012; **60**: 1778–86.

7 Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. *Circulation* 2003; **108**: 2093–8.

8 Careri G, Nerla R, di Monaco A et al. Clinical correlates and prognostic value of flow mediated dilation in patients with non-ST segment elevation acute coronary syndromes. *Am J Cardiol* 2013; **111**: 51–7.

9 Corrado E, Rizzo M, Coppola G, Muratori I, Carella M, Novo S. Endothelial dysfunction and carotid lesions are strong predictors of clinical events in patients with early stages of atherosclerosis: a 24-month follow-up study. *Coron Artery Dis* 2008; **19**: 139–44.

10 Fichtlscherer S, Breuer S, Zeiher AM. Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the "vulnerable" patient. *Circulation* 2004; **110**: 1926–32.

11 Fischer D, Rossa S, Landmesser U et al. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. *Eur Heart J* 2005; **26**: 65–9.

12 Frick M, Suessenbacher A, Alber HF et al. Prognostic value of brachial artery endothelial function and wall thickness. *J Am Coll Cardiol* 2005; **46**: 1006–10.

13 Gokce N, Keaney JF Jr, Hunter LM et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* 2003; **41**: 1769–75.

14 Guazzi M, Reina G, Gripari P, Tumminello G, Vicenzi M, Arena R. Prognostic value of flow-mediated dilatation following myocardial infarction. *Int J Cardiol* 2009; **132**: 45–50.

15 Heitzer T, Baldus S, von Kodolitsch Y, Rudolph V, Meinertz T. Systemic endothelial dysfunction as an early predictor of adverse outcome in heart failure. *Arterioscler Thromb Vasc Biol* 2005; **25**: 1174–9.

16 Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001; **104**: 2673–8.

17 Hu R, Wang WQ, Lau CP, Tse HF. Gender differences on brachial flow-mediated dilation and carotid intima-media thickness for prediction of spontaneous cardiovascular events. *Clin Cardiol* 2008; **31**: 525–30.

18 Huang PH, Chen JW, Lu TM, Yu-An Ding P, Lin SJ. Combined use of endothelial function assessed by brachial ultrasound and high-sensitive C-reactive protein in predicting cardiovascular events. *Clin Cardiol* 2007; **30**: 135–40.

19 Karatzis EN, Ikonomidis I, Vamvakou GD et al. Long-term prognostic role of flow-mediated dilatation of the brachial artery after acute coronary syndromes without st elevation. *Am J Cardiol* 2006; **98**: 1424–8.

20 Kitta Y, Obata JE, Nakamura T et al. Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease. *J Am Coll Cardiol* 2009; **53**: 323–30.

21 von Mering GO, Arant CB, Wessel TR et al. Abnormal coronary vasomotion as a prognostic indicator of cardiovascular events in women: results from the national heart, lung, and blood institute-sponsored women's ischemia syndrome evaluation (wise). *Circulation* 2004; **109**: 722–5.

22 Muiesan ML, Salvetti M, Paini A et al. Prognostic role of flow-mediated dilatation of the brachial artery in hypertensive patients. *J Hypertens* 2008; **26**: 1612–8.

23 Neunteufl T, Heher S, Katzenschlager R et al. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *Am J Cardiol* 2000; **86**: 207–10.

24 Perticone F, Ceravolo R, Pujia A et al. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001; **104**: 191–6.

25 Rossi R, Nuzzo A, Origliani G, Modena MG. Prognostic role of flow-mediated dilation and cardiac risk factors in post-menopausal women. *J Am Coll Cardiol* 2008; **51**: 997–1002.

26 Sanchez-Recalde A, Galeote G, Moreno R et al. Long-term prognostic value of endothelial dysfunction in patients with chest pain and angiographically normal coronary arteries. *Rev Port Cardiol* 2009; **28**: 785–91.

27 Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000; **101**: 1899–906.

28 Schindler TH, Hornig B, Buser PT et al. Prognostic value of abnormal vasoreactivity of epicardial coronary arteries to sympathetic stimulation in patients with normal coronary angiograms. *Arterioscler Thromb Vasc Biol* 2003; **23**: 495–501.

29 Schindler TH, Nitzsche EU, Schelbert HR et al. Positron emission tomography-measured abnormal responses of myocardial blood flow to sympathetic stimulation are associated with the risk of developing cardiovascular events. *J Am Coll Cardiol* 2005; **45**: 1505–12.

30 Shimbo D, Grahame-Clarke C, Miyake Y et al. The association between endothelial dysfunction and cardiovascular outcomes in a population-based multi-ethnic cohort. *Atherosclerosis* 2007; **192**: 197–203.

31 Suessenbacher A, Dorler J, Wunder J et al. Comparison of brachial artery wall thickness versus endothelial function to predict late cardiovascular events in patients undergoing elective coronary angiography. *Am J Cardiol* 2013; **111**: 671–5.

32 Suzuki T, Hirata K, Elkind MS et al. Metabolic syndrome, endothelial dysfunction, and risk of cardiovascular events: the northern manhattan study (nomas). *Am Heart J* 2008; **156**: 405–10.

33 Takase B, Matsushima Y, Uehata A, Ishihara M, Kurita A. Endothelial dysfunction, carotid artery plaque burden, and conventional exercise-induced myocardial ischemia as predictors of coronary artery disease prognosis. *Cardiovasc Ultrasound* 2008; **6**: 61.

34 Takase B, Hamabe A, Satomura K et al. Comparable prognostic value of vasodilator response to acetylcholine in brachial and coronary arteries for predicting long-term cardiovascular events in suspected coronary artery disease. *Circ J* 2006; **70**: 49–56.

35 Takishima I, Nakamura T, Hirano M et al. Predictive value of serial assessment of endothelial function in chronic heart failure. *Int J Cardiol* 2012; **158**: 417–22.

36 Ulriksen LS, Malmqvist BB, Hansen A, Friberg J, Jensen GB. Flow-mediated dilatation has no independent prognostic effect in patients with chest pain with or without ischaemic heart disease. *Scand J Clin Lab Invest* 2009; **69**: 475–80.

37 Wang X, Guo F, Li G, Cao Y, Fu H. Prognostic role of brachial reactivity in patients with st myocardial infarction after percutaneous coronary intervention. *Coron Artery Dis* 2009; **20**: 467–72.

38 Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the cardiovascular health study. *Circulation* 2007; **115**: 2390–7.

39 Yeboah J, Folsom AR, Burke GL et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation* 2009; **120**: 502–9.

40 Yilmaz MI, Stenvinkel P, Sonmez A et al. Vascular health, systemic inflammation and progressive reduction in kidney function; clinical determinants and impact on cardiovascular outcomes. *Nephrol Dial Transplant* 2011; **26**: 3537–43.

41 Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the world health organization monica project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation* 1994; **90**: 583–612.

42 The World Health Organization Monica Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO Monica Project Principal Investigators. *J Clin Epidemiol* 1988; **41**: 105–14.

43 Hollenberg SM, Klein LW, Parrillo JE et al. Coronary endothelial dysfunction after heart transplantation predicts allograft vasculopathy and cardiac death. *Circulation* 2001; **104**: 3091–6.

44 Chong AY, Freestone B, Patel J et al. Endothelial activation, dysfunction, and damage in congestive heart failure and the relation to brain natriuretic peptide and outcomes. *Am J Cardiol* 2006; **97**: 671–5.

45 Mallamaci F, Tripepi G, Cutrupi S, Malatino LS, Zoccali C. Prognostic value of combined use of biomarkers of inflammation, endothelial dysfunction, and myocardiopathy in patients with ESRD. *Kidney Int* 2005; **67**: 2330–7.

46 Shechter M, Marai I, Marai S et al. The association of endothelial dysfunction and cardiovascular events in healthy subjects and patients with cardiovascular disease. *Isr Med Assoc J* 2007; **9**: 271–6.

47 Meyer B, Mortl D, Strecker K et al. Flow-mediated vasodilation predicts outcome in patients with chronic heart failure: comparison with B-type natriuretic peptide. *J Am Coll Cardiol* 2005; **46**: 1011–8.

48 Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. *J Am Coll Cardiol* 2003; **42**: 1037–43.

49 Ghiadoni L, Faita F, Salvetti M et al. Assessment of flow-mediated dilation reproducibility: a nationwide multicenter study. *J Hypertens* 2012; **30**: 1399–405.

50 Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term followup of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000; **101**: 948–54.

51 Bugiardini R, Manfrini O, Pizzi C, Fontana F, Morgagni G. Endothelial function predicts future development of coronary artery disease: a study of women with chest pain and normal coronary angiograms. *Circulation* 2004; **109**: 2518–23.

52 Halcox JP, Schenke WH, Zalos G et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002; **106**: 653–8.

53 Cardona A, Ambrosio G, Mengoni A et al. Pulse pressure affects the relationship between flow-mediated dilatation and cardiovascular risk. *Int J Cardiol* 2014; **175**(3): 581–4.

54 Anderson TJ, Uehata A, Gerhard MD et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995; **26**: 1235–41.

55 Matsuo S, Matsumoto T, Takashima H et al. The relationship between flow-mediated brachial artery vasodilation and coronary vasomotor responses to bradykinin: comparison with those to acetylcholine. *J Cardiovasc Pharmacol* 2004; **44**: 164–70.

56 Kavousi M, Elias-Smale S, Rutten JH et al. Evaluation of newer risk markers for coronary heart disease risk classification: a cohort study. *Ann Intern Med* 2012; **156**(6): 438–44.

57 Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. *Heart* 2012; **98**(3): 177–84.

58 den Ruijter HM, Peters SA, Anderson TJ et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. *JAMA* 2012; **308**(8): 796–803.

59 Ben-Shlomo Y, Spears M, Boustred C et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol* 2014; **63**(7): 636–46

60 Sitia S, Tomasoni L, Atzeni F et al. From endothelial dysfunction to atherosclerosis. *Autoimmun Rev* 2010; **9**: 830–4.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Overall unstandardised risk ratios for the association between endothelial dysfunction and CVD.

Figure S2. Overall standardised risk ratios for the association between (top vs. bottom third of) endothelial dysfunction and CVD.

Figure S3. Standardised risk ratios for the association between top vs. bottom third of endothelial dysfunction and CVD using fixed-effect model meta-analysis.

Figure S4. Standardised risk ratios for the association between top vs. bottom third of endothelial dysfunction and CVD in studies that did not include heart failure in their composite CVD end-point.

Figure S5. Association between top vs. bottom third of endothelial dysfunction and CVD based on the approach used for endothelial function assessment, after excluding the studies by Shindler and Takase (see text for details).

Figure S6. Funnel plot for the assessment of publication bias.

Table S1. PRISMA-based data extraction protocol for the meta-analysis.