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Early View

Review

Endothelial dysfunction in chronic obstructive pulmonary disease: a systematic review and meta-analysis of studies using different functional assessment methods

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TITLE PAGE

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COPD is significantly associated with endothelial dysfunction of both conduit vessels and microvasculature. This association is further strengthened when patients with COPD are compared to non-smoking controls.

ABSTRACT

Background: Cardiovascular disease is a major cause of morbidity and mortality in chronic obstructive pulmonary disease (COPD). Endothelial dysfunction is suggested to be one of the pathogenetic mechanisms involved. This is a systematic review and meta-analysis of studies using any available functional method to examine differences in endothelial function between patients with COPD and individuals without COPD (controls).

Methods: Literature search involved PubMed and Scopus databases. Eligible studies included adult patients and evaluated endothelial damage via functional methods. Newcastle-Ottawa Scale was applied to evaluate the quality of retrieved studies. Subgroup analyses were performed to explore heterogeneity across the studies. Funnel-plots were constructed to evaluate publication bias.

Results: Of the 21 initially identified reports, 19 studies with a total of 968 participants were included in the final meta-analysis. A significantly impaired response in endothelium-dependent (weighted mean between-group difference, WMD: -2.59%, 95%CI [-3.75, -1.42]) and –independent vasodilation (WMD: -3.13, 95%CI [-5.18, -1.09]) was observed in patients with COPD compared to controls. When pooling all studies together, regardless of the technique used for assessment of vascular reactivity, pronounced endothelial dysfunction was observed in COPD compared to controls (standardised-mean-difference, SMD: -1.19, 95%CI [-1.69, -0.68]). Subgroup analysis showed that the difference was larger when patients with COPD were compared with non-smoking controls (SMD: -1.75, 95%CI [-2.58, -0.92]. Sensitivity analyses confirmed the above results.

Conclusions: Patients with COPD have significantly impaired endothelial function compared to controls without COPD. Future studies should delineate the importance of endothelial dysfunction towards development of cardiovascular disease in COPD.

Keywords: chronic obstructive pulmonary disease, endothelial dysfunction, flow-mediated dilatation, reactive hyperemia index, cardiovascular risk

TEXT

Introduction

Chronic obstructive pulmonary disease (COPD) is chronic inflammatory pulmonary disease characterized by partially reversible airflow obstruction, affecting about 12% of the global population [1]. COPD is a major source of morbidity and mortality; death rates from COPD have been rapidly rising over the last decades, and it is now considered to be the third leading cause of death worldwide [2]. Cardiovascular disease contributes significantly to mortality and disease severity [3]. The degree of airflow obstruction is an independent predictor of adverse cardiovascular outcomes, such as myocardial infarction, stroke, congestive heart failure, and sudden cardiovascular death [4, 5], insinuating a causal relationship between airflow limitation and cardiovascular disease [4].

Although cardiovascular disease and COPD share a major risk factor, that is smoking, and various common systemic manifestations, including diabetes mellitus, hypertension and obesity [6, 7], the underlying mechanisms have not been fully established. Among the latter, chronic systemic inflammation, oxidative stress, chronic hypoxia, arterial stiffness and endothelial dysfunction are proposed to significantly affect the link between these two entities [8]. In fact, endothelial dysfunction is shown not only to contribute to the development of cardiovascular disease in this population, but it is also related to COPD severity [9]. Moreover, ageing could also be another potential link. Vascular endothelial dysfunction occurs during the human aging process and is accompanied by deterioration in the balance between vasodilator and vasoconstriction substances produced by the endothelium; pathophysiologic mechanisms include alterations related to oxidative stress, changes in pro-inflammatory cytokines levels and senescence of endothelial cells [10]. On the other hand, as hallmarks of accelerated ageing and lung cell senescence, including telomere shortening, genomic instability, mitochondrial dysfunction, and stem cell exhaustion, are all observed in various proportions in COPD lungs, “the aging hypothesis for COPD” has been

developed, suggesting that this syndrome, with both respiratory and systemic manifestations, represents a manifestation of accelerated aging [11, 12]. Furthermore, endothelial dysfunction manifested in the pulmonary vessels plays a central role in pulmonary arterial hypertension development [9], a condition that further exacerbates morbidity and mortality in COPD [13].

Endothelial dysfunction, defined as a state of imbalance between endothelium-derived relaxing and contracting factors, is the earliest stage of atherosclerosis [14]. Starting from the very invasive method of the epicardial coronary angiography after intracoronary infusion of vasoactive drugs, several less invasive functional techniques [i.e. venous occlusion plethysmography (VOP), forearm flow-mediated dilatation (FMD), peripheral arterial tonometry (PAT), nailfold capillaroscopy, laser-speckle contrast imaging/analysis (LSCI/LASCA), etc.] and biomarkers [i.e. Asymmetric dimethylarginine (ADMA), endothelial microparticles, inflammation markers, etc.] have been used to evaluate peripheral endothelial function in individuals with high cardiovascular risk [14]. Despite the fact that all these techniques have boosted the research in this field, none of them has been established as a diagnostic tool for cardiovascular events prediction in daily clinical practice so far [15]. In COPD, FMD is the most widespread used functional method for peripheral endothelial function assessment, whereas in the recent years the application of PAT gains more ground due to its non-invasive and operator-independent nature [16].

Previous meta-analyses in the field conducted some years ago demonstrated that patients with COPD had impaired endothelial function compared to controls, and that this decline was proportionally associated with the degree of airway obstruction [17, 18]. Despite their interesting results, these works carried some important methodological errors in the design and execution of the meta-analysis (e.g. double counts and units of analysis errors) and included studies that used only FMD for endothelial function assessment [17, 18]. FMD examines the function of conduit arteries, but does not provide information about microvascular function and hyperemia within the tissue itself (assessed by other methods e.g. PAT, LASCA, NIRS, etc.) [15, 19]. Furthermore,

although FMD is a correlated with coronary endothelial function, it has been suggested that microvascular dysfunction may be an earlier indicator of cardiovascular risk [15]. In the light of the above, we conducted an updated systematic review and meta-analysis of studies using any available functional method to examine differences in endothelial function between patients with COPD and individuals without COPD.

Materials and methods

This systematic review and meta-analysis was conducted in accordance with the Preferred-Reporting-Items-for-Systematic Reviews-and-Meta-Analyses (PRISMA) and the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines (Supplementary Table 1). All research was conducted according to a protocol submitted in PROSPERO database (CRD42021225836).

Search strategy and Eligibility Criteria

A systematic literature search was conducted in PubMed and Scopus databases (from database inception to 25 November 2020), using a combination of free text terms and relevant Medical Subject Headings (MeSH). Keywords and an example of our search strategy used in PubMed are presented in the Supplementary Table 2. Manual checking of reference lists of retrieved articles and reports, including relevant reviews and meta-analyses, was performed to identify additional and potentially relevant articles. Observational studies (cohorts, case-control and cross-sectional studies) assessing endothelial function in patients with COPD compared to controls (healthy individuals or patients with concomitant diseases other than COPD), as well as clinical trials (if a control group was included at baseline and relevant baseline comparisons were available) were considered eligible. All types of semi-invasive and non-invasive functional methods based on Doppler ultrasound, plethysmography, Laser Doppler, Near-infrared spectroscopy or novel, based on optical coherence techniques for assessment of endothelial damage [FMD, nitroglycerine-

mediated dilatation (NMD), PAT, laser-doppler flowmetry (LDF), VOP, LASCA, nailfold capillaroscopy, near-infrared spectroscopy (NIRS), arterial glycocalyx) were included. In our inclusion criteria, we accepted studies evaluating endothelial function of both conduit arteries and microvessels. Preclinical studies, studies with non-adult patients, studies evaluating endothelial dysfunction via serum biomarkers or invasive methods, and studies evaluating endothelial function during acute exacerbation, were excluded. The search strategy was developed with English language restriction.

Study Selection and Data Extraction

Two authors (DB, MT) examined thoroughly titles and abstracts of records retrieved throughout search and then performed independently full text assessment to identify eligible studies, being unblinded to the records' authors and institutions. A data extraction form designed according to the Cochrane checklist of Items, containing fields for all important data on study design, demographics, outcome measurements and details relevant to quality assessment was completed for each eligible study by the two authors (DB, MT). All disagreements on study selection and data collection were solved by a third senior reviewer (MA). In case of missing data, study authors were contacted by e-mail to try to retrieve original data.

Quality assessment tool

Assessment of the quality of the eligible studies was performed by the two reviewers (DB, MT) according to Newcastle-Ottawa-Scale (NOS), a tool developed for quality assessment of non-randomized studies, with a different scale corresponding to every study's design (cohort or case-control studies) [20]. NOS is a 9-point scale that involves the appraisal of methodological issues and their reporting. The scoring system encompasses three major domains (participant selection,

group comparability and ascertainment of exposure); scores range from 0 to 9, with scores ≥7 indicating high quality studies (Supplementary Table 3).

Statistical analyses

For studies assessing endothelial function using the same method, the weighted mean between-group difference (WMD) was calculated with pertinent 95% confidence intervals (95%CI) when data were expressed in the same measurement scale (proportional change from baseline, ml/min per 100ml tissue). When data from different studies corresponding to the same method were expressed in different measurement scales or when pooling all available data from all types of methods of functional evaluation of endothelial damage, the respective standardized-mean-difference (SMD, with 95%CI) was used. For the total of the studies (including all methods of assessment), subgroup analysis was performed based on the basis of sex, presence of coronary artery disease (CAD) and controls' smoking status. Finally, we planned to explore robustness of our findings by means of a sensitivity analysis excluding studies judged as of poor quality (NOS<7). For studies reporting median and range or interquartile range values, we calculated mean and SDs values based on relevant formulas [21]. For studies including multiple comparator groups (e.g. Group 1: Patients with COPD and coronary artery disease [CAD]; Group 2: Patients with COPD without CAD; Group 3: controls with CAD; Group 4: controls without CAD), all relevant groups were combined to create a single pair-wise comparison in order to avoid a unit-of-analysis error [22]. Similarly for subgroup analysis with a shared group (e.g. patients with COPD) and different comparator groups (smokers and non-smokers controls), shared group was divided out approximately evenly among subgroup comparisons [22]. When pooling all available data from the total of studies in order to calculate the SMD, for those studies assessing endothelial function with more than one methods, data reported from exclusively one method were included.

We evaluated statistical heterogeneity across studies using the Cochran's Q-test ($p<0.1$ indicating existence of heterogeneity) along with the I^2 statistic (with a result >50% suggesting significant heterogeneity). Funnel plot of all studies assessing endothelial function were examined for presence of asymmetry. The random-effects meta-analytic model was used to combine our data, due to the existence of clinical and methodological high between-study heterogeneity. The inverse variance method was used to estimate study weights, but with shared intervention groups divided out approximately evenly among the comparisons. Statistical analyses were performed with Review Manager (RevMan) Version5.3.

Results

Search results

Study selection process is presented as flow diagram in Supplementary Figure 1. The searches identified in total 1,726 reports; after removing duplicates (n=395), 1,331 studies were screened at a title/abstract level. Following assessment of 50 reports at full text, we excluded 29. Hence, 21 studies enrolling 638 patients with COPD and 595 controls were included in this systematic review. From the 21 studies, only 19 studies (with 968 participants) [23–41] were included in the quantitative analysis, since available data for the rest 2 were inadequate [42, 43]. Authors were contacted by email requesting supplemental data, with one of them responding [35].

Quality assessment

Our search did not identify any cohort or cross-sectional studies, so the NOS for case-control studies was used. The overall study quality assessment for studies included in this analysis is depicted in Supplementary Table 3. According to the NOS score, 16 studies where classified as high quality (NOS≥7) and the remaining 3 studies as low quality [25, 28, 29].

Publication bias

As presented in Supplementary Figure 2, asymmetry in the funnel plot suggests that some small studies with non-significant results might be missing, therefore indicating that the possibility of publication bias could not be excluded.

Study characteristics

Of the 21 studies included in this systematic review, 16 studies evaluating endothelial function only via FMD (8 NMD) [28–43], 2 only via VOP [forearm blood flow (FBF) assessment after bradykinin infusion] [23, 24], 1 study via FMD and VOP (FBF after a typical post-occlusion reactive hyperemia protocol) [25], 1 study via PAT [26] and 1 study via FMSF [27]. Seven studies included patients with COPD, without overt cardiovascular disease [23, 26, 31–33, 39, 40] and 1 study included patients with COPD and CAD co-existence [25]. Regarding sex distribution, 4 studies including only male participants [23–25, 43], whilst no study was conducted only in female patients. Table 1 and Supplementary Table 4 show the characteristics of the included studies.

Endothelial function assessment via FMD

Across 15 studies evaluating endothelial function by measuring FMD of the brachial artery, a significantly lower endothelium-dependent vasodilatation of WMD -2.59% (95%CI -3.75 to -1.42) was observed in patients with COPD compared to controls, but with high heterogeneity across studies (I^2=96%, p<0.00001) (Figure 1A).

Endothelial function assessment via PAT

Only one study explored endothelial function via PAT in patients with COPD and healthy controls, showing a markedly impaired reactive hyperemia index (RHI) in the former (Figure 1B).

Endothelial function assessment via VOP in the forearm

Only one study evaluated FBF during reactive hyperemia (Figure 1C), without noting significant differences between patients with COPD and controls (COPD: 9.8±4.6 vs control: 8.9±3.8 ml/min per 100 ml tissue; p=0.577). Across the 2 studies evaluating FBF after bradykinin infusion, calculation of a WMD between patients with COPD and controls could not be performed due to differences in measurement scales of reported results, so data had to be pooled using SMD. No significant differences in FBF were observed between the patients with COPD and controls (SMD: -2.31, 95%CI [-7.08, 2.44], I^2=96%, p<0.00001) (Figure 1C). In overall, endothelium-dependent vasodilation (expressed via FBF after reactive hyperemia or bradykinin infusion) was non-significantly lower in COPD, compared to controls (SMD: -1.31, 95%CI [-3.28, 0.67]) (Figure 1C).

Endothelial function assessment via FMSF

As expected due to the novelty of the method, only one study has used FMSF to assess endothelial function in COPD, reporting that hyperemic response did not differentiate between the two study groups (-3.70%, 95%CI [-9.01, 1.61]) (Figure 1D).

Endothelial function assessment via NMD

Across 6 studies evaluating endothelium-independent vasodilation by the use of NMD, a significantly impaired response by WMD -3.13% (95%CI -5.18 to -1.09) was observed in patients with COPD compared to controls, with moderate heterogeneity (I^2=61%, p=0.02). (Figure 2)

Endothelial function assessment via all methods (pooled analysis)

When pooling all studies together, regardless of the type of method used for assessment of vascular reactivity, pronounced endothelial dysfunction was observed in patients with COPD

compared to non-COPD controls (SMD: -1.19, 95%CI -1.69 to -0.68) but with high heterogeneity (I^2=92%, p<0.00001) (Figure 3).

Subgroup analysis

In order to explore the heterogeneity across the included studies, subgroup analysis comparing endothelial function according to smoking status of controls was performed (Figure 4). In the 12 studies comparing patients with COPD and non-smoking controls, a more prominent endothelial dysfunction was evident in patients with COPD compared to non-smoking controls (SMD: -1.75, 95%CI [-2.58, -0.92], I^2=93%, p<0.00001), while no significant differences were observed between patients with COPD and smoking controls (SMD: -0.78, 95%CI [-1.87, 0.32], I^2=89%, p<0.0001).

Moreover, we performed subgroup analyses according to the presence of CAD. Supplementary Table 4 includes definitions used in the various studies for CAD and CVD, whether CAD or CVD were inclusion/exclusion criteria, and the percentage of patients receiving common vasoactive medications. Analysis of 9 studies including patients with COPD and controls without CAD showed a marginal, but not significant impairment in endothelial function in COPD compared to controls (SMD: -0.61, 95%CI [-1.23, 0.01], I^2=89%, p<0.00001), while data from one study including patients with COPD and controls with CAD report a more prominent impairment in endothelial function in COPD than controls (Supplementary Figure 3). Finally, when pooling studies including only male participants, a marginally impaired endothelial function in patients with COPD compared to controls was noted (Supplementary Figure 4).

Sensitivity analysis

We have repeated the main analysis by including only the high-quality studies (NOS score≥7) in order to explore the robustness of our findings. Of interest, after excluding studies

classified as of low quality, presence of a similarly impaired endothelial function in patients with COPD compared to controls was confirmed (SMD: -1.20, 95%CI [-1.76, -0.65]) (Supplementary Figure 5).

Discussion

This is the first systematic review of the assessment of endothelial function using almost all available functional methods in patients with COPD. The main finding of the present analysis is that patients with COPD have significantly impaired endothelial function compared with non-COPD controls. Sensitivity analysis excluding poor quality studies confirmed the main results. The observed difference in endothelial function is more pronounced when patients with COPD are compared to controls that are non-smokers. Furthermore, the difference was slightly more pronounced when patients with COPD and CAD were compared to controls.

Endothelium is the single cell layer that lines the interior surface of the vascular system and it is involved in multiple mechanisms of vascular homeostasis, including regulation of vasomotor tone, vascular permeability, hemostasis, angiogenesis and innate and adaptive immunity [44]. Endothelial dysfunction is the basis of atherosclerosis and a trigger of cardiovascular outcomes in several cohort studies [15, 45–47]. Reduced nitric oxide (NO) is the hallmark of endothelial dysfunction; it may result either from decreased endothelial NO-synthase (eNOS) activity [due to endo/exogenous inhibitors (e.g. asymmetric-dimethyl-arginine) or due to reduction in L-arginine] or from decreased NO-bioavailability (e.g. due to endothelin-1 overexpression) [48–50]. Existing evidence supports that oxidative stress and inflammation lead also to decreased NO-bioavailability and endothelial dysfunction [51], and probably, this pathway plays central role in endothelial damage in patients with COPD [9]. In particular, several studies indicated a significant association between COPD and inflammatory biomarkers (i.e. hs-CRP, fibrinogen, TNF-a, etc.) [52], even in moderate COPD [53]. Worsening systemic inflammation is related to COPD severity, as well as

greater morbidity and mortality [7]. Moreover, preliminary evidence showed that angiotensin-2 induces endothelial damage and vascular inflammation, suggesting that renin-angiotensin-system plays also a significant role in endothelial damage [15, 48, 54]. Finally, insulin resistance is another pathway that is disturbed in patients with COPD [55] and is suggested to play a crucial role in endothelial dysfunction [48]. In states of insulin resistance, insulin signaling is altered, resulting in a dramatical downregulation of eNOS activity, whereas hyperglycemia leads to increase of advanced glycation end-products, which are shown to promote vascular inflammation and oxidative excess, quench NO and impair endothelial function [48, 49].

As mentioned above, several functional techniques have been used in research works to evaluate endothelial integrity in populations with high-burden of cardiovascular disease, including those with COPD [56]. VOP was one of the first techniques for endothelial function assessment, but it is currently rarely used due to its semi-invasive nature [56]. FMD is considered for several years the reference method, as it is non-invasive, cheap and strongly correlated with coronary function and cardiovascular outcomes [15, 56, 57]. However, its application in everyday clinical practice can be challenging, as it requires good standardization, adherence to strict protocols, experienced operators, and controlled environment (quiet room, stable temperature, etc.) [15, 57, 58]. PAT is used for assessment of endothelial function of the microvasculature; it is non-invasive, reproducible, operator-independent, and also shows strong correlation with outcomes [15]. However, it can be affected by environmental factors (temperature, light, etc.), whereas increased sympathetic tone –something common among patients with COPD- has been also suggested to impact the PAT signal [59]. More recent technologies evaluating skin or sublingual microcirculation are promising, as they are non-invasive and can be combined with several reactivity tests or exercise; however, only a few studies examined their correlations with coronary endothelial function and adverse outcomes [14]. It should be also noted that all the above techniques require patient preparation (abstinence from smoking, caffeine, etc.) and collaboration

during the test (lying still for some minutes); although quite simple, these tasks may be demanding for some patients with COPD, like those with frequent cough, the very obese and others.

In line with our review, other studies have established a link between endothelial damage and COPD. In a previous systematic review, Ye et al. showed that patients with COPD had higher markers of endothelial function, arterial stiffness and other markers of subclinical cardiovascular disease, independently of smoking status [3]. In the aforementioned systematic review and meta-analysis from Ambrosino et al., patients with COPD showed a significantly lower FMD and NMD; FMD impairment was associated with age and FEV1% [17]. In another meta-analysis of similar design, Vaes et al. confirmed the above results, showing a decline in both endothelial-dependent and –independent vasodilation of the forearm, as assessed by FMD. [18]. Overall, our results extend the previous evidence, as they are indicative of a large difference in endothelial function between patients with COPD and non-COPD controls (SMD=-1.19, 95%CI [-1.19, -0.68]), not only in the conduit arteries (as assessed by FMD), but also in the microvasculature (as assessed by the other above-mentioned methods). The association between FMD and coronary endothelial function is well-established [15]; however, more recent original works demonstrated that microvascular dysfunction is also strongly associated with cardiovascular risk factors [60], suggesting that these methods should be used complementary, as they measure different aspects of vascular biology [15].

In addition, our subgroup analysis showed significantly worse endothelial function in patients with COPD compared to non-smoking controls, but this association was less prominent when patients with COPD were compared to smoking individuals. Smoking is closely associated with endothelial damage, as oxidative stress, systemic inflammation and impaired nitric oxide bioavailability were considered to be related with cigarette smoking [61]. In fact, Cui et al. demonstrated that current smokers have significantly lower FMD compared with never-smokers, and this association was dependent from the total packyears [62]. Although smoking is a major cardiovascular factor that plays a predominant role in the atherosclerotic process, it might not fully

explain the high cardiovascular risk in COPD [63]. Moreover, in the previous work from Ambrosino et al. [17], the relationship between COPD and endothelial function was independent of baseline smoking status.

To our knowledge, the present systematic review and meta-analysis is the largest effort in this field, including 19 studies and using the vast majority of the available functional methods of endothelial function evaluation in COPD. It followed a careful literature search and a rigorous methodology; we attempted to elucidate design errors detected in previous meta-analyses (e.g. double counts and units of analysis errors) in this field. However, our work has also some limitations that have to be acknowledged. First, there was significant heterogeneity across the included studies; we attempted to minimize the extent that it might affect our results by using the random-effects model, as well as by performing a number of subgroup analyses. In some of our subgroup analyses, such as three of the four analyses by the specific functional method used (PAT, VOP, FMSF) or the analysis in male patients, the number of included studies were small and, thus, did not allow us to draw firm conclusions. There was also a difference in the percentages of patients receiving vasoactive medications in some of the included studies and could not know to what extent these mismatches interfere with our findings. Our search was restricted in English-language journals; hence we may have introduced publication bias. Finally, although we extensively tried to retrieve missing data by contacting authors of the primary studies, we could not use data from a few studies due to missing values.

In conclusion, the present meta-analysis showed that patients with COPD have impaired endothelial function compared to controls without COPD. Considering the bidirectional relationship between endothelial damage and cardiovascular disease, future large and properly designed studies are needed to shed more light in this field, first by examining associations of endothelial function with adverse cardiovascular outcomes specifically in patients with COPD and, second, by assessing the feasibility of performing these assessments in everyday clinical practice in this population.

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TABLES

Table 1. Characteristics of studies included in this systematic review and meta-analysis

Study ID	Assessment method	Measurements and scale	COPD					Controls			
			N	Age (y)	Males (%)	FEV1(%)	CVD (%)*	N	Age (y)	Males (%)	Smoking status
Barak et al. 2017 [30]	FMD	FMD% (% proportional change from baseline)	17	69.0±8.1	64.7	31.8±11.0	5.9	10	65.3±7.3	70.0	N=4 former smokers N=6 non-smokers
Barr et al. 2007 [42]	FMD	FMD% (% proportional change from baseline)	44	n/a	54.5	n/a	n/a	63	70.0±5.0	54.0	Former smokers
Blum et al. 2014 [28]	FMD	FMD% (% proportional change from baseline)	23	64.4±8.4	56.0	45.0±14.0	26.0	22	44.7±11.7	46.0	non-smokers
Costanzo et al. 2017 [29]	FMD	FMD% (% proportional change from baseline)	41	74.0±5.8	56.1	61.9±16.6	n/a (0% CAD, but data about PAD and stroke missing)	35	73.8±6.6	45.7	n/a
Eickhoff et al. 2008 [31]	FMD and NMD	FMD% and NMD% (% proportional change from baseline)	60	62.0±8.0	56.6	41.0±18.0	0	40	60.9±10.4	37.5	N=20 non-smokers N=20 smokers
Gelinas et al. 2017 [32]	FMD and NMD	FMD% and NMD% (% proportional change from baseline)	24	69.9±2.8	54.2	68.0±19.0	0	20	62.6±1.1	50.0	n/a (excluded if >10 packyears)
Hartmann et al. 2016 [33]	FMD and NMD	FMD% and NMD% (% proportional	10	67.0±3.0	40.0	60.0±5.0	0	10	66.0±2.0	40.0	Non-smokers

		change from baseline)									
Ives et al. 2014 [34]	FMD	FMD% (% proportional change from baseline)	30	66.0±2.0	50.0	55.0±4.0	6.66	30	66.0±2.0	50.0	Non-smokers
Keymel et al. 2018 [25]	FMD and NMD, VOP	FMD%, NMD% (% proportional change from baseline) and FBF after reactive hyperemia (ml/min per 100ml tissue)	17	66.0±8.0	100	59.0±17.0	100.0	16	64±10.0	100	N=16 former smokers
Kuzubova et al. 2013 [43]	FMD	FMD% (% proportional change from baseline)	63	60.4±1.0	100	45.1±2.4	n/a	95	57.3±1.7	100	57% former or current smokers
Maclay et al. 2009 [23]	VOP	FBF after bradykinin infusion (ml/min per 100ml tissue)	18	65.0±5.4	100	47.6±20.1	0	17	63.0±6.0	100	Non-smokers
Majewsky et al. 2020 [27]	FMSF	Reactive hyperemia (% proportional change from baseline)	26	66.9±8.3	42.3	63.7±13.1	7.69	20	52.5±13.2	60.0	N=2 smokers N=3 former smokers N= 15 non-smokers
Malerba et al. 2018 [26]	PAT	RHI	16	74.2±8.6	62.5	69.5±19.0	0	16	75.1±3.2	62.5	N=3 smokers N=7 former smokers N=6 non-smokers
Marchetti et al. 2011 [35]	FMD and NMD	FMD% and NMD% (% proportional change from baseline)	8	61.0±8.0	50.0	33.0±22.0	0	9	53.0±6.0	66.6	Non-smokers

Moro et al. 2008 [36]	FMD and NMD	FMD% and NMD% (% proportional change from baseline)	44	76.7	61.4	n/a	15.9	48	73.4	27.1	N=7 smokers N=15 former smokers N=26 non-smokers
Ozben et al. 2010 [37]	FMD and NMD	FMD% and NMD% (% proportional change from baseline)	30	64.2±10.9	73.3	51.0±15.0	33.3	20	61.9±7.4	75.0	Non-smokers
Piccari et al. 2020 [38]	FMD	FMD% (% proportional change from baseline)	61[#]	62.5±4.7	83.6	43.6±19.7	n/a	47[#]	55.2±8.1	44.7	N=26 non-smokers N=20 smokers
Pizarro et al. 2014 [39]	FMD and NMD	FMD% and NMD% (% proportional change from baseline)	62	62.0±8.0	93.5	53.0±18.0	0	35	58.5±7.1	19.0	N=18 non-smokers N=17 smokers
Rodriguez-Miguelez et al. 2018 [40]	FMD	FMD% (% proportional change from baseline)	17	56.0±7.0	35.3	58.0±15.0	0	15	58.0±7.0	33.3	N=13 non-smokers N=2 smokers
Yang et al. 2018 [24]	VOP	FBF after bradykinin infusion (% proportional change from baseline)	12	63.0±6.0	100	53.0±13.0	n/a	12	64.0±7.0	100	Non-smokers
Zelt et al. 2018 [41]	FMD	FMD% (% proportional change from baseline)	16	66.0±8.0	31.3	86.2±13.8	12.5	16	64.0±8.0	43.8	N=1 smoker N=4 former smokers N=11 non-smokers

Variables are presented as mean ± SD.
CVD: Cardiovascular diseases, FBF: forearm blood flow, FEV1: Forced expiratory volume in the first second, FMD: flow mediated dilatation, FMSF: Flow mediated skin fluorescence, NMD: Nitroglycerin- mediated dilatation, PAT: peripheral arterial tonometry, RHI: reactive hyperemia index, VOP: Venous occlusion plethysmography.
*The term CVD includes: coronary artery disease (CAD), peripheral artery disease (PAD) and stroke
[#] 1 participant excluded from analysis due to missing data

FIGURES LEGENDS

Figure 1. Forest plot of the difference in (A) flow-mediated dilatation (FMD%), (B) reactive hyperemia index (RHI), assessed by peripheral arterial tonometry (PAT), (C) forearm blood flow (FBF) assessed by venous occlusion plethysmography (VOP) and (D) reactive hyperemia assessed by flow mediated skin fluorescence (FMSF), among patients with COPD and non-COPD controls.

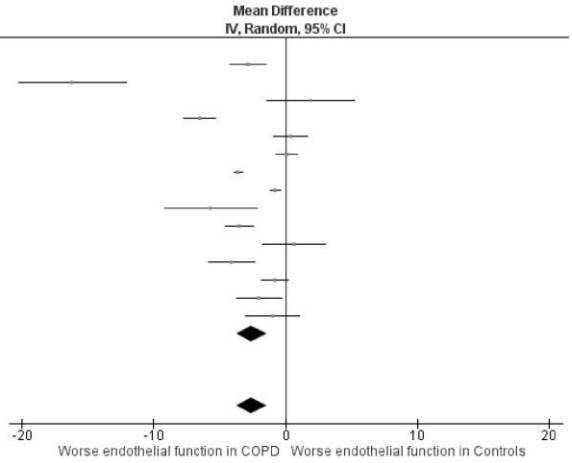
Figure 2. Forest plot of the difference in nitroglycerine-mediated dilatation (NMD%) among patients with COPD and non-COPD controls.

Figure 3. Forest plot of the difference in endothelial function among patients with COPD and non-COPD controls (all methods).

Figure 4. Subgroup analysis comparing endothelial function of patients with COPD with non-smoking and smoking controls.

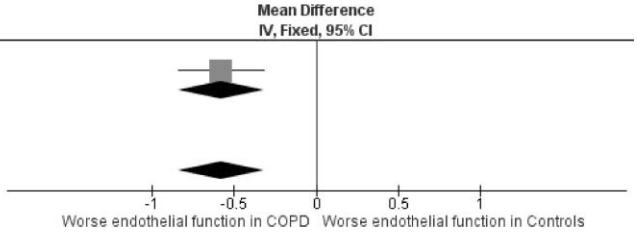
A

Study or Subgroup	COPD Mean	COPD SD	COPD Total	Controls Mean	Controls SD	Controls Total	Weight	Mean Difference IV, Random, 95% CI
1.1.1 Endothelial function assessment via FMD								
Barak et al. 2017	5.03	1.51	17	7.88	1.81	10	7.2%	-2.85 [-4.18, -1.52]
Blum et al. 2014	-0.6	6.3	23	15.6	7.6	22	4.0%	-16.20 [-20.29, -12.11]
Costanzo et al. 2017	14.2	8	41	12.3	6.8	35	4.8%	1.90 [-1.43, 5.23]
Eickhoff et al. 2008	11	3	60	17.5	2.9396	40	7.4%	-6.50 [-7.69, -5.31]
Gelinas et al. 2017	4.7	1.9	24	4.3	2.3	20	7.3%	0.40 [-0.86, 1.66]
Hartmann et al.2016	6	0.9	10	5.9	1	10	7.7%	0.10 [-0.73, 0.93]
Ives et al. 2014	3.1	0.5	30	6.7	0.6	30	8.0%	-3.60 [-3.88, -3.32]
Keymel et al. 2018	3.4	0.5	17	4.2	0.6	16	7.9%	-0.80 [-1.18, -0.42]
Marchetti et al. 2011	5.1	2.4	8	10.8	4.7	9	4.6%	-5.70 [-9.19, -2.21]
Moro et al. 2008	5.4	3.1	44	8.9	2.1	48	7.5%	-3.50 [-4.59, -2.41]
Ozben et al. 2010	10.42	4.86	30	9.77	3.83	20	5.9%	0.65 [-1.77, 3.07]
Piccari et al. 2020	4.6425	3.2181	60	8.7496	5.3461	46	6.8%	-4.11 [-5.85, -2.36]
Pizarro et al. 2014	0.63	2.67	62	1.4323	2.2953	35	7.5%	-0.80 [-1.81, 0.21]
Rodriguez-Miguelez et al. 2018	5.0765	2.2208	17	7.1	2.6	15	6.8%	-2.02 [-3.71, -0.34]
Zelt et al. 2018	4.11	3.16	16	5.08	2.79	16	6.4%	-0.97 [-3.04, 1.10]
Subtotal (95% CI)			**459**			**372**	**100.0%**	**-2.59 [-3.75, -1.42]**
Heterogeneity: Tau² = 4.40; Chi² = 317.98, df = 14 (P < 0.00001); I² = 96%								
Test for overall effect: Z = 4.36 (P < 0.0001)								
Total (95% CI)			**459**			**372**	**100.0%**	**-2.59 [-3.75, -1.42]**
Heterogeneity: Tau² = 4.40; Chi² = 317.98, df = 14 (P < 0.00001); I² = 96%								
Test for overall effect: Z = 4.36 (P < 0.0001)								
Test for subgroup differences: Not applicable								



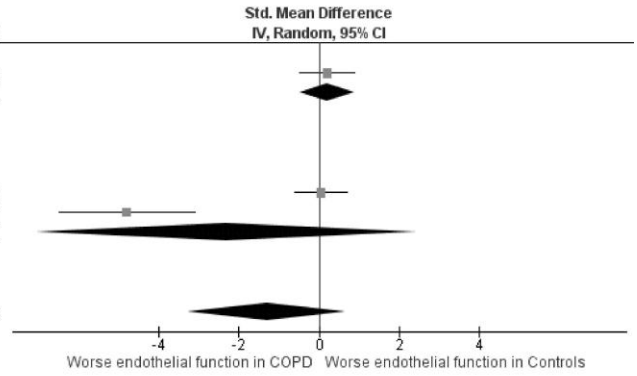
B

Study or Subgroup	COPD Mean	COPD SD	COPD Total	Controls Mean	Controls SD	Controls Total	Weight	Mean Difference IV, Fixed, 95% CI
2.1.1 Endothelial assessment via PAT (RHI)								
Malerba et al. 2018	0.27	0.52	16	0.85	0.13	16	100.0%	-0.58 [-0.84, -0.32]
Subtotal (95% CI)			**16**			**16**	**100.0%**	**-0.58 [-0.84, -0.32]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 4.33 (P < 0.0001)								
Total (95% CI)			**16**			**16**	**100.0%**	**-0.58 [-0.84, -0.32]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 4.33 (P < 0.0001)								
Test for subgroup differences: Not applicable								



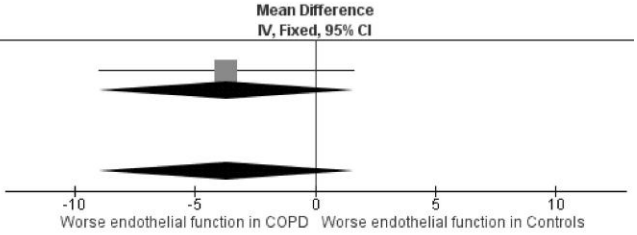
C

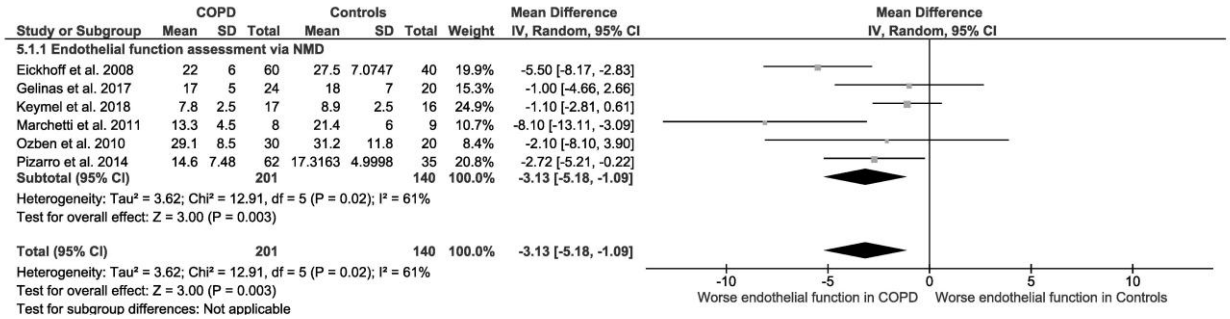
Study or Subgroup	COPD Mean	COPD SD	COPD Total	Controls Mean	Controls SD	Controls Total	Weight	Std. Mean Difference IV, Random, 95% CI
3.1.1 FBF during reactive hyperemia (VOP)								
Keymel et al. 2018	9.8	4.6	17	8.9	3.8	16	35.4%	0.21 [-0.48, 0.89]
Subtotal (95% CI)			**17**			**16**	**35.4%**	**0.21 [-0.48, 0.89]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.59 (P = 0.55)								
3.1.2 FBF during bradykinin infusion (VOP)								
Maclay et al. 2009	16	9	18	15.6	6.4	17	35.5%	0.05 [-0.61, 0.71]
Yang et al. 2017	552	103	12	1,314	191	12	29.1%	-4.79 [-6.48, -3.11]
Subtotal (95% CI)			**30**			**29**	**64.6%**	**-2.31 [-7.05, 2.44]**
Heterogeneity: Tau² = 11.31; Chi² = 27.48, df = 1 (P < 0.00001); I² = 96%								
Test for overall effect: Z = 0.95 (P = 0.34)								
Total (95% CI)			**47**			**45**	**100.0%**	**-1.31 [-3.28, 0.67]**
Heterogeneity: Tau² = 2.75; Chi² = 30.42, df = 2 (P < 0.00001); I² = 93%								
Test for overall effect: Z = 1.29 (P = 0.20)								
Test for subgroup differences: Chi² = 1.06, df = 1 (P = 0.30), I² = 5.4%								



D

Study or Subgroup	COPD Mean	COPD SD	COPD Total	Controls Mean	Controls SD	Controls Total	Weight	Mean Difference IV, Fixed, 95% CI
4.1.1 Endothelial function assessment via FMSF								
Majewsky et al. 2020	9.7	13.4	26	13.4	2.9	20	100.0%	-3.70 [-9.01, 1.61]
Subtotal (95% CI)			**26**			**20**	**100.0%**	**-3.70 [-9.01, 1.61]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.37 (P = 0.17)								
Total (95% CI)			**26**			**20**	**100.0%**	**-3.70 [-9.01, 1.61]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.37 (P = 0.17)								
Test for subgroup differences: Not applicable								




COPD
Controls
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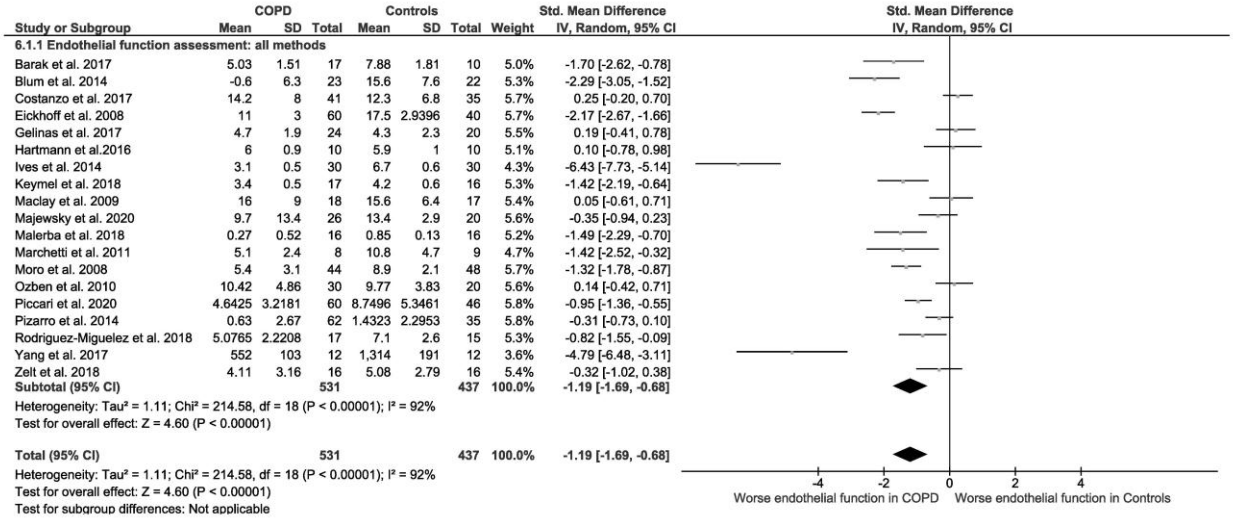
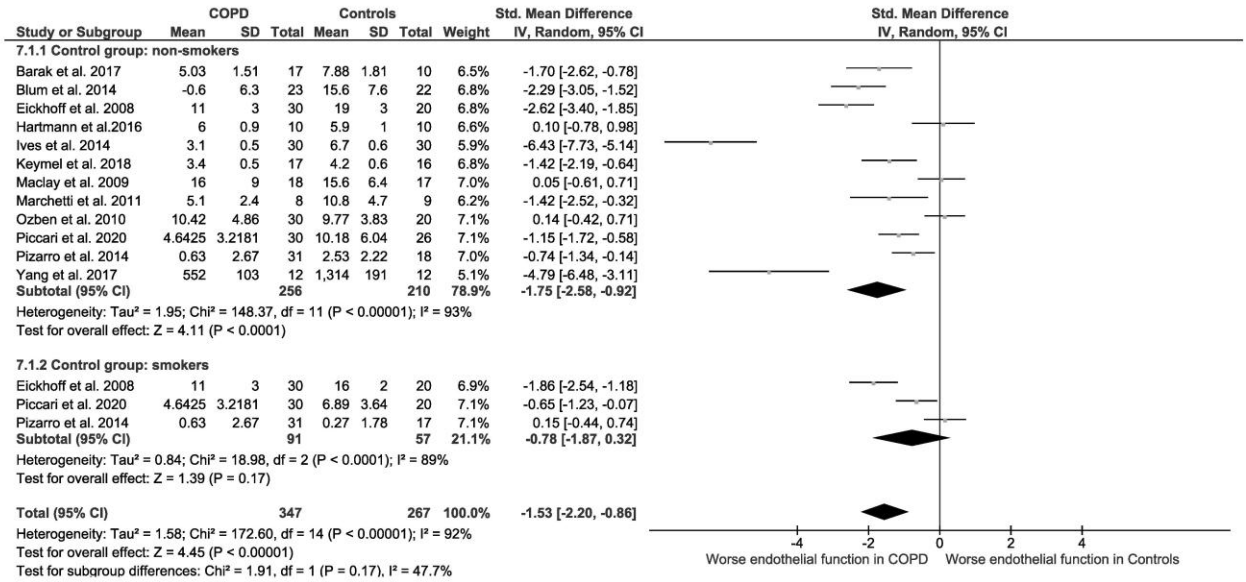
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
5.1.1 Endothelial function assessment via NMD
Eickhoff et al. 2008 22 6 60 27.5 7.0747 40 19.9% -5.50 [-8.17, -2.83]
Gelinas et al. 2017 17 5 24 18 7 20 15.3% -1.00 [-4.66, 2.66]
Keymel et al. 2018 7.8 2.5 17 8.9 2.5 16 24.9% -1.10 [-2.81, 0.61]
Marchetti et al. 2011 13.3 4.5 8 21.4 6 9 10.7% -8.10 [-13.11, -3.09]
Ozben et al. 2010 29.1 8.5 30 31.2 11.8 20 8.4% -2.10 [-8.10, 3.90]
Pizarro et al. 2014 14.6 7.48 62 17.3163 4.9998 35 20.8% -2.72 [-5.21, -0.22]
Subtotal (95% CI) 201 140 100.0% -3.13 [-5.18, -1.09]
Heterogeneity: Tau² = 3.62; Chi² = 12.91, df = 5 (P = 0.02); I² = 61%
Test for overall effect: Z = 3.00 (P = 0.003)
Total (95% CI) 201 140 100.0% -3.13 [-5.18, -1.09]
Heterogeneity: Tau² = 3.62; Chi² = 12.91, df = 5 (P = 0.02); I² = 61%
Test for overall effect: Z = 3.00 (P = 0.003)
Test for subgroup differences: Not applicable
-10
-5
0
5
10
Worse endothelial function in COPD
Worse endothelial function in Controls


COPD
Controls
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
6.1.1 Endothelial function assessment: all methods
Barak et al. 2017 5.03 1.51 17 7.88 1.81 10 5.0% -1.70 [-2.62, -0.78]
Blum et al. 2014 -0.6 6.3 23 15.6 7.6 22 5.3% -2.29 [-3.05, -1.52]
Costanzo et al. 2017 14.2 8 41 12.3 6.8 35 5.7% 0.25 [-0.20, 0.70]
Eickhoff et al. 2008 11 3 60 17.5 2.9396 40 5.7% -2.17 [-2.67, -1.66]
Gelinas et al. 2017 4.7 1.9 24 4.3 2.3 20 5.5% 0.19 [-0.41, 0.78]
Hartmann et al.2016 6 0.9 10 5.9 1 10 5.1% 0.10 [-0.78, 0.98]
Ives et al. 2014 3.1 0.5 30 6.7 0.6 30 4.3% -6.43 [-7.73, -5.14]
Keymel et al. 2018 3.4 0.5 17 4.2 0.6 16 5.3% -1.42 [-2.19, -0.64]
Maclay et al. 2009 16 9 18 15.6 6.4 17 5.4% 0.05 [-0.61, 0.71]
Majewsky et al. 2020 9.7 13.4 26 13.4 2.9 20 5.6% -0.35 [-0.94, 0.23]
Malerba et al. 2018 0.27 0.52 16 0.85 0.13 16 5.2% -1.49 [-2.29, -0.70]
Marchetti et al. 2011 5.1 2.4 8 10.8 4.7 9 4.7% -1.42 [-2.52, -0.32]
Moro et al. 2008 5.4 3.1 44 8.9 2.1 48 5.7% -1.32 [-1.78, -0.87]
Ozben et al. 2010 10.42 4.86 30 9.77 3.83 20 5.6% 0.14 [-0.42, 0.71]
Piccari et al. 2020 4.6425 3.2181 60 8.7496 5.3461 46 5.8% -0.95 [-1.36, -0.55]
Pizarro et al. 2014 0.63 2.67 62 1.4323 2.2953 35 5.8% -0.31 [-0.73, 0.10]
Rodriguez-Miguelez et al. 2018 5.0765 2.2208 17 7.1 2.6 15 5.3% -0.82 [-1.55, -0.09]
Yang et al. 2017 552 103 12 1,314 191 12 3.6% -4.79 [-6.48, -3.11]
Zelt et al. 2018 4.11 3.16 16 5.08 2.79 16 5.4% -0.32 [-1.02, 0.38]
Subtotal (95% CI) 531 437 100.0% -1.19 [-1.69, -0.68]
Heterogeneity: Tau² = 1.11; Chi² = 214.58, df = 18 (P < 0.00001); I² = 92%
Test for overall effect: Z = 4.60 (P < 0.00001)
Total (95% CI) 531 437 100.0% -1.19 [-1.69, -0.68]
Heterogeneity: Tau² = 1.11; Chi² = 214.58, df = 18 (P < 0.00001); I² = 92%
Test for overall effect: Z = 4.60 (P < 0.00001)
Test for subgroup differences: Not applicable
-4
-2
0
2
4
Worse endothelial function in COPD
Worse endothelial function in Controls


COPD
Controls
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
7.1.1 Control group: non-smokers
Barak et al. 2017 5.03 1.51 17 7.88 1.81 10 6.5% -1.70 [-2.62, -0.78]
Blum et al. 2014 -0.6 6.3 23 15.6 7.6 22 6.8% -2.29 [-3.05, -1.52]
Eickhoff et al. 2008 11 3 30 19 3 20 6.8% -2.62 [-3.40, -1.85]
Hartmann et al.2016 6 0.9 10 5.9 1 10 6.6% 0.10 [-0.78, 0.98]
Ives et al. 2014 3.1 0.5 30 6.7 0.6 30 5.9% -6.43 [-7.73, -5.14]
Keymel et al. 2018 3.4 0.5 17 4.2 0.6 16 6.8% -1.42 [-2.19, -0.64]
Maclay et al. 2009 16 9 18 15.6 6.4 17 7.0% 0.05 [-0.61, 0.71]
Marchetti et al. 2011 5.1 2.4 8 10.8 4.7 9 6.2% -1.42 [-2.52, -0.32]
Ozben et al. 2010 10.42 4.86 30 9.77 3.83 20 7.1% 0.14 [-0.42, 0.71]
Piccari et al. 2020 4.6425 3.2181 30 10.18 6.04 26 7.1% -1.15 [-1.72, -0.58]
Pizarro et al. 2014 0.63 2.67 31 2.53 2.22 18 7.0% -0.74 [-1.34, -0.14]
Yang et al. 2017 552 103 12 1,314 191 12 5.1% -4.79 [-6.48, -3.11]
Subtotal (95% CI) 256 210 78.9% -1.75 [-2.58, -0.92]
Heterogeneity: Tau² = 1.95; Chi² = 148.37, df = 11 (P < 0.00001); I² = 93%
Test for overall effect: Z = 4.11 (P < 0.0001)
7.1.2 Control group: smokers
Eickhoff et al. 2008 11 3 30 16 2 20 6.9% -1.86 [-2.54, -1.18]
Piccari et al. 2020 4.6425 3.2181 30 6.89 3.64 20 7.1% -0.65 [-1.23, -0.07]
Pizarro et al. 2014 0.63 2.67 31 0.27 1.78 17 7.1% 0.15 [-0.44, 0.74]
Subtotal (95% CI) 91 57 21.1% -0.78 [-1.87, 0.32]
Heterogeneity: Tau² = 0.84; Chi² = 18.98, df = 2 (P < 0.0001); I² = 89%
Test for overall effect: Z = 1.39 (P = 0.17)
Total (95% CI) 347 267 100.0% -1.53 [-2.20, -0.86]
Heterogeneity: Tau² = 1.58; Chi² = 172.60, df = 14 (P < 0.00001); I² = 92%
Test for overall effect: Z = 4.45 (P < 0.00001)
Test for subgroup differences: Chi² = 1.91, df = 1 (P = 0.17), I² = 47.7%
-4
-2
0
2
4
Worse endothelial function in COPD
Worse endothelial function in Controls

SUPPLEMENTARY MATERIAL

Title: Endothelial dysfunction in chronic obstructive pulmonary disease: a systematic review and meta-analysis of studies using different functional assessment methods

Authors: Marieta P. Theodorakopoulou,[1] Maria Eleni Alexandrou,[1] Dimitra Rafailia Bakaloudi,[1] Georgia Pitsiou,[2] Ioannis Stanopoulos,[2] Theodoros Kontakiotis,[3] Afroditi K. Boutou[4]

Affiliations: 1) Department of Nephrology, Hippokration Hospital, Aristotle University of Thessaloniki, Greece; 2) Department of Respiratory Failure, Aristotle University of Thessaloniki, Greece; 3) Department of Respiratory Medicine, Aristotle University of Thessaloniki, Greece; 4) Department of Respiratory Medicine, G. Papanikolaou Hospital, Thessaloniki, Greece

Supplementary Table 1. Quality assessment of the present meta-analysis according to MOOSE checklist for observational studies (From: Stroup DF, Berlin JA, Morton SC, et al, for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. Meta-analysis of Observational Studies in Epidemiology. A Proposal for Reporting. JAMA. 2000;283(15):2008-2012. doi: 10.1001/jama.283.15.2008.)

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	4-5
2	Hypothesis statement	5-6
3	Description of study outcome(s)	7-8
4	Type of exposure or intervention used	6-8
5	Type of study designs used	6-8
6	Study population	6-8
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	7
8	Search strategy, including time period included in the synthesis and key words	6-7
9	Effort to include all available studies, including contact with authors	7
10	Databases and registries searched	6-7
11	Search software used, name and version, including special features used (eg, explosion)	7-8
12	Use of hand searching (eg, reference lists of obtained articles)	6-7
13	List of citations located and those excluded, including justification	9, Supplementary Figure 1
14	Method of addressing articles published in languages other than English	6
15	Method of handling abstracts and unpublished studies	n/a
16	Description of any contact with authors	7, 10
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	7-9

18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	6-7
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	7
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	8-9
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	8-9
22	Assessment of heterogeneity	8-9
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	8-9
24	Provision of appropriate tables and graphics	Figures 1-4, Supplementary Material
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	9-12, Figures 1-3
26	Table giving descriptive information for each study included	Table 1, Supplementary Figure 4
27	Results of sensitivity testing (eg, subgroup analysis)	11-12, Figure 4, Supplementary Figures 3-5
28	Indication of statistical uncertainty of findings	n/a
Reporting of discussion should include		
29	Quantitative assessment of bias (eg, publication bias)	9, Supplementary Figure 2
30	Justification for exclusion (eg, exclusion of non-English language citations)	Supplementary Figure 1
31	Assessment of quality of included studies	7, 9, Supplementary Table 3
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	14-16
33	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature	14-16

	review)	
34	Guidelines for future research	16
35	Disclosure of funding source	1

Supplementary Table 2. Search term used for this systematic review and meta-analysis

Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/) [All Fields] #1 COPD AND endothelial dysfunction #2 COPD AND endothelial function #3 COPD AND VOP #4 COPD AND venous occlusion plethysmography #5 COPD AND FMD #6 COPD AND Flow mediated dilation #7 COPD AND LDF #8 COPD AND Laser Doppler flowmetry #9 COPD AND glycocalyx #10 COPD AND LSCI #11 COPD AND Laser speckle contrast imaging #12 COPD AND LASCA #13 COPD AND laser speckle contrast analysis #14COPD AND nailfold capillaroscopy #15 COPD AND NIRS #16 COPD AND near-infrared spectroscopy #17 COPD AND PAT #18 COPD AND peripheral arterial tonometry #19 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18
Scopus(https://www.scopus.com/home.uri) ((COPD AND "endothelial dysfunction") OR (COPD AND "endothelial function") OR (COPD AND VOP) OR (COPD AND FMD) OR (COPD AND glycocalix) OR (COPD AND LASCA) OR (COPD AND RIS) OR (COPD AND PAT))

Supplementary Table 3. Quality evaluation of the included studies according to Newcastle-Ottawa Scale (NOS).

Study	Selection	Comparability	Exposure	NOS score
Barak et al., 2017	****	**	**	8
Blum et al., 2014	***	*	**	6
Costanzo et al., 2016	**	**	**	6
Eickhoff et al., 2007	****	**	***	9
Gelinas et al., 2017	****	**	***	9
Hartmann et al., 2016	****	**	**	8
Iveset al., 2020	****	**	***	9
Keymel et al., 2016	**	**	**	6
Maclay et al., 2009	****	**	**	8
Majewski et al., 2020	****	**	**	8
Malerba et al., 2018	****	**	***	9
Marchetti et al., 2011	**	**	***	7
Moro et al., 2008	****	**	***	9
Özbenet al., 2010	****	**	*	7
Piccari et al., 2020	***	**	***	8
Pizarro et al., 2014	****	**	***	9
Rodriguez-Miguelez et al., 2018	****	**	***	9
Yang et al., 2017	****	**	**	8
Zelt et al., 2018	****	**	**	8

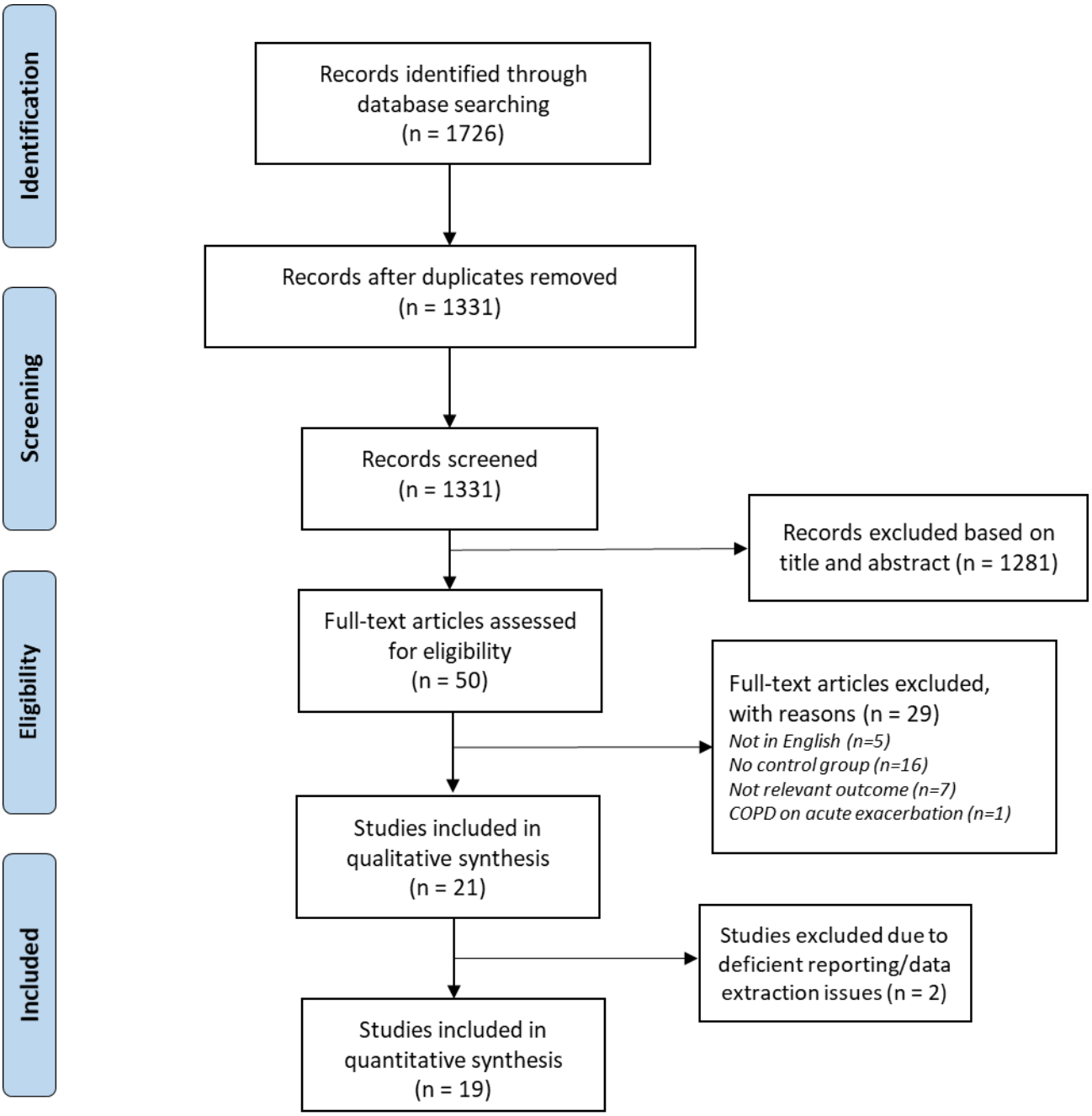
Supplementary Table 4. Study characteristics regarding presence of coronary artery disease (CAD) and cardiovascular disease (CVD) and use of common vasoactive medications.

Study	CVD/CAD definition	Inclusion/exclusion criteria	Patients with CVD	b-blocker	RAAS inhibitors	CCBs	Nitrate
Barak et al., 2017	No clear definition	-	CAD: COPD 5.9%, Controls 0% PAD: COPD 11.8%, Controls 0%, no information about stroke	COPD 23.5%, Controls 10%	COPD 47.1%, Controls 10%	COPD 17.6%, Controls 0%	n/a
Blum et al., 2014	No clear definition	-	CAD: COPD: 26%, Controls 0%, no information about PAD, stroke	n/a	n/a	n/a	n/a
Costanzo et al., 2016	CVD defined as: history of ischemic heart disease, heart failure, severe valvular heart disease, cardiomyopathy, arrhythmias	CVD was an exclusion criterion	CAD: 0%, no information about PAD or stroke	COPD 15% Controls 11%	COPD 54% Controls 60%	n/a	n/a
Eickhoff et al., 2008	CVD defined as: cerebrovascular disease, chest pain on exertion, congestive heart failure, coronary heart disease, peripheral artery occlusive disease, acute pulmonary embolism or revascularization within the past 24 months	CVD was an exclusion criterion	0%	n/a	0%	n/a	n/a
Gelinas et al., 2017	CVD defined as: myocardial infarction, stroke, heart failure	CVD was an exclusion criterion	0%	n/a	COPD 54.2%, Controls 6.0%,	n/a	n/a
Hartmann et al., 2016	CVD defined as: cerebrovascular disease, myocardial infarction, angina, arrhythmia, valvular heart disease, chronic heart failure, peripheral arterial disease	CVD was an exclusion criterion	0%	n/a	n/a	n/a	n/a
Ives et al., 2020	CAD from patients' history	-	CAD: COPD: 6.7% Controls 3.3%, no information about PAD, stroke	COPD: 6.7% Controls 13.3%	COPD: 40.0% Controls 10.0%	COPD: 30.0% Controls 6.7%	n/a
Keymel et al., 2016	CAD was diagnosed by coronary angiography	CAD was an inclusion criterion	100%	n/a	n/a	n/a	n/a
Maclay et al., 2009	CVD defined as: cardiovascular, cerebrovascular, and peripheral vascular disease	CVD was an exclusion criterion	0%	0%	0%	n/a	n/a
Majewski et al., 2020	No clear definition	-	CAD: COPD: 7.7% Controls 0%, no information about PAD, stroke	n/a	n/a	n/a	n/a

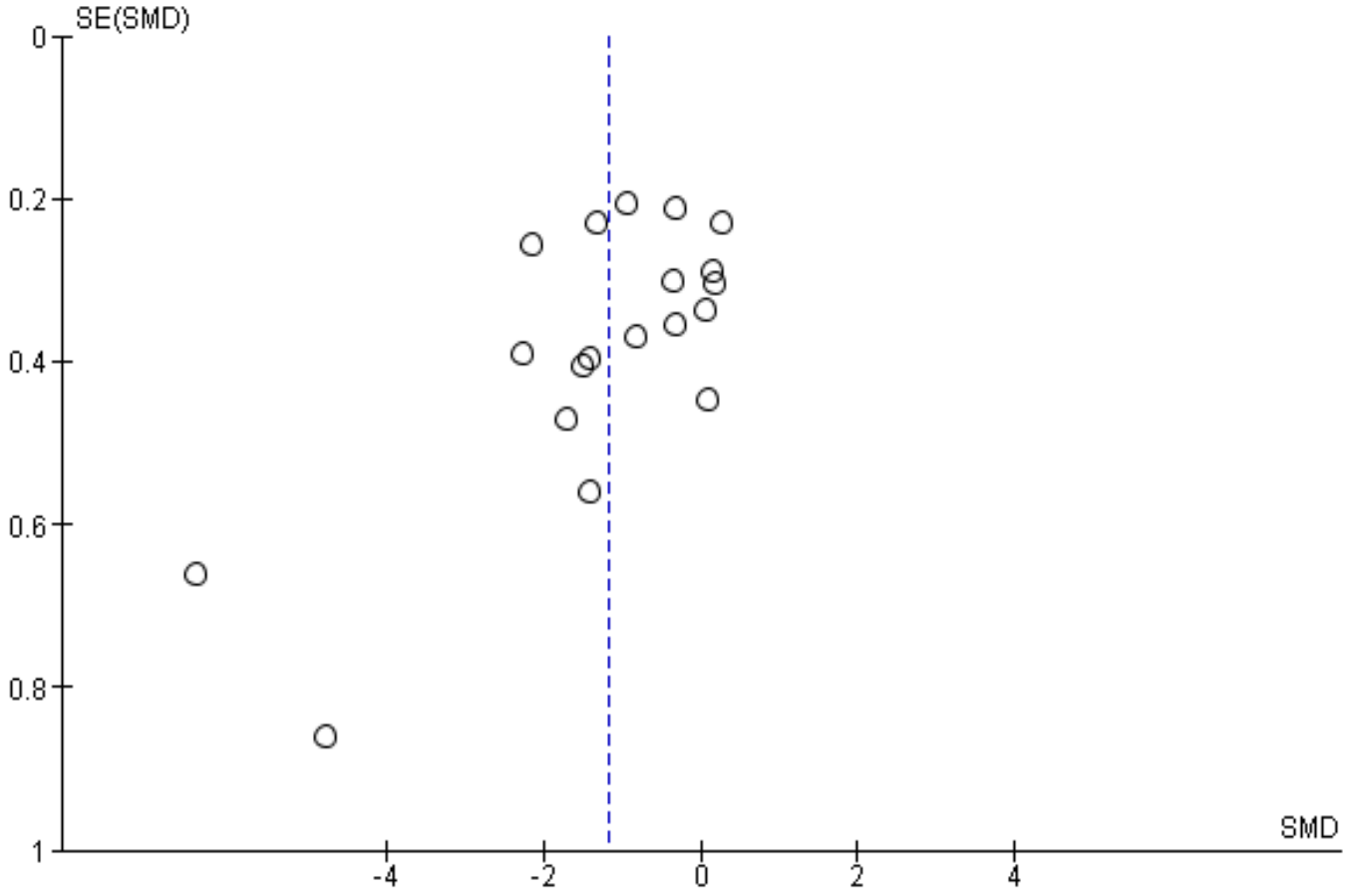
Malerba et al., 2018	CVD defined as history of any cardiovascular disease (except hypertension)	CVD was an exclusion criterion	0%	COPD: 25.0% Controls: 37.5%	COPD: 50.0% Controls: 62.5%	COPD: 13.3% Controls: 37.5%	n/a
Marchetti et al., 2011	No clear definition. (PAD, CAD, stroke reported as distinct conditions)	PAD was an exclusion criterion	0%	0%	COPD: 25% Controls 0%	COPD: 12.5% Controls 0%	n/a
Moro et al., 2008	No clear definition	-	CAD: COPD: 22.9%, Controls: 15.9% PAD: COPD 10.4% Controls 22.7% Cerebrovascular: COPD 20.8% Controls 36.4%	COPD: 22.9%, Controls: 11.4%	COPD: 75.0%, Controls: 58.1%	COPD: 16.7%, Controls: 15.9%	COPD: 10.4%, Controls: 18.2%
Özben et al., 2010	No clear definition	-	No information about stroke/PAD	n/a	n/a	n/a	n/a
Piccari et al., 2020	n/a	-	n/a	n/a	n/a	n/a	n/a
Pizarro et al., 2014	CVD was defined as: established cardiovascular or cerebral-vascular disease	CVD was an exclusion criterion	0%	n/a	n/a	0%	0%
Rodriguez-Miguelez et al., 2018	No clear definition (CVD only referred as not clinical diagnosis of overt CVD)	CVD was an exclusion criterion	0%	n/a	COPD 5.8%, Controls 6.7%	COPD 23.5%, Controls 0%	0%
Yang et al., 2017	n/a	-	n/a	n/a	n/a	n/a	n/a
Zelt et al., 2018	No clear definition	-	CAD: COPD: 12.5% Controls 6.3%, no information about PAD, stroke	n/a	n/a	n/a	n/a

Abbreviations: CAD, coronary artery disease, CCB, calcium channel blocker; COPD ,chronic obstructive pulmonary disease; CVD, cardiovascular disease; n/a, not applied; PAD, peripheral arterial disease; RAAS, renin-angiotensin aldosterone system

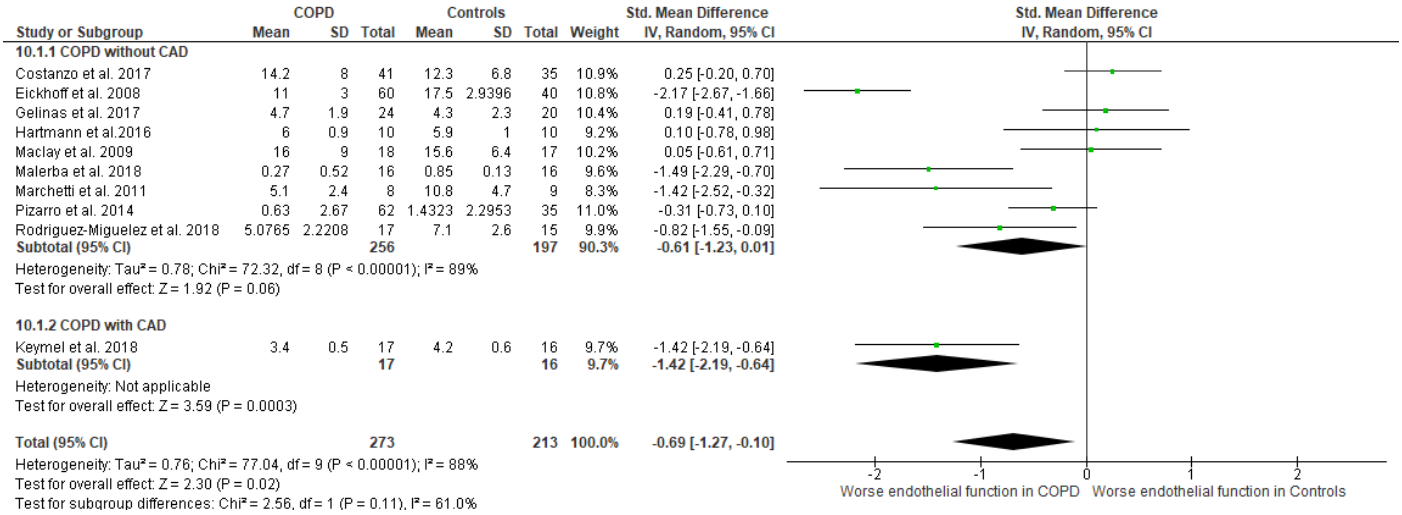
Supplementary Figure 1. Flow diagram of study selection process



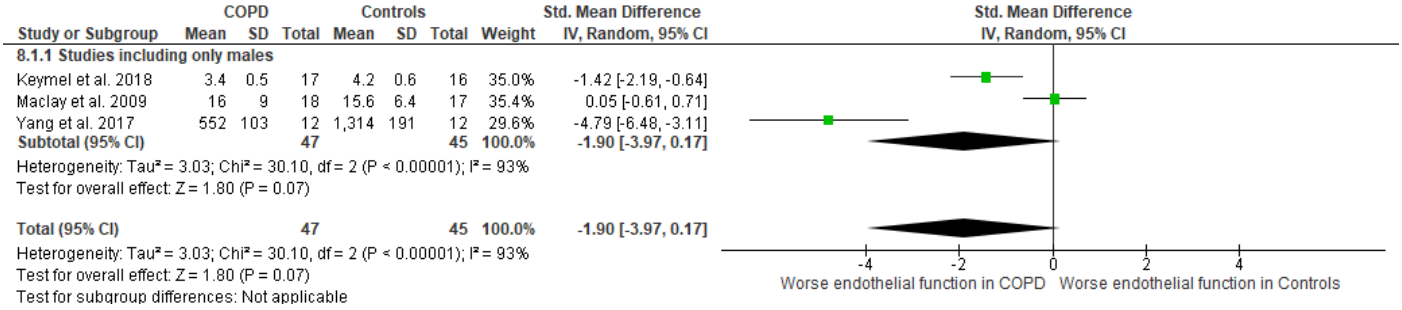
Supplementary Figure 2. Funnel plot assessing publication bias for the total of the studies.



Supplementary Figure 3. Subgroup analysis comparing endothelial function between patients with COPD and with/without coronary artery disease (CAD) and controls



Supplementary Figure 4. Forest plot of the difference in endothelial function among male patients with COPD and non-COPD controls.



Supplementary Figure 5. Sensitivity analysis (included studies with NOS score ≥7): Forest plot of the difference in endothelial function among patients with COPD and non-COPD controls