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Effect of prolonged treatment with phosphodiesterase-5-inhibitors on endothelial dysfunction in vascular diseases and vascular risk conditions: a systematic review analysis and meta-analysis of randomized double-blind placebo-controlled trials

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Abstract

Objective: To challenge the argument that continuous use of phosphodiesterase–5 selective inhibitors may reduce endothelial cell dysfunction in patients with vascular diseases or vascular risk conditions.

Design: This study included systematic reviews and meta-analysis of randomized double-blind placebo-controlled trials dealing with a prolonged use of phosphodiesterase–5 selective inhibitors. The risk of bias and quality of trials were assessed by the Cochrane algorithm. Fixed or random effect models, standardized mean differences, and heterogeneity were estimated in the study.

Data sources: Systematic search for randomized double-blind placebo-controlled trials was done in PubMed, Scopus, CINAHL, Science direct and the Cochrane Library.

Eligibility criteria for selecting studies: Randomized double-blind placebo-controlled trials reporting measures of endothelial cell dysfunction and/or endothelial cell activation were included.

Results: On the whole, 469 subjects were allocated to the phosphodiesterase–5 selective inhibitor group while 463 were assigned to the placebo group in 13 randomized double-blind placebo-controlled trials. Flow-mediated dilation of the brachial artery was found to improve after the administration of phosphodiesterase–5 selective inhibitors ($p<0.0001$). The results were questioned by the elevated and uncorrectable heterogeneity ($I^2=92\%$) and the asymmetry of the funnel plot suggested a publication bias. Phosphodiesterase–5 selective inhibitors have no effect on endothelial cell dysfunction, as assessed in the resistance vessels by digital arterial tonometry. The blood level of endothelin–1 was observed to be decreased in phosphodiesterase–5 selective inhibitors arm ($p=0.03$), although the effect disappeared once the publication bias and heterogeneity were corrected. The effect of phosphodiesterase–5 selective inhibitors on biomarkers of endothelial cell activation was found to be inconsistent.

Conclusions: The results on the benefits of a prolonged use of phosphodiesterase–5 selective inhibitors, with the objective of lowering endothelial cell dysfunction in patients with vascular diseases or vascular risk conditions are not convincing. This is due to the overall low quality of evidence, giving an unclear scientific support to this treatment.

Systematic review registration: - PROSPERO registration: CRD42017055399.

Keywords: Phosphodiesterase–5, Endothelial dysfunction, Flow-mediated dilation, Type–2 Diabetes, Vascular diseases, Vascular risk conditions.

Short Title: PDE5-inhibitors and endothelial dysfunction

Review Criteria

The criteria for study selection were 1) randomized double-blind placebo-controlled trials; 2) continuous use of phosphodiesterase–5 selective inhibitors, at the therapeutic dosage for at least four weeks; 3) enrollment of patients (males and/or females) with vascular diseases or vascular risk conditions; 4) outcomes including those of biomarkers of endothelial cell dysfunction and/or activation as well as of the non-invasive techniques to measure endothelial function.

This study was conducted in accordance with the Cochrane Collaboration and PRISMA statement.

Message for the clinics

The benefits of a prolonged use of phosphodiesterase–5 selective inhibitors, in patients with vascular diseases or vascular risk conditions, with the objective of lowering endothelial cell dysfunction are not persuasive. This may be due to the overall low quality of the evidence giving an unclear scientific support to this treatment.

Introduction

Endothelial cell dysfunction (EcDy), resulting in disruption of the vasoactive role of endothelium in regulation of tissue perfusion, is due to an imbalance caused by a decrease in the release of relaxing factors and an increase in the release of contracting factors, including nitric oxide (NO) and endothelin–1 (ET–1), respectively [1, 2]. EcDy is preceded by endothelial cell activation (EcAc) of

gene transcription and the *de novo* synthesis of proteins including adhesion molecules, cytokines, chemokines, and pro-coagulant factors [3, 4]. Cardiovascular risk conditions are associated with EcDy [2], manifested at the same time in the coronary and peripheral arteries [5]. Flow-mediated dilation (FMD) of the brachial arteries is a non-invasive measure of EcDy in conduit arteries [6]; its impairment has helped predict cardiovascular events in patients with cardiovascular diseases and vascular risk factors (VRFs) [7, 8, 9]. EcDy precedes vascular changes and contributes to atherosclerotic lesion development and progression [10, 11, 12]. The evaluation of EcDy may, therefore, represent a relevant intermediate endpoint to assess the efficacy of treatments on clinical outcomes that require a longer time as compared to that of the effect of treatment on endothelial function, which is substantially observed in only a few days or weeks [13].

The selective inhibitors (i) of phosphodiesterase type–5 (PDE5) competitively inhibit the hydrolysis of cyclic guanosine 5'-monophosphate (cGMP) by PDE5, thereby fostering NO-dependent cGMP accumulation and the consequent relaxation of vascular smooth muscle cells (SMCs) [14–16]. Although proposed as the first line treatment of erectile dysfunction [17], the wide cellular expression of PDE5 [18] fostered the possible use of PDE5 inhibitors (PDE5-i) in different clinical conditions including primary pulmonary artery hypertension [19, 20], systolic heart failure with reduced ejection fraction [21], and Raynaud's phenomenon [22]. Previous meta-analyses of randomized double-blind placebo-controlled trials (RCTs) revealed a positive effect of a continuous treatment of PDE5i on endothelial function in type–2 diabetic as well as non-diabetic males with ED, based on changes of FMD in the brachial artery [23, 24]. The high heterogeneity of reported studies has restricted the validity of the findings. The availability of previously unanalyzed trials suggested that a systematic review and meta-analysis of RCTs dealing with the prolonged use of PDE5i in all areas of medicine reporting changes of EcDy and/or of EcAc, should be performed. This research aimed to explore the use of PDE5i in reducing EcDy and/or EcAc in patients with vascular diseases or vascular risk conditions.

Methods

The study was conducted in accordance with the Cochrane Collaboration and PRISMA statement [25]. The study protocol was registered in the "PROSPERO International" prospective register of 'systematic reviews' at a website (link- https://www.crd.york.ac.uk/PROSPERO/) and bears a registration number CRD42017055399.

Data sources and searches

A complete literature search was carried out for the studies in English-language, in the MEDLINE (PubMed), Cochrane Library, SCOPUS, CINAHL, and Science direct, by using the Boolean function AND/OR. The search sentences are shown in **Table 1**.

Study selection

The eligibility criteria for selection of the studies included 1) RCTs; 2) continuous use of PDE5i at the therapeutic dosages for at least four weeks; 3) enrollment of patients (males and/or females) with VRFs or vascular diseases; 4) evaluation of EcDy and/or EcAc. Trials measuring outcomes with co-administration of PDE5i and drugs with a potential effect on endothelium were excluded from this research. Two independent reviewers (SDA and AM) evaluated the selected abstracts and full texts. In cases, where any disagreement resulted, a third reviewer (SF) took a decision after conducting an open discussion (**Figure 1)**.

Data extraction and quality assessment

One investigator (SDA) extracted data from the selected RCTs, considering the number of subjects, missing subjects at follow-up, mean age of subjects, study design, year of publication, financial disclosure, type of PDE5i, and dosage and duration of treatment. The quality of the RCTs was assessed by the Jadad scale [26] and a score of ≥3 identified high-quality studies. End-of-treatment mean ±standard deviation (SD) in the treated arm and in the placebo arm was considered for comparing the studies on the effect of treatment (**Table 2**). Summary statistics were calculated [27] and authors were contacted in cases of missing or inconsistent data. An investigator (SN) performed quality control checks on the extracted data and assessed the risk of bias through the Cochrane risk-of-bias algorithm [28] (**Table 3**).

Outcomes

Biomarkers of EcDy and EcAc, as well as non-invasive techniques to measure endothelial function, were included as outcomes, provided that each outcome was evaluated by two or more studies. Besides FMD of the brachial arteries, as a non-invasive measure of EcDy in conduit arteries [6], determination of peripheral (digital) arterial tonometry (EndoPAT, Itamar-Medical, Caesarea, Israel)

was included as an outcome since it represents a non-invasive method to assess EcDy in the resistance vessels and its deterioration correlates with cardiovascular risk [29].

Data synthesis and analysis

Data were meta-analyzed by the Review Manager (RevMan) Software (Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014) and the 'metafor' package in the R statistical software (version 3.0.3; The R Foundation for Statistical Computing, Vienna, Austria). The available data were combined using fixed or random effect models, as appropriate [30]. Standardized (std) mean differences with a 95% coefficient interval (CI) were estimated for each endpoint. Heterogeneity in the results of different studies was assessed by the Cochrane Chi-square χ^2 (Cochrane Q) statistic and the I^2 test. An I^2 <25% indicated no heterogeneity; whereas, I^2 values >50% and/or P values <0.05 indicated substantial heterogeneity [31]. Ninety-five percent prediction intervals (PIs) were included, as previously reported [32], when significant overall estimates in random-effect models were present, in order to illustrate heterogeneity that might not be completely conveyed by 95% CI. Meta-regression models and sub-group analyses were conducted to investigate study heterogeneity [33] by looking at the possible covariates that could affect the estimates: publication year, geographic region, age of participants, other disease (categorized as diabetic and non diabetic patients), type of PDE5i, Jadad quality score of the studies, and the length of the treatment (from 4 to 24 weeks). The publication bias was graphically explored using the Funnel plots [34].

Results

Study Selection

The search was conducted between January 2016 and March 2016 and was updated in November 2017 (**Figure 1**). One thousand and seventy-one studies were retrieved from the electronic source while five articles from manual search. One thousand and fifty-nine studies were selected after removing the duplicate studies. Three meta-analyses, 202 reviews and 776 non-human studies were excluded among these. One RCT was performed with a PDE3i, while two RCTs were non-English language studies and were thus excluded. A total of 75 studies were identified, out of which only 28 met the inclusion criteria. The excluded studies included 14 placebo-crossover trials, 7 trials with no

placebo-control group, 9 trials evaluating the effect of acute administration of PDE5i and 17 cohort studies. Thirteen studies [35–47], on the whole, were included in the meta-analysis (**Table 2**).

Study characteristics

The details of 932 subjects were obtained: 469 were allocated to PDE5i while 463 were assigned to the placebo group (**Table 2**). Four hundred and one patients were randomized to Sildenafil, 42 to Tadalafil, and 26 to Vardenafil. Ten studies enrolled only male patients, two studies enrolled male and female patients [35, 36], while one study enrolled only female patients [37]. The length of the treatment ranged from four weeks to 24 weeks. Dosage included: 50–150 mg/day for Sildenafil, 10–20 mg/day for Tadalafil and 20 mg/day for Vardenafil (**Table 2**). Four multicenter trials were included [35, 36, 37, 38].

Synthesis of results

Endothelial Cell Dysfunction: ***FMD of the brachial artery*** showed a significant improvement after PDE5i treatment, as assessed in eight RCTs, enrolling 150 patients treated with PDE5i and 148 patients treated with placebo (Std mean difference IV, random: 2.02; 95% CI: 0.99, 3.05; p <0.0001) (**Figure 2A**). ***EndoPAT*** was measured in three trials [36, 37, 39] enrolling 52 patients in the PDE5i arm and 56 in the placebo arm. The difference between treated and placebo arms was not significant (std mean difference IV, random: 1.92; 95% CI: –0.22, 4.07; p = 0.08) (**Figure 2A**). ***ET–1*** blood level was reported in six RCTs enrolling 213 patients in the treated arm and 204 patients in the placebo arm (**Figure 2B**). PDE5i treatment was associated with a significantly lower level of ET–1 as compared to placebo (std mean difference IV, fixed: –0.21; 95% CI: –0.41, –0.02; p = 0.03).

Endothelial Cell Activation: RCTs were performed in males affected by type 2 diabetes mellitus (**Figure 3**). IL–6, assessed in three trials, was found to be significantly lower in the treatment arm (189 treated, 190 placebo) (std mean difference IV, random: –0.67; 95% CI: –1.20, PI: –2.65, 1.31, –0.13; p = 0.01) [38, 39, 40]. No difference between PDE5i and placebo was observed in the C reactive protein (hCRP) level in two trials (36 treated and 38 placebo) (std mean difference IV, random: –0.03; 95% CI: –0.40, 0.33; p = 0.85) [39, 40]. The levels of intercellular adhesion molecule (ICAM)–1 were lower in the placebo arm of two RCTs (34 treated, 36 placebo arms) [40, 41]; the lower baseline level of ICAM–1 in the placebo groups probably explains the finding.

Risk of bias and publication bias

The studies showed a risk of selection bias (**Table 3**). Random sequence generation was appropriately described in seven trials [35, 36, 37, 40, 41, 42, 43]. Allocation concealment was rightly reported in five studies [35, 36, 37, 40, 43]. Eight trials showed a low risk of bias for blinding of participants and personnel [37, 39, 40, 43, 44, 45, 46] while three studies showed a low risk of bias for blinding of outcome assessment [43, 44, 46]. Three other studies showed a low risk of attrition bias [35, 37, 43]. The risk of reporting bias was, however, unclear in all trials.

Funnel plot depicted an asymmetry, which indicated substantial publication bias in the overall analysis of both FMD (**Fig. 4A**) and ET–1 (**Fig.4B**), with positive effects restricted only to the smaller studies.

Heterogeneity evaluation

A meta-regression analysis was applied to explore possible covariates causing the between-study high heterogeneity observed in the pooled comparative analyses. Predictors of potential sources of heterogeneity, such as publication year, geographic region, age of participants, concomitant diseases, type of PDE5i, Jadad quality score of the studies, and length of the treatment, were included in univariate meta-regression models.

The analysis for FMD showed that only the age of participants and the sample size of the studies could significantly contribute to the sources of heterogeneity; the improvement of FMD was observed to be higher in older populations (β= 0.25; 95% CI: 0.04, 0.45; p = 0.01) and in studies with smaller sample size (β= –0.31; 95% CI: –0.58, –0.04; p = 0.02) (**Figure 5**). Consequently, a sub-group analysis was performed and two trials that exhibited both predictors of heterogeneity were excluded from this trial [39, 47]. FMD was improved by PDE5i; although the pooled std mean difference was 0.92 (95% CI: 0.23, 1.60; p = 0.009), yet the heterogeneity was still high and severe (P for heterogeneity <0.0001, I^2: 84%). Thus, the studies presented undefined sources of heterogeneity which were uncorrectable.

Predictors of Std mean differences in case of ET–1 levels included publication year (β = 0.087; 95% CI: 0.018, 0.155; p = 0.01), the Jadad quality score of the included studies (β = 0.157; 95% CI: 0.025, 0.288; p = 0.02), and the length of treatment (β = –0.028; 95% CI: 0.004, 0.051; p = 0.02). A higher ET–1-lowering effect of PDE5i was reported in older trials with poor Jadad quality score and a short treatment length. Accordingly, a sub-group analysis which excluded the oldest trial, also exhibiting

the lowest Jadad quality score [47], did not show a significant overall decrease in ET–1 serum levels in PDE5i arm when compared to placebo (Std mean difference IV, fixed: –0.16; 95% CI: –0.36, 0.05; p = 0.12) with no heterogeneity ($P_{for\ heterogeneity}$ = 0.5, I^2: 0%) **(Figure 6).**

DISCUSSION

PDE5i has a beneficial effect on endothelial cells, which is related to the stimulated NO availability [15, 16]; this fosters the potential use of this class of drugs in reducing EcDy [48]. This was the first study to analyze RCTs in all areas of medicine, dealing, in particular, with a prolonged use of PDE5i to improve EcDy and/or EcAc as treatment outcome. The meta-analysis included trials of male patients affected by type 2 diabetes, with or without ED; male and female patients with chronic heart diseases and male patients with VRFs and ED. This analysis suggests a beneficial effect of a prolonged treatment with PDE5i on EcDy in patients with vascular diseases or VRFs. However, these findings are not conclusive as the overall quality of evidence is low. The data on EcAc biomarkers were very limited in number and were insufficient to assess any treatment effect of PDE5i.

Endothelial Cell Dysfunction

EcDy was analyzed in the conduit arteries by evaluating FMD of the brachial artery, and in resistance microvessels by EndoPAT [29], while blood level of ET–1 was used as a biomarker of EcDy [2].

Improved **FMD** was observed in treated patients as compared to the placebo group, independent of the type of PDE5-i and duration of treatment. The effect, however, is questioned by the result of the funnel plot (**Figure 4A**) that showed a publication bias and a positive effect of PDE5-i in smaller studies only. A significant effect of PDE5i on FMD is also questioned by the elevated heterogeneity, which is probably related to the paucity of trials. Only two of the studies [42, 44] reported a baseline FMD that was significantly lower in the patients as compared to that in healthy controls. The improved FMD after treatment was more relevant in patients with a lower baseline FMD [44] suggesting that the beneficial effect of PDE5i might be dependent on the presence of a baseline documented EcDy in the conduit arteries. The available data were therefore not conclusive of the effect of PDE5i on EcDy assessed in conduit arteries in males with vascular disease and vascular risk related conditions. The effect of PDE5i on EcDy in resistance vessels, as assessed by peripheral (digital) arterial tonometry, was reviewed in this study for the first time in the literature. Meta-analysis restricted to three RCTs showed no significant effect of Sildenafil on resistance vessels (**Figure 2A**). The results of the meta-analysis were confirmed by two multicenter RCTs, which were

initially excluded from this meta-analysis study because of insufficient data [49, 50] and by a RCT in patients with chronic heart disease [51], excluded from the analysis as it was the only trial assessing vascular effects of PDE5i treatment by strain-gauge plethysmography. Overall, the data suggest that PDE5i has no effect on endothelial function in the resistance vessels. The observation that arteriolar volume changes after transitory ischemia is not entirely dependent on NO [52], probably helped to explain the limited effect of PDE5i on resistance vessels. The low number of trials suggests caution on the conclusion. Level of **ET–1** as a biomarker for EcDy has resulted to be significantly reduced (**Figure 2B**), although the effect was no more present after correcting the publication bias and heterogeneity. Overall, available data showed a positive effect of PDE5–1 on the circulating levels of ET–1, nevertheless the low quality of evidence has questioned the conclusion.

Endothelial cell Activation

Although numerous biomarkers of EcAc have been proposed, yet, most of them are not specific for endothelial cells and may derive from multiple types of activated cells, including neutrophils, platelets, mast cells, or macrophages [1]. Meta-analysis was restricted to a limited number of trials carried out in males with type 2 diabetes**,** assessing the PDE5i effect of EcAc on circulating levels of non-specific markers (IL–6, hCRP, ICAM–1). Three RCTs, excluded from the meta-analysis, reported that treatment with Tadalafil [51, 53], or with Sildenafil [50], was ineffective in reducing blood levels of more specific (E-selectin and von Willebrand factor) and less specific biomarkers of EcAc (hCRP, plasminogen activator inhibitor, VCAM–1, and ICAM–1). On the whole, PDE5i were not effective in reducing the blood levels of biomarkers of EcAc.

The added value of this research to previous meta-analyses on the same topic

Two meta-analyses have been produced on the same topic. One evaluated four RCTs (two trials on males with chronic heart disease and two trials on males with VRFs and ED) measuring the effect of a prolonged administration of PDE5i on FMD of the brachial artery [23]. A positive effect was observed on ED patients only; however, the high heterogeneity limited the validity of the findings. The second meta-analysis evaluated the effect of a continuous treatment with Sildenafil on FMD and on different blood biomarkers (ET–1, hCRP and IL–6) in six RCTs done on male patients affected by type–2 diabetes [24]. Authors concluded that Sidenafil treatment resulted in a positive effect on FMD in the brachial artery and on reduced circulating levels of IL–6. Nine RCTs were cumulatively analyzed in the two previous meta-analyses which also included a crossover study [54] and an RCT

with incompletely reported data [55]. The latter two studies were excluded from the present quantitative analysis which included 13 RCTs involving a continuous PDE5i treatment in all areas of medicine, including diabetes, chronic heart disease, VRFs associated to ED, and female obesity associated either with metabolic syndrome or hyperinsulinemia. Besides the evaluation of FMD in the brachial artery, the authors of this study have considered for the first time, the effect of PDE5i on endothelial cell function in resistance vessels, as well as the effects of this class of medications on ET–1 levels and on all available biomarkers of EcAc. This represents the first attempt to comprehensively analyze the effect of a prolonged use of PDE5i on endothelium.

Limitations

The present meta-analysis has a number of limitations. The baseline severity of EcDy has seldom been reported, probably increasing the heterogeneity of data substantially, due to the differences in baseline clinical characteristics, type and length of treatment. The paucity of studies did not allow the authors to perform subgroup analysis according to type and length of treatment, as well as on different clinical characteristics. The trials including male and female patients did not report outcome related to gender differences; therefore, the gender contribution to different results was also left undetermined. Vascular function was assessed either by FMD in the brachial artery or by measurement of EndoPAT hyperemic response in resistance vessels. The latter, however, was restricted to three trials only. A high risk of selection bias and publication bias was present and the risk of the reporting bias was unclear in all RCTs. On the contrary, industrial bias was apparently low, since all RCTs were spontaneous only, with occasional external support. Conclusions on different outcomes and subgroup analyses were assessed only in two trials, suggesting concern on their relevance.

Conclusions

The results on the benefits of a prolonged use of phosphodiesterase–5 selective inhibitors, with the objective of lowering endothelial cell dysfunction are not convincing. This is due to the overall low quality of available evidence. The analysis highlights the necessity of promoting uniform, multicenter trials having enough time to determine the effect of treatment of PDE5 selective inhibitors in order to support their clinical utility over vascular risk. The use of PDE5 selective inhibitors to reduce endothelial cell dysfunction in patients with vascular diseases or vascular risk conditions does not have a clear scientific support as per the literature available. Yet, hygienic measures and lifestyle changes must be considered for their potential ability to reduce cardiovascular risk.

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Data sharing. No additional data available.

Ethical approval. Ethical approval and patient consent were not required as this study was a meta-analysis based on published data.

Authors' contributions. SDA acquisition of data, analysis and interpretation of data, drafting the article; AB analysis and interpretation of data, drafting the article; AM acquisition of data; SN statistical analysis and interpretation of data; FF critical revision for important intellectual content; SF conception and design, analysis and interpretation of data, critical revision for important intellectual content, drafting the article, final approval of the version to be published. All authors read and approved the final manuscript.

Transparency declaration. Arcangelo Barbonetti and D'Andrea Settimio, on behalf of all authors, affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; no important aspects of the studies have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained

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FIGURE LEGENDS

FIGURE 1: Study flow diagram. See text for excluded studies.

FIGURE 2: Effects of PDE5i over placebo, A: on brachial artery flow-mediated dilation (FMD) and on EndoPAT; B: on the blood level of Endothelin (ET)–1.

ET–1 values were expressed in pg/mL; FMD was expressed as a percentage change in the maximal brachial artery diameter after reactive hyperemia; EndoPAT was expressed as reactive hyperemic change in digital blood flow in response to upper arm cuff occlusion; a reactive hyperemic index was determined. * trials with male and female patients; ° trials with female patients only.

FIGURE 3: **Effects of PDE5i over placebo on blood levels of Interleukin (IL)–6, Intercellular adhesion molecule (ICAM)–1, and human C-reactive protein (hCRP).**

IL–6 levels were expressed in pg/mL; ICAM–1 levels were expressed in ng/mL; hCRP levels were expressed in mg/dL.

FIGURE 4: Funnel plot; A: brachial artery flow-mediated dilation (FMD); B: Endothelin (ET)–1 serum level.

FIGURE 5: Meta-regression bubble plot: standardized mean differences (SMD) in flow-mediated dilation (FMD) of brachial artery between PDE5i treated and placebo-treated groups as a function of mean age and sample size. SMD values above zero indicate higher FMD in the PDE5i-treated group; SMD values below zero indicate higher FMD in the control group. The predicted effects (solid line) with corresponding confidence intervals (dashed line) are also shown.

FIGURE 6: Forest plot including the results of sub-group analysis on Endothelin (ET)–1.

ET–1 values were expressed in pg/mL.

Table 1. Sentences for the search in the literature.

MEDLINE
("phosphodiesterase 5" OR "phosphodiesterase 5 inhibitors" OR "phosphodiesterase 5 inhibitor" OR "phosphodiesterase type 5 inhibitors" OR "phosphodiesterase type 5 inhibitor" OR "phosphodiesterase-5 inhibitors" OR "phosphodiesterase-5 inhibitor" OR "inhibitors of phosphodiesterase" OR ""inhibitor of phosphodiesterase" OR ""inhibitors of phosphodiesterase 5" OR "inhibitor of phosphodiesterase 5" OR "inhibitors of phosphodiesterase-5" OR "inhibitor of phosphodiesterase-5" OR "inhibitors of phosphodiesterase type 5" OR "inhibitor of phosphodiesterase type5" OR "PDE5i" OR "PDE5 inhibitors" OR "PDE5 inhibitor" OR "PDE type 5 inhibitors" OR "PDE type 5 inhibitor" OR "PDE-5 inhibitors" OR "PDE-5 inhibitor" OR "sildenafil" OR "vardenafil" OR "tadalafil" OR "avanafil" OR "udenafil") AND ("endothelial activation" OR "endothelium activation" OR "endothelial dysfunction" OR "endothelium dysfunction" OR "endothelium repair" OR "endothelial repair" OR "endothelial cell" OR "endothelial cells" OR "endothelial stem cells" OR "endothelial stem cell" OR "endothelium cell" OR "endothelium cells" OR "endothelium stem cells" OR "endothelium stem cell" OR "angiogenic cells" OR "angiogenic cell" OR "circulating angiogenic cells" OR "circulating angiogenic cell" OR "vascular function" OR "vascular dysfunction" OR "vascular repair" OR "flow mediated dilation" OR "flow mediated vasodilation" OR "flow mediated dilatation" OR "brachial artery flow mediated dilation" OR "selectin" OR "e-selectin" OR "VCAM" OR "ICAM" OR "ET-1" OR "endothelin 1" OR "endothelial activation factor" OR "endothelial activation factors" OR "plasminogen activator inhibitor-1" OR "PAI-1" OR "CRP" OR "c reactive protein")
SCOPUS
("phosphodiesterase type 5 inhibitors"/exp OR "phosphodiesterase 5" OR "phosphodiesterase 5 inhibitors" OR "phosphodiesterase 5 inhibitor" OR "phosphodiesterase type 5 inhibitor" OR "phosphodiesterase-5 inhibitors" OR "phosphodiesterase-5 inhibitor" OR "inhibitors of phosphodiesterase" OR ""inhibitor of phosphodiesterase" OR ""inhibitors of phosphodiesterase 5" OR "inhibitor of phosphodiesterase 5" OR "inhibitors of phosphodiesterase-5" OR "inhibitor of phosphodiesterase-5" OR "inhibitors of phosphodiesterase type 5" OR "inhibitor of phosphodiesterase type5" OR "PDE5i" OR "PDE5 inhibitors" OR "PDE5 inhibitor" OR "PDE type 5 inhibitors" OR "PDE type 5 inhibitor" OR "PDE-5 inhibitors" OR "PDE-5 inhibitor" OR "sildenafil" OR "vardenafil" OR "tadalafil" OR "avanafil" OR "udenafil") AND ("endothelial activation"/exp OR "endothelium activation" OR "endothelial dysfunction" OR "endothelium dysfunction" OR "endothelium repair" OR "endothelial repair" OR "endothelial cell" OR "endothelial cells" OR "endothelial stem cells" OR "endothelial stem cell" OR "endothelium cell" OR "endothelium cells" OR "endothelium stem cells" OR "endothelium stem cell" OR "angiogenic cells" OR "angiogenic cell" OR "circulating angiogenic cells" OR "circulating angiogenic cell" OR "vascular function" OR "vascular dysfunction" OR "vascular repair" OR "flow mediated dilation" OR "flow mediated vasodilation" OR "flow mediated dilatation" OR "brachial artery flow mediated dilation" OR "selectin" OR "e-selectin" OR "VCAM" OR "ICAM" OR "ET-1" OR "endothelin 1" OR "endothelial activation factor" OR "endothelial activation factors" OR "plasminogen activator inhibitor-1" OR "PAI-1" OR "CRP" OR "c reactive protein") AND [humans]/lim AND [scopus]/lim

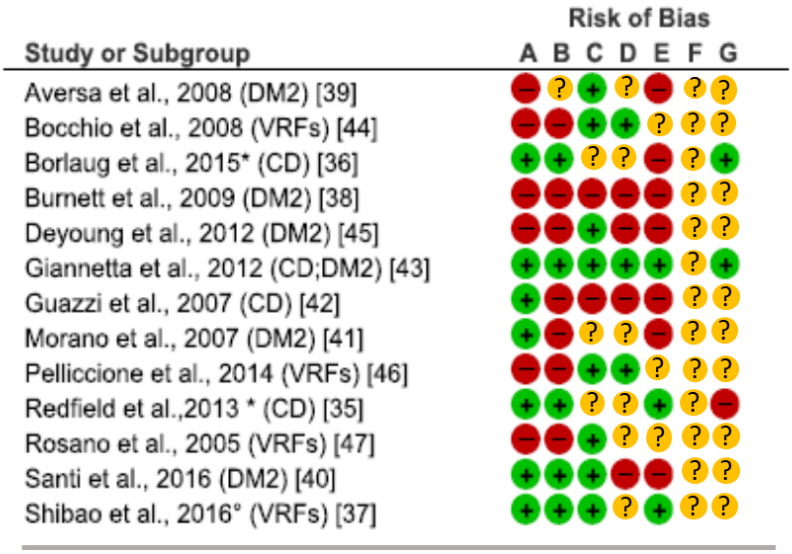
Table 2. Features of study included in the meta-analysis.

References	N° patients PDE5i (placebo)	Mean age PDE5i (placebo)	Sex	Drugs	Duration (weeks)	Condition	Outcomes	Jadad Score	Concurrent therapy
Aversa *et al*., 2008 [39]	10(10)	64,2±5,4 (63,6±5,7)	M	Sildenafil (75 mg/d)	4	DM2	Brachial artery FMD, EndoPAT, IL-6, hCRP, VCAM-1, ICAM-1	3	hypoglycemic oral drugs; statins; diuretics, Ca-antagonist, beta-blockers; Ace-inhibitors; Angiotensin-inhibitors;
Bocchio *et al*., 2008 [44]	18(18)	52,1±8,9 (49,6±12,8)	M	Tadalafil (20mg/od)	4	VRFs	Brachial artery FMD, Carotid artery IMT, CACs	1	Hypoglycemic agents, aspirin, diuretics, insulin, ACE-inhibitors, Ca-antagonist
Borlaug *et al*., 2015 [36]	23(25)	67,6±7,9 (71,0±9,4)	M/F	Sildenafil (20 mg/tid 60mg/tid)	24	CD	EndoPAT, Echocardiographic assessment	5	Beta-blockers, ACE-inhibitor, angiotensin rec blockers, diuretic
Burnett *et al*., 2009 [38]	153(152)	NA (NA)	M	Sildenafil (100mg/d)	4	DM2	cGMP,IL-6, IL-8, 8-isoprostane	1	Not reported
Deyoung *et al*., 2012 [45]	12(12)	59,4 (59,8)	M	Sildenafil (50mg/d)	10	DM2	IIEF-5, Brachial artery FMD	1	Not reported
Giannetta *et al*., 2012 [43]	30(29)	60,7±7,6 (60,2±8,3)	M	Sildenafil (100mg/d)	12	DM2, CD	Cardiac Kinetics, ET-1, VEGF, BNP, PINP, TGF-β	5	Diuretics, ACE-inhibitors, hypoglycemic oral drugs, beta-blockers, statins, angioantensina blocker
Guazzi *et al*., 2007 [42]	23(23)	62,0±3,0 (63,0±4,0)	M	Sildenafil (50mg/td)	24	CD	Brachial artery FMD, CPET	1	Diuretic, Ace-inhibitors, Angiotensin receptor blocker, digoxin, beta-blocker
Morano *et al*., 2007 [41]	8(8)	54,0±7,4 (57,6±4,3)	M	Sildenafil (50mg/tw)	12	DM2	IIEF-5, AGE, P-selectin, Monocyte	4	hypoglycemic oral drugs, other not reported

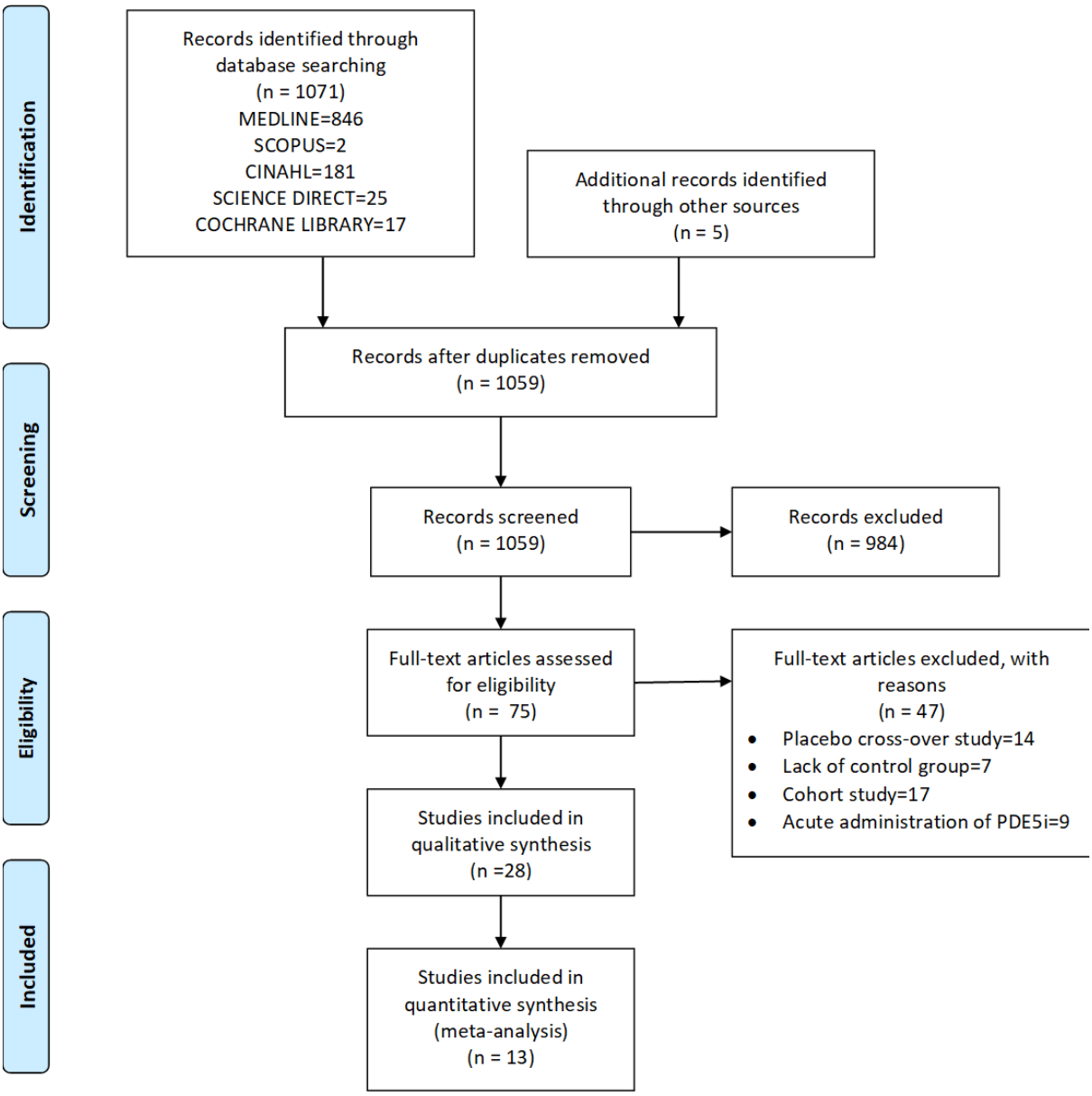
							Oxidative Activity, ICAM-1		
Pelliccione *et al*., 2014 [46]	18(18)	52,1±8,9 (49,6±12,8)	M	Tadalafil (20mg/od)	4	VRFs	Carotid artery IMT, CACs, ET-1, E-selectin, tPA	1	Aspirin, beta-blocker, diuretic, ACE-inhibitors, insulin,
Redfield *et al*., 2013 [35]	113(103)	69,3±11,3 (69,0±11,3)	M/F	Sildenafil (20 mg/tid 60mg/tid)	24	CD	Et-1, peak oxygen consumption, six-minute walk distance test	5	hypoglycemic oral drugs, diuretics, Ace-inhibitors, Angiotensin receptor blocker
Rosano *et al*., 2005 [47]	16(16)	65,4±6,3 (65,4±6,3)	M	Tadalafil (20mg/od)	4	VRFs	Brachial artery FMD, ET-1, Nitrates serum level	1	Statin, hypoglycemic oral drugs, anti-hypertensive drugs (ca-antagonist, Ace-inhibitors, angiotensin blocker)
Santi *et al*., 2016 [40]	26(28)	55,8±5,0 (55,0±5,0)	M	Vardenafil (10mg/td)	12	DM2	IIEF-15,LH, FSH, TT, Brachial artery FMD, IL-6, ET-1, ICAM-1, VCAM-1, hCRP, Fibrinogen	5	hypoglycemic oral drugs, insulin, anti-hypertensive drugs
Shibao et al., 2016 [37]	19(21)	42.0±8.6 (43.0±10.4)	F	Sildenafil (20mg/tid)	4	VRFs	Insulin sensitivity, Brachial artery FMD, EndoPAT, gene variant	3	anti-hypertensive drugs (excluded adrenergic α-blocking)

AGE= advanced glycation end product. BNP= B-type natriuretic peptide. CACS= Circulating Angiogenic Cells. CD= chronic heart disease. CPET= cardiopulmonary exercise testing. hCRP= C-reactive protein. d= once day. od= every other day. DM2= diabetes mellitus 2 . E-selectin = endothelial selectin. ET-1= endothelin-1. FMD= Flow mediated Dilation. ICAM-1= Intercellular adhesion molecule. IL-6= Interleukin-6. IL-8= Interleukin-8. IMT = Intima-Media Thickness. IIEF-5= International Index of Erectile Function. MCP1= monocyte chemotactic protein-1. MPO= myeloperoxidase. P-selectin = platelet selectin. PINP= procollagen aminoterminal propeptide. td= twice daily. TGF-β= transforming growth factor-β. tid= three time a day. tPA= tissue-type plasminogen activator. TT= total testosteron. tw= twice weekly. VCAM-1= Vascular adhesion molecule. VEGF= vascular endothelial growth factor. VRFs= vascular risk factors.

Table 3. Risk of bias.

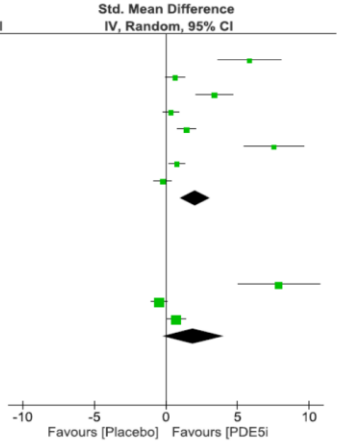


CD= chronic heart disease. DM2= diabetes mellitus 2. VRFs= vascular risk factors. * trials with male and female patients; ° ° trials with female patients only



A

Study or Subgroup	PDE5i Mean	PDE5i SD	PDE5i Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Std. Mean Difference IV, Random, 95% CI
FMD								
Aversa et al., 2008 (DM2) [39]	12.5	0.7	10	6.5	1.2	10	8.8%	5.85 [3.65, 8.05]
Bocchio et al., 2008 (VRFs) [44]	8.19	2.67	18	6.14	3.55	18	13.9%	0.64 [-0.03, 1.31]
Deyoung et al., 2012 (DM2) [45]	4.5	0.1	12	4.15	0.1	12	11.9%	3.38 [2.06, 4.70]
Giannetta et al., 2012 (CD;DM2) [43]	15.67	11.25	26	11.08	14.97	20	14.1%	0.35 [-0.24, 0.94]
Guazzi et al., 2007 (CD) [42]	12.7	0.7	23	11.6	0.8	23	13.9%	1.44 [0.78, 2.09]
Rosano et al., 2005 (VRFs) [47]	9.3	0.3	16	4.1	0.9	16	9.2%	7.56 [5.46, 9.65]
Santi et al., 2016 (DM2) [40]	8.57	2.84	26	6.41	2.77	28	14.2%	0.76 [0.21, 1.31]
Shibao et al., 2016° (VRFs) [37]	5.3	2.8	19	6	3.2	21	14.0%	-0.23 [-0.85, 0.40]
Subtotal (95% CI)			**150**			**148**	**100.0%**	**2.02 [0.99, 3.05]**
Heterogeneity: Tau² = 1.87; Chi² = 91.15, df = 7 (P < 0.00001); I² = 92%								
Test for overall effect: Z = 3.85 (P = 0.0001)								
EndoPAT								
Aversa et al., 2008 (DM2) [39]	1.98	0.05	10	1.33	0.1	10	23.2%	7.87 [5.01, 10.74]
Borlaug et al., 2015* (CD) [36]	1.55	0.3	23	1.73	0.43	25	38.5%	-0.47 [-1.05, 0.10]
Shibao et al., 2016° (VRFs) [37]	2.6	0.77	19	2.1	0.6	21	38.2%	0.71 [0.07, 1.36]
Subtotal (95% CI)			**52**			**56**	**100.0%**	**1.92 [-0.22, 4.07]**
Heterogeneity: Tau² = 3.02; Chi² = 35.38, df = 2 (P < 0.00001); I² = 94%								
Test for overall effect: Z = 1.75 (P = 0.08)								



B

Study or Subgroup	PDE5i Mean	PDE5i SD	PDE5i Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Endothelin 1								
Aversa et al., 2008 (DM2) [39]	2.8	0.7	10	3.4	0.8	10	4.5%	-0.76 [-1.68, 0.15]
Giannetta et al., 2012 (CD;DM2) [43]	510	150	30	550	150	29	14.3%	-0.26 [-0.78, 0.25]
Pelliccione et al., 2014 (VRFs) [46]	2.5	1.8	18	3.5	2.6	18	8.6%	-0.44 [-1.10, 0.22]
Redfield et al.,2013 * (CD) [35]	2.47	1.03	113	2.51	0.83	103	52.6%	-0.04 [-0.31, 0.22]
Rosano et al., 2005 (VRFs) [47]	2.9	0.7	16	3.6	0.7	16	6.9%	-0.97 [-1.71, -0.24]
Santi et al., 2016 (DM2) [40]	1.38	0.38	26	1.42	0.4	28	13.2%	-0.10 [-0.64, 0.43]
Subtotal (95% CI)			**213**			**204**	**100.0%**	**-0.21 [-0.41, -0.02]**
Heterogeneity: Chi² = 7.70, df = 5 (P = 0.17); I² = 35%								
Test for overall effect: Z = 2.14 (P = 0.03)								
Total (95% CI)			**213**			**204**	**100.0%**	**-0.21 [-0.41, -0.02]**
Heterogeneity: Chi² = 7.70, df = 5 (P = 0.17); I² = 35%								
Test for overall effect: Z = 2.14 (P = 0.03)								
Test for subgroup differences: Not applicable