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Does Statin therapy Reduce Plasma VEGF levels in humans? A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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28 **Abstract**

29 **Background:** The effect of statins on plasma concentrations of vascular endothelial growth factor (VEGF),
30 the main angiogenic growth factor with pro-inflammatory and atherogenic properties, is controversial. A
31 systematic review and meta-analysis of randomized controlled trials (RCTs) was conducted to obtain a
32 conclusive result in humans.

33 **Methods:** PubMed-Medline, SCOPUS, Web of Science and Google Scholar databases were searched to
34 identify RCTs investigating the impact of statins on plasma VEGF concentrations. A random-effects model
35 and the generic inverse variance method were used for quantitative data synthesis. Meta-regression,
36 sensitivity analysis and publication bias assessments were performed using standard methods.

37 **Results:** Eight RCTs examining the effects of statins on plasma VEGF concentrations were included. Meta-
38 analysis suggested a significant reduction of plasma VEGF levels following statin therapy (weighed mean
39 difference: -19.88 pg/mL, 95% CI: -35.87, -3.89, $p=0.015$). VEGF reductions were observed in the subsets of
40 trials with treatment durations ≥ 4 weeks (-19.54, -37.78, -1.30, $p=0.036$), LDL-C reductions ≥ 50 mg/dL
41 (-28.59, -43.68, -13.50, $p<0.001$), lipophilic statins (-22.31, -40.65, -3.98, $p=0.017$), and diseased populations
42 (-21.08, -39.97, -2.18, $p=0.029$), but not in the opposite subsets. Meta-regression also suggested a
43 significant association between changes in plasma VEGF levels and LDL-C changes, treatment duration, but
44 not molar dose of statins.

45 **Conclusions:** These results suggest a significant reduction in plasma VEGF concentrations following statin
46 therapy. This effect depends on duration of treatment, LDL-lowering activity, lipophilicity of statins, and
47 health status of studied individuals. Further RCTs are needed to explore if the VEGF reduction is implicated
48 in the statin benefits on cardiovascular outcomes.

49

50 **Keywords:** Angiogenesis; Atherosclerosis; Cholesterol; Ischemic heart diseases; Pleiotropic effect; Statins

51 **Abbreviations:** BMI (body mass index) CI (confidence interval) EPCs (circulating endothelial progenitor cells) hs-CRP
52 (high sensitivity C-reactive protein) HDL-C (high density lipoprotein cholesterol) LDL-C (low density lipoprotein
53 cholesterol) RCT (randomized controlled trial) VEGF (vascular endothelial growth factor) WMD (weighed mean
54 difference)

55 **Introduction**

56 Vascular permeability, vasculogenesis and angiogenesis are regulated by a complex interplay among several
57 growth factors and their associated receptors. In this process, vascular endothelial growth factor (VEGF)
58 family and its receptors play an essential role [1-2]. The VEGF family consists of different isoforms with
59 several subtypes; each isoform performs a different role in the endothelial and vascular physiology and
60 pathology, as comprehensively reviewed [1-4]. In particular, VEGF is involved in vascular development,
61 integrity, homeostasis, thrombogenicity modulation, recruitment of hematopoietic precursors and
62 migration of monocytes and macrophages. The angiogenic, permeability-enhancing and pro-inflammatory
63 properties of VEGF determine its role in pathological conditions, such as cancer, ischemia and inflammation
64 [1-4]. At a cardiovascular level, VEGF is implicated in the progression of atherosclerosis, instability of
65 atherosclerotic plaque through induction of neoangiogenesis inside the plaque, prediction of worse clinical
66 outcomes in acute coronary syndromes, and cardiac hypertrophy through a nitric oxide (NO)-dependent
67 mechanism [1-7].

68 Hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins) are known to exert beneficial effects on
69 the clinical outcomes of cardiovascular diseases both by their lipid-lowering, anti-inflammatory, antioxidant
70 and antithrombotic effects and by improving endothelial function, attenuating vascular/myocardial
71 remodeling and stabilizing atherosclerotic plaques [8-9]. Alternative additional mechanisms by which
72 statins may reduce cardiovascular events beyond their lipid reduction effects may be the modulation of
73 angiogenesis by reducing VEGF levels, as suggested by some case-control human studies performed almost
74 a decade ago [10-11]. More recently, the effects of different statins on the reduction of VEGF levels have
75 been shown [12-19]; however, the results of human studies have not been fully conclusive [20-26]. In
76 addition, some experimental *in-vitro* and animal studies have suggested a statin-induced stimulation of
77 VEGF expression after endothelial and vascular injuries [27-32]. Furthermore, there is evidence indicating
78 that statins could directly augment circulating endothelial progenitor cells (EPCs) through mechanisms
79 independent of VEGF [17,19,21-22,33]. Therefore, at present the role of statins on the VEGF homeostasis is
80 very controversial.

81 The aim of the present study was to conduct a systematic review and meta-analysis of randomized
82 controlled trials (RCTs) to clarify the effect of statin treatment on plasma concentrations of VEGF in
83 humans.

84

85 **Methods**

86 *Search Strategy*

87 This study was designed according to the guidelines of the 2009 preferred reporting items for systematic
88 reviews and meta-analysis (PRISMA) statement [34]. PubMed-Medline, SCOPUS, Web of Science and
89 Google Scholar databases were searched using the following search terms in titles and abstracts (also in
90 combination with MESH terms): (atorvastatin OR simvastatin OR rosuvastatin OR fluvastatin OR pravastatin
91 OR pitavastatin OR lovastatin OR cerivastatin OR “statin therapy” OR statins) AND (VEGF OR “vascular
92 endothelial growth factor” OR VEGF-A). The wild-card term “*” was used to increase the sensitivity of the
93 search strategy. No language restriction was used in the literature search. The search was limited to studies
94 in humans. The literature was searched from inception to January 08, 2015.

95

96 *Study Selection*

97 Original studies were included if they met the following inclusion criteria: (i) being a randomized controlled
98 trial with either parallel or cross-over design, (ii) investigating the impact of statin therapy on
99 plasma/serum concentrations of VEGF, (iii) treatment duration of at least two weeks, (iv) presentation of
100 sufficient information on VEGF concentrations at baseline and at the end of follow-up in each group or
101 providing the net change values. Exclusion criteria were (i) non-randomized trials, (ii) lack of an appropriate
102 control group for statin therapy, (iii) observational studies with case-control, cross-sectional or cohort
103 design, and (iv) lack of sufficient information on baseline or follow-up VEGF concentrations.

104

105 *Data extraction*

106 Eligible studies were reviewed and the following data were abstracted: 1) first author's name; 2) year of
107 publication; 3) study location; 4) study design; 5) number of participants in the statin and control (in case of

108 randomized design) groups; 5) age, gender and body mass index (BMI) of study participants; 6) baseline
 109 levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol
 110 (HDL-C), triglycerides, high-sensitivity C-reactive protein (hs-CRP) and glucose; 7) systolic and diastolic
 111 blood pressures; and 8) data regarding baseline and follow-up concentrations of VEGF.

112

113 *Quality assessment*

114 A systematic assessment of bias in the included studies was performed using the Cochrane criteria [35]. The
 115 items used for the assessment of each study were as follows: adequacy of sequence generation, allocation
 116 concealment, blinding, addressing of dropouts (incomplete outcome data), selective outcome reporting,
 117 and other potential sources of bias. According to the recommendations of the Cochrane Handbook, a
 118 judgment of “yes” indicated low risk of bias, while “no” indicated high risk of bias. Labeling an item as
 119 “unclear” indicated an unclear or unknown risk of bias.

120

121 *Quantitative Data Synthesis*

122 Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ) [36]. Net
 123 changes in measurements (change scores) were calculated as follows: measure at end of follow-up –
 124 measure at baseline. For single-arm cross-over trials, net change in plasma concentrations of VEGF were
 125 calculated by subtracting the value after control intervention from that reported after treatment. Standard
 126 deviations (SDs) of the mean difference were calculated using the following formula: $SD = \text{square root}$
 127 $[(SD_{\text{pre-treatment}})^2 + (SD_{\text{post-treatment}})^2 - (2R \times SD_{\text{pre-treatment}} \times SD_{\text{post-treatment}})]$, assuming a correlation coefficient (R)
 128 = 0.5. Where standard error of the mean (SEM) was only reported, standard deviation (SD) was estimated
 129 using the following formula: $SD = SEM \times \text{sqrt}(n)$, where n is the number of subjects.

130 A random-effects model (using DerSimonian-Laird method) and the generic inverse variance method were
 131 used to compensate for the heterogeneity of studies in terms of demographic characteristics of populations
 132 being studied and also differences in study design and type of statin being studied [37]. Heterogeneity was
 133 quantitatively assessed using I^2 index. Effect sizes were expressed as weighed mean difference (WMD) and
 134 95% confidence interval (CI). Subgroup analyses were carried out to explore the impact of duration (< 4

weeks versus ≥ 4 weeks) of statin therapy and type (lipophilic versus hydrophilic) of statin therapy as well as magnitude of reduction in plasma LDL-C concentrations (< 50 mg/dL versus ≥ 50 mg/dL) on plasma VEGF alterations. To avoid the problem of double-counting in RCTs with multiple treatment arms and a common control group, the number of subjects in the control group was splitted among the required comparisons. In order to evaluate the influence of each study on the overall effect size, sensitivity analysis was conducted using leave-one-out method, i.e. removing one study each time and repeating the analysis [38-39].

Meta-regression

Random-effects meta-regression was performed using unrestricted maximum likelihood method to evaluate the association between calculated WMD and potential confounders including duration of treatment with statins, molar dose of statins, and magnitude of LDL-C reduction by statin therapy.

Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, and Begg's rank correlation and Egger's weighted regression tests. Duval & Tweedie "trim and fill" and "fail-safe N" methods were used to adjust the analysis for the effects of publication bias [40].

Results

Flow and characteristics of included studies

With the initial literature search, 235 articles were found (**Figure 1**). All these records were screened, and 219 did not meet the inclusion criteria. The full text of the remaining 16 was carefully assessed for eligibility and 8 were selected for the meta-analysis because they satisfied the inclusion criteria. Reasons for rejecting the other 8 articles were: lack of measurements of VEGF concentrations, non-interventional design, short (< 2 weeks) treatment duration, lack of control for statin therapy, and incomplete data on VEGF concentrations.

161 A total number of 330 subjects were included in the 8 eligible studies, comprising 185 individuals treated
 162 with statins and 145 individuals treated with placebo; subjects in the only cross-over trial were counted 3-
 163 times, because they were sequentially treated with 3 different statins (**Table 1**). Overall, we have evaluated
 164 8 eligible studies with 10 treatment arms. The largest study had a population size of 65 subjects [15], while
 165 the smallest study recruited only 12 subjects [18]. Included studies were published between 2006-2014 and
 166 were conducted in the United States [18], Japan [12-13,15,21-23], Greece [17], and Italy [22]. The following
 167 statins were used: pravastatin [18,21], atorvastatin [12-13,18,22], rosuvastatin [17-18] and pitavastatin
 168 [15,23]. The duration of statin therapy was variable, ranging from 14 days [13] to 6 months [12]. Most of
 169 these randomized trials were placebo-controlled and had a parallel design [12,15,17,21-23], one was a
 170 single-arm triple crossover trial [18], and one compared aspirin plus statin with aspirin alone, without
 171 placebo [13]. The inclusion criteria were heterogeneous: healthy subjects [18]; healthy subjects with low
 172 HDL-cholesterol [21]; chronic smokers with mild hypercholesterolemia [23]; patients with acute coronary
 173 syndromes [12,15]; subjects undergoing coronary artery bypass grafting [13,22]; and subjects with
 174 congestive heart failure [17]. The demographic and baseline biochemical parameters of the included
 175 studies are shown in Table 1.

176

177 *Risk of bias assessment*

178 A half of the analyzed studies provided insufficient information about randomization procedures (**Table 2**).
 179 Similarly, blinding of participants or researchers was often inadequate, since blinding of the study
 180 personnel [17] or of the participants [18] was unknown. Furthermore, some study designs did not include a
 181 placebo arm [13,23], and most authors did not report about missing data. However, all studies appeared to
 182 be free of selective outcome reporting and of other sources of bias.

183

184 *Effect of statin therapy on plasma VEGF concentrations*

185 Meta-analysis of data from 10 RCT arms revealed a significant reduction of plasma VEGF concentrations
 186 following treatment with statins (WMD: -19.88 pg/mL, 95% CI: -35.87, -3.89, $p = 0.015$). This effect was
 187 robust in the sensitivity analysis (**Figure 2**). In subgroup analysis, VEGF reduction was observed in the

subsets of trials with treatment durations ≥ 4 weeks (WMD: -19.54 pg/mL, 95% CI: -37.78, -1.30, $p = 0.036$), and LDL-C reductions ≥ 50 mg/dL (WMD: -28.59 pg/mL, 95% CI: -43.68, -13.50, $p < 0.001$), but not those with treatment durations < 4 weeks (WMD: -53.70 pg/mL, 95% CI: -120.47, 13.07, $p = 0.115$), and LDL-C reductions < 50 mg/dL (WMD: -15.04 pg/mL, 95% CI: -33.08, 3.00, $p = 0.102$) (**Figures 1S and 2S**). In addition, whilst plasma VEGF concentrations were significantly reduced by lipophilic statins (WMD: -22.31 pg/mL, 95% CI: -40.65, -3.98, $p = 0.017$), no significant change was observed in RCTs administering hydrophilic statins (WMD: -29.58 pg/mL, 95% CI: -83.03, 23.87, $p = 0.278$) (**Figure 3S**). A separate analysis was also performed to ascertain the effect size in healthy and diseased population groups. This analysis revealed a significant VEGF-lowering effect of statin therapy in the subset of studies in diseased populations (WMD: -21.08 pg/mL, 95% CI: -39.97, -2.18, $p = 0.029$), but not in the subset of studies in healthy populations (WMD: -32.26 pg/mL, 95% CI: -74.73, 10.21, $p = 0.137$) (**Figure 4S**).

199

200 *Meta-regression*

Random-effects meta-regression was performed to evaluate the impact of potential moderators on the estimated effect size. Consistent with the findings of subgroup analysis, changes in plasma VEGF concentrations were dependent to duration of treatment (slope: -1.82; 95% CI: -3.62, -0.02; $p = 0.047$) and magnitude of LDL-C reduction (slope: 0.61; 95% CI: 0.28, 0.94; $p = 0.0003$) by statins. However, there was no significant association between changes in plasma VEGF concentrations and molar dose of statins administered (slope: -375.66; 95% CI: -906.54, 155.22; $p = 0.165$) (**Figure 3**).

207

208 *Publication bias*

The funnel plot of standard error versus effect size (mean difference) was slightly asymmetric. Using “trim and fill” correction, two potentially missing studies on the right side of funnel plot were imputed leading to a corrected effect size that was still significant (WMD: -17.01 pg/mL, 95% CI: -33.02, -1.00) (**Figure 4**). The results of Begg’s rank correlation (Kendall’s Tau with continuity correction = -0.13, $Z = 0.54$, two-tailed p -value = 0.592) and Egger’s linear regression (intercept = -1.39, standard error = 0.67; 95% CI = -2.93, 0.14, $t = 2.09$, $df = 8.00$, two-tailed $p = 0.070$) tests excluded the possibility of publication bias in the analysis of

215 statins' effects on plasma VEGF concentrations. The "fail-safe N" test showed that 35 studies would be
 216 needed to bring the WMD down to a non-significant ($p > 0.05$) value.

217

218 Discussion

219 The results of the present meta-analysis of RCTs showed that statin treatment was associated with a
 220 significant reduction in circulating VEGF concentrations. This effect was greater with lipophilic statins and in
 221 patients with cardiac diseases, and found to be associated with treatment duration and LDL-lowering effect
 222 of statins.

223 At present, the potential implication of the VEGF family of growth factors in human cardiovascular health is
 224 highly controversial. Both vasculogenesis, the *in-situ* formation of blood vessels from migrated EPCs
 225 differentiating into endothelial cells, and angiogenesis, the sprouting of new capillaries by migrating
 226 endothelial cells extending pre-existing vasculature, are implicated in adult neovascularization and both are
 227 stimulated by VEGF [41].

228 Therefore, VEGF has been proposed as a potential therapeutic strategy for neovascularization in patients
 229 with ischemic heart disease [41-42]. However, the balance between hazards and benefits of VEGF is
 230 delicate [42]: VEGF-mediated neovascularization and microvascular permeability enhancement; its pro-
 231 inflammatory effects have been implicated in the exacerbation and progression of atherosclerotic plaque
 232 deposition, restenosis and negative remodeling following injury [1-7,42-43]; clinical trials with VEGF-A have
 233 not yielded the expected results [44-45]. To complicate matters, the role of circulating VEGF concentrations
 234 might be questioned since either the expression of VEGF in smooth muscle cells and atherosclerotic vessels
 235 seems implicated in the progression of atherosclerotic lesions, or elevated VEGF levels may be a surrogate
 236 marker of myocardial injury rather than the cause [5,12,46].

237 However, there seems to be a gradual increase in VEGF concentrations by worsening of atherosclerosis
 238 [10]; increased circulating levels of VEGF have been correlated with adverse prognosis in acute coronary
 239 syndromes [7]; an increasing number of studies reported a major contribution for VEGF to plaque
 240 development and progression, and to calcification processes [5-7,43,47-48]. Finally, inhibitors of VEGF
 241 receptors can reduce arteriosclerosis induced by abdominal aorta transplantation in animals [49].

242 Statins have several pleiotropic beneficial properties that are important for the treatment of micro- and
 243 macro-vascular diseases [8-9]. Repair of the damaged endothelial surface of atherosclerotic lesions may
 244 occur as the result of adjacent cell migration or the mobilization of circulating EPCs derived from bone
 245 marrow. Statins accelerate re-endothelialization by increasing EPC proliferation, an effect that is
 246 independent of the putative lipid-lowering activity [9]. Modulation of VEGF, one of the key growth factors
 247 involved in angiogenesis, is another potential mechanism through which statins may improve endothelial
 248 function [8,10-32]. Available data about this latter mechanism are highly controversial. Our meta-analysis
 249 suggested a significant reduction of plasma VEGF concentrations by statins in RCTs while, in experimental
 250 settings, statins have been reported to induce the release of VEGF from injured endothelium and vascular
 251 surface [27-32]. However, the results of *in-vitro* or animal studies are difficult to translate into clinical
 252 practice [8].

253 Several potential mechanisms may account for the VEGF-lowering effects of statins, including increased
 254 activity of the VEGF receptor Fms-like tyrosine kinase 1, resulting in a decrease in the free VEGF levels [12];
 255 inhibition of factors that up-regulate VEGF expression, such as the transcription factors sterol regulatory
 256 element-binding proteins (SREBPs), nuclear factor-kappa B (NF- κ B) and hypoxia-inducible factor (HIF),
 257 reactive oxygen species, and pro-inflammatory cytokines [8,11,14,50-52]; suppression of apolipoprotein
 258 CIII-induced vascular endothelial activation and inflammation [53]; and reduction of LDL-C and oxidized LDL
 259 with subsequent down-regulation of VEGF expression [11].

260 Our subgroup analysis revealed different effects according to the LDL-lowering effect, duration of
 261 treatment, lipophilicity of statins and basic condition of populations studied. A greater reduction of VEGF
 262 levels by statins was observed in studies that employed lipophilic statins [11-14,16,19], recruited subjects at
 263 a high risk of cardiovascular disease, and had longer duration of treatment [10-12,14,16].

264 Lipophilic statins (such as atorvastatin, simvastatin, lovastatin, fluvastatin) can be passively diffused
 265 through the lipid bilayer of the cellular membrane and can therefore be up-taken by a larger number of
 266 cells compared with hydrophilic statin (such as rosuvastatin, pravastatin). Accordingly, apoptosis and
 267 reversion of neointimal thickening in vascular smooth muscle cells are induced by the lipophilic statins, but
 268 not by pravastatin [8]. Furthermore, lipophilic statins beneficially impact on markers of endothelial

dysfunction and oxidative stress [53-55], and rosuvastatin was shown to be less effective than simvastatin in improving endothelium-dependent vasodilatation, despite its powerful lipid-lowering action [56]. It appears that at equipotent LDL-C lowering effects of lipophilic and hydrophilic statins, the former can exert more pronounced effects on endothelial dysfunction [53,55-56]. Intriguingly, simvastatin exerts protective effects during acute ischemia in the lipophilic (but not hydrophilic) form [57]. It was therefore hypothesized that lipophilic statins may affect endothelial dysfunction by different pleiotropic mechanisms unshared with other statins, and possess stronger lipid-independent effects [53-54,58]. A debate about the clinical impact of statin lipophilicity exists [54,59], but further investigations are needed, before lipophilicity is considered in the choice of statins.

Pitavastatin has a different metabolism [60] and its role on vascular protection is less defined at present. Its effects on plasma VEGF levels are even more controversial: either reduction [15], no change [23] or an increase [25] have been reported. This suggests that rather than a class effect, the impact of statins on plasma VEGF levels may depend on the specific molecular structure.

A significant VEGF-lowering effect of statin therapy in the subset of studies performed in diseased cohorts was evident when compared to the subset of healthy individuals; differences in baseline VEGF concentrations and the more than 2-fold higher number of individuals in the former subset might explain the lower 95%CI values found in the patients with cardiac diseases.

Finally, differences in the cholesterol-lowering efficacy of statins may be another factor that could be potentially responsible for the controversial results of literature. We found that greater LDL-C reductions were associated with significant VEGF reductions in the RCTs evaluated. Overall, studies with ≥ 50 mg/dL reductions in plasma LDL-C concentrations were associated with a greater decrease in VEGF concentrations [11-12,14-15,18] compared with studies with a lower decrease in LDL-C concentrations [21-23]. This finding suggests that the hypocholesterolemic effect of statins might affect the VEGF-lowering effect of these drugs. Indeed, the excess of circulating lipids is an important independent cause of endothelial dysfunction, a condition known as lipotoxicity [61]. However, we did not observe any associations between the effect and molar dose of statins; indeed, the LDL-C lowering activity is dependent not only to the statin

295 dose, but also to the statin type. In addition, doses of different statins are not directly comparable even
296 after conversion into molar doses.

297 The translational value of the results of this meta-analysis is evidencing a new potential pleiotropic action
298 of statin therapy that may be important in the prevention of cardiovascular and non-vascular diseases. In
299 this context, the impact of newer lipid-lowering therapies on plasma VEGF concentrations merits
300 investigation [62-66].

301

302 *Strengths and limitations*

303 To the best of our knowledge, this is the first systematic review and meta-analysis investigating the effect
304 of statin therapy on plasma VEGF levels, that could contribute to advancing knowledge and generating new
305 studies in the field. However, a number of limitations deserve mentioning. First of all, findings of the
306 present meta-analysis do not provide any proof on the relationship between the reduction in VEGF levels
307 and improvement of arterial stiffness, atherosclerotic lesions or cardiovascular events in humans. Second,
308 the heterogeneity of studies included in the meta-analysis should be considered as another limitation, since
309 either patients with mild hypercholesterolemia or patients with chronic or acute coronary artery diseases
310 were enrolled in the RCTs included. Part of this inter-study heterogeneity was addressed by choosing a
311 random-effects model for meta-analysis. As another limitation, studies included in this analysis were not
312 primarily designed to assess the effects of statins on VEGF concentrations or expression. Finally, the
313 number of trials included and the number of individual studied in the present meta-analysis was small.
314 However, the current pooled population size was sufficient to detect a significant VEGF-lowering effect of
315 statins. Nevertheless, additional studies are required to ascertain the impact of each statin type separately,
316 and compare the impact of different statins on plasma VEGF levels.

317

318 *Conclusions*

319 Findings from the present meta-analysis of RCTs suggested a significant reduction of plasma VEGF
320 concentrations following statin therapy. This effect was found to be dependent on the duration of
321 treatment, health status of the cohort, LDL-lowering activity, and lipophilicity of statins. However, further

322 studies are required to ascertain the presence of any dose-response association for the VEGF-lowering
323 effect of each statin. Future RCTs are also warranted to explore if reduction of plasma VEGF levels play a
324 role in the established effects of statins in reducing cardiovascular outcomes. Finally, the inhibitory effects
325 of statins on VEGF may justify the proposed indications of these drugs in the management of other diseases
326 that are mechanistically related to augmented angiogenesis, a hypothesis that merits further investigation.

327

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329

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331

332 **Author contribution:** AS contributed to conception of the design of the study, acquisition and analysis of
333 data, drafting and revising the paper; MCP contributed to interpretation of data, writing and revising the
334 paper; IG contributed to interpretation of data, writing and revising the paper; SB contributed to
335 interpretation of data writing and revising the paper.

336 All authors have approved the final version of the manuscript.

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Table 1. Demographic characteristics of the included studies.

Study	Cimato ¹⁸ , 2014	Higashi ²¹ , 2010	Kodama ¹² , 2006	Nakamura ¹³ , 2006	Nakamura ^{15,2} , 2008	Spadaccio ²² , 2010	Tousoulis ¹⁷ , 2011	Yoshida ²³ , 2010
Location Design	USA Randomized cross-over trial	Japan Randomized placebo-controlled trial	Japan Randomized placebo-controlled trial	Japan Randomized double-blind trial	Japan Randomized placebo-controlled trial	Italy Randomized double-blind placebo-controlled trial	Greece Randomized double-blind placebo-controlled trial	Japan Randomized placebo-controlled trial
Duration	6 weeks	4 weeks	6 months	14 days	1 month	3 weeks	1 month	4 weeks
Inclusion criteria	Healthy subjects	Healthy subjects with low HDL-c	Subjects within 3-days after acute myocardial infarction	Subjects undergoing coronary artery bypass grafting	Acute coronary syndrome plus carotid plaques	Patients undergoing elective coronary artery bypass grafting	Subjects with systolic heart failure	Chronic smokers with mild hypercholesterolemia
Statins	Atorvastatin Pravastatin Rosuvastatin	Pravastatin	Atorvastatin	Atorvastatin	Pitavastatin	Atorvastatin	Rosuvastatin	Pitavastatin
Participants	12	Cases 15 Controls 14	Cases 25 Controls 25	Cases 15 Controls 16	Cases 33 Controls 32	Cases 25 Controls 25	Cases 21 Controls 18	Cases 15 Controls 15
Age (years)	43±13	Cases 46±8 Controls 45±9	Cases 65±2 Controls 63±2	Cases 60±13 Controls 63±8	Cases 60±9 Controls 59±9	Cases 66±8 Controls 65±7	Cases 65±11 Controls 66±11	Cases 40 (3) Controls 38 (2)
Gender (M/F)	7/5	Cases 11/4 Controls 11/3	Cases 17/8 Controls 19/6	Cases 15/0 Controls 14/2	Cases 25/8 Controls 23/9	Cases 13/12 Controls 14/11	Cases 19/2 Controls 16/2	Cases 15/0 Controls 15/0
BMI (kg/m²)	24.9±7.2	Cases 23.5±3.2 Controls 23.8±3.3	Cases 23.2±0.6 Controls 24.5±0.6	NS	Cases 25.5±3.0 Controls 25.9±3.0	NS	Cases 27.8±4.3 Controls 28.0±5.0	Cases 23.6 (0.8) Controls 23.0 (0.9)
Smokers (%)	0	Cases 0 Controls 0	Cases 56 Controls 48	NS	Cases 36 Controls 34	Cases 48 Controls 44	Cases 19 Controls 22	Cases 100 Controls 100
Glucose (mg/dL)	NS	Cases 88±17 Controls 91±18	NS	NS	Cases 123±23 Controls 128±23	NS	NS	Cases 99 (2) Controls 95 (2)
Insulin (pmol/L)	NS	Cases 88±17 Controls 91±18	NS	NS	Cases 54±18 Controls 52±18	NS	NS	NS

Diabetes (%)	0	Cases 0 Controls 0	Cases 60 Controls 64	Cases 33 Controls 25	Cases 30 Controls 32	Cases 0 Controls 0	Cases 0 Controls 0	Cases 0 Controls 0
Total cholesterol (mg/dL)	211±28	Cases 178±13 Controls 175±24	Cases 196±8 Controls 202±8	Cases 190±35 Controls 190±40	Cases 240±21 Controls 238±18	Cases 240±39 Controls 244±27	Cases 223±38 Controls 217±38	Cases 199 (9) Controls 190 (10)
LDLc (mg/dL)	136±23	Cases 101±15 Controls 104±18	Cases 120±6 Controls 116±7	NS	Cases 164±27 Controls 156±23	NS	Cases 147±40 Controls 152±33	Cases 125 (8) Controls 116 (10)
HDLc (mg/dL)	54±13	Cases 34±3 Controls 34±5	Cases 46±3 Controls 45±3	NS	Cases 43±6 Controls 42±5	Cases 46±15 Controls 46±23	Cases 47±9 Controls 41±9	Cases 51 (3) Controls 57 (3)
Triglycerides (mg/dL)	NS	Cases 88±27 Controls 85±40	Cases 133±17 Controls 143±12	NS	Cases 165±19 Controls 163±18	Cases 151±80 Controls 159±27	Cases 113;106-177 Controls 120;80-148	Cases 151 (24) Controls 118 (14)
Systolic blood pressure (mmHg)	NS	Cases 116±11 Controls 114±11	NS	NS	NS	NS	NS	Cases 119 (4) Controls 120 (3)
Diastolic blood pressure (mmHg)	NS	Cases 67±7 Controls 68±7	NS	NS	NS	NS	NS	NS
Hypertension (%)	17	Cases 0 Controls 0	Cases 72 Controls 64	Cases 47 Controls 50	Cases 48 Controls 38	Cases 52 Controls 48	Cases 43 Controls 67	Cases 0 Controls 0
Coronary artery diseases (%)	0	Cases 0 Controls 0	Cases 100 Controls 100	Cases 100 Controls 100	Cases 100 Controls 100	Cases 100 Controls 100	Cases 67 Controls 86	Cases 0 Controls 0
C-reactive protein (mg/L)	1.1±1.3	Case 1.0±2.1 Control 1.1±2.0	NS	NS	Cases 0.6±0.3 Controls 0.7±0.3	NS	Cases 2.4;0.9-4.0 Controls 2.5;1.1-7.6	Cases 1.8 (0.8) Controls 1.0 (0.3)
VEGF (pg/mL)	200 (45)	Case 85±12 Controls 86±13	Cases 163±19 Controls 143±10	Cases 84±31 Controls 84±36	Cases 176±35 Controls 175±30	Cases 120±40 Controls 120±26	Cases 313±129 Controls 278±221	Cases 63 (11) Controls 57 (8)

Data are expressed as: mean ± SD mean (SEM) median; 25th-75th percentiles

Abbreviations: BMI = body mass index; HDLc = HDL cholesterol; LDLc = LDL cholesterol; NS = non stated;
VEGF = Vascular Endothelial Growth Factor

Table 2. Risk of bias assessment in the studies included in this meta-analysis.

Study	Random sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective reporting	Free of other bias
Nakamura ¹⁵ , 2008	L	L	L	U	L	L
Yoshida ²³ , 2010	U	U	H	U	L	L
Cimato ¹⁸ , 2014	L	L	H	L	L	L
Kodama ¹² , 2006	L	L	L	U	L	L
Nakamura ¹³ , 2006	L	L	H	U	L	L
Tousoulis ¹⁷ , 2011	U	U	H	U	L	L
Spadaccio ²² , 2010	U	U	L	U	L	L
Higashi ²¹ , 2010	U	U	U	L	L	L

Criteria defined for quality assessment are based on the Cochrane guidelines.

Abbreviations: H, high risk of bias; L low risk of bias; U unclear or unrevealed risk of bias

Figure legends

Figure 1. Flow chart of the number of studies identified and included into the meta-analysis.

Figure 2. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of statin therapy on plasma VEGF concentrations. Lower plot shows leave-one-out sensitivity analysis.

Figure 3. Meta-regression plots of the association between mean changes in plasma VEGF concentrations with duration of statin therapy, magnitude of LDL-C reduction, and molar dose of statins.

Figure 4. Funnel plot displaying publication bias in the studies reporting the impact of statin therapy on plasma VEGF concentrations. Open diamond represents observed effect size; closed diamond represents imputed effect size.

Figure legends

Figure 1S. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of statin therapy on plasma VEGF concentrations in trials with treatment durations of < 4 weeks (upper plot) and ≥ 4 weeks (lower plot).

Figure 2S. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of statin therapy on plasma VEGF concentrations in trials with LDL-cholesterol reductions < 50 mg/dL (upper plot) and ≥ 50 mg/dL (lower plot).

Figure 3S. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of statin therapy on plasma VEGF concentrations in trials with lipophilic (upper plot) and hydrophilic statins (lower plot).

Figure 4S. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of statin therapy on plasma VEGF concentrations in trials with diseased (upper plot) and healthy populations (lower plot).