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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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**Abstract**

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

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

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

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

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

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

## Introduction

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

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

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

## Methods

*Search Strategy*

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

*Study Selection*

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

*Data extraction*

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

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

*Quality assessment*

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

*Quantitative Data Synthesis*

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

A random-effects model (using DerSimonian-Laird method) and the generic inverse variance method were used to compensate for the heterogeneity of studies in terms of demographic characteristics of populations being studied and also differences in study design and type of statin being studied [37]. Heterogeneity was quantitatively assessed using $I^2$ index. Effect sizes were expressed as weighed mean difference (WMD) and 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.

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

*Risk of bias assessment*

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

*Effect of statin therapy on plasma VEGF concentrations*

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

188 subsets of trials with treatment durations ≥ 4 weeks (WMD: -19.54 pg/mL, 95% CI: -37.78, -1.30, $p$ = 0.036),
189 and LDL-C reductions ≥ 50 mg/dL (WMD: -28.59 pg/mL, 95% CI: -43.68, -13.50, $p$ < 0.001), but not those
190 with treatment durations < 4 weeks (WMD: -53.70 pg/mL, 95% CI: -120.47, 13.07, $p$ = 0.115), and LDL-C
191 reductions < 50 mg/dL (WMD: -15.04 pg/mL, 95% CI: -33.08, 3.00, $p$ = 0.102) (**Figures 1S** and **2S**). In
192 addition, whilst plasma VEGF concentrations were significantly reduced by lipophilic statins (WMD: -22.31
193 pg/mL, 95% CI: -40.65, -3.98, $p$ = 0.017), no significant change was observed in RCTs administering
194 hydrophilic statins (WMD: -29.58 pg/mL, 95% CI: -83.03, 23.87, $p$ = 0.278) (**Figure 3S**). A separate analysis
195 was also performed to ascertain the effect size in healthy and diseased population groups. This analysis
196 revealed a significant VEGF-lowering effect of statin therapy in the subset of studies in diseased populations
197 (WMD: -21.08 pg/mL, 95% CI: -39.97, -2.18, $p$ = 0.029), but not in the subset of studies in healthy
198 populations (WMD: -32.26 pg/mL, 95% CI: -74.73, 10.21, $p$ = 0.137) (**Figure 4S**).
199
200 *Meta-regression*
201 Random-effects meta-regression was performed to evaluate the impact of potential moderators on the
202 estimated effect size. Consistent with the findings of subgroup analysis, changes in plasma VEGF
203 concentrations were dependent to duration of treatment (slope: -1.82; 95% CI: -3.62, -0.02; $p$ = 0.047) and
204 magnitude of LDL-C reduction (slope: 0.61; 95% CI: 0.28, 0.94; $p$ = 0.0003) by statins. However, there was
205 no significant association between changes in plasma VEGF concentrations and molar dose of statins
206 administered (slope: -375.66; 95% CI: -906.54, 155.22; $p$ = 0.165) (**Figure 3)**.
207
208 *Publication bias*
209 The funnel plot of standard error versus effect size (mean difference) was slightly asymmetric. Using "trim
210 and fill" correction, two potentially missing studies on the right side of funnel plot were imputed leading to
211 a corrected effect size that was still significant (WMD: -17.01 pg/mL, 95% CI: -33.02, -1.00) (**Figure 4**). The
212 results of Begg's rank correlation (Kendall's Tau with continuity correction = -0.13, $Z$ = 0.54, two-tailed $p$-
213 value = 0.592) and Egger's linear regression (intercept = -1.39, standard error = 0.67; 95% CI = -2.93, 0.14, $t$
214 = 2.09, df = 8.00, two-tailed $p$ = 0.070) tests excluded the possibility of publication bias in the analysis of

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

**Discussion**

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

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

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

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

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

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

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

Lipophilic statins (such as atorvastatin, simvastatin, lovastatin, fluvastatin) can be passively diffused through the lipid bilayer of the cellular membrane and can therefore be up-taken by a larger number of cells compared with hydrophilic statin (such as rosuvastatin, pravastatin). Accordingly, apoptosis and reversion of neointimal thickening in vascular smooth muscle cells are induced by the lipophilic statins, but 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

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

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

*Strengths and limitations*

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

*Conclusions*

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

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

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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],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 ($kg/m^2$)** | 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 Controla 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) |

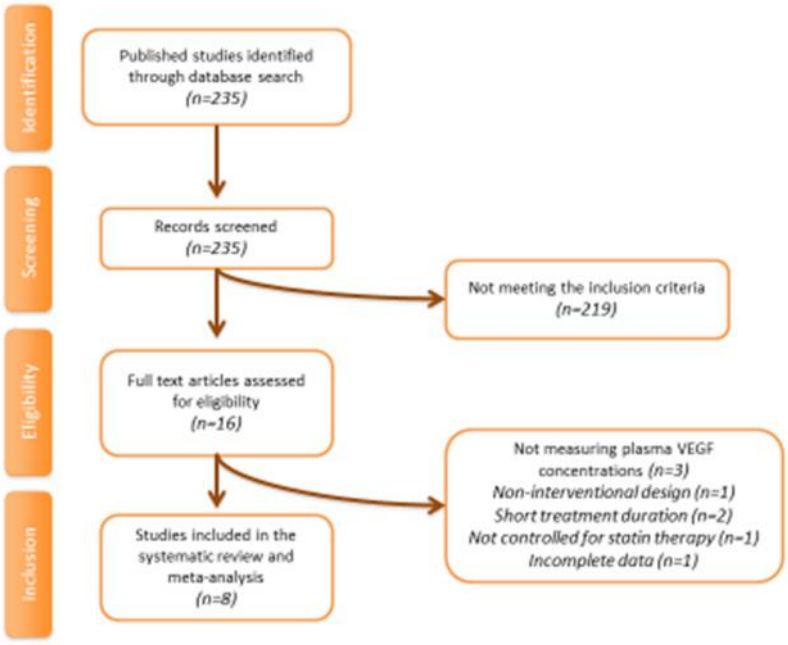
Data are expressed as: mean ± SD mean (SEM) median; $25^{th}$-$75^{th}$ percentiles

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

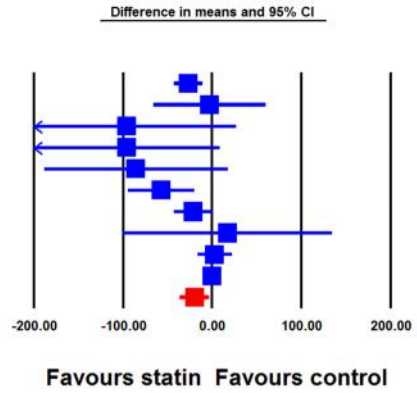
**Table 2. Risk of bias assessment in the studies included in this meta-analysis.**

| 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 |

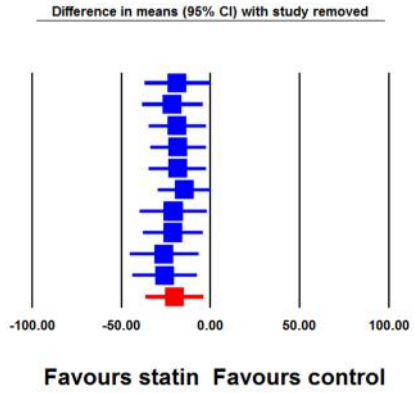
Criteria defined for quality assessment are based on the Cochrane guidelines.
Abbreviations: H, high risk of bias; L low risk of bias; U unclear or unrevealed risk of bias

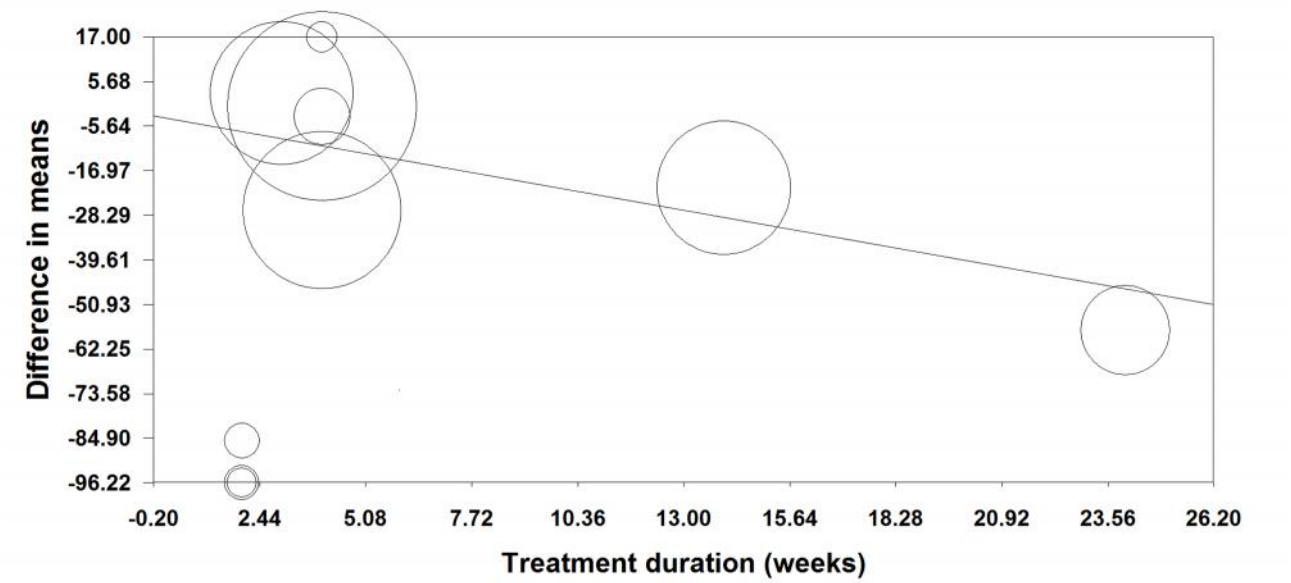

Identification
Screening
Eligibility
Inclusion
Published studies identified through database search (n=235)
Records screened (n=235)
Not meeting the inclusion criteria (n=219)
Full text articles assessed for eligibility (n=16)
Not measuring plasma VEGF concentrations (n=3)
Non-interventional design (n=1)
Short treatment duration (n=2)
Not controlled for statin therapy (n=1)
Incomplete data (n=1)
Studies included in the systematic review and meta-analysis (n=8)

| Study name | Difference in means | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---|---|---|---|---|---|---|---|
| Nakamura et al., 2008 | -27.000 | 7.913 | 62.617 | -42.509 | -11.491 | -3.412 | 0.001 |
| Yoshida et al., 2010 | -3.230 | 31.876 | 1016.079 | -65.706 | 59.246 | -0.101 | 0.919 |
| Cimato et al., 2014a | -96.220 | 62.422 | 3896.450 | -218.564 | 26.124 | -1.541 | 0.123 |
| Cimato et al., 2014b | -96.220 | 53.319 | 2842.968 | -200.724 | 8.284 | -1.805 | 0.071 |
| Cimato et al., 2014c | -85.610 | 52.380 | 2743.635 | -188.272 | 17.052 | -1.634 | 0.102 |
| Kodama et al., 2006 | -57.460 | 18.955 | 359.299 | -94.611 | -20.309 | -3.031 | 0.002 |
| Nakamura et al., 2006 | -21.290 | 10.873 | 118.231 | -42.601 | 0.021 | -1.958 | 0.050 |
| Tousoulis et al., 2011 | 17.000 | 59.872 | 3584.618 | -100.346 | 134.346 | 0.284 | 0.776 |
| Spadaccio et al., 2010 | 2.690 | 9.653 | 93.177 | -16.229 | 21.609 | 0.279 | 0.780 |
| Higashi et al., 2010 | -0.600 | 4.527 | 20.494 | -9.473 | 8.273 | -0.133 | 0.895 |
| | -19.878 | 8.159 | 66.572 | -35.870 | -3.886 | -2.436 | 0.015 |




| Study name | Point | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---|---|---|---|---|---|---|---|
| Nakamura et al., 2008 | -18.403 | 9.250 | 85.554 | -36.532 | -0.275 | -1.990 | 0.047 |
| Yoshida et al., 2010 | -21.161 | 8.627 | 74.422 | -38.069 | -4.252 | -2.453 | 0.014 |
| Cimato et al., 2014a | -18.468 | 8.072 | 65.163 | -34.290 | -2.647 | -2.288 | 0.022 |
| Cimato et al., 2014b | -17.914 | 7.964 | 63.425 | -33.523 | -2.305 | -2.249 | 0.024 |
| Cimato et al., 2014c | -18.200 | 8.070 | 65.117 | -34.016 | -2.384 | -2.255 | 0.024 |
| Kodama et al., 2006 | -14.435 | 7.594 | 57.665 | -29.318 | 0.449 | -1.901 | 0.057 |
| Nakamura et al., 2006 | -20.526 | 9.566 | 91.504 | -39.275 | -1.778 | -2.146 | 0.032 |
| Tousoulis et al., 2011 | -20.771 | 8.392 | 70.432 | -37.220 | -4.323 | -2.475 | 0.013 |
| Spadaccio et al., 2010 | -25.772 | 9.692 | 93.941 | -44.768 | -6.775 | -2.659 | 0.008 |
| Higashi et al., 2010 | -25.405 | 9.239 | 85.356 | -43.512 | -7.297 | -2.750 | 0.006 |
| | -19.878 | 8.159 | 66.572 | -35.870 | -3.886 | -2.436 | 0.015 |




Difference in means
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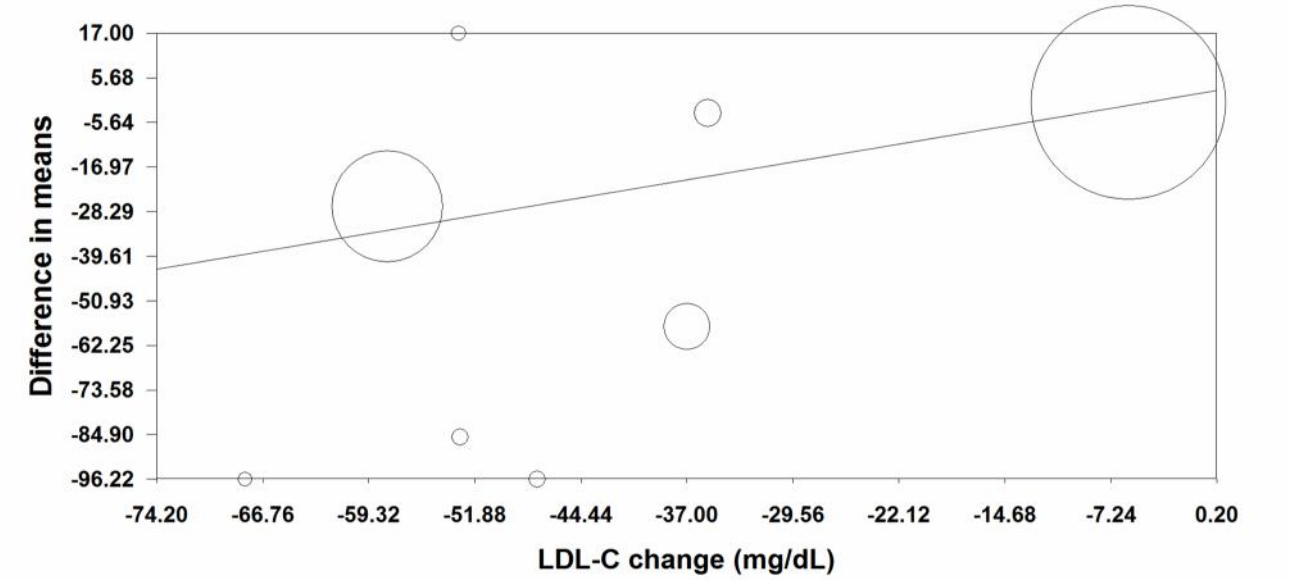
5.68
-5.64
-16.97
-28.29
-39.61
-50.93
-62.25
-73.58
-84.90
-96.22
-0.20
2.44
5.08
7.72
10.36
13.00
15.64
18.28
20.92
23.56
26.20
Treatment duration (weeks)


Difference in means
17.00
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-5.64
-16.97
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-62.25
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-84.90
-96.22
-74.20
-66.76
-59.32
-51.88
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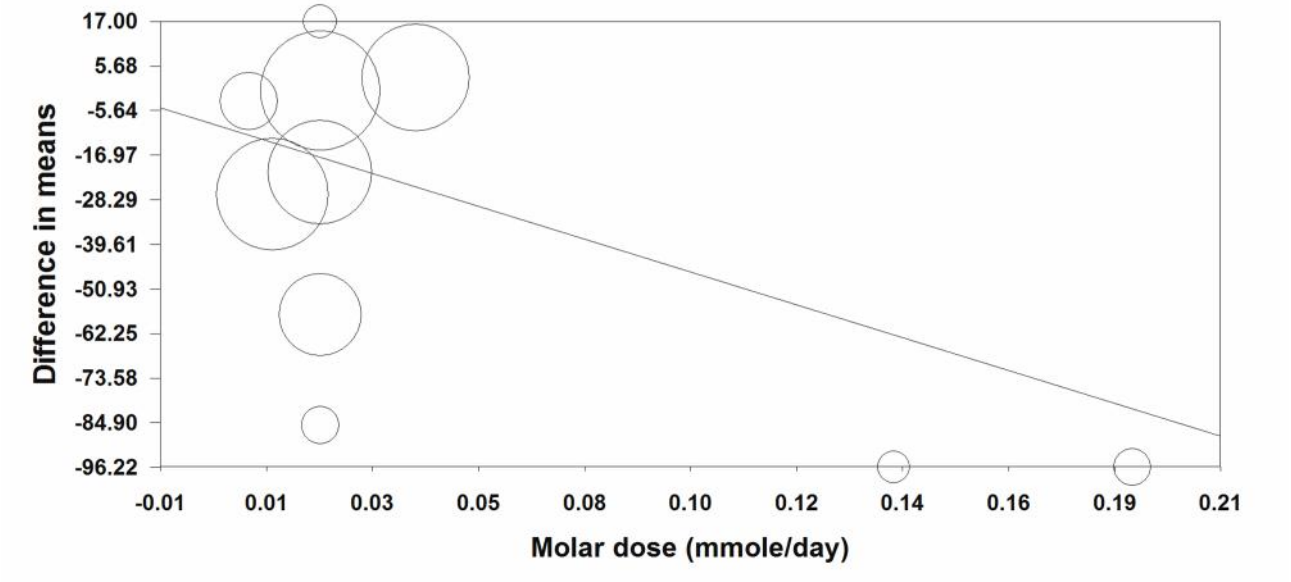
-37.00
-29.56
-22.12
-14.68
-7.24
0.20
LDL-C change (mg/dL)


Difference in means
17.00
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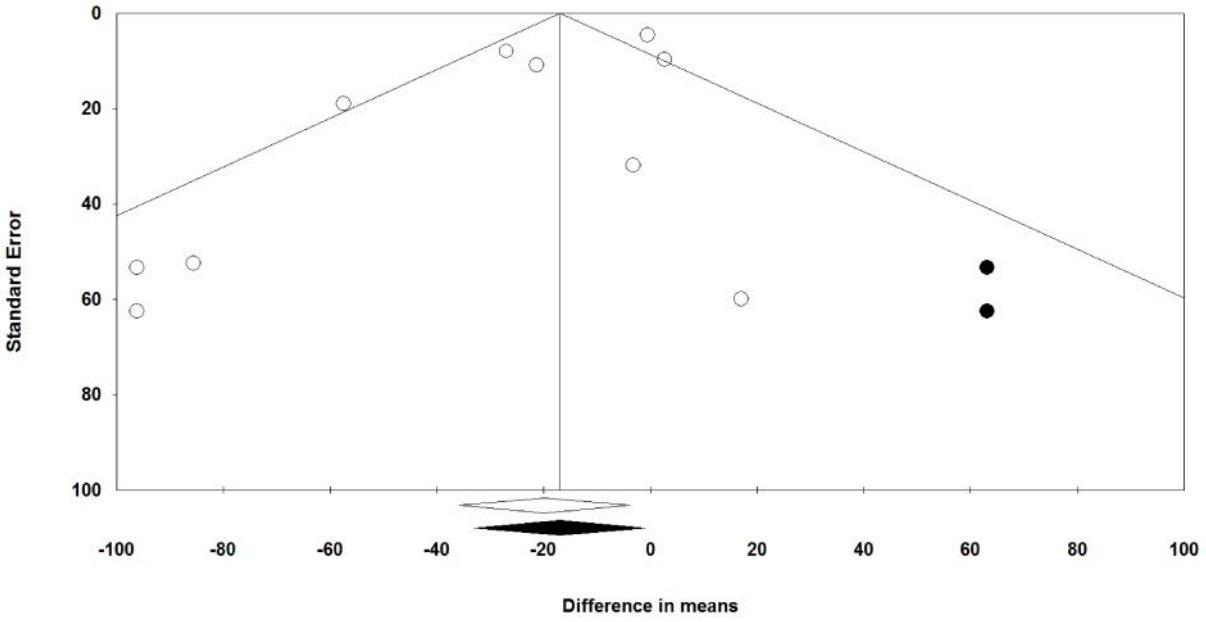
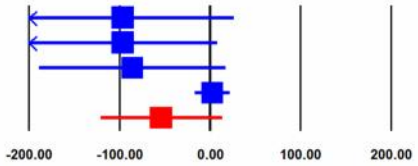
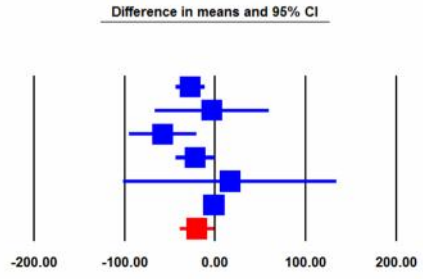
-5.64
-16.97
-28.29
-39.61
-50.93
-62.25
-73.58
-84.90
-96.22
-0.01
0.01
0.03
0.05
0.08
0.10
0.12
0.14
0.16
0.19
0.21
Molar dose (mmole/day)


0
20
40
60
80
100
Standard Error
-100
-80
-60
-40
-20
0
20
40
60
80
100
Difference in means

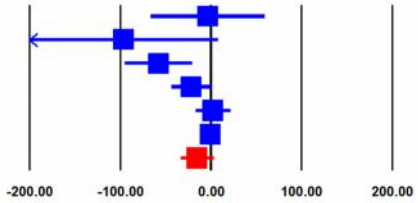
| Study name | Difference in means | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---|---|---|---|---|---|---|---|
| Cimato et al., 2014a | -96.220 | 62.422 | 3896.450 | -218.564 | 26.124 | -1.541 | 0.123 |
| Cimato et al., 2014b | -96.220 | 53.319 | 2842.968 | -200.724 | 8.284 | -1.805 | 0.071 |
| Cimato et al., 2014c | -85.610 | 52.380 | 2743.635 | -188.272 | 17.052 | -1.634 | 0.102 |
| Spadaccio et al., 2010 | 2.690 | 9.653 | 93.177 | -16.229 | 21.609 | 0.279 | 0.780 |
| | -53.699 | 34.068 | 1160.659 | -120.472 | 13.074 | -1.576 | 0.115 |




| Study name | Difference in means | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---|---|---|---|---|---|---|---|
| Nakamura et al., 2008 | -27.000 | 7.913 | 62.617 | -42.509 | -11.491 | -3.412 | 0.001 |
| Yoshida et al., 2010 | -3.230 | 31.876 | 1016.079 | -65.706 | 59.246 | -0.101 | 0.919 |
| Kodama et al., 2006 | -57.460 | 18.955 | 359.299 | -94.611 | -20.309 | -3.031 | 0.002 |
| Nakamura et al., 2006 | -21.290 | 10.873 | 118.231 | -42.601 | 0.021 | -1.958 | 0.050 |
| Tousoulis et al., 2011 | 17.000 | 59.872 | 3584.618 | -100.346 | 134.346 | 0.284 | 0.776 |
| Higashi et al., 2010 | -0.600 | 4.527 | 20.494 | -9.473 | 8.273 | -0.133 | 0.895 |
| | -19.543 | 9.306 | 86.596 | -37.782 | -1.304 | -2.100 | 0.036 |



| Study name | Difference in means | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---|---|---|---|---|---|---|---|
| Yoshida et al., 2010 | -3.230 | 31.876 | 1016.079 | -65.706 | 59.246 | -0.101 | 0.919 |
| Cimato et al., 2014b | -96.220 | 53.319 | 2842.968 | -200.724 | 8.284 | -1.805 | 0.071 |
| Kodama et al., 2006 | -57.460 | 18.955 | 359.299 | -94.611 | -20.309 | -3.031 | 0.002 |
| Nakamura et al., 2006 | -21.290 | 10.873 | 118.231 | -42.601 | 0.021 | -1.958 | 0.050 |
| Spadaccio et al., 2010 | 2.690 | 9.653 | 93.177 | -16.229 | 21.609 | 0.279 | 0.780 |
| Higashi et al., 2010 | -0.600 | 4.527 | 20.494 | -9.473 | 8.273 | -0.133 | 0.895 |
| | -15.042 | 9.205 | 84.734 | -33.084 | 3.000 | -1.634 | 0.102 |

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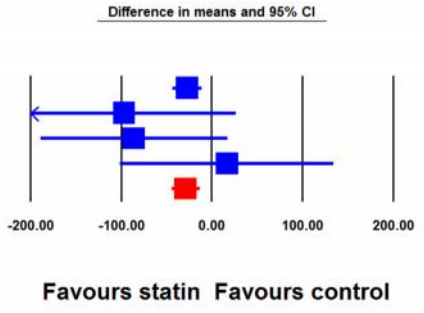
| Tousoulis et al., 2011 | 17.000 | 59.872 | 3584.618 | -100.346 | 134.346 | 0.284 | 0.776 |
| | -28.592 | 7.699 | 59.276 | -43.682 | -13.502 | -3.714 | 0.000 |



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|---|---|---|---|---|---|---|---|
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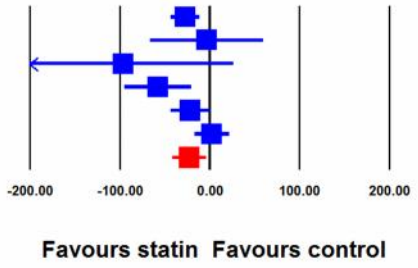
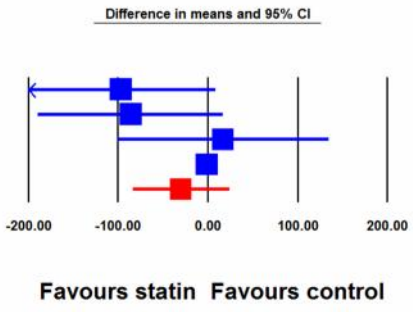
| Yoshida et al., 2010 | -3.230 | 31.876 | 1016.079 | -65.706 | 59.246 | -0.101 | 0.919 |
| Cimato et al., 2014a | -96.220 | 62.422 | 3896.450 | -218.564 | 26.124 | -1.541 | 0.123 |
| Kodama et al., 2006 | -57.460 | 18.955 | 359.299 | -94.611 | -20.309 | -3.031 | 0.002 |
| Nakamura et al., 2006 | -21.290 | 10.873 | 118.231 | -42.601 | 0.021 | -1.958 | 0.050 |
| Spadaccio et al., 2010 | 2.690 | 9.653 | 93.177 | -16.229 | 21.609 | 0.279 | 0.780 |
| | -22.313 | 9.354 | 87.507 | -40.648 | -3.979 | -2.385 | 0.017 |




| Study name | Difference in means | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---|---|---|---|---|---|---|---|
| Cimato et al., 2014b | -96.220 | 53.319 | 2842.968 | -200.724 | 8.284 | -1.805 | 0.071 |
| Cimato et al., 2014c | -85.610 | 52.380 | 2743.635 | -188.272 | 17.052 | -1.634 | 0.102 |
| Tousoulis et al., 2011 | 17.000 | 59.872 | 3584.618 | -100.346 | 134.346 | 0.284 | 0.776 |
| Higashi et al., 2010 | -0.600 | 4.527 | 20.494 | -9.473 | 8.273 | -0.133 | 0.895 |
| | -29.579 | 27.270 | 743.680 | -83.028 | 23.870 | -1.085 | 0.278 |



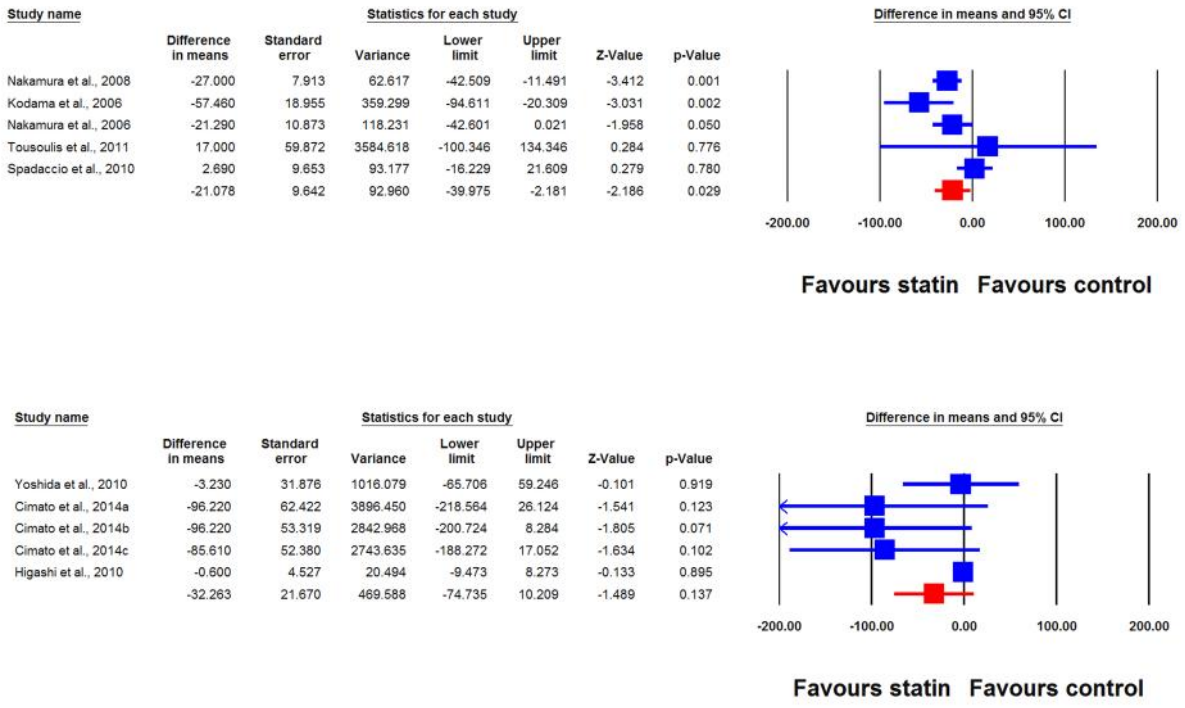



**Figure legends**

**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 heathy populations (lower plot).