A systematic review and meta-analysis of the effect of statins on plasma asymmetric dimethylarginine concentrations

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The impact of statin therapy on plasma asymmetric dimethylarginine (ADMA) levels has not been conclusively studied. Therefore the aim of the meta-analysis was to assess the effect of statins on circulating ADMA levels. We searched selected databases (up to August 2014) to identify randomized controlled trials (RCTs) that investigate the effect of statins on plasma ADMA concentrations. A weighted meta-regression (WMD) using unrestricted maximum likelihood model was performed to assess the impact of statin dose, duration of statin therapy and baseline ADMA concentrations as potential variables on the WMD between statin and placebo group. In total, 1134 participants in 9 selected RCTs were randomized; 568 were allocated to statin treatment and 566 were controls. There was a significant reduction in plasma ADMA concentrations following statin therapy compared with placebo (WMD: −0.104 μM, 95% confidence interval: −0.131 to −0.077, Z = −7.577, p < 0.0001). Subgroups analysis has shown a significant impact of hydrophilic statins (WMD: −0.207 μM, 95%CI: −0.427 to +0.013, Z = −7.250, p < 0.0001) and a non-significant effect of hydrophobic statins (WMD: −0.101 μM, 95%CI: −0.128 to −0.074, Z = −1.845, p = 0.065). In conclusion, this meta-analysis of available RCTs showed a significant reduction in plasma ADMA concentrations following therapy with hydrophilic statins.

Endothelial dysfunction is an early event in atherogenesis characterized by decreased availability of nitric oxide (NO), which diffuses towards the vascular smooth muscle tissues (VSMCs), triggers a rise of intracellular cyclic guanosine monophosphate (cGMP), leading to vasorelaxation1. Endothelial dysfunction may be associated with increased circulating asymmetric dimethylarginine (ADMA) levels - an

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L-arginine analogue, which inhibits NO formation. ADMA is a pan-inhibitor of all 3 NO synthases (NOS) isoforms (potent noncompetitive inhibitor of neuronal NOS and week inhibitor of inducible and endothelial NOS) and its plasma concentrations in the general population is 0.4–0.7 μM.

The first study that showed that middle-aged smoking men in the highest quartile of ADMA levels were at an almost 4-fold risk for acute coronary events was conducted in 2001. Since then, it has been shown that higher ADMA levels are related to increased mortality and adverse clinical outcomes in patients with coronary artery disease (CAD), diabetes, renal disease and ischemic stroke. Moreover, in the Coronary Artery Risk Determination investigating the Influence of ADMA Concentration (CARDIAC) study, ADMA was shown to be a risk factor for CAD, independently of traditional predictors.

Formation of NO is regulated by both substrate availability (L-arginine) and the presence of the inhibitor (ADMA), which in turn may be represented by their ratio. However, the application of L-arginine/ADMA ratio is much limited due to the fact that L-arginine varies much stronger that ADMA levels in the circulation, and therefore the ratio need not reflect the intracellular situation. The Hoorn Study showed that systemic inflammation was associated with decreased arginine and increased ADMA plasma levels resulting in an unfavorable NOS substrate-to-inhibitor ratio.

The interplay of inflammation, endothelial dysfunction, and oxidative stress might play a crucial role in ADMA pathophysiology, and reduction of ADMA levels might be a significant target for preventing endothelial dysfunction. Statins may provide an effective response to reverse endothelial dysfunction via reduction of ADMA levels; however, the available evidence is not conclusive. Therefore, the aim of this systematic review and meta-analysis was to assess the impact of statins on circulating ADMA levels.

METHODS
Data Sources. This study was designed in conformity to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. Our search included PubMed, Web of Science, Cochrane Library, Scopus and EMBASE databases and was limited to randomized controlled trials (RCTs) carried out from January 1, 1970 to August 1, 2014, investigating the potential effects of statins on circulating ADMA levels. The references of relevant publications were searched and articles of interest were retrieved. The databases were searched using the following search terms in titles and abstracts (also in combination with MESH terms): (rosuvastatin OR pravastatin OR fluvastatin OR simvastatin OR atorvastatin OR pitavastatin OR lovastatin OR cerivastatin OR "statin therapy" OR statins) AND (ADMA OR "asymmetric dimethylarginine"). The wild-card term “*” was used to increase the sensitivity of the search strategy. Two reviewers (CS and AS) evaluated each article separately. Disagreements were resolved by agreement and discussion with a third party (MB). Uncontrolled studies or those with results that did not consider the main objectives of the meta-analysis were omitted.

Study selection. Inclusion criteria. Study design had to meet the following criteria: (1) randomized, placebo-controlled parallel or cross-over trial, (2) population enrolled: adults ≥ 18 years, and, (3) plasma ADMA levels at baseline and after statin administration were available.

Exclusion criteria. The studies were excluded if: (1) had a non-randomized or uncontrolled design, (2) the study was not conducted in statin-treated subjects, (3) no numerical values were presented concerning plasma ADMA levels at baseline and at the end of the study, (3) had duplicate data on ADMA concentrations, (4) we were unable to obtain adequate details of study methodology or results from the article or the investigators, and, (5) the study was an ongoing trial.

Quality assessment. The quality of involved studies in this meta-analysis was evaluated using Jadad scale. This scale includes randomization (0–2 points), blinding (0–2 points), and dropouts and withdrawals (0–1 point). The overall score of a study in accordance with this scale varies among 0-5, with greater scores as a measure of better quality. Studies with Jadad scale of ≤2 and ≥3 were considered as low- and high-quality, respectively.

Quantitative Data Synthesis. Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biosstat, NJ). Since all studies used the same methods for the measurement of ADMA levels (plasma levels measured in μM), weighted raw mean difference and 95% confidence interval (CI) was used as summary statistic. Weighting of results was performed using the inverse variance method (Borenstein M, et al. Comprehensive meta-analysis version 2. Engelwood, NJ: Biosstat, 2005). Mean difference in measurements was calculated as follows: (measure at end of follow-up in the statin group – measure at baseline in the statin group) – (measure at end of follow-up in the placebo group – measure at baseline in the placebo group). Standard deviations (SDs) of the mean difference were calculated using the following formula: SD = √[(SDpre-treatment)² + (SDpost-treatment)² – (2R × SDpre-treatment × SDpost-treatment)], assuming a correlation coefficient (R) = 0.5. A random-effect model and the generic inverse variance method were used for quantitative data synthesis in order to address the inter-study variations in time of statin type, statin dose and duration of treatment. Pooled effect size was expressed as weighted mean difference (WMD) with 95%CI. In order to evaluate the influence of each study on the overall effect size, sensitivity analysis was conducted using the one-study remove (leave-one-out) approach. In case the values were only presented as graph, the software GetData Graph Digitizer 2.24 (http://getdata-graph-digitizer.com/)
was applied to digitize and extract the data; otherwise the authors of the article were contacted to provide numerical values of ADMA concentrations in statin and/or placebo group.

A weighted meta-regression using unrestricted maximum likelihood model was performed to assess the impact of statin dose, duration of statin therapy and baseline ADMA concentrations as potential moderator variables on the WMD in ADMA concentrations between statin and placebo group.

Presence of publication bias was explored graphically using funnel plots of precision (1/standard error) by study effect size (mean difference). Asymmetric funnel plot was further assessed for publication bias using Duval & Tweedie trim-and-fill and classic “fail-safe N” methods, as well as Begg’s rank correlation and Egger’s weighted regression tests.

RESULTS
Search results and trial flow. A summary of the study selection process is shown in Fig. 1. The initial screening for potential relevance excluded articles whose titles and/or abstracts were clearly irrelevant. After removing the trials not assessing the effects of statins in reducing plasma ADMA concentrations, only 17 RCTs met the inclusion criteria and the full-texts were obtained. After assessment 9 articles met the inclusion criteria and were selected for the final meta-analysis.

Description of studies. In total, 1134 participants in the 9 selected RCTs were randomized; 568 were allocated to statin treatment and 566 were controls. The number of participants in these trials ranged from 53 to 650. The included studies were published between 2003 and 2012, and were conducted in Norway, Taiwan, the Netherlands, Bulgaria, Italy, Turkey, New Zealand, China and Finland. Statins (pravastatin, rosuvastatin, simvastatin, fluvastatin, and atorvastatin) were administered at doses from 10 to 80 mg/day. Duration of trials ranged between 6 weeks and 24 months. Six trials were designed as parallel-group studies and 2 trials as cross-over studies. One study was a prospective follow-up trial conducted in 3 stages. Demographic and baseline parameters of the included studies are shown in Table 1.

Quantitative data synthesis. Combining results of retrieved RCTs indicated a significant reduction in plasma ADMA concentrations following treatment with statins compared with placebo (WMD: -0.104 μM, 95%CI: -0.131 to -0.077, Z = -7.577, p < 0.0001). Forest plots detailing the meta-analysis of RCTs assessing the impact of statin therapy on plasma ADMA levels is illustrated in Fig. 2. Subgroup analysis revealed a significant impact of hydrophilic statins (rosuvastatin, pravastatin and fluvastatin; n = 403; WMD: -0.207 μM, 95%CI: -0.427 to +0.013, Z = -7.250, p < 0.0001), and a
Table 1. Demographic characteristics of the included studies. Values are expressed as mean ± SD. *Median values and 25, 75 percentiles are given; ABBREVIATIONS: BMI: body mass index; LDL-C: low-density lipoprotein cholesterol; NA: not applicable; NS: not stated.

| Statin intervention | Simvastatin 40 mg/day | Pravastatin 40 mg/day | Rosuvastatin 10 mg/day | Pravastatin 40 mg/day | Simvastatin 40 mg/day | Simvastatin 80 mg/day | Fluvastatin 80 mg/day | Atorvastatin 40 mg/day | Rosuvastatin 10 mg/day | Pravastatin 40 mg/day |
|---------------------|------------------------|-----------------------|------------------------|------------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Participants | Treatment | 20 | 32 | 23 | 46 | 325 | 120 | 42 | 23 | 32 | 25 |
| Control | 15 | 32 | 23 | 47 | 325 | 120 | 43 | 23 | 32 | 26 |
| Age (years) | Treatment | 60 ± 12 | 35–71 | 62.8 ± 11.2 | 54 ± 11 | 46 ± 4 | 46 ± 3 | 55.50 ± 10.46 | 60.7 ± 10.4 | 62.28 ± 8.55 | 35.7 ± 4.3 |
| Control | 58 ± 11 | 35–71 | 59.8 ± 11.8 | 52 ± 13 | 46 ± 2 | 46 ± 2 | 56.16 ± 7.56 | 60.7 ± 10.4 | 60.72 ± 8.21 | 34.6 ± 4.3 |
| Male (%) | Treatment | 70.0 | 100.0 | 43.5 | 52.1 | 51.1 | 46.7 | 38.1 | NS | 68.7 | 100.0 |
| Control | 60.0 | 100.0 | 73.9 | 61.7 | 52.3 | 49.2 | 37.2 | NS | 62.5 | 100.0 |
| BMI (kg/m²) | Treatment | 25.1 ± 3.0 | NS | 25.3 ± 2.7 | 27.5 ± 5 | 25 ± 2 | 24.4 ± 4 | NS | NS | 23.74 ± 2.26 | 25.3 ± 2.8 |
| Control | 24.9 ± 2.3 | NS | 24.8 ± 2.9 | 26 ± 4 | 25 ± 3 | 25 ± 2 | NS | NS | 23.49 ± 2.20 | 24.6 ± 1.8 |
| Baseline plasma ADMA concentration (μM) | Treatment | 0.90 ± 0.10 | 1.50 (1.18, 1.75)* | 0.60 ± 0.19 | 0.63 ± 0.06 | 1.17 ± 0.15 | 1.26 ± 0.38 | 1.57 ± 1.07 | NS | 1.60 ± 0.41 | 0.38 ± 0.18 |
| Control | 0.74 ± 0.12 | 1.64 (1.24, 1.75)* | 0.54 ± 0.14 | 0.53 ± 0.09 | 1.16 ± 0.17 | 1.25 ± 0.21 | 1.17 ± 0.41 | NS | 1.58 ± 0.40 | 0.42 ± 0.15 |
non-significant overall effect. Likewise, Begg's rank correlation test (Kendall's Tau with continuity correction = −0.167, Z = 0.626, two-tailed p = 0.532) and Egger's linear regression tests suggested no evidence of publication bias (intercept = −0.478, 95%CI = −1.150 to 0.205, t = 1.679, df = 7.00, two-tailed p = 0.137).
Discussion
To our knowledge this meta-analysis is the first that assessed the effects of statin therapy on plasma levels of ADMA. The findings provide a thorough synthesis of results from available RCTs and showed a significant reduction in plasma ADMA concentrations. Additionally, statin therapy was examined by class: hydrophobic (simvastatin and atorvastatin), that might be dispersed at low levels throughout human tissues and hydrophilic (pravastatin, rosuvastatin and fluvastatin) that functions mainly in the liver and are present in the circulation. In our meta-analysis hydrophilic statins (rosuvastatin, pravastatin and fluvastatin) had a significant impact on ADMA levels while hydrophobic statins (simvastatin and atorvastatin) non-significantly reduced ADMA levels.

Currently ADMA is considered a prognostic marker of cardiovascular disease and mortality. The available data also suggests that ADMA has been involved in systemic vascular inflammation through induction of reactive oxygen species (ROS) in endothelial cells. In patients undergoing coronary bypass surgery, it was observed that ADMA levels were correlated with elevated NOS-derived generation of ROS. Furthermore, it has been shown that ROS upregulate ADMA synthesis and protein arginine N-methyltransferase expression. In cell culture studies, it has been shown that pro-oxidant and pro-inflammatory stimuli inhibit dimethylarginine dimethylaminohydrolase (DDAH) activity. Decreased DDAH, the enzyme responsible for ADMA degeneration, is generally followed by the consecutive decrease of NOS activity, increase of ADMA concentrations and development of atherosclerosis. However, it should also be mentioned that there are some doubts on the ADMA/DDAH association – e.g. DDAH activity is not associated with oxidative stress in the elderly patients with peripheral arterial occlusive disease. In human monocyctic cells, ADMA induces tumor necrosis factor (TNF)-α production via the inhibitory effect of reinioside C and ROS/nuclear factor (NF)-κB dependent pathways. Since both ROS and systemic inflammation are responsible for increased ADMA levels, and statins are recognized as anti-inflammatory and antioxidant agents, the hypothesis was that statin therapy might decrease ADMA levels. Indeed, several smaller studies have shown that statin therapy reduces ADMA levels, however other studies with high dose statins (e.g. simvastatin 80 mg/day or atorvastatin 40 mg/day) did not decrease plasma ADMA levels. It seems that our meta-analysis provides the answer to the question on the role of statins on ADMA levels (mainly hydrophilic), irrespective of the statins doses and therapy duration. These results also show the marginal or lack of effect of simvastatin and atorvastatin (hydrophobic statins) on plasma ADMA levels.

There are few hypotheses on how statins influence ADMA levels. One of them concerns the inhibition of ADMA-induced inflammatory reaction, modulated by mitogen-activated protein kinase (MAPK) pathway in human endothelial cells. Statins also activate the transcription factor sterol response element binding protein (SREBP) through decreasing content of the cholesterol in the membrane. Specifically enhances the expression of more than 30 genes associated with the synthesis and uptake of fatty acids, phospholipids, cholesterol and triglycerides. One of its isoforms - nuclear SREBP-2 increases the transcription of proprotein convertase subtilisin/kexin type 9 (PCSK9). It has been shown that statins upregulate both PCSK9 mRNA levels and LDLR via activation of sterol-mediated SREBP-2, an important activator of DDAH transcription and activity. Since reduced DDAH activity is linked to endothelial dysfunction, we speculate that statin therapy might decrease ADMA levels through multiple mechanisms such as activation of sterol-mediated SREBP-2, increasing of transcription of PCSK9 or by decreasing ADMA-induced inflammatory reaction, modulated by MAPK.

This meta-analysis has several limitations. Most importantly, the eligible RCTs usually had small populations and short follow-up (up to 6 months in 8/9 included studies). The included studies were also heterogeneous with regards to population characteristics (there were patients with hyperlipidemia, renal failure or atrial fibrillation), study design, and statin preparation and dose. In order to cover these variabilities we used a more conservative random-effects model and performed the sensitivity analysis. The meta-regression analysis also revealed that none of the moderator parameters i.e. statin dose, duration of statin therapy and baseline ADMA concentrations were significantly associated with the pooled estimate of statin effect on plasma ADMA concentrations. Finally, the smoking status, an important determinant of ADMA levels (as well as other variables, such as: hyperhomocysteinemia, hypertension, coronary artery disease, heart failure, and administration of the following drugs: antioxidants, estrogen, vitamin A, angiotensin converting enzyme inhibitors, angiotensin AT1 receptor antagonists, and beta-adrenergic receptor blocking drugs), could not be considered in this meta-analysis due to lack of data.

In conclusion, this meta-analysis of RCTs showed a significant reduction in plasma ADMA concentrations following hydrophilic statin therapy. These results might reveal an additional benefit of statins, which might contribute to the observed reduction of cardiovascular risk. Larger, well-designed studies involving smoking status are needed to validate our findings.

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CS - designed the study, made the literature search, drafted the manuscript, prepared the revised version; AS - designed the study, made the statistical analysis, corrected the draft of the paper; SU - made the statistical analysis, drafted the manuscript; DPM, MR, GYHL, GKH, JJPK, LK, JR - corrected the draft of the paper and the revised version; MB - designed the study, made the literature search, drafted the manuscript, prepared the revised version, submitted the paper.

Additional Information
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