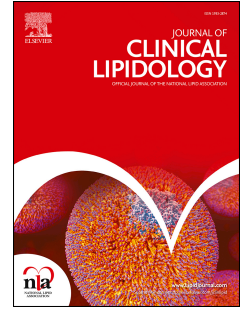# Accepted Manuscript

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Effect of statin therapy on plasma apolipoprotein C-III concentrations: a Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Running title: Statins reduce plasma ApoC-III

Abstract

Background: Statins are well established LDL-cholesterol-lowering drugs. Elevated Apo CIII levels are associated with elevated triglyceride-rich particles which are also considered to be a possible risk factor for CVD.

Objective: The aim of this meta-analysis of randomized placebo-controlled clinical trials was to assess the effect of statins on Apo CIII concentrations.

Methods: Randomized placebo-controlled trials investigating the impact of statin treatment on cholesterol lowering that include lipoprotein measurement were searched in PubMed-Medline, SCOPUS, Web of Science and Google Scholar databases (up to July 31, 2017). A random-effects model and generic inverse variance method were used for quantitative data synthesis. Sensitivity analysis was conducted using the leave-one-out method. A weighted random-effects meta-regression was performed to evaluate the impact of potential confounders on Apo CIII concentrations.

Results: This meta-analysis of data from 6 randomized placebo-controlled clinical trials (10 statin arms) involving 802 subjects showed that statin therapy significantly decreased circulating Apo CIII concentrations (weighted mean difference [WMD]: -2.71, 95% CI: -3.74, -1.68, $p<0.001$; I^2: 73.83%). The effect size was robust in the leave-one-out sensitivity analysis and not driven by any single study. Subgroup analysis showed a reduction of Apo CIII concentrations by atorvastatin (WMD: -4.74, 95% CI: -3.74, -1.68, $p=0.002$; I^2: 84.02%), rosuvastatin (WMD: -2.68, 95% CI: -4.52, -0.84, $p=0.004$; I^2: 0%) and lovastatin (WMD: -1.64, 95% CI: -2.22, -1.07, $p<0.001$; I^2: 0%).

Conclusion: This meta-analysis suggests that statin treatment significantly reduces plasma Apo

CIII levels.

Introduction

Statins are well established low density lipoprotein cholesterol (LDL-C)-lowering drugs with proven efficacy in reducing cardiovascular disease (CVD) morbidity and mortality, both in primary and secondary prevention.[1-3] Statins also reduce triglycerides (TG) levels and moderately raise high density lipoprotein cholesterol (HDL-C) levels.[4] This statin effect depends on the pre-treatment TG level and the LDL-C lowering effect of the statin.

In addition to plasma lipids and lipoproteins, statins also exert several pleiotropic effects relevant to the reduction of CVD risk.[5-7] Apolipoprotein (Apo) CIII, described almost half a century ago, is crucial in regulating TG metabolism because it inhibits lipoprotein lipase and hepatic lipase activity as well as hepatic uptake of TG-rich lipoproteins.[4,8,9,10]

Elevated Apo CIII levels are therefore associated with elevated TG levels which are considered to be a possible risk factor for CVD.[4,11] Elevated plasma TG and low HDL-C levels are not only predictors for macrovascular atherosclerotic disease and CVD events but they are also risk factors for microvascular disease in type 2 diabetes mellitus (T2DM).[12] Because of these effects, elevated Apo CIII levels are considered as markers/predictors of CVD risk.[13-15] This concept has been supported relatively recently by finding that loss-of-function in APOC3 mutations are associated with low TG concentrations and reduced CVD risk.[16-18]

There are still some open questions concerning the effect of statin treatment on Apo CIII levels. Furthermore, the results of some trials that evaluated this relationship are contradictory. Therefore, we performed a meta-analysis of randomized controlled trials that assessed this relationship.

Methods

Search Strategy

This study was designed according to the guidelines of the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement.[19] PubMed-Medline, Scopus and ISI Web of Knowledge databases were searched using the following search terms in titles and abstracts: Atorvastatin OR mevastatin OR lovastatin OR simvastatin OR fluvastatin OR rosuvastatin OR pitavastatin OR pravastatin) AND (Apo CIII OR Apo C-III OR "apo CIII" OR "apo C-III" OR apoC3 OR "apo C3") AND placebo. The wild-card term ''*'' was used to increase the sensitivity of the search strategy by considering all interchangeable formats of search terms. The search was limited to articles published in English. The literature was searched from inception to July 31, 2017.

Study Selection

Original studies were included if they met the following criteria: (i) randomized placebo-controlled clinical trial with either parallel or cross-over design, (ii) investigated the impact of statins versus placebo on circulating concentrations of Apo CIII, and, (iii) presentation of sufficient information on Apo CIII concentrations at baseline and at study end in both intervention and control groups or providing the net change values. Exclusion criteria were: (i) non-clinical studies, (ii) uncontrolled OR non-placebo-controlled studies, (iii) observational studies with case-control, cross-sectional or cohort design, and, (iv) lack of sufficient information on baseline or follow-up total circulating Apo CIII levels.

Data extraction

Eligible studies were reviewed and the following data were abstracted: 1) first author's name, 2) year of publication, 3) country where the study was performed, 4) study design, 5) number of participants in the statin and control groups, 6) type of statin used, 7) statin dose, 9) age, gender

and body mass index (BMI) of study participants, and, 10) baseline and follow-up concentrations of plasma lipids, lipoproteins and apolipoproteins (including Apo CIII).

Quality assessment

The quality of involved studies in this meta-analysis was evaluated using the Cochrane criteria.[20]

Quantitative Data Synthesis

Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ).[21] A random-effects model (using DerSimonian-Laird method) and the generic inverse variance weighting method were used to compensate for the heterogeneity of studies in terms of study design, treatment duration, and the characteristics of populations being studied.[22] Standard deviations (SDs) of the mean difference were calculated using the following formula: SD = square root $[(SD_{\text{pre-treatment}})^2 + (SD_{\text{post-treatment}})^2 - (2R \times SD_{\text{pre-treatment}} \times SD_{\text{post-treatment}})]$, assuming a correlation coefficient (R) = 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. All Apo CIII values were collated in mg/dL. Effect sizes were expressed as standardized mean difference (WMD) and 95% confidence interval (CI). In order to evaluate the influence of each study on the overall effect size, a sensitivity analysis was conducted using the leave-one-out method (i.e. removing one study each time and repeating the analysis).[23,24]

Meta-regression

As potential confounders of treatment response, changes in plasma lipid, lipoproteins and apolipoproteins levels were entered into a random-effects meta-regression model to explore their association with the estimated effect size on plasma Apo CIII levels.

Publication bias

Evaluation of funnel plot, Begg's rank correlation and Egger's weighted regression tests were employed to assess the presence of publication bias in the meta-analysis. When there was an evidence of funnel plot asymmetry, potentially missing studies were imputed using the "trim and fill" method.[25] In case of significant result, the number of potentially missing studies required to

make the *p*-value non-significant was estimated using the "fail-safe N" method as another marker of publication bias.

Results

Overall, 58 articles were found following multi-database search. After screening of titles and abstracts, 16 articles were assessed in full text. Of these, 10 articles were excluded because of lack of reporting serum/plasma total Apo CIII concentrations (n=5), duplicate data report (n=4) and not being placebo-controlled (n=1), leaving 6 eligible articles with 10 treatment arms for meta-analysis.

Study characteristics

Data were pooled from 6 randomized placebo-controlled trials comprising a total of 802 subjects, including 444 and 358 participants in the statin and placebo arms (individuals in cross-over trials were considered in the treatment and control groups), respectively. The trials used different types and doses of statins.[26-31] Selected studies were published between 1994[26,29] and 2008.[30] The range of treatment duration was from 5 weeks[27,28] to 30 weeks.[28] Study designs of included trials were parallel[26,28,29] and cross-over group.[27,30,31] Selected studies enrolled subjects with hypercholesterolemia,[26,29] coronary artery disease,[26,29] obesity,[27] type 2 diabetes[28] and metabolic syndrome.[30,31] The characteristics of the included clinical trials are shown in **Table 1**.

Risk of bias assessment

Almost all of the selected studies were characterized by lack of information about random sequence generation. All of the included trials had insufficient information regarding to allocation concealment. With respect to blinding of participants, personnel and outcome assessors, one study had a high risk of bias.[26] However, all evaluated trials showed a low risk of bias for incomplete outcome data and selective outcome reporting.

Quantitative data synthesis

Meta-analysis of data from 10 statin treatment arms suggested a significant reduction of

circulating Apo CIII concentrations (WMD: -2.71, 95% CI: -3.74, -1.68, $p<0.001$; I^2: 73.83%) (**Figure 1**). The effect size was robust in the leave-one-out sensitivity analysis (**Figure 1**) and not mainly driven by any single study. Subgroup analysis showed reduction of Apo CIII concentrations by atorvastatin (WMD: -4.74, 95% CI: -3.74, -1.68, $p=0.002$; I^2: 84.02%), rosuvastatin (WMD: -2.68, 95% CI: -4.52, -0.84, $p=0.004$; I^2: 0%) and lovastatin (WMD: -1.64, 95% CI: -2.22, -1.07, $p<0.001$; I^2: 0%) (**Figure 2**).

Meta-regression

Random-effects meta-regression was performed to assess the impact of potential confounders on the effects of statins on plasma Apo CIII levels. The results suggested a significant positive association between the changes in plasma concentrations of Apo CIII and those of LDL-C (slope: 0.06; 95% CI: 0.001, 0.13; $p=0.047$), apoB (slope: 0.12; 95% CI: 0.01, 0.22; $p=0.026$) and TGs (slope: 0.03; 95% CI: 0.01, 0.05; $p=0.015$), and a borderline non-significant association with changes in plasma Apo AI levels (slope: 0.27; 95% CI: -0.001, 0.55; $p=0.051$). No significant association was found between changes in plasma Apo CIII and HDL-C concentrations (slope: -0.47; 95% CI: -1.85, 0.90; $p=0.500$) (**Figure 3**).

Publication bias

Visual inspection of Begg's funnel plots revealed a slight asymmetry in the meta-analyses of statin's effects on plasma Apo CIII levels. Begg's rank correlation ($p=0.074$) but not Egger's regression test ($p=0.116$) also suggested the presence of potential but slight publication bias. Using "trim and fill" method, one potentially missing study was imputed yielding an adjusted effect size of -2.92 (-3.94, -1.89) (**Figure 4**). The results of "fail-safe N" test suggested that 252 missing studies would be required to make the observed significant result non-significant.

Discussion

The results of this meta-analysis suggest that treatment with statins significantly reduces plasma Apo CIII levels.

Although elevated LDL-C is the primary target for treatment and the drugs of choice are statins, many patients have atherogenic dyslipidemia. This lipoprotein disturbance is characterized by increased levels of TGs usually accompanied by decreased levels of HDL-C and normal or only

moderately raised concentrations of LDL-C.[32,33] LDL particles in this lipoprotein disturbance are smaller and more dense and are considered to be more atherogenic than larger buoyant LDL particles.[34,35]

Atherogenic dyslipidemia often occurs in patients with metabolic syndrome and/or type 2 diabetes mellitus (T2DM). Apo CIII is contained in TG-rich particles such as very low density lipoprotein (VLDL) and TG-rich remnant particles but also in some LDL particles which are enriched with TGs.[36-39] Therefore, in patients with elevated TGs, especially in those with metabolic syndrome and/or T2DM, the levels of Apo CIII are also elevated.[40,41] This may be attributed to increased production of Apo CIII in subjects with obesity and hypertriglyceridemia which might be related to the effect of insulin resistance in increasing the expression of Apo CIII mediated by forkhead box O1.[40,42] The role of Apo CIII has also been implicated in beta-cell dysfunction and influence on pancreatic microvasculature thus participating in etiopathogenesis of T2DM[43-45] but also in atherogenesis by directly activating pro-inflammatory and atherogenic mechanisms in endothelial cells of the arterial wall and monocytes.[46-48]

Fibrates are the treatment of choice for hypertriglyceridemia and they have been demonstrated to reduce apoC-III as a possible mechanism of TG reduction.[49] Statins also decrease elevated TG levels. The mechanism involved in the TG lowering effect of statins has not been fully elucidated. It may involve decreasing the production rate of VLDLs; these effects seem to be dependent on pre-treatment circulating VLDL concentrations.

It has also been demonstrated that statins decrease hepatic apoB mRNA and apoB secretion, leading to reduced plasma VLDL apoB levels. In addition, statin therapy can enhance plasma clearance of TG-rich lipoproteins. This increased clearance may explain the effect of statins on plasma Apo CIII levels as shown in this meta-analysis. Particularly a combination treatment with a statin and a fibrate, usually fenofibrate, was advocated, especially for atherogenic dyslipidemia.[50,51] In this context, ezetimibe, another LDL-C lowering drug (acting at the level of intestinal cholesterol transport), can, on the other hand, increase the TG-lowering effect of statins and this effect may be more obvious at higher baseline TG levels.[52-55]

More recently a new drug - volanesorsen - a second generation antisense oligonucleotide that reduces the levels of Apo CIII mRNA is being evaluated.[56,57] Nevertheless, statins are and will be

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used for treatment of atherogenic dyslipidemia for quite some time because of their price which will be considerably less than for volanesorsen. It has been shown in experimental conditions that statins decrease Apo Apo CIII synthesis in human HepG2 cells due to activating the peroxisome proliferator-activated receptor-α pathway via inhibition of ρ-signaling, thereby repressing Apo CIII mRNA expression.[58,59] However, such an effect of statins on Apo CIII in humans has not been confirmed so far.

Our study has some limitations including the heterogeneity with respect to the use of different statins, doses, duration of treatment, patient characteristics and also baseline TG levels. The number of studies was too low to allow subgroup analysis of the effects of individual statins.

Conclusions

This meta-analysis suggests that treatment with statins significantly reduces plasma Apo CIII levels. This can, at least to some extent, explain why apart from an LDL-C lowering effect, statins also have a TG-lowering effect. More research should address the effect of different statins on Apo CIII levels/activity in order to identify possible differences between these drugs.

Conflict of interests: MB has served on the speaker's bureau and as an advisory board member for Amgen, Sanofi, Aventis and Lilly. DPM has given talks and attended conferences sponsored by MSD, Libytec and AstraZeneca. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Author contribution statement:
AS and ŽR have given the idea and concept of the manuscript. All the authors IE, AS, LES, ETR, DPM, SLA, MB and ŽR have participating in making calculations and writing the article.

All the authors have approved the final article.

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TABLES

Table 1. Demographic characteristics of the included studies.

FIGURE LEGENDS

Figure 1. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of statin therapy on circulating Apo C-III concentrations. Lower plot shows the results of leave-one-out sensitivity analysis. Symbols "a" and "b" denote different statin treatment arms reported in a single study.

Figure 2. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of different statins on circulating Apo C-III concentrations. Symbols "a" and "b" denote different statin treatment arms reported in a single study.

Figure 3. Meta-regression bubble plots of the association between mean changes in plasma Apo C-III concentrations and changes in plasma concentrations of LDL-C, HDL-C, triglycerides, apoB and apo A-I following treatment with statins. The size of each circle is inversely proportional to the variance of change.

Figure 4. Funnel plot detailing publication bias in the studies reporting the impact of statins on plasma Apo C-III concentrations. Open and closed circles represent reported studies and potentially missing studies imputed using "trim and fill" method.

Table 1. Demographic characteristics of the included studies.

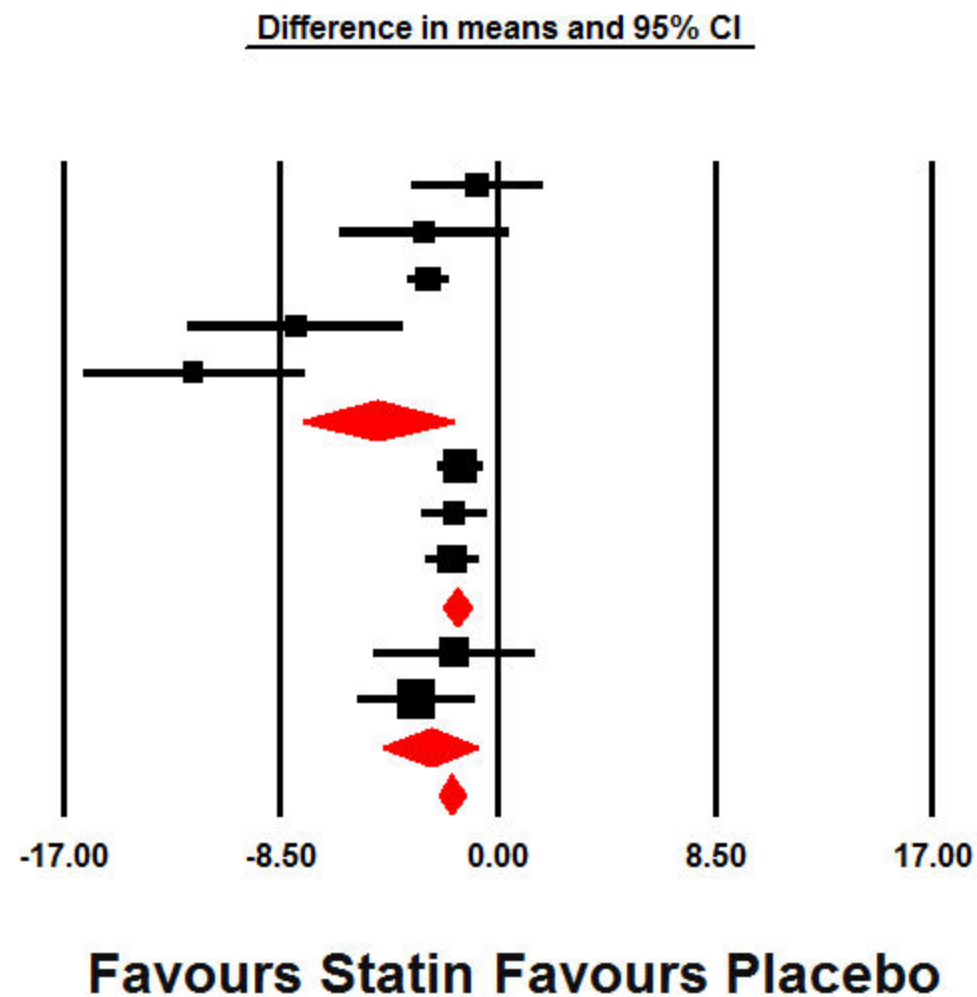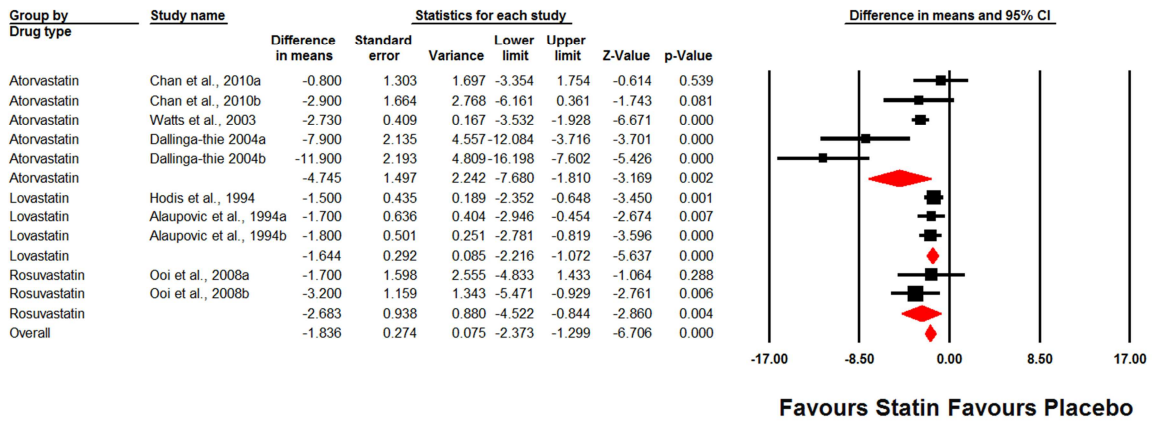| Author	Study design	Target Population	Treatment duration	n	Study groups	Age, years	Female (n, %)	BMI, (kg/m²)	Total cholesterol (mg/dl)	LDL cholesterol (mg/dl)*	HDL cholesterol (mg/dl)	Triglycerides (mg/dl)	ApoC-III (mg/dl)	% Change vs baseline**	% Change vs placebo	ApoC-III Assay method
Alaupovic et al. (1994)	Randomized, placebo-controlled	Hypercholesterolemia and CAD	2 years		Lovastatin 80 mg/day											Electroimmuno-assay
				32	Lp	58.7±6.7	3 (10)	ND	226.3±24.3	151.6±24.3	42.0±7.3	163.9±81.4	12.3±3.9	-10.5%	Lp	
				102	No Lp	ND	ND	ND	232.3±23.2	158.1±24.2	42.7±11.1	159.0±9.0	12.7±4.0	-14.4%	-18%	
					Placebo											
				31	Lp	58.5±6.1	3 (10)	ND	233.5±21.1	156.1±26.1	43.1±8.3	172.7±91.3	13.0±3.8	3.0%		
				105	No Lp	ND	ND	ND	230.5±24.5	156.7±20.4	42.7±9.2	155.5±67.6	13.2±7.1	0.0%	No Lp -17%	
Chan et al. (2010)	Randomized, double-blind, placebo-controlled, cross-over	Obese men	6 weeks	11	Placebo	50±12	0 (0.0)	32.0±2.8	228.2±3.9	146.9±3.9	ND	150.6±17.7	15.9±1.0	-10.0%		Immunoturbido-metry
				9	Atorvastatin 40 mg/day	54±9	0 (0.0)	33.7±4.5	224.3±7.7	150.8±7.7	ND	150.6±8.9	15.7±7.0	-15.2%	-6.9%	
				11	Fish oil	58±8	0 (0.0)	35.3±4.6	232.0±7.7	154.7±7.7	ND	177.1±35.4	15.9±1.2	-10.6%	-0.6%	
				8	Atorvastatin 40 mg/day + fish oil	56±8	0 (0.0)	32.3±2.7	228.2±7.7	146.9±11.6	ND	177.1±17.7	16.4±1.3	-27.4%	-16.7%	
Dallinga-Thie et al. (2006)	Randomized, double-blind, placebo-controlled	Type 2 diabetes	30 weeks	71	Placebo	59±8	ND	32.2±6.1	232.0±34.8	ND	40.6±8.1	248.0±88.6	41.2±9.0	-0.5%		Electroimmuno-assay
				72	Atorvastatin 10 mg/day	60±8	ND	30.0±3.8	228.2±34.8	ND	40.6±10.1	239.1±79.7	39.8±9.4	-21.1%	-22.4%	
				72	Atorvastatin 80 mg/day	60±8	ND	30.4±4.5	232.0±34.8	ND	39.8±9.3	265.7±97.4	43.2±11.2	-27.2%	-24.1%	
Hodis et al. (1994)	Randomized, double-blind, placebo-controlled	Hypercholesterolemia and CAD	2 years	220	Overall	ND	ND	ND	230.1±14.8	156.0±23.7	42.3±10.3	160.4±75.6	12.7±5.9			Electroimmuno-assay
				114	Lovastatin 80 mg/day	ND	ND	ND	ND	ND	ND	ND	ND	-11.0%	-10.9%	
				106	Placebo	ND	ND	ND	ND	ND	ND	ND	ND	0.7%		
Ooi et al. (2008)	Randomized, double-blind, placebo-controlled, cross-over	Men with metabolic syndrome	5 weeks	12	Overall	48.6±8.5	0 (0.0)	33.6±4.9	220±29	ND	38±4	202±66	ND			Electroimmuno-diffusion
				12	Placebo	ND	ND	ND	ND	ND	ND	ND	ND	NC		
				12	Rosuvastatin 10 mg/day	ND	ND	ND	ND	ND	ND	ND	ND	NC	-12.2%	
				12	Rosuvastatin 40 mg/day	ND	ND	ND	ND	ND	ND	ND	ND	NC	-23.0%	
Watts et al. (2003)	Randomized, double-blind, placebo-controlled, cross-over	Men with metabolic syndrome	5 weeks	11	Overall	46.3±6.9	0 (0.0)	30.5±2.6	227.4±19.3	152.4±26.7	36.3±5.4	215.2±92.1	ND			Immunoturbido-metry
				11	Atorvastatin 40 mg/day	ND	ND	ND	ND	ND	ND	ND	ND	NC	-18.0%	
				11	Placebo	ND	ND	ND	ND	ND	ND	ND	ND	NC		

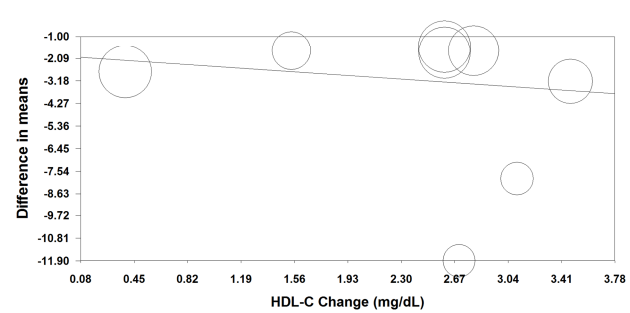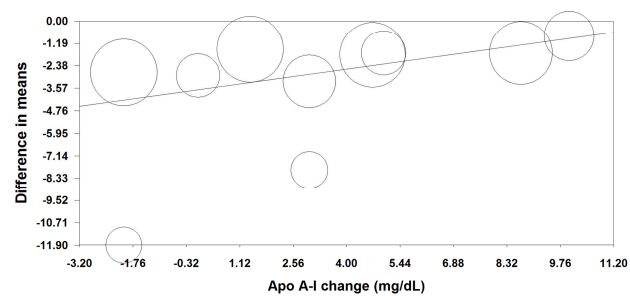Values are expressed as mean ± SD

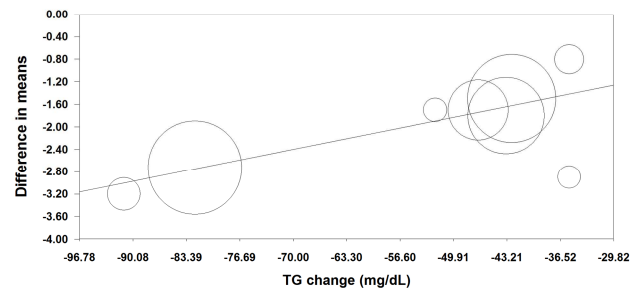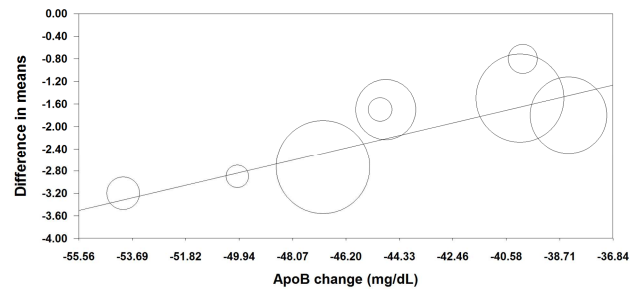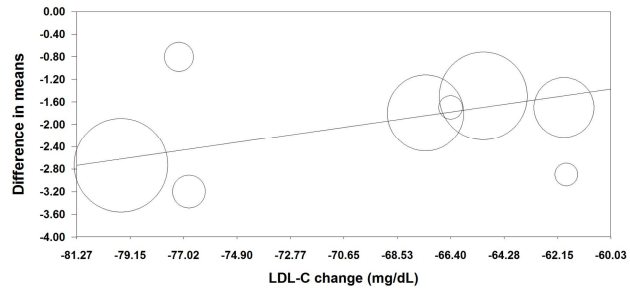** Values are expressed as mean ± SEM

* LDL values were calculated by the Friedwald equation

Abbreviations: ND, no data; BMI, body mass index; CAD, coronary artery disease; NC, not calculable.

Group by Drug type	Study name	Statistics for each study							Difference in means and 95% CI
		Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value	
Atorvastatin	Chan et al., 2010a	-0.800	1.303	1.697	-3.354	1.754	-0.614	0.539	
Atorvastatin	Chan et al., 2010b	-2.900	1.664	2.768	-6.161	0.361	-1.743	0.081	
Atorvastatin	Watts et al., 2003	-2.730	0.409	0.167	-3.532	-1.928	-6.671	0.000	
Atorvastatin	Dallinga-thie 2004a	-7.900	2.135	4.557	-12.084	-3.716	-3.701	0.000	
Atorvastatin	Dallinga-thie 2004b	-11.900	2.193	4.809	-16.198	-7.602	-5.426	0.000	
Atorvastatin		-4.745	1.497	2.242	-7.680	-1.810	-3.169	0.002	
Lovastatin	Hodis et al., 1994	-1.500	0.435	0.189	-2.352	-0.648	-3.450	0.001	
Lovastatin	Alaupovic et al., 1994a	-1.700	0.636	0.404	-2.946	-0.454	-2.674	0.007	
Lovastatin	Alaupovic et al., 1994b	-1.800	0.501	0.251	-2.781	-0.819	-3.596	0.000	
Lovastatin		-1.644	0.292	0.085	-2.216	-1.072	-5.637	0.000	
Rosuvastatin	Ooi et al., 2008a	-1.700	1.598	2.555	-4.833	1.433	-1.064	0.288	
Rosuvastatin	Ooi et al., 2008b	-3.200	1.159	1.343	-5.471	-0.929	-2.761	0.006	
Rosuvastatin		-2.683	0.938	0.880	-4.522	-0.844	-2.860	0.004	
Overall		-1.836	0.274	0.075	-2.373	-1.299	-6.706	0.000	

-17.00   -8.50   0.00   8.50   17.00

Favours Statin Favours Placebo

Group by	Study name	Statistics for each study							Difference in means and 95% CI
Drug type		Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value	
Atorvastatin	Chan et al., 2010a	-0.800	1.303	1.697	-3.354	1.754	-0.614	0.539	
Atorvastatin	Chan et al., 2010b	-2.900	1.664	2.768	-6.161	0.361	-1.743	0.081	
Atorvastatin	Watts et al., 2003	-2.730	0.409	0.167	-3.532	-1.928	-6.671	0.000	
Atorvastatin	Dallinga-thie 2004a	-7.900	2.135	4.557	-12.084	-3.716	-3.701	0.000	
Atorvastatin	Dallinga-thie 2004b	-11.900	2.193	4.809	-16.198	-7.602	-5.426	0.000	
Atorvastatin		-4.745	1.497	2.242	-7.680	-1.810	-3.169	0.002	
Lovastatin	Hodis et al., 1994	-1.500	0.435	0.189	-2.352	-0.648	-3.450	0.001	
Lovastatin	Alaupovic et al., 1994a	-1.700	0.636	0.404	-2.946	-0.454	-2.674	0.007	
Lovastatin	Alaupovic et al., 1994b	-1.800	0.501	0.251	-2.781	-0.819	-3.596	0.000	
Lovastatin		-1.644	0.292	0.085	-2.216	-1.072	-5.637	0.000	
Rosuvastatin	Ooi et al., 2008a	-1.700	1.598	2.555	-4.833	1.433	-1.064	0.288	
Rosuvastatin	Ooi et al., 2008b	-3.200	1.159	1.343	-5.471	-0.929	-2.761	0.006	
Rosuvastatin		-2.683	0.938	0.880	-4.522	-0.844	-2.860	0.004	
Overall		-1.836	0.274	0.075	-2.373	-1.299	-6.706	0.000	

Favours Statin Favours Placebo

Highlights:

- Results suggest that treatment with statins reduces plasma Apo CIII levels

- This can explain why statins also have a TG-lowering effect

- This effect of statins might contribute to their antiatherosclerotic effects