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Evaluation of the Pleiotropic Effects of Statins: A re-analysis of the randomized trial evidence using Egger regression

Short title: Pleiotropic effects of statins

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Abstract

Objective: To re-analyze data from recent randomized trials of statins to assess whether the benefits and risks of statins are mediated primarily via their LDL-C lowering effects or via other mechanisms.

Approach and Results: We adapted Egger regression, a technique frequently used in Mendelian randomization studies to detect genetic pleiotropy, to re-analyze the available randomized trial (RCT) data of statin therapy. For cardiovascular endpoints, each 1 mmol/L change in LDL-C with statin therapy was associated with a hazard ratio (HR) of 0.77 (95% CI 0.71-0.84) with an intercept that was indistinguishable from zero [intercept = -0.032 (p=0.93)], indicating no pleiotropy. For incident diabetes, a 1 mmol/L change in LDL-C with statin therapy was associated with a HR 1.07 (95%CI 0.99-1.16) and an intercept non-distinguishable from zero, [intercept = -0.015 (p=0.91)], again indicating no pleiotropy.

Conclusion: Our re-analysis of the RCT data using Egger regression adds to the existing evidence that the cardiovascular benefits of statins and their association with incident diabetes are mediated primarily, if not entirely, via their LDL-C lowering properties rather than by any pleiotropic effects.

Abbreviations

cardiovascular (CV)

low density lipoprotein (LDL)

low density lipoprotein cholesterol (LDL-C)

inverse-variance weights (IVW)

instrument strength independent of direct effect (InSIDE)

no measurement error (NOME)

single nucleotide polymorphism (SNP)

Statins directly inhibit HMG-CoA reductase, a critical enzyme regulating *de novo* intracellular cholesterol synthesis. This leads to upregulation of hepatic low-density lipoprotein (LDL) receptors and lower circulating LDL, which has been presumed to be the primary mechanism producing cardiovascular benefit. Despite prior analyses suggesting that the cardiovascular benefits of statins are mediated mainly by the reduction in low density lipoprotein cholesterol (LDL-C)[1], there has been ongoing debate as to whether the observed benefits and risks of statins are mediated exclusively via their LDL lowering properties or whether other pleiotropic mechanisms come into play[2,3,4]. The findings from the JUPITER trial[5] and subsequent meta-analyses,[6,7] which demonstrated that statins also increase the risk of new onset diabetes by yet undetermined mechanisms, as well as the recently completed FOURIER trial,[8] , have reignited this debate.

Herein, we present a new approach to address whether the benefits and risks of statins are mediated primarily via their LDL-C lowering effects or via other mechanisms. By adapting Egger regression, a technique frequently used in Mendelian randomization studies to detect genetic pleiotropy, we re-analyze the available randomized trial data of statin therapy.

Methods

We performed a literature review of randomized trials of statin therapy for either primary or secondary prevention. In our analysis, we only included trials that provided data for a per mmol change in LDL for our two main outcomes of interest: major cardiovascular events and new onset diabetes. We compared our results to the recent meta-analyses by Sattar et al. [7] Silverman et al.[9], and Chou et al.[10] to review for any missing studies or data. We included all relevant statin trials performed for either primary and secondary prevention.

To assess the potential pleiotropic effects of statins we used a modified form of Egger regression. Briefly, Egger regression was initially developed to assess for small study effects and publication bias in meta-analyses. This method has been recently adapted by Bowden *et al* for the Mendelian randomization context to assess for genetic pleiotropy[11]. Herein, we further adapt Egger regression to the RCT context, to assess whether studies with small effects in the mediator of interest, in this case, change in LDL-C, (plotted on the x-axis) have the expected small effect on the outcomes of interest, in this case, reduction in cardiovascular (CV) outcomes or incident diabetes (plotted on y-axis). Egger regression estimates the y-intercept of such a linear plot to evaluate whether the effect of statins is zero when the LDL-C lowering effect is zero, which would indicate no pleiotropy.

First, we performed standard linear meta-regression using the log transformed outcome measure from the published studies as the dependent variable and the mean LDL change with therapy as the independent variable. For each outcome of interest (e.g. reduction in CV events or new-onset diabetes), we performed two such linear regressions, first using inverse-variance weights (IVW) and forcing the

intercept through zero, which assumes no pleiotropy is present, as previously performed by Sattar et al.[7] and Silverman et al.[9] We then repeated the analysis using Egger regression with the intercept unconstrained which allows for possible pleiotropy. We compared the goodness of fit of the inverse variance weighted approach to the Egger regression using the Q_R statistic described by Bowden et al.[12] A Q_R statistic of ~ 1 indicates no or minimal evidence of pleiotropy. A funnel plot for each endpoint was also constructed and is available in the online appendix.

Egger regression makes several assumptions. In this context, the "instrument strength independent of direct effect" (InSIDE) assumption requires that any pleiotropic effects must be independent of the strength of the LDL-C change from statins. Furthermore, analogous to the No Measurement Error (NOME) assumption used in MR which assumes that the majority of the variation in the single nucleotide polymorphism (SNP)-exposure associations are due to true differences in exposures across SNPs as opposed to other sources of variation (i.e. measurement error for a given SNP). In our context, we assume that the variation in LDL-C change across studies is explained by true interstudy differences in LDL-C lowering by statins not by other sources of variation (i.e. intrastudy variability, in this case, measurement error and/or variable individual response to statins within a study).

Results

We included 25 primary and secondary prevention statin trials that provided information on cardiovascular endpoints (see supplementary table 1), as well as 12 statin trials that provided data on incident diabetes. (see supplementary table 2) A list of the included trials and the data included in this analysis are available in the online appendix. For the cardiovascular endpoints, the log-transformed hazard ratio for every 1 mmol/L change in LDL was -0.26, which translates into a hazard ratio of 0.77 per mmol/L change in LDL-C (95%confidence interval [CI] 0.71-0.84). (Figure 1) There was no difference in the slope of the intercept line between the IVW and Egger regression and the intercept in the Egger regression was not distinguishable from zero [intercept = -0.0320 (p=0.932)]. The QR statistic approximated 1 demonstrating no meaningful improvement to the goodness of fit of the data when the Egger approach is used (Table 1) and suggesting no directional pleiotropy.

Table 1: Results of the IVW and MR-Egger regressions for cardiovascular endpoints*

	IVW		Egger	
	Estimate (se)	p-value	Estimate (se)	p-value
Beta	-0.26 (0.017)	<0.001	-0.26 (0.042)	<0.001
Intercept	n/a‡		-0.032 (0.038)	P=0.93
Q_R	0.999			

IVW = inverse variance weight, se=standard error

*estimates are reported on the logarithmic scale

‡ with IV weight, the intercept term is forced through zero and therefore fixed

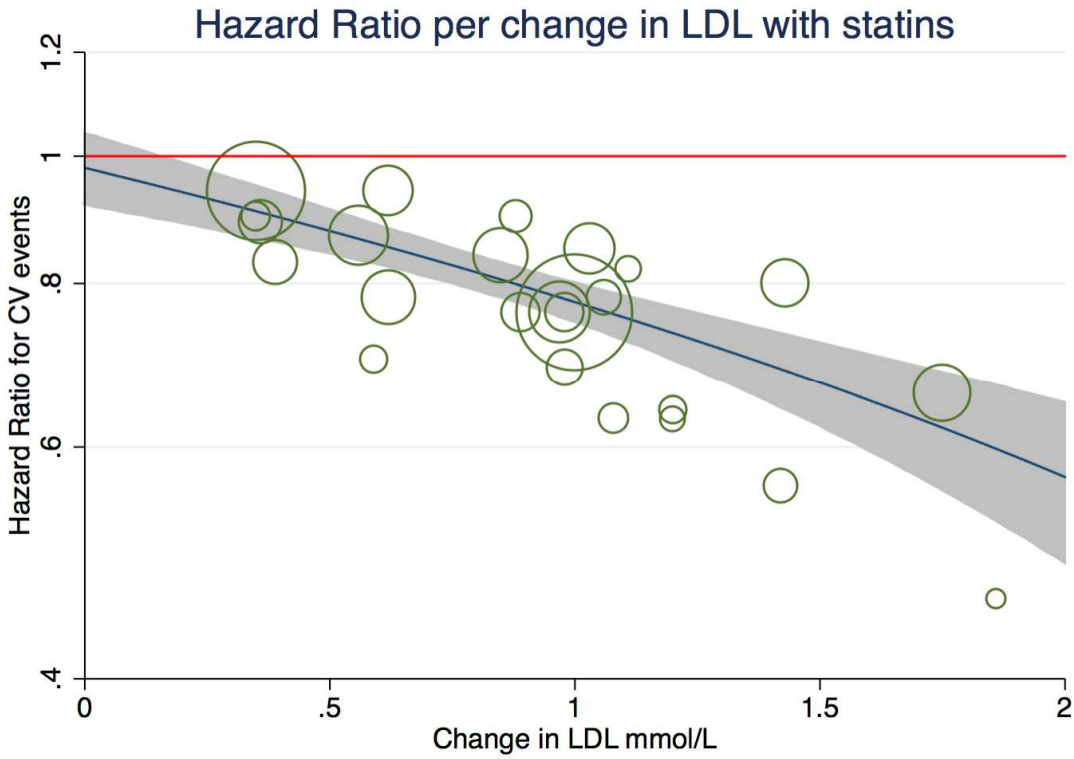

Figure 1: Hazard ratio for CV events per mmol/L change in LDL.
Shaded area reflects 95% confidence interval of regression line

For the diabetes endpoints, a per mmol change in LDL was associated with log-transformed risk ratio of 0.070, which translates into a risk ratio of 1.07 (95%CI 0.99-1.16). Figure 2. Again there was no distinguishable change when the Egger regression technique was used. The intercept using Egger regression was not statistically different from zero [intercept = -0.015 (p=0.91)]. Again, the QR statistic was very close to the null value of 1 and showed no improvement in the goodness of fit when using Egger regression (Table 2), which was again consistent with no pleiotropy.

Table 2: Results of the IVW and MR-Egger regressions for incident diabetes*

	IVW		Egger	
	Estimate (se)	p-value	Estimate (se)	p-value
Beta	0.070 (0.037)	0.089	0.081 (0.12)	0.51
Intercept	n/a‡		-0.015 (0.13)	P=0.91
Q_R	0.999			

IVW = inverse variance weight, se=standard error
*estimates are reported on the logarithmic scale

‡ with IV weight, the intercept term is forced through zero and therefore fixed

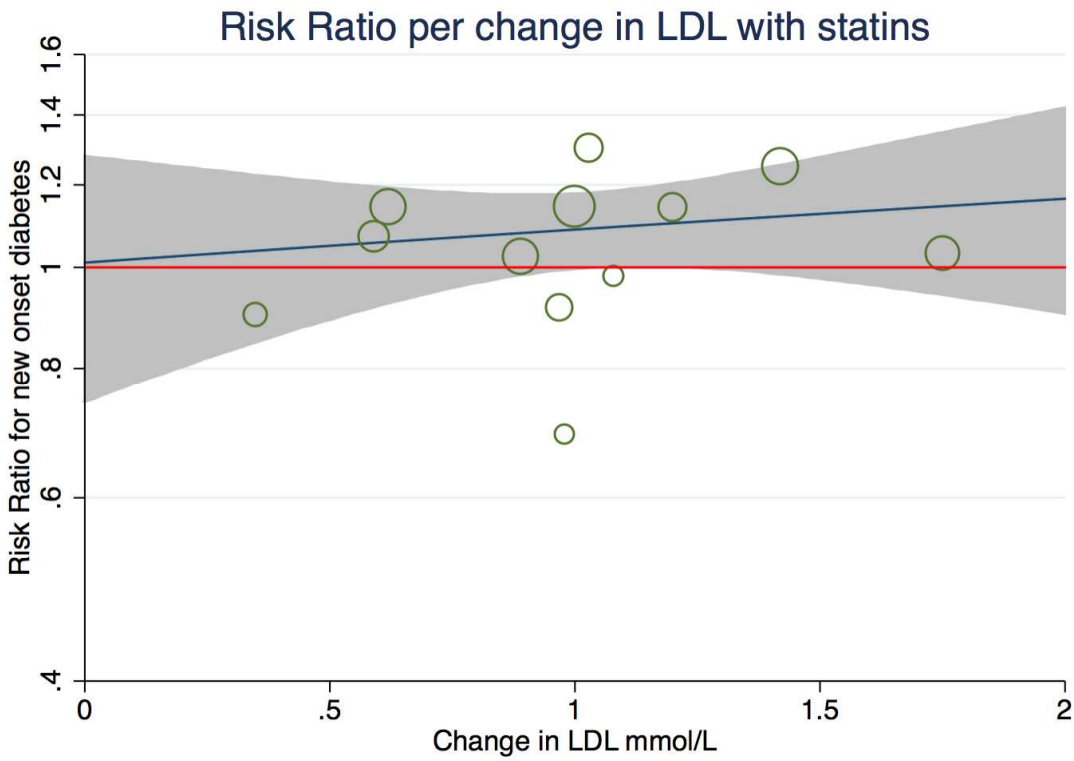

Figure 2: Hazard ratio for incident diabetes per mmol/L change in LDL.
Shaded area reflects 95% confidence interval of regression line

Discussion

Our re-analysis of the available RCTs suggests that statins do not exert any observable pleiotropic effects in mediating their benefits of reducing cardiovascular outcomes or in their risks of incident diabetes. In fact, the evidence suggests that most, if not all, of their effects are mediated through their LDL lowering mechanism. Our results using standard inverse variance weighting with a fixed intercept, mirror closely the results obtained by Silverman et al.[9] in terms of the effects per mmol change in LDL for the reduction in CV events. It is more difficult to directly compare our results on diabetes to the analysis by Sattar et al. since they reported only a graphical representation of their meta-regression and assessed LDL change as a percent change rather than as an absolute change. Nevertheless, graphically, our results appear similar. In both settings however, we extend these previous analyses by demonstrating that the intercepts of the Egger's regression are not statistically different from zero, which is consistent with no directional pleiotropy in mediating either of these outcomes. Also, the Q_R statistic for both settings is very close to unity, which suggests that the Egger's regression provides no improvement on the goodness of fit for either outcome and is consistent with a lack of pleiotropy.

Although recently used in the context of Mendelian randomization studies, Egger regression can also be applied to the current RCT context. Indeed, Egger regression was initially conceived to evaluate small study bias in meta-analyses of RCTs.[13] In MR-Egger regression, the intercept indicates whether estimates from instruments with a small effect are skewed towards either positive or negative outcomes, due to directional pleiotropy. Whereas Mendelian randomization studies are concerned with the small effects of the genetic instrumental variable, here we substitute the small effect of the genetic instrument with the small effect of statins on LDL in certain studies, i.e. studies in which statins led to a small change in LDL.

Put another way, in studies where the effects of statins on LDL are small, the risks and benefits should also be small if no pleiotropy is present. By extending the MR-Egger approach to the meta-regression context across several statin RCTs, the near zero intercept in our analysis is therefore consistent with the lack of any measurable pleiotropy from statins. If pleiotropy was operational, larger than expected benefits and/or risks would be observed when LDL change was minimal. Our analysis is therefore in keeping with the notion that the *primary* effects of statins are mediated nearly entirely via LDL-C.

It is important to highlight that our results do not exclude the potential role of downstream secondary effects of directly lowering LDL-C (e.g. lower inflammation as a result of directly lowering LDL-C). In our context, directional pleiotropy refers to effects of statins that are independent of their effect of LDL-C. Thus our results do not imply that statins only affect LDL-C. Indeed statins have been shown to have an effect on venous thromboembolic events in JUPITER [14] and may have a role in coagulation. [15] A recent genetic analysis has shown that HMGCR inhibition led not only to a lowering of LDL-C, but also to increased body weight, waist circumference, plasma insulin concentrations and plasma glucose levels.[16] These results strongly support the notion that several statin effects that may appear pleiotropic, are in fact mediated by their primary "on target" effect which is to lower LDL-C by upregulating the LDL receptor, and are therefore, entirely consistent with our findings.

Our results are dependent on the instrument strength independent of direct effect (InSIDE) assumption, which assumes that any independent pleiotropic effects are independent of the strength of the effect on LDL-C. To put simply, the InSIDE assumption holds that a causal effect, in this case between statins and CV outcomes or incident diabetes, can still be estimated if pleiotropy is present, as long as the magnitude of the pleiotropy is independent of the statin effect on LDL-C. For example, if one speculated that statins exerted independent pleiotropic effects via their lowering of C-reactive protein (CRP), the InSIDE assumption would hold true if the change in CRP mediated by statins was independent of the change in LDL-C. Although this assumption cannot be directly tested across all possible pleiotropic mechanisms, analyses from the JUPITER trial have shown that the LDL-C and CRP

are only weakly correlated and that the InSIDE assumption likely does hold true in this setting.[17]

Our results that statins exert their effects in reducing CV events and in increasing the risk of diabetes primarily via a LDL lowering mechanism is in keeping with several lines of evidence. Mendelian randomization studies have shown that, per unit change in LDL, any genetic mechanism of LDL lowering leads to similar effects on outcomes.[18,19] In addition, randomized trial evidence demonstrates that lipid lowering agents with different mechanisms of action (e.g. statins, ezetimide, and PCSK9 inhibitors) all lead to similar reductions in CV outcomes per mmol/L change in LDL-C[9]. Using Mendelian randomization, similar results have also recently been predicted for incident diabetes, with similar risks of diabetes (per unit change in LDL-C) observed across several variants in *HMGCR* and/or *PCSK9* genes.[19,20,21] Although the exact mechanism for statin-related diabetes has not been elucidated and many theories have been proposed, [22,23] increased uptake of LDL particles due to upregulation of pancreatic LDL receptor activity with subsequent tissue injury has been hypothesized, and would be consistent with these findings.

Our analysis has some limitations. First, the Egger regression results are admittedly more robust for the cardiovascular endpoints than for the incident diabetes risk. This is due to the fact that more trials have available data on cardiovascular endpoints than new cases of diabetes. Second, we utilized data on change in LDL-C due to the availability of this measure in all relevant RCTs. Our analysis focused on excluding pleiotropic effects beyond LDL lowering not differentiating whether these effects are mediated primarily by lowering the cholesterol content or the number of LDL particles. We have previously shown that statin benefit is more strongly correlated to lowering apoB, as opposed to LDL-C[24], and this has been recently corroborated using Mendelian randomization.[25] Third, it was not possible to estimate the I^2 statistic, which would have allowed us to test the NOME assumption since not all studies reported the variance of the LDL change. However, given that sample sizes of each included study was large and the effect of statins on LDL-C is also known to be relatively strong, the NOME assumption is likely reasonable in this case. Therefore, based on our results, the evidence is suggestive, if not definitive, that that the benefits of statins on CV disease reduction and incident diabetes are mediated primarily, if not entirely by their LDL lowering effect.

Conclusion

While there remains some debate regarding statin pleiotropy and off target effects, a re-analysis of the available RCT data using the technique of Egger regression suggests that the cardiovascular benefits and the risk of diabetes from statins are not mediated through pleiotropic effects but rather through their primary LDL lowering mechanism of action.

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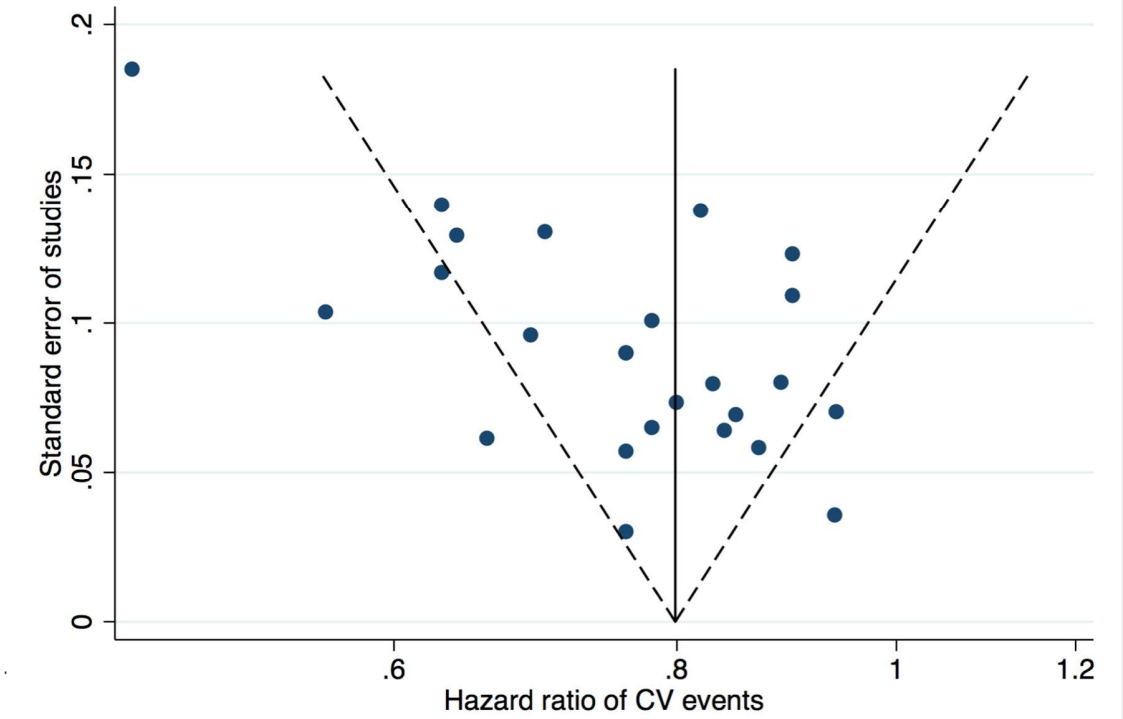
Highlights:

- Our re-analysis of the available RCTs suggests that statins do not exert any observable pleiotropic effects in mediating their benefits of reducing cardiovascular outcomes.
- The association between statins and incident diabetes is also not mediated by any observable pleiotropic effects.
- The evidence suggests that most, if not all, of statins' effects are mediated through their LDL lowering mechanism.

Supplemental figure 1: Funnel plot of studies for CV endpoint



Supplemental figure 2: Funnel plot of studies for incident diabetes endpoint