Statin therapy and plasma free fatty acids: A systematic review and meta-analysis of controlled clinical trials

Statin therapy and Plasma Free Fatty Acids: A Systematic Review and Meta-Analysis of Controlled Clinical Trials

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Abstract

Background. It has been suggested that free fatty acids (FFAs) may contribute to coronary heart disease through promoting atherogenesis and thrombogenesis. The use of statins has been shown to reduce cardiovascular outcomes in primary and secondary prevention. However, studies evaluating the effects of statins on circulating FFA concentrations in humans are scarce and contradictory.

Objective. To evaluate the effect of statin therapy on plasma FFA concentrations in a systematic review and meta-analysis of controlled clinical trials.

Methods. PubMed-Medline, SCOPUS, Web of Science and Google Scholar databases were searched **(**from inception to February 16, 2015) to identify controlled trials evaluating the impact of statins on plasma FFA concentrations. A systematic assessment of bias in the included studies was performed using the Cochrane criteria. 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. Random-effects meta-regression was performed using unrestricted maximum likelihood method to evaluate the impact of potential moderators.

Results. Meta-analysis of data from 14 treatment arms indicated a significant reduction in plasma FFA concentrations following treatment with statins (WMD: -19.42%, 95% CI: -23.19, -15.64, $p < 0.001$). Subgroup analysis confirmed the significance of the effect with both atorvastatin (WMD: -20.56%, 95% CI: -24.51, -16.61, $p < 0.01$) and simvastatin (WMD: -18.05%, 95% CI: -28.12, -7.99, $p < 0.001$). Changes in plasma FFA concentrations were independent of treatment duration (slope: -0.10; 95% CI: -0.30, 0.11; $p = 0.354$) and magnitude of reduction in plasma low-density lipoprotein cholesterol concentrations (slope: 0.55; 95% CI: -0.17, 1.27; $p = 0.133$) by statins.

Conclusion. Results of the present study suggest that statin therapy significantly reduces plasma FFA concentrations.

Keywords: Statins; Fatty acid; Dyslipidemia; Cholesterol; Meta-analysis

Introduction

Inhibitors of 3-hydroxy-3methyl glutaryl coenzyme A (HMG-CoA), known as statins, are the most frequently administered type of cholesterol-lowering drugs. The use of statins has been consistently shown to decrease cardiovascular outcomes and mortality in both primary and secondary prevention trials (1-4). The main effect of statins is decreasing plasma low-density lipoprotein cholesterol (LDL-C) levels by inhibition endogenous cholesterol biosynthesis leading to overexpression of hepatic LDL receptors (5). In addition, statin therapy can reduce triglycerides and increase high-density lipoprotein (HDL) cholesterol levels (6,7).

Thus, it has been suggested that free fatty acids (FFAs) may contribute to coronary heart disease by either atherogenesis or promoting thrombogenesis (8).

FFAs are non-esterified fatty acids that are released from adipocyte triglyceride stores by lipolysis, and from phospholipids after hydrolysis by phospholipases (9). The availability of FFAs is an important signal to trigger the formation of cholesterol in the liver (10). In addition, FFAs promote the formation and release of triglycerides by the liver that leads to an overproduction of very low-density lipoprotein (VLDL) triglyceride (11, 12) and consequently development of atherogenic dyslipidemia characterized by high triglycerides and low HDL cholesterol. On the other hand, overproduction of VLDL and LDL is associated with reduction of fatty acid trapping and retention by adipose tissue leading to elevated plasma FFA levels and increased FFA flux to the liver causing hepatic insulin resistance and inflammation (13).

Fatty acid metabolism was once considered to be unchanged by statin therapy (14-16); however, mild elevations in fatty acid synthesis were subsequently reported in animal models (17) cultured cells (18), and after statin treatment in mice (19). The main effect of statins is a decrease in circulating LDL cholesterol via inhibition of hepatic cholesterol synthesis and consequent regulation of hepatic LDL receptors; however, studies evaluating the statin effects on plasma lipid

and lipoprotein metabolism have revealed more complex mechanisms (20, 21). In this regard, the statin therapy increases receptor-mediated clearance of LDL in subjects with familial hypercholesterolemia (5, 22) and normal individuals (23), finding consistent with the expected response of LDL receptors to inhibition of hepatic cholesterol synthesis (24). Nonetheless, these drugs also modify other lipid components (6, 7). Also statins may inhibit hepatic synthesis of apolipoprotein B-100 and decrease the synthesis and secretion of triglyceride-rich lipoproteins (25, 26). Hitherto, the molecular mechanism by which statins reduce triglycerides levels is not known with certainty. In this regard, statins could affect hepatic FFA metabolism (27). However, studies evaluating the effects of statins on serum fatty acid metabolism in humans are scarce and contradictory (28). Therefore, the aim of this study was to evaluate the effect of statin therapy on plasma FFA concentrations and calculate the size of this effect using a systematic review and meta-analysis of controlled clinical trials.

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 (29). 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 (“free fatty acid” OR "free fatty acids” OR FFA OR FFAs). 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 human. The literature was searched from inception to February 16, 2015.

Study Selection

Original studies were included if they met the following inclusion criteria: (i) being a controlled trial with either parallel or cross-over design, (ii) investigating the impact of statin therapy, either as monotherapy or combination therapy, on plasma/serum concentrations of FFAs, (iii) treatment duration of at least two weeks, (iv) presentation of sufficient information on FFA concentrations at baseline and at the end of follow-up in each group or providing the net change values. Exclusion criteria were (i) non-interventional 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 FFA 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 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), and triglycerides; 7) systolic and diastolic blood pressures; and 8) data regarding baseline and follow-up concentrations of FFAs.

Quality assessment

A systematic assessment of bias in the included studies was performed using the Cochrane criteria (30). 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) (31). 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 FFA were calculated by subtracting the value after control intervention from that reported after treatment. All values were collated as percent change from baseline in each group, or percent change in the statin group relative to control group. 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. If the outcome measures were reported in median and range (or 95% confidence interval [CI]), mean and standard SD values were estimated using the method described by Hozo et al. (32). 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. To avoid the problem of double-counting in randomized controlled trials with multiple treatment arms and

a common control group, the number of subjects in the control group was divided by the required comparisons.

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 (33). 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 statin type and treatment duration (< 12 weeks versus ≥ 12 weeks) on plasma FFA concentrations. 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 (34, 35).

Meta-regression

Random-effects meta-regression was performed using unrestricted maximum likelihood method to evaluate the association between calculated WMD and potential moderators including duration of treatment with 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 method was used to adjust the analysis for the effects of publication bias (36).

Results

Flow and characteristics of included studies

Firstly, 189 published studies were identified following database search. After reviewing the titles and abstracts, 117 studies did not meet the inclusion criteria and were excluded. Then, 19 full text articles were carefully assessed and reviewed; of which 10 studies were excluded for non-interventional design (n=1), being non-original (n=1), presenting incomplete data (n=2), not measuring plasma/serum FFA concentrations (n=4), and not being controlled for statin therapy (n=2). Finally, 9 eligible studies with 14 treatment arms were included in the systematic review and meta-analysis. The study selection process is shown in **Figure 1**.

A total of 764 individuals were incorporated in the 9 eligible controlled trials, including 462 and 302 subjects in the statin and control groups (participants from the cross-over trials were counted in both treatment and control groups), respectively. Included studies were published between 1991 and 2014. The clinical trials used only atorvastatin and simvastatin but with different doses. Two studies used atorvastatin 10 mg/day (37, 38), one study atorvastatin 20 mg/day (39), one study atorvastatin 80 mg/day (38), one study simvastatin 10 mg/day (40), one study simvastatin 20 mg/day (8), one study simvastatin 30 mg/day (41), three studies simvastatin 40 mg/day (8, 42, 43) and one study simvastatin 80 mg/day (44). The range of intervention periods was from 3 weeks (41) to 2 years (8). Study designs of included studies were parallel (8, 38-40, 42-44) and cross-over (37, 41). Selected trials enrolled subjects with primary hypercholesterolemia (8, 42, 43), type 2 diabetes (38, 41, 44), metabolic syndrome (37, 40), and mixed dyslipidemia (39). Finally, FFA concentrations were determined by an enzymatic microfluorimetric method.

Risk of bias assessment

Seven included studies were characterized by lack of information about the sequence generation, allocation concealment, and blinding of participants, personnel and outcome assessors (8, 37, 38, 41-44). In addition, two studies had high risk of bias for sequence generation and allocation concealment (42, 43). However, six evaluated studies showed low risk of bias with respect to selective outcome reporting and other sources of bias (8, 38-40, 42, 44). Finally, all trials had low risk of bias for incomplete outcome data. Details of the quality of bias assessment are shown in **Table 2**.

Effect of statin therapy on plasma FFA concentrations

Meta-analysis of data from 14 treatment arms revealed a significant reduction in plasma FFAs following treatment with statins (WMD: -19.42%, 95% CI: -23.19, -15.64, $p < 0.001$). This effect was robust in the sensitivity analysis (**Figure 2**). In subgroup analysis, reductions in plasma FFA levels were observed in both subsets of trials with treatment durations < 12 weeks (WMD: -7.38%, 95% CI: -13.36, -1.40, $p = 0.016$) and ≥ 12 weeks (WMD: -23.51%, 95% CI: -25.88, -21.14, $p < 0.001$), though the effect size was numerically greater in the latter group (**Figure 3**). With respect to the type of statin, there were significant reductions in plasma FFA concentrations with both atorvastatin (WMD: -20.56%, 95% CI: -24.51, -16.61, $p < 0.01$) and simvastatin (WMD: -18.05%, 95% CI: -28.12, -7.99, $p < 0.001$) (**Figure 4**).

Meta-regression

Random-effects meta-regression was performed to evaluate the impact of potential moderators on the estimated effect size. Changes in plasma FFA concentrations were independent of treatment

duration (slope: -0.10; 95% CI: -0.30, 0.11; $p = 0.354$) and magnitude of LDL-C reduction (slope: 0.55; 95% CI: -0.17, 1.27; $p = 0.133$) by statins (**Figure 5**).

Publication bias

The funnel plot of standard error versus effect size (mean difference) was asymmetric and suggested potential publication bias. Presence of publication bias was also suggested by Egger's linear regression (intercept = 1.05, standard error = 0.48; 95% CI = 0.004, 2.10, $t = 2.19$, df = 12.00, two-tailed $p = 0.049$) but not Begg's rank correlation test (Kendall's Tau with continuity correction = 0, $z = 0$, two-tailed p-value = 1.000). After adjustment of effect size for potential publication bias using "trim and fill" correction, five potentially missing studies on the left side of funnel plot were imputed leading to a corrected effect size that was greater than the initial estimate (WMD: -21.54%, 95% CI: -25.44, -17.64) (**Figure 6**). The "fail-safe N" test showed that 1417 studies would be needed to bring the WMD down to a non-significant ($p > 0.05$) value.

Discussion

The findings of the present meta-analysis suggested a significant reduction in plasma FFA levels following treatment with statins, an effect that was independent of statin type, treatment duration, and magnitude of changes in plasma LDL-C concentrations. Although the effect of statins on FFA metabolism are unclear, there are several mechanism that may be involved. In this regard, acetyl coenzyme A carboxylase and fatty acid synthase, two important regulatory enzymes in fatty acid biosynthesis, could be regulated simultaneously by HMG-CoA reductase enzyme at the genomic level. Therefore, activity of both enzymes could be influenced by statin therapy (45, 46). Since HMG-CoA reductase and acetyl coenzyme A carboxylase are reversibly inactivated through

phosphorylation by AMP-activated protein kinase (47), down-regulation of this enzyme by sterol deficiency would increase acetyl coenzyme A carboxylase activity after statin therapy when regulatory sterols are absent (48). In addition, a pleiotropic effect of statins on peroxisome proliferator-activated receptor-α expression has been described. Statins activate the peroxisome proliferator-activated receptor-α which increases the hepatic fatty acid uptake promoting the transformation of fatty acids to acyl-coenzyme A increasing the beta-oxidation of fatty acids resulting in a reduced availability of fatty acids (49). However, the information on the effects of statins on plasma FFA concentrations in humans is still limited. Therefore, we conducted a random-effects model and the generic inverse variance method to compensate for the heterogeneity of studies and sensitivity analysis to evaluate the influence of each study on the overall effect size, after which remained a significant reduction in plasma FFAs following treatment with statins.

Circulating FFAs can mainly derive from adipose tissue lipolysis. Hepatic FFAs are available from *de novo* lipogenesis and uptake of triglycerides-rich lipoproteins, cholesteryl esters, and plasma FFAs. The most likely mechanism for the FFA-reducing effect of statins is inhibition of intrahepatic cholesterol biosynthesis (50) resulting in increased removal and decreased hepatic secretion of VLDL (51). Interestingly, lipid-lowering drugs have shown a reduction of serum total fatty acid concentration while simultaneously increasing the proportion of long-chain polyunsaturated fatty acids and precursor fatty acids for eicosanoid production (52). Circulating total fatty acids are found in different forms: 45% in triacylglycerols, 15% in cholesteryl esters, 35% in phospholipids, and about 5% as nonesterified free fatty acids. Between 75% and 80% of serum cholesterol is esterified with fatty acids and only 20% to 25% is nonesterified cholesterol (52). Nonetheless, statins appear to exert their effects on the metabolism and serum composition

of FFAs through disturbing the biogenesis of isolated fatty acids independently of the mechanism that regulates lipoprotein synthesis and secretion. In this context, the results of meta-regression analysis revealed that changes in plasma FFA concentrations were independent of the magnitude of LDL-C reduction; hence, other mechanisms of statin therapy may be involved.

There is evidence indicating that treatment with statins is a risk factor for the development of new-onset type 2 diabetes (53), though the causes of this negative effect remain unexplained. In this regard, it has been suggested that statins may generate insulin resistance and impair β-cell function (54). In animal models, long-term treatment with statins increased insulin resistance in the adipose tissue (55).

Both increased efflux of free fatty acids from adipose tissue and impaired insulin-mediated skeletal muscle uptake of free fatty acids, increase fatty acid flux to the liver promoting the development of peripheral insulin resistance (56, 57). The association between plasma FFA levels and insulin resistance has been supported by epidemiological studies (58). Controversial results have been reported as to the differential effects of statins on insulin sensitivity. While pravastatin appears to improve insulin sensitivity, simvastatin has been reported to have negative effects (59). On the other hand, atorvastatin and rosuvastatin had no significant effect on insulin sensitivity (59). According to the present results, statins are unlikely to exert a negative effect on insulin sensitivity through a FFA-mediated mechanism. Thus, further studies are needed to elucidate the mechanisms underlying modulation of insulin sensitivity by statins

Some limitations of this study should be mentioned. The most important one was the small sample size in several of the included studies. As another limitation, changes in plasma FFA levels were not among the primary objectives of any of the included studies. Thus, further studies are

warranted to evaluate the effect of statin therapy on FFA concentrations as primary outcome to obtain more robust evidence about the effects of statins on circulating FFA status.

In conclusion, results of this meta-analysis, being the first of its kind, showed that statin therapy significantly reduces plasma FFA concentrations. Future investigations are required to clarify if this effect of statin therapy accounts, at least in part, for the established cardiovascular benefits of these drugs. Also, the association of this effect of statins with the hepatic content of FFAs and risk of hepatic insulin resistance needs to be elucidated in future studies.

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TABLES

Table 1. Demographic characteristics of the included studies.

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

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 FFA concentrations. Lower plot shows leave-one-out sensitivity analysis.

Figure 3. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of statin therapy on plasma FFA concentrations in trials with treatment durations of < 12 weeks (upper plot) and ≥ 12 weeks (lower plot).

Figure 4. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of atorvastatin (upper plot) and simvastatin (lower plot) on plasma FFA concentrations.

Figure 5. Meta-regression plots of the association between mean changes in plasma FFA concentrations with duration of statin therapy (upper plot) and magnitude of LDL-C reduction (lower plot).

Figure 6. Funnel plot displaying publication bias in the studies reporting the impact of statin therapy on plasma FFA concentrations. Open diamond represents observed effect size; closed diamond represents imputed effect size.