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Protocol for systematic review and meta-analysis: impact of statins as immune-modulatory agents on inflammatory markers in adults with chronic diseases

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ABSTRACT

Introduction: Statins, also known as 3-Hydroxy-3-Methylglutaryl Co-A (HMG-CoA) reductase inhibitors, are lipid-lowering agents that are central in preventing or reducing the complications of atherosclerotic cardiovascular disease. Because statins have anti-inflammatory properties, there is considerable interest in their therapeutic potential in other chronic inflammatory conditions. We aim to identify the statin with the greatest ability to reduce systemic inflammation, independent of the underlying disease entity.

Methods and analysis: We aim to conduct a comprehensive search of published and peer-reviewed randomized controlled clinical trials (RCT), with at least one intervention arm of an FDA or EMA-licensed statin and a minimum treatment duration of 12 weeks. Our objective is to investigate the effect of statins (atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin) on lipid profile, particularly, cholesterol low-density lipoprotein (LDL-C) and inflammation markers such as hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16 in adults, published in the last 20 years (between January 1999 and December 2019). We aim to identify the most potent statin to reduce systemic inflammation and optimal dosing. The following databases will be searched: Medline, Scopus, Web of Science, and Cochrane Library of Systematic Reviews. Risk of bias of included studies will be assessed by Cochrane Risk of Bias tool and Quality Assessment Tool for Quantitative Studies. The quality of studies will be assessed, to show uncertainty, by Jadad score. If sufficient evidence is identified, a meta-analysis will be conducted with risk ratios or odds ratios with 95% confidence intervals (CI) in addition to mean differences.

Ethics and dissemination: Ethics approval is not required as no primary data will be collected. Results will be presented at conferences and published in a peer-review journal.

PROSPERO registration number: Pending

Strengths and limitations of this study

- This study will include randomized controlled clinical trials to determine the most effective statin on the combined reduction of lipid profile and inflammatory biomarkers.
- High-quality clinical trials will be reviewed accurately to generate reliable evidence.
- This study will be conducted following Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P) guidelines.
- Variation of statin doses among included studies will likely produce heterogeneity that will subsequently reduce the sample size of the meta-analysis.

INTRODUCTION

Statins are US Food & Drug Administration (FDA) approved lipid-lowering drugs (Table 1) that have been on the market for more than 30 years[1] and are widely prescribed to patients who are at high risk of cardiovascular diseases.[2] Statins exert their function via inhibiting 3-Hydroxy-3-Methylglutaryl Coenzyme-A (HMG-CoA) reductase which converts HMG-CoA into L-mevalonate resulting in reduced cholesterol biosynthesis.[3] The cholesterol biosynthesis inhibition via statins results in the upregulation of Low-Density Lipoprotein (LDL) receptors on the cell surface which consequently leads to increased uptake and clearance of LDL in the circulating blood. This ultimately lowers LDL-C and decreases the risk associated with lipoprotein deposition in the arterial wall and progression to atherosclerosis and vascular disease. In addition to statins' LDL-C lowering ability, statins also inhibit protein prenylation,[4] which is an important biological process that mediates protein-protein interaction and anchoring of cell membrane proteins.[5] The ability of statins to inhibit isoprenoids, important metabolites in the protein prenylation pathway, account for their lipid-independent pleiotropic effects.[6 7] Indeed statins have been reported to have anti-inflammatory, antioxidant, anti-proliferative and immunomodulatory effects independent of their cholesterol-lowering ability.[8] The reported vascular effects of statins are wide-ranging and include improvement of endothelial functioning, decreasing oxidative stress and maintenance of coronary artery plaque stability.[8] Statins may also lower the risk of liver cancer.[9] The anti-inflammatory effects vary among the different types of currently licensed statins with various meta-analyses reporting differential efficacy in reducing inflammation in chronic obstructive pulmonary disease (COPD).[10] Statins (Table 1) are categorized into two main groups according to their solubility: 1) hydrophilic statins which include pravastatin and rosuvastatin and these display high hepato-selectivity with increased first-pass effect; and 2) lipophilic statins which are characterized by passive diffusion into cells; these include atorvastatin, simvastatin, lovastatin, fluvastatin, pitavastatin and cerivastatin.[11]

Statins and inflammation

Inflammatory responses to various clinical conditions result in elevated secretion and activity of acute inflammatory proteins such as C-reactive protein (CRP). In the liver, CRP is mainly secreted by hepatocytes in response to IL-6[12]. Increased secretion of IL-6 and CRP further exacerbate the inflammatory milieu through secretion of pro-inflammatory cytokines such as TNF, activation of the complement pathway, apoptosis, phagocytosis, and nitric oxide release.[13] Previous clinical trials have reported statin therapy to reduce CRP levels through an LDL cholesterol-independent mechanism,[14 15] resulting in better clinical outcomes in patients with reduced CRP.[16] In addition, atorvastatin therapy was shown to reduce inflammatory biomarkers such as high sensitive CRP (hsCRP) and IL-6 in patients with unstable angina who received the percutaneous coronary intervention and furthermore reduced cardiac troponin I (cTnl) and Creatine Kinase muscle/brain (CK-MB) suggesting a reduction in cardiac myocyte necrosis.[17] Additionally, the PRINCE clinical trial reported pravastatin (40 mg/day) therapy to have a significant reduction in CRP levels following 12 and 24 weeks of treatment.[14] Statin therapy further resulted in the downregulation of other inflammatory biomarkers, such as IL-8 and sCD14, in patients with coronary artery inflammation.[18 19] Currently it is not fully elicited on how different types of statins (hydrophilic or lipophilic, Table 1) or the treatment duration differentially affect immune responses.

Mechanisms to reduce inflammation

Statins are selectively taken up by hepatocytes and decrease inflammatory responses by regulating the expression of various cell surface molecules/receptors, transcription factors, cytokines, chemokines and other soluble inflammatory mediators.[20] Furthermore, their ability to be taken up by other cell types, including immune cells, depend on the expression of cell membrane transport proteins and their chemical properties.[11 21] Statins can enter their target cells either through passive diffusion,[11] or active transport which involves transmembrane proteins within the organic anionic-transporting polypeptide (OATP)[21 22] and Na+ taurocholate co-transporting polypeptides (NTCP) groups.[23]

Effects on cell surface receptor

Even though statins were shown to have no effect on peripheral frequencies of circulating $CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{++}$ monocyte subsets, statins were shown to reduce expression of cell surface receptors such as vascular endothelial growth factor receptor-2 (VEGFR-2), Toll-like receptor (TLR)-4 and tyrosine kinase receptor Tie2 which are involved in proliferation, migration and pathogen recognition within all monocyte populations.[24] Furthermore, statins downregulate the expression of Toll-like receptor (TLR)-2, human leukocyte antigen (HLA)-DR and CC-Chemokine Receptor-2 (CCR-2) on monocytes, while increasing Peroxisome Proliferator Activated Receptor-gamma (PPAR-γ) activity, which enhances their anti-inflammatory properties.[17 25] The ability of statins to reduce chemokine and chemokine receptor expression on human vascular endothelial cells and human primary macrophages are achieved via inhibition of the isoprenoid geranylgeranyl pyrophosphate pathway.[26]

Effect on cell signalling

Statins are documented to affect cellular functionality of both monocytes and T cells through altering activation of lymphocyte function-associated antigen (LFA)-1 integrin molecules that are involved in lymphocyte adhesion, migration and transduction of co-stimulatory signals to T cells during antigen presentation.[27] Activation of LFA-1 integrin molecules leads to conformational changes on their structures, thus increasing their binding affinity for their respective substrates, which further enhances pro-inflammatory responses.[28] However, cellular uptake of statins is reported to inhibit these conformational changes in LFA-1 molecules and further enhance their anti-inflammatory properties.[27] Statins also modulate immune responses through alteration of cell-to-cell interaction. Here statins suppress monocyte-derived dendritic cells resulting in reduced T cell activation, proliferation and T helper differentiation.[25]

Downstream effects on soluble biomarkers

Statins inhibit monocyte chemoattractant protein-1 (MCP-1) secretion, resulting in decreased leukocyte recruitment during inflammation.[29] Statins suppress the production of pro-inflammatory cytokines such as IL-6 and IL-8 in IL-1β-stimulated synoviocytes from rheumatoid arthritis patients via interference in protein prenylation and NF-κβ pathway.[30]

Classification of statins

Statins are classified based on several different factors:

Source of origin: They are classified as natural, semi-synthetic or fully synthetic (Table 1). Natural statins are acquired from fungal fermentation and these include lovastatin and pravastatin. Simvastatin is classified as a semi-synthetic statin because it is produced through direct alkylation of lovastatin. Fully synthetic statins are produced from different substrates and these include pitavastatin, rosuvastatin, fluvastatin, atorvastatin and cerivastatin.[11]

Pharmacological properties: Two pharmacological properties differentiate statins; they are either prodrugs or active drugs (Table 1). Prodrug statins include lovastatin and simvastatin; they are administered in an inactive state and are activated through hydrolysis by liver enzymes. Atorvastatin, cerivastatin, fluvastatin, and pravastatin are administered as active drugs.[11]

Physiochemical properties: Statins are classified as lipophilic or hydrophilic (Table 1). Atorvastatin, simvastatin, lovastatin, fluvastatin, cerivastatin, and pitavastatin are relatively lipophilic statins as they dissolve efficiently in lipid/fat solution. Cytochrome P450 enzyme mediates complete metabolism of all lipophilic statins except pitavastatin.[11] Pravastatin and rosuvastatin are classified as hydrophilic statins as they dissolve efficiently in water. Pravastatin and rosuvastatin are excreted largely as the parent compound into faeces, urine and bile.[31 32]

Liver selectivity: The hepato-selective processing of statins is defined by their solubility profile; therefore, lipophilic statins diffused passively through hepatocyte cell membranes, whereas hydrophilic statins' uptake occurs through carrier transmembrane proteins.[11]

Statins in clinical conditions other than cardiovascular disease

Inflammation

Statin therapy has been reported to have a wide range of potentially beneficial effects. These include the improved clinical outcome of chronic kidney disease in patients presenting with the acute coronary syndrome.[33] Statins also reduced mortality in cirrhotic patients with bacteraemia and pneumonia.[34] Additionally, a 2-year treatment period with atorvastatin was associated with milder disease progression in relapsing-remitting multiple sclerosis patients.[35] However, a study by Birnbaum et al. reported that disease progression was exacerbated by atorvastatin combined with beta interferon in multiple sclerosis patients.[36] Moreover, statin users developed significantly less uveitis.[37] Atorvastatin and rosuvastatin also inhibited the micro inflammatory state and improved the nutritional status in maintenance haemodialysis patients.[38] Rosuvastatin therapy was shown to reduce the levels of inflammatory markers, such as IL-6 and hsCRP, leading to resolved systemic inflammation and improved endothelial-dependent vascular function in COPD patients.[39] Furthermore, a 6-month atorvastatin (80 mg) therapy improved cough on a quality-of-life scale in patients with bronchiectasis.[40]

Cancer

The *Reduction by Dutasteride of Prostate Cancer Events* (REDUCE) clinical trial reported the effect of statins; specifically simvastatin, lovastatin, atorvastatin and fluvastatin in the reduction of inflammatory responses in both acute and chronic prostate inflammation.[41] Furthermore, COPD patients had a lower risk of prostate cancer following simvastatin, atorvastatin, pravastatin, fluvastatin and lovastatin therapy.[42]

Central nervous system (CNS)

Statins have a major effect on the Central Nervous System (CNS), particularly on cognition and neurological disorders, and may decrease the risk of Alzheimer's Disease (AD) and Parkinson's disease through direct impact on neurodegeneration and microglia, respectively.[43]

Infection

Statins are reported to have a great effect on vaginal microbiome via reduced proportions of *Gardnerella vaginalis* and increased proportions of beneficial lactobacilli.[44] In addition, statins diminished the risk of infections in type 2 diabetes patients.[45] Inversely, in patients with dementia statin therapy was associated with increased risk of infection.[46] However, it was reported that statin use in asthma chronic pulmonary disease overlap syndrome (ACOS) patients, was associated with lower TB and pneumonia risks after adjustment for multiple confounding factors.[47] Statin use was also associated with a lower risk of active tuberculosis.[48 49] Statin therapy also reduced the mycobacterial growth in human macrophages and mice by induction of autophagy and phagosome maturation.[50] Furthermore, many studies have stated the potential use of statins as host-directed therapy against infectious diseases caused by viruses, protozoa, fungi and bacteria.[51]

Most of the data on statins as therapeutic agents originate from observational studies. This further highlights the need to perform randomized controlled trials to evaluate statins' immunomodulatory effects independent of their cholesterol-lowering ability. This protocol describes the investigation of commonly available statins (Table 1) atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin focusing on their effect to reduce systemic inflammation in humans. Here, cerivastatin and lovastatin will be excluded due to decommissioned status from the market and the lack of license in Great Britain and Switzerland, respectively. This systematic review will address the hypothesis that pravastatin and rosuvastatin are inferior to other statin in reducing systemic inflammation due to increased first-pass effect.

OBJECTIVES

Primary objective

To identify the type of statin with the best potential to reduce systemic inflammation (statin type stratification).

Secondary objective

To identify the optimal dose for each statin to reduce systemic inflammation (statin dose stratification).

METHODS AND DESIGN

Population

The systematic review will include high-quality randomized-controlled trials (RCT) on adults of at least 18 years of age who have been treated with either atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, or simvastatin, and in whom LDL-C, and at least one of the following markers of systemic inflammation: hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16 are measured before and after statin treatment.

Patient and Public Involvement

This is a systemic review and meta-analysis protocol which will address the anti-inflammatory effects of stains. This study does not involve patients and/ or the public at any stage as primary data will not be collected.

Study design

This systematic review will consider published and peer-reviewed randomized controlled clinical trials with at least one intervention arm of an FDA or EMA-licensed statin and a minimum treatment duration of 12 weeks.

Search strategy

The search strategy (Supplement 1) aims to identify published and peer-reviewed articles with available full-text. A stepwise approach will identify the selected articles. As indicated in Figure 1, an initial limited search of Medline and Scopus will be undertaken; this will be followed by the analysis of the text words contained in the titles and abstracts, and of the index terms used to describe each article. A second search, using all identified keywords and index terms, will then be undertaken across all included databases. In the third step, the reference lists of key articles will be searched for additional studies. Studies will be restricted to the English language and to those published from 1999 to 2019, inclusive. The databases that will be searched are Medline, Scopus, Web of Science, and Cochrane Library of Systematic Reviews.

Eligibility criteria

Inclusion criteria

1. Randomized-controlled trials (RCT) in humans.
2. Adults of at least 18 years of age.
3. At least one intervention arm including an FDA or EMA-licensed statin.
4. Minimum treatment duration of 12 weeks.
5. Studies that report the effects of statins on lipid profile LDL-C and inflammation markers hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16.

6. Publication year- January 1999 to December 2019.

Exclusion criteria

1. RCT including participants with malignancies.
2. RCT including participants with autoimmune diseases.
3. RCT with cerivastatin (decommissioned from the market) or lovastatin (not commonly prescribed anymore and its usage is associated with more risks than beneficial effects) as intervention therapy.
4. Genetic studies.

Study selection

The primary selection of publications will depend on the information contained in their titles and abstracts and will be conducted by two independent investigators and reported using PRISMA-P guidelines (Supplement 2). When the reviewers disagree, the article will be re-assessed by a third reviewer.

Quality assessment

Two reviewers will independently verify selected articles to reduce the source of bias. All selected RCT will be graded for their quality based on the Jadad scale (Supplement 3), the Oxford quality scoring system which is a widely used checklist for classification of quality of evidence.[52]

Risk of bias assessment

Two reviewers will assess the risk of bias, based on the Cochrane Risk of Bias Tool for randomized controlled trials (Supplement 4). The source of bias will be judged as high, low or unclear for the following domains: random sequence generation, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting of outcome and other sources of bias.

Data extraction

Quantitative and qualitative data will be extracted from selected papers; higher scores in grading, low risk in the evaluation and depending on publication bias tool used. The data extracted will include three domains: (1) Identification of the study (year publication, first author's name, PubMed identification number, title, journal name and impact factor); (2) Methodology (study type, co-medication with statin intervention, target population (median/mean age, gender distribution, race, target condition, comorbidities, statin, type, dose, duration; (3) Outcomes (change [or relevant data to estimate change] in lipid profile LDL-C and inflammation markers hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16). For data extraction, two independent Microsoft Excel spreadsheets will be compiled by two reviewers to summarize the data from the included studies. The spreadsheets will then be combined into one. The overall agreement rate between the two investigators will be calculated using Cohen's κ statistic. Disagreements will be resolved by a third investigator.

Management of missing data

The investigator will be contacted via email in the case that key study specifics or outcome data are missing. If a response is not received within two weeks, a reminder email will be sent. A further two weeks waiting period will be allowed for responses; if no response or connection is established with the investigator, these studies will be excluded from the analysis.

Data management

Data management will be the responsibility of investigators. A Google Drive folder with shared access amongst the investigators will be provided for the systematic review which will encompass the protocol, manuscripts and supplementary files from included and excluded studies, as well as documentation of steps in data extraction and analysis, risk of bias and quality assessment. A back-up of the records will be stored on a second hard drive. Endnote X9 reference management software will be used in the study.

Outcomes

The primary outcome is the mean difference between study arms at the end of the statin intervention. The outcomes of the systemic review will be classified into two sections.

1. Lipid profile: Data will be provided as a change in percent over time for total cholesterol (TC), LDL, high-density lipoprotein (HDL) and triglycerides (TG).
2. Systemic inflammatory markers: Data will be provided as a change in percent over time for hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16.

ANALYSIS

Descriptive analysis

Studies will be categorized by each type of statin intervention and comparison, with data tabulated in narrative form to illustrate the study populations, interventions, durations and outcomes. The outcomes from included studies will provide the following:

1. Type of intervention (statin) and sample size.
2. Intervention outcomes will include the change in lipid profile and other inflammatory biomarkers such as hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16.

The outcomes will be analysed together using the Cochrane Review Manager Version 5.3 software, according to the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. If statistical heterogeneity is detected, the random-effects model will be adopted. In terms of considerable statistical

heterogeneity, a qualitative summary will be provided by a table, as described above. This will be done by the lead investigator in liaison with a second investigator for accuracy.

Statistical analysis

We will analyse dichotomous data as risk ratios or odds ratios with 95% confidence intervals and continuous data as mean differences or standardized mean differences. We will perform meta-analyses only if the treatment participants, the underlying clinical question and outcomes are similar enough. If a randomized control trial consists of multiple arms, we will include only the relevant arms. A meta-analysis on LDL-C will be performed to assess the potency of statins; for each study, this will be reported as standardized mean differences with its 95% CI. A scatter plot of the percentage change in LDL-C against percentage change in inflammatory biomarkers over a specific time period will be performed to assess the correlation between lipid profile and inflammation. Heterogeneity and potential sources of heterogeneity will be assessed and quantified using I^2 and Q statistics. Funnel plot and Egger's test will be used to assess publication and small sample size bias. Subgroup analysis of identified studies will be stratified based on statin type, concentration and intervention period. Univariable and multivariable meta-regression analysis will be used to investigate the potential sources of heterogeneities. Potential outliers will be investigated in a sensitivity analysis by dropping each study at a time. The Duval and Tweedie trim-and-fill will be used to adjust estimates for the effects of publication bias, if any.

CONCLUSION

This systematic review will provide further insight into the effectiveness of statins to reduce systemic inflammation in various stages of chronic disease conditions, inform on the most potent statin to reduce systemic inflammation, and optimal dosing. Hence, the study will guide future decision making for the use of statins to treat a wide variety of diseases.

Contribution

RG and FT conceived and planned the idea; BM, SS and MO wrote the study protocol; SS, BM designed the figure and wrote the first draft; RG, FT and MO revised the protocol; All authors have approved and contributed to the final written manuscript. No patients and/ or the public was involved in this manuscript.

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Competing interest

We declare no conflict of interest.

TABLES, FIGURES AND SUPPLEMENTS

Table 1: List of statins as a single-ingredient product licensed by the FDA and EMA.

Statin	**Common brand name**	**Solubility**	**Type**	**Pharmacological properties**
Atorvastatin	Lipitor	Lipophilic	Fully synthetic	Active drug
Cerivastatin	*Lipobay**	*Lipophilic*	*Fully synthetic*	*Active drug*
Fluvastatin	Lescol	Lipophilic	Natural statin	Active drug
Lovastatin	*Mevacor***	*Lipophilic*	*Natural statin*	*Pro-drug*
Pitavastatin	Livalo	Lipophilic	Fully synthetic	Active drug
Pravastatin	Pravachol	Hydrophilic	Natural statin	Active drug
Rosuvastatin	Crestor	Hydrophilic	Fully synthetic	Active drug
Simvastatin	Zocor	Lipophilic	Semi-synthetic	Pro-drug

*withdrawn from the market due to rhabdomyolysis in 2001

**not commonly prescribed anymore and not licensed in Great Britain and Switzerland

Figure 1: A schematic process of the systemic review.

Supplement 1: PubMed search strategy.

Supplement 2: PRISMA-P guidelines.

Supplement 3: Oxford quality scoring system (Jadad scale).

Supplement 4: Cochrane Risk of Bias Tool.

REFERENCES

1. Endo A. A historical perspective on the discovery of statins. *Proceedings of the Japan Academy, Ser B,Physics and Biological Science* 2010;86(5):484-93. doi: 10.2183/pjab.86.484
2. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;366(9493):1267-78. doi: 10.1016/S0140-6736(05)67394-1
3. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 2001;292(5519):1160-4. doi: 10.1126/science.1059344
4. Stancu C, Sima A. Statins: mechanism of action and effects. *Journal of cellular and molecular medicine* 2001;5(4):378-87. doi: 10.1111/j.1582-4934.2001.tb00172.x
5. Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 1996;65:241-69. doi: 10.1146/annurev.bi.65.070196.001325
6. Kavalipati N, Shah J, Ramakrishan A, et al. Pleiotropic effects of statins. *Indian J Endocrinol Metab* 2015;19(5):554-62. doi: 10.4103/2230-8210.163106
7. Liao JK, Laufs U. Pleiotropic effects of statins. *Annual review of pharmacology and toxicology* 2005;45:89-118. doi: 10.1146/annurev.pharmtox.45.120403.095748
8. Blanco-Colio LM, Tunon J, Martin-Ventura JL, et al. Anti-inflammatory and immunomodulatory effects of statins. *Kidney Int* 2003;63(1):12-23. doi: 10.1046/j.1523-1755.2003.00744.x
9. Tran KT, McMenamin UC, Coleman HG, et al. Statin use and risk of liver cancer: Evidence from two population-based studies. *International journal of cancer* 2019 doi: 10.1002/ijc.32426
10. Lu Y, Chang R, Yao J, et al. Effectiveness of long-term using statins in COPD - a network meta-analysis. *Respir Res* 2019;20(1):17. doi: 10.1186/s12931-019-0984-3
11. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundamental & clinical pharmacology* 2005;19(1):117-25. doi: 10.1111/j.1472-8206.2004.00299.x
12. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990;265(3):621-36. doi: 10.1042/bj2650621
13. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol* 2018;9:754. doi: 10.3389/fimmu.2018.00754
14. Albert MA, Danielson E, Rifai N, et al. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *Jama* 2001;286(1):64-70.
15. Schaefer EJ, McNamara JR, Asztalos BF, et al. Effects of atorvastatin versus other statins on fasting and postprandial C-reactive protein and lipoprotein-associated phospholipase A2 in patients with coronary heart disease versus control subjects. *Am J Cardiol* 2005;95(9):1025-32. doi: 10.1016/j.amjcard.2005.01.023
16. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med* 2005;352(1):20-8. doi: 10.1056/NEJMoa042378
17. Yang J, Liu C, Zhang L, et al. Intensive Atorvastatin Therapy Attenuates the Inflammatory Responses in Monocytes of Patients with Unstable Angina Undergoing Percutaneous Coronary Intervention via

Peroxisome Proliferator-Activated Receptor gamma Activation. *Inflammation* 2015;38(4):1415-23. doi: 10.1007/s10753-015-0116-2

18. Toribio M, Fitch KV, Sanchez L, et al. Effects of pitavastatin and pravastatin on markers of immune activation and arterial inflammation in HIV. *AIDS* 2017;31(6):797-806. doi: 10.1097/QAD.0000000000001427
19. Waehre T, Damas JK, Gullestad L, et al. Hydroxymethylglutaryl coenzyme a reductase inhibitors down-regulate chemokines and chemokine receptors in patients with coronary artery disease. *J Am Coll Cardiol* 2003;41(9):1460-7. doi: 10.1016/s0735-1097(03)00263-8
20. McKenney JM. Pharmacologic characteristics of statins. *Clin Cardiol* 2003;26(4 Suppl 3):III32-8. doi: 10.1002/clc.4960261507
21. Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* 2009;158(3):693-705. doi: 10.1111/j.1476-5381.2009.00430.x
22. Grube M, Kock K, Oswald S, et al. Organic anion transporting polypeptide 2B1 is a high-affinity transporter for atorvastatin and is expressed in the human heart. *Clin Pharmacol Ther* 2006;80(6):607-20. doi: 10.1016/j.clpt.2006.09.010
23. Greupink R, Dillen L, Monshouwer M, et al. Interaction of fluvastatin with the liver-specific Na+ -dependent taurocholate cotransporting polypeptide (NTCP). *Eur J Pharm Sci* 2011;44(4):487-96. doi: 10.1016/j.ejps.2011.09.009
24. Jaipersad AS, Shantsila E, Blann A, et al. The effect of statin therapy withdrawal on monocyte subsets. *Eur J Clin Invest* 2013;43(12):1307-13. doi: 10.1111/eci.12183
25. Yilmaz A, Reiss C, Weng A, et al. Differential effects of statins on relevant functions of human monocyte-derived dendritic cells. *J Leukoc Biol* 2006;79(3):529-38. doi: 10.1189/jlb.0205064
26. Veillard NR, Braunersreuther V, Arnaud C, et al. Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. *Atherosclerosis* 2006;188(1):51-8. doi: 10.1016/j.atherosclerosis.2005.10.015
27. Schramm R, Menger MD, Harder Y, et al. Statins inhibit lymphocyte homing to peripheral lymph nodes. *Immunology* 2007;120(3):315-24. doi: 10.1111/j.1365-2567.2006.02505.x
28. Fraemohs L, Koenen RR, Ostermann G, et al. The functional interaction of the beta 2 integrin lymphocyte function-associated antigen-1 with junctional adhesion molecule-A is mediated by the I domain. *J Immunol* 2004;173(10):6259-64. doi: 10.4049/jimmunol.173.10.6259
29. Romano M, Diomede L, Sironi M, et al. Inhibition of monocyte chemotactic protein-1 synthesis by statins. *Lab Invest* 2000;80(7):1095-100.
30. Lazzerini PE, Lorenzini S, Selvi E, et al. Simvastatin inhibits cytokine production and nuclear factor-kB activation in interleukin 1beta-stimulated synoviocytes from rheumatoid arthritis patients. *Clin Exp Rheumatol* 2007;25(5):696-700.
31. Hatanaka T. Clinical pharmacokinetics of pravastatin: mechanisms of pharmacokinetic events. *Clin Pharmacokinet* 2000;39(6):397-412. doi: 10.2165/00003088-200039060-00002
32. Martin PD, Warwick MJ, Dane AL, et al. Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. *Clin Ther* 2003;25(11):2822-35. doi: 10.1016/s0149-2918(03)80336-3

33. Natanzon SS, Matetzky S, Beigel R, et al. Statin therapy among chronic kidney disease patients presenting with acute coronary syndrome. *Atherosclerosis* 2019;286:14-19. doi: 10.1016/j.atherosclerosis.2019.05.002

34. Hung TH, Tsai CC, Lee HF. Statin use in cirrhotic patients with infectious diseases: A population-based study. *PLoS One* 2019;14(4):e0215839. doi: 10.1371/journal.pone.0215839

35. Lanzillo R, Moccia M, Russo CV, et al. Therapeutic lag in reducing disability progression in relapsing-remitting multiple sclerosis: 8-year follow-up of two randomized add-on trials with atorvastatin. *Mult Scler Relat Disord* 2019;28:193-96. doi: 10.1016/j.msard.2018.12.042

36. Birnbaum G, Cree B, Altafullah I, et al. Combining beta interferon and atorvastatin may increase disease activity in multiple sclerosis. *Neurology* 2008;71:1390–95.

37. Borkar DS, Tham VM, Shen E, et al. Association between statin use and uveitis: results from the Pacific Ocular Inflammation study. *Am J Ophthalmol* 2015;159(4):707-13. doi: 10.1016/j.ajo.2015.01.009

38. Tian J, Hou X, Hu L, et al. Efficacy comparison of atorvastatin versus rosuvastatin on blood lipid and microinflammatory state in maintenance hemodialysis patients. *Ren Fail* 2017;39(1):153-58. doi: 10.1080/0886022X.2016.1256309

39. Neukamm A, Hoiseth AD, Einvik G, et al. Rosuvastatin treatment in stable chronic obstructive pulmonary disease (RODEO): a randomized controlled trial. *J Intern Med* 2015;278(1):59-67. doi: 10.1111/joim.12337

40. Mandal P, Chalmers JD, Graham C, et al. Atorvastatin as a stable treatment in bronchiectasis: a randomised controlled trial. *Lancet Respir Med* 2014;2(6):455-63. doi: 10.1016/S2213-2600(14)70050-5

41. Allott EH, Howard LE, Vidal AC, et al. Statin Use, Serum Lipids, and Prostate Inflammation in Men with a Negative Prostate Biopsy: Results from the REDUCE Trial. *Cancer prevention research* 2017;10(6):319-26. doi: 10.1158/1940-6207.CAPR-17-0019

42. Lin HW, Lin LF, Chen HC, et al. Chronic obstructive pulmonary disease with short-acting inhaled pharmacotherapy increases the risk of prostate cancer: A two-stage database approach. *PLoS One* 2018;13(9):e0203377. doi: 10.1371/journal.pone.0203377

43. Willey JZ, Elkind MS. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in the treatment of central nervous system diseases. *Arch Neurol* 2010;67(9):1062-7. doi: 10.1001/archneurol.2010.199

44. Abdelmaksoud AA, Girerd PH, Garcia EM, et al. Association between statin use, the vaginal microbiome, and Gardnerella vaginalis vaginolysin-mediated cytotoxicity. *PLoS One* 2017;12(8):e0183765. doi: 10.1371/journal.pone.0183765

45. Pouwels KB, Widyakusuma NN, Bos JH, et al. Association between statins and infections among patients with diabetes: a cohort and prescription sequence symmetry analysis. *Pharmacoepidemiol Drug Saf* 2016;25(10):1124-30. doi: 10.1002/pds.4052

46. Yeh L-T, Tang C-Y, Yang S-F, et al. Association between Statin Use and Sepsis Risk in Patients with Dementia: A Retrospective Cohort Study. *International Journal of Environmental Research and Public Health* 2019;16(9):1626. doi: 10.3390/ijerph16091626

47. Yeh JJ, Lin CL, Hsu CY, et al. Statin for Tuberculosis and Pneumonia in Patients with Asthma(-)Chronic Pulmonary Disease Overlap Syndrome: A Time-Dependent Population-Based Cohort Study. *Journal of clinical medicine* 2018;7(11) doi: 10.3390/jcm7110381

48. Su VY, Su WJ, Yen YF, et al. Statin Use Is Associated With a Lower Risk of TB. *Chest* 2017;152(3):598-606. doi: 10.1016/j.chest.2017.04.170

49. Lai CC, Lee MT, Lee SH, et al. Statin treatment is associated with a decreased risk of active tuberculosis: an analysis of a nationally representative cohort. *Thorax* 2016;71(7):646-51. doi: 10.1136/thoraxjnl-2015-207052

50. Parihar SP, Guler R, Khutlang R, et al. Statin therapy reduces the mycobacterium tuberculosis burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. *The Journal of infectious diseases* 2014;209(5):754-63. doi: 10.1093/infdis/jit550

51. Parihar SP, Guler R, Brombacher F. Statins: a viable candidate for host-directed therapy against infectious diseases. *Nature Reviews Immunology* 2019;19(2):104-17. doi: 10.1038/s41577-018-0094-3

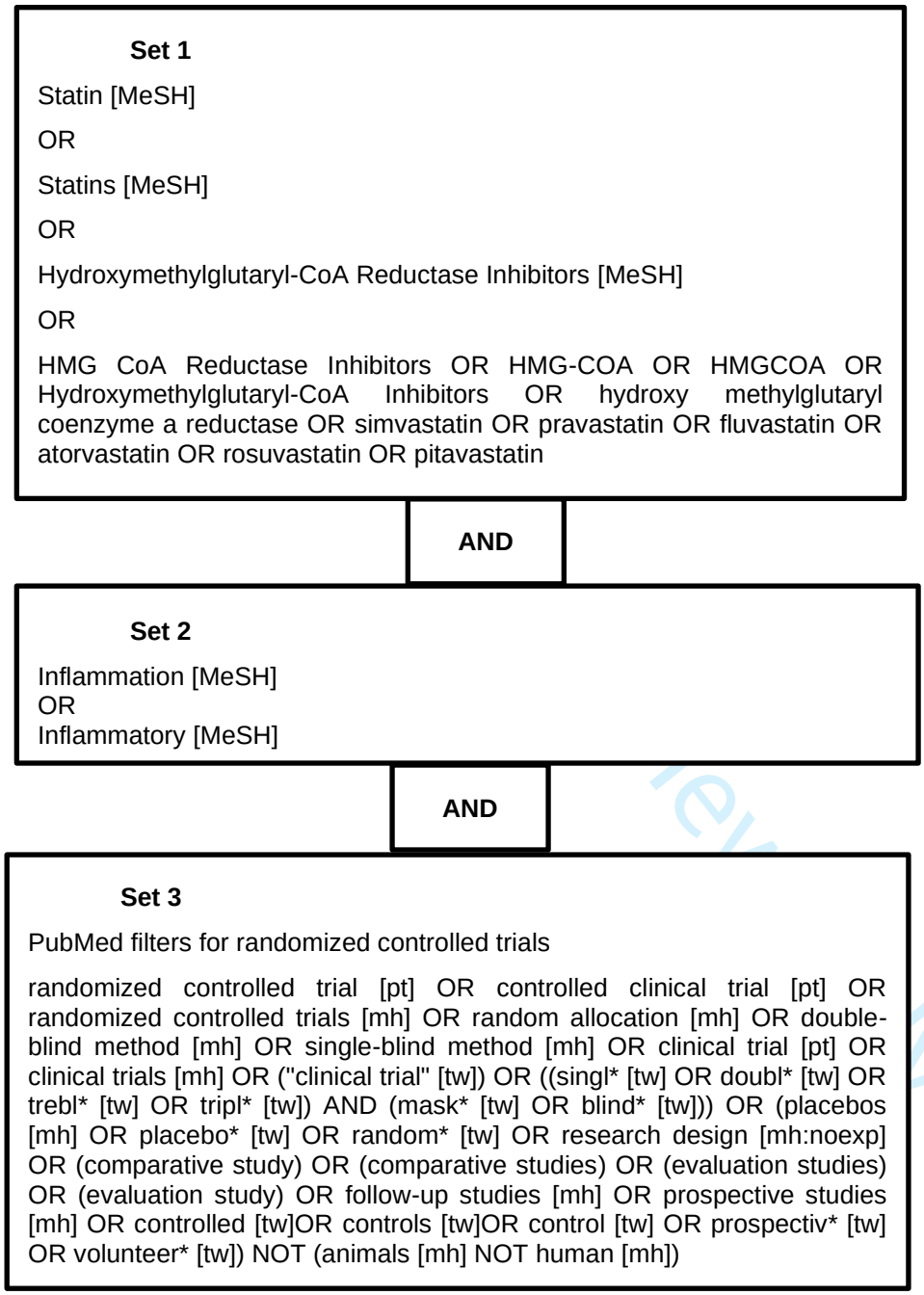
52. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996;17(1):1-12. doi: 10.1016/0197-2456(95)00134-4

1. Initial Medline and Scopus search will be performed to identify article on statins and inflammation.
2. A key word list will be generated by analysing text words in each article.

1. Using these identified key words a complete search will be conducted.
2. Medline, Scopus, Web of Science, and Cochrane Library of Systematic Reviews Databases will be searched.

1. The reference lists of key articles will be used to search for additional studies.
2. Selection of studies will follow PRISMA-P guidelines.
3. RCT studies in humans for ≥ 12 weeks intervention duration will be included.
4. English-language restriction will be applied.
5. Studies will be searched that are published from 1999 to 2019 inclusive.
6. Studies with effects of statins on lipid profile LDL-C and inflammation markers hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16
7. All selected RCT will be judged for their quality based on the Jadad scale ≥3.

Supplement 1: Search strategy in PubMed



Supplement 2: PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	(Page No.#)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	10
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	10
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	7-8
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	7
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	9
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8
Data	11c	Describe planned method of extracting data from reports (such as piloting forms,	8

collection process		done independently, in duplicate), any processes for obtaining and confirming data from investigators	
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	9
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9-10
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	10

Supplement 3: Quality assessment according to the Jadad scale.

Quality assessment questionnaire for RCTs	**Evaluation**
Was the study described as randomized (this includes the use of words such as randomly, random, and randomization)?	□ Yes (1 Point) □ No
Was the method to generate the sequence of randomization described and was it appropriate (table of random numbers, computer generated, etc.).	□ Yes (1 Point) □ No
Was the study described as double blind?	□ Yes (1 Point) □ No
The method of double blinding was described, and it was appropriate (identical placebo, active placebo, dummy, etc.)	□ Yes (1 Point) □ No
Was there a description of withdrawals and dropouts?	□ Yes (1 Point) □ No
Assessment	□ <3 → Low Quality □ ≥3 → High Quality

Supplement 4: Cochrane Risk of Bias Tool

Domain	Description	High Risk of Bias	Low Risk of Bias	Unclear Risk of Bias	Reviewer Assessment
Selection bias **Random sequence generation**	Described the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups	Selection bias (biased allocation to interventions) due to inadequate generation of a randomized sequence	Random sequence generation method should produce comparable groups	Not described in sufficient detail	**High** **Low** **Unclear**
Selection bias **Allocation concealment**	Described the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrollment	Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment	Intervention allocations likely could not have been foreseen in before or during enrollment	Not described in sufficient detail	**High** **Low** **Unclear**
Reporting bias **Selective reporting**	Stated how the possibility of selective outcome reporting was examined by the authors and what was found	Reporting bias due to selective outcome reporting	Selective outcome reporting bias not detected	Insufficient information to permit judgment†	**High** **Low** **Unclear**
Other bias **Other sources of bias**	Any important concerns about bias not addressed above*	Bias due to problems not covered elsewhere in the table	No other bias detected	There may be a risk of bias, but there is either insufficient information to assess whether an important risk of bias exists or insufficient rationale or evidence that an identified problem will introduce bias	**High** **Low** **Unclear**
Performance bias **Blinding (participants and personnel)**	Described all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provided any information relating to whether the intended blinding was	Performance bias due to knowledge of the allocated interventions by participants and personnel during the study.	Blinding was likely effective.	Not described in sufficient detail	**High** **Low** **Unclear**

	effective.				
Detection bias **Blinding (outcome assessment)**	Described all measures used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Provided any information relating to whether the intended blinding was effective.	Detection bias due to knowledge of the allocated interventions by outcome assessors.	Blinding was likely effective.	Not described in sufficient detail	**High** **Low** **Unclear**
Attrition bias **Incomplete outcome data**	Described the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. Stated whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported.	Attrition bias due to amount, nature or handling of incomplete outcome data.	Handling of incomplete outcome data was complete and unlikely to have produced bias	Insufficient reporting of attrition/exclusions to permit judgment (e.g., number randomized not stated, no reasons for missing data provided)	**High** **Low** **Unclear**

BMJ Open

Protocol for systematic review and meta-analysis: impact of statins as immune-modulatory agents on inflammatory markers in adults with chronic diseases

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Protocol for systematic review and meta-analysis: impact of statins as immune-modulatory agents on inflammatory markers in adults with chronic diseases

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ABSTRACT

Introduction: Statins, also known as 3-Hydroxy-3-Methylglutaryl Co-A (HMG-CoA) reductase inhibitors, are lipid-lowering agents that are central in preventing or reducing the complications of atherosclerotic cardiovascular disease. Because statins have anti-inflammatory properties, there is considerable interest in their therapeutic potential in other chronic inflammatory conditions. We aim to identify the statin with the greatest ability to reduce systemic inflammation, independent of the underlying disease entity.

Methods and analysis: We aim to conduct a comprehensive search of published and peer-reviewed randomized controlled clinical trials (RCT), with at least one intervention arm of an FDA or EMA-licensed statin and a minimum treatment duration of 12 weeks. Our objective is to investigate the effect of statins (atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin) on lipid profile, particularly, cholesterol low-density lipoprotein (LDL-C) and inflammation markers such as hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16 in adults, published in the last 20 years (between January 1999 and December 2019). We aim to identify the most potent statin to reduce systemic inflammation and optimal dosing. The following databases will be searched: Medline, Scopus, Web of Science, and Cochrane Library of Systematic Reviews. The risk of bias of included studies will be assessed by Cochrane Risk of Bias tool and Quality Assessment Tool for Quantitative Studies. The quality of studies will be assessed, to show uncertainty, by the Jadad score. If sufficient evidence is identified, a meta-analysis will be conducted with risk ratios or odds ratios with 95% confidence intervals (CI) in addition to mean differences.

Ethics and dissemination: Ethics approval is not required as no primary data will be collected. Results will be presented at conferences and published in a peer-review journal.

PROSPERO registration number: Pending

Strengths and limitations of this study

- This study will include randomized controlled clinical trials to determine the most effective statin on the combined reduction of lipid profile and inflammatory biomarkers.
- High-quality clinical trials will be reviewed accurately to generate reliable evidence.
- This study will be conducted following Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P) guidelines.
- Variation of statin doses among included studies will likely produce heterogeneity that will subsequently reduce the sample size of the meta-analysis.

INTRODUCTION

Statins are US Food & Drug Administration (FDA) approved lipid-lowering drugs (Table 1) that have been on the market for more than 30 years[1] and are widely prescribed to patients who are at high risk of cardiovascular diseases.[2] Statins exert their function via inhibiting 3-Hydroxy-3-Methylglutaryl Coenzyme-A (HMG-CoA) reductase which converts HMG-CoA into L-mevalonate resulting in reduced cholesterol biosynthesis.[3] The cholesterol biosynthesis inhibition via statins results in the upregulation of Low-Density Lipoprotein (LDL) receptors on the cell surface which consequently leads to increased uptake and clearance of LDL in the circulating blood. This ultimately lowers LDL-cholesterol (LDL-C) and decreases the risk associated with lipoprotein deposition in the arterial wall and progression to atherosclerosis and vascular disease. In addition to statins' LDL-C lowering ability, statins also inhibit protein prenylation,[4] which is an important biological process that mediates protein-protein interaction and anchoring of cell membrane proteins.[5] The ability of statins to inhibit isoprenoids, important metabolites in the protein prenylation pathway, account for their lipid-independent pleiotropic effects.[6] [7] Indeed statins have been reported to have anti-inflammatory, antioxidant, anti-proliferative and immunomodulatory effects independent of their cholesterol-lowering ability.[8] The reported vascular effects of statins are wide-ranging and include improvement of endothelial functioning, decreasing oxidative stress and maintenance of coronary artery plaque stability.[8] Statins may also lower the risk of liver cancer.[9] The anti-inflammatory effects vary among the different types of currently licensed statins with various meta-analyses reporting differential efficacy in reducing inflammation in chronic obstructive pulmonary disease (COPD).[10] Statins (Table 1) are categorized into two main groups according to their solubility: 1) hydrophilic statins which include pravastatin and rosuvastatin and these display high hepato-selectivity with increased first-pass effect; and 2) lipophilic statins which are characterized by passive diffusion into cells; these include atorvastatin, simvastatin, lovastatin, fluvastatin, pitavastatin and cerivastatin.[11]

Statins and inflammation

Inflammatory responses to various clinical conditions result in elevated secretion and activity of acute inflammatory proteins such as C-reactive protein (CRP). In the liver, CRP is mainly secreted by hepatocytes in response to IL-6[12]. Increased secretion of IL-6 and CRP further exacerbate the inflammatory milieu through secretion of pro-inflammatory cytokines such as TNF, activation of the complement pathway, apoptosis, phagocytosis, and nitric oxide release.[13] Previous clinical trials have reported statin therapy to reduce CRP levels through an LDL-C independent mechanism,[14] [15] resulting in better clinical outcomes in patients with reduced CRP.[16] In addition, atorvastatin therapy was shown to reduce inflammatory biomarkers such as high sensitive CRP (hsCRP) and IL-6 in patients with unstable angina who received the percutaneous coronary intervention and furthermore reduced cardiac troponin I (cTnl) and Creatine Kinase muscle/brain (CK-MB) suggesting a reduction in cardiac myocyte necrosis.[17] Additionally, the PRINCE randomised controlled trial reported pravastatin (40 mg/day) therapy to have a significant reduction in CRP levels following 12 and 24 weeks of treatment.[14] Statin therapy further resulted in the downregulation of other inflammatory biomarkers, such as IL-8 and sCD14, in patients with coronary artery inflammation.[18] [19] Currently it is not fully elicited on how different types of statins (hydrophilic or lipophilic, Table 1) or the treatment duration differentially affect immune responses.

Mechanisms to reduce inflammation

Statins are selectively taken up by hepatocytes and decrease inflammatory responses by regulating the expression of various cell surface molecules/receptors, transcription factors, cytokines, chemokines and other soluble inflammatory mediators.[20] Furthermore, their ability to be taken up by other cell types, including immune cells, depending on the expression of cell membrane transport proteins and their chemical properties.[11 21] Statins can enter their target cells either through passive diffusion,[11] or active transport which involves transmembrane proteins within the organic anionic-transporting polypeptide (OATP)[21 22] and Na+ taurocholate co-transporting polypeptides (NTCP) groups.[23]

Effects on cell surface receptor

Even though statins were shown to have no effect on peripheral frequencies of circulating $CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{++}$ monocyte subsets, statins were shown to reduce expression of cell surface receptors such as vascular endothelial growth factor receptor-2 (VEGFR-2), Toll-like receptor (TLR)-4 and tyrosine kinase receptor Tie2 which are involved in proliferation, migration and pathogen recognition within all monocyte populations.[24] Furthermore, statins downregulate the expression of Toll-like receptor (TLR)-2, human leukocyte antigen (HLA)-DR and CC-Chemokine Receptor-2 (CCR-2) on monocytes, while increasing Peroxisome Proliferator Activated Receptor-gamma (PPAR-γ) activity, which enhances their anti-inflammatory properties.[17 25] The ability of statins to reduce chemokine and chemokine receptor expression on human vascular endothelial cells and human primary macrophages are achieved via inhibition of the isoprenoid geranylgeranyl pyrophosphate pathway.[26]

Effect on cell signalling

Statins are documented to affect cellular functionality of both monocytes and T cells through altering activation of lymphocyte function-associated antigen (LFA)-1 integrin molecules that are involved in lymphocyte adhesion, migration and transduction of co-stimulatory signals to T cells during antigen presentation.[27] Activation of LFA-1 integrin molecules leads to conformational changes in their structures, thus increasing their binding affinity for their respective substrates, which further enhances pro-inflammatory responses.[28] However, cellular uptake of statins is reported to inhibit these conformational changes in LFA-1 molecules and further enhance their anti-inflammatory properties.[27] Statins also modulate immune responses through alteration of cell-to-cell interaction. Here statins suppress monocyte-derived dendritic cells resulting in reduced T cell activation, proliferation and T helper differentiation.[25]

Downstream effects on soluble biomarkers

Statins inhibit monocyte chemoattractant protein-1 (MCP-1) secretion, resulting in decreased leukocyte recruitment during inflammation.[29] Statins suppress the production of pro-inflammatory cytokines such as IL-6 and IL-8 in IL-1β-stimulated synoviocytes from rheumatoid arthritis patients via interference in protein prenylation and NF-κβ pathway.[30]

Classification of statins

Statins are classified based on several different factors:

Source of origin: They are classified as natural, semi-synthetic or fully synthetic (Table 1). Natural statins are acquired from fungal fermentation and these include lovastatin. Simvastatin and pravastatin are classified as a semi-synthetic statin because they are produced through direct alkylation of lovastatin and hydroxylation of mevastatin, respectively. Fully synthetic statins are produced from different substrates and these include pitavastatin, rosuvastatin, fluvastatin, atorvastatin and cerivastatin.[11]

Pharmacological properties: Two pharmacological properties differentiate statins; they are either prodrugs or active drugs (Table 1). Prodrug statins include lovastatin and simvastatin; they are administered in an inactive state and are activated through hydrolysis by liver enzymes. Atorvastatin, cerivastatin, fluvastatin, and pravastatin are administered as active drugs.[11]

Physiochemical properties: Statins are classified as lipophilic or hydrophilic (Table 1). Atorvastatin, simvastatin, lovastatin, fluvastatin, cerivastatin, and pitavastatin are relatively lipophilic statins as they dissolve efficiently in lipid/fat solution. Cytochrome P450 enzymes metabolize most lipophilic statins except pitavastatin, which is only partially metabolized by this pathway. Hydrophilic statins such as rosuvastatin and pravastatin are not significantly metabolized by the cytochrome P450 system.[11] Pravastatin and rosuvastatin are classified as hydrophilic statins as they dissolve efficiently in water. Pravastatin and rosuvastatin are excreted largely as the parent compound into faeces, urine and bile.[31 32]

Liver selectivity: The hepato-selective processing of statins is defined by their solubility profile; therefore, lipophilic statins diffused passively through hepatocyte cell membranes, whereas hydrophilic statins' uptake occurs through carrier transmembrane proteins.[11]

Statins in clinical conditions other than cardiovascular disease

Inflammation

Statin therapy has been reported to have a wide range of potentially beneficial effects. These include the improved clinical outcome of chronic kidney disease in patients presenting with acute coronary syndrome.[33] Statins also reduced mortality in cirrhotic patients with bacteraemia and pneumonia.[34] Additionally, a 2-year treatment period with atorvastatin was associated with milder disease progression in relapsing-remitting multiple sclerosis patients.[35] However, a study by Birnbaum et al. reported that disease progression was exacerbated by atorvastatin combined with beta interferon in multiple sclerosis patients.[36] Moreover, statin users developed significantly less uveitis.[37] Atorvastatin and rosuvastatin also inhibited the micro inflammatory state and improved the nutritional status in maintenance haemodialysis patients.[38] In a retrospective observational study, pitavastatin usage significantly decreased the mortality risk in Japanese hemodialysis patients[39]. However, Palmer et al. published a systemic review of randomised controlled trial and reported statins to be associated with uncertain adverse events in adults treated with dialysis regardless of serum cholesterol levels; furthermore, statin treatment showed no beneficial effects on mortality and cardiovascular events for dialysis patients[40]. Rosuvastatin therapy was shown to reduce the levels of inflammatory markers, such as IL-6 and hsCRP, leading to resolved systemic

inflammation and improved endothelial-dependent vascular function in COPD patients.[41] Furthermore, a 6-month atorvastatin (80 mg) therapy improved cough on a quality-of-life scale in patients with bronchiectasis.[42]

Cancer

The *Reduction by Dutasteride of Prostate Cancer Events* (REDUCE) randomised controlled trial reported the effect of statins; specifically simvastatin, lovastatin, atorvastatin and fluvastatin in the reduction of inflammatory responses in both acute and chronic prostate inflammation.[43] Furthermore, in a retrospective cohort study, COPD patients had a lower risk of prostate cancer following simvastatin, atorvastatin, pravastatin, fluvastatin and lovastatin therapy.[44] Inversely, an observational study by Emilsson et al. that used observational data from the Surveillance, Epidemiology, and End Results (SEER)-Medicare databases on 17372 cancer patients, reported that treatment with statins within 6-months after cancer diagnosis did not improve patients survival rates when followed up for 3 years[45].

Central nervous system (CNS)

Statins have a major effect on the Central Nervous System (CNS), particularly on cognition and neurological disorders, and may decrease the risk of Alzheimer's Disease (AD) and Parkinson's disease through direct impact on neurodegeneration and microglia, respectively.[46] However, the Lipitor's Effect in Alzheimer's Dementia (LEADe) randomised controlled trial showed that even though atorvastatin (80 mg/day) treatment was well tolerated without unexpected adverse events in Alzheimer disease patients, this treatment did not have significant beneficial effects on Alzheimer disease over 72 weeks period.[47] Additionally, Sano et al. further showed in a randomised controlled trial that despite a significant reduction in cholesterol, simvastatin (20 mg/day) treatment did not prevent the progression of symptoms in individuals with mild to moderate Alzheimer disease.[48]

Infection

Statins are reported to have a great effect on vaginal microbiome via reduced proportions of *Gardnerella vaginalis* and increased proportions of beneficial lactobacilli.[49] In addition, statins diminished the risk of infections in type 2 diabetes patients.[50] Inversely, in patients with dementia statin therapy was associated with increased risk of infection.[51] However, it was reported that statin use in asthma chronic pulmonary disease overlap syndrome (ACOS) patients, was associated with lower TB and pneumonia risks after adjustment for multiple confounding factors.[52] Statin use was also associated with a lower risk of active tuberculosis.[53] [54] Statin therapy also reduced the mycobacterial growth in human macrophages and mice by induction of autophagy and phagosome maturation.[55] Furthermore, many studies have stated the potential use of statins as host-directed therapy against infectious diseases caused by viruses, protozoa, fungi and bacteria.[56]

Most of the data on statins as therapeutic agents originate from observational studies. This further highlights the need to perform randomized controlled trials to evaluate statins' immunomodulatory effects independent of their cholesterol-lowering ability. This protocol describes the investigation of commonly available statins (Table 1) atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin focusing on their effect to reduce systemic inflammation in humans. Here, cerivastatin and lovastatin will be excluded due to decommissioned status from the market and the lack of license in Great Britain and Switzerland, respectively. This systematic

review will address the hypothesis that pravastatin and rosuvastatin are inferior to other statins in reducing systemic inflammation due to increased first-pass effect.

OBJECTIVES

Primary objective

To identify the type of statin with the best potential to reduce systemic inflammation (statin type stratification).

Secondary objective

To identify the optimal dose for each statin to reduce systemic inflammation (statin dose stratification).

METHODS AND DESIGN

Population

The systematic review will include high-quality randomized-controlled trials (RCT) on adults of at least 18 years of age who have been treated with either atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, or simvastatin, and in whom LDL-C, and at least one of the following markers of systemic inflammation: hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16 are measured before and after statin treatment.

Patient and Public Involvement

This is a systemic review and meta-analysis protocol which will address the anti-inflammatory effects of statins. This study does not involve patients and/ or the public at any stage as primary data will not be collected.

Study design

This systematic review will consider published and peer-reviewed randomized controlled clinical trials with at least one intervention arm of an FDA or EMA-licensed statin and a minimum treatment duration of 12 weeks.

Search strategy

The search strategy (Supplement 1) aims to identify published and peer-reviewed articles with available full-text. A stepwise approach will identify the selected articles. As indicated in Figure 1, an initial limited search of Medline and Scopus will be undertaken; this will be followed by the analysis of the text words contained in the titles and abstracts, and of the index terms used to describe each article. A second search, using all identified keywords and index terms, will then be undertaken across all included databases. In the third step, the reference lists of key articles will be searched for additional studies. Studies will be restricted to the English language and

to those published from 1999 to 2019, inclusive. The databases that will be searched are Medline, Scopus, Web of Science, and Cochrane Library of Systematic Reviews.

Eligibility criteria

Inclusion criteria

1. Randomized-controlled trials (RCT) in humans.
2. Adults of at least 18 years of age.
3. At least one intervention arm including an FDA or EMA-licensed statin.
4. Minimum treatment duration of 12 weeks.
5. Studies that report the effects of statins on lipid profile LDL-C and inflammation markers hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16.
6. Publication year- January 1999 to December 2019.

Exclusion criteria

1. RCT including participants with malignancies.
2. RCT including participants with autoimmune diseases.
3. RCT with cerivastatin (decommissioned from the market) or lovastatin (not commonly prescribed anymore and its usage is associated with more risks than beneficial effects) as intervention therapy.
4. Genetic studies.

Study selection

The primary selection of publications will depend on the information contained in their titles and abstracts and will be conducted by two independent investigators and reported using PRISMA-P guidelines (Supplement 2). When the reviewers disagree, the article will be re-assessed by a third reviewer.

Quality assessment

Two reviewers will independently verify selected articles to reduce the source of bias. All selected RCT will be graded for their quality based on the Jadad scale (Supplement 3), the Oxford quality scoring system which is a widely used checklist for classification of quality of evidence.[57]

Risk of bias assessment

Two reviewers will assess the risk of bias, based on the Cochrane Risk of Bias Tool for randomized controlled trials (Supplement 4). The source of bias will be judged as high, low or unclear for the following domains: random

sequence generation, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting of outcome and other sources of bias.

Data extraction

Quantitative and qualitative data will be extracted from selected papers; higher scores in grading, low risk in the evaluation and depending on publication bias tool used. The data extracted will include three domains: (1) Identification of the study (year publication, first author's name, PubMed identification number, title, journal name and impact factor); (2) Methodology (study type, co-medication with statin intervention, target population (median/mean age, gender distribution, race, target condition, comorbidities, statin, type, dose, duration; (3) Outcomes (change [or relevant data to estimate change] in lipid profile LDL-C and inflammation markers hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16). For data extraction, two independent Microsoft Excel spreadsheets will be compiled by two reviewers to summarize the data from the included studies. The spreadsheets will then be combined into one. The overall agreement rate between the two investigators will be calculated using Cohen's κ statistic. Disagreements will be resolved by a third investigator.

Management of missing data

The investigator will be contacted via email in the case that key study specifics or outcome data are missing. If a response is not received within two weeks, a reminder email will be sent. A further two weeks waiting period will be allowed for responses; if no response or connection is established with the investigator, these studies will be excluded from the analysis.

Data management

Data management will be the responsibility of investigators. A Google Drive folder with shared access amongst the investigators will be provided for the systematic review which will encompass the protocol, manuscripts and supplementary files from included and excluded studies, as well as documentation of steps in data extraction and analysis, risk of bias and quality assessment. A back-up of the records will be stored on a second hard drive. Endnote X9 reference management software will be used in the study.

Outcomes

The primary outcome is the mean difference in systemic inflammatory markers and the secondary outcome is the change in lipid profile between study arms at the end of the statin intervention. The outcomes of the systemic review will be classified into primary and secondary outcomes as follows:

1. Systemic inflammatory markers: Data will be provided as a change in percent over time for hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16.

2. Lipid profile: Data will be provided as a change in percent over time for total cholesterol (TC), LDL, high-density lipoprotein (HDL) and triglycerides (TG).

ANALYSIS

Descriptive analysis

Studies will be categorized by each type of statin intervention and comparison, with data tabulated in narrative form to illustrate the study populations, interventions, durations and outcomes. The outcomes from included studies will provide the following:

1. Type of intervention (statin) and sample size.
2. Intervention outcomes will include the change in lipid profile and other inflammatory biomarkers such as hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16.

The outcomes will be analysed together using the Cochrane Review Manager Version 5.3 software, according to the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. If statistical heterogeneity is detected, the random-effects model will be adopted. In terms of considerable statistical heterogeneity, a qualitative summary will be provided by a table, as described above. This will be done by the lead investigator in liaison with a second investigator for accuracy.

Statistical analysis

We will analyse dichotomous data as risk ratios or odds ratios with 95% confidence intervals and continuous data as mean differences or standardized mean differences. We will perform meta-analyses only if the treatment participants (age group), the underlying clinical question (disease type) and outcomes (assessed inflammatory markers) are similar enough. If a randomized control trial consists of multiple arms, we will include only the relevant arms. A meta-analysis on LDL-C will be performed to assess the potency of statins; for each study, this will be reported as standardized mean differences with its 95% CI. A scatter plot of the percentage change in LDL-C against percentage change in inflammatory biomarkers over a specific time period will be performed to assess the correlation between lipid profile and inflammation. Heterogeneity and potential sources of heterogeneity will be assessed and quantified using I^2 and Q statistics. Funnel plot and Egger's test will be used to assess publication and small sample size bias. Subgroup analysis of identified studies will be stratified based on statin type, concentration and intervention period. Univariable and multivariable meta-regression analysis will be used to investigate the potential sources of heterogeneities. Potential outliers will be investigated in a sensitivity analysis by dropping each study at a time. The Duval and Tweedie trim-and-fill will be used to adjust estimates for the effects of publication bias, if any.

This systematic review will provide further insight into the effectiveness of statins to reduce systemic inflammation in various stages of chronic disease conditions, inform on the most potent statin to reduce systemic inflammation, and optimal dosing. In addition, this study will add and improve the existing knowledge of the

effects of statins on inflammatory markers and may further provide a basis for future clinical trials in specific diseases.

Contribution

RG and FT conceived and planned the idea; BM, SS and MO designed the study protocol. SS and BM designed the figure and wrote the first draft; RG, FT and MO revised the protocol. APK and DB provided valuable insight in data acquisition and statistical analysis. DB and RJW revised and designed the reporting of literature. SM, KS, EN, GG and CS critically reviewed the protocol. All authors have approved and contributed to the final written manuscript. No patients and/ or the public was involved in this manuscript.

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Competing interest

We declare no conflict of interest

TABLES, FIGURES AND SUPPLEMENTS

Statin	Solubility	Type	Synthetic state	Pharmacokinetic parameters								
				Cytochrome P450 subclass	Half-life (hrs)	Clearance (L/hr)				Hepatic extraction	Excretion	
						Systemic	Oral	Hepatic	Renal		Urine	Fecal
Atorvastatin Lipitor [a]	Lipophilic	Fully synthetic	Active drug	CYP3A4	Mean [b] 17.8 Range [c] 13.8 - 20.7 Dose [d]: 20, 40, 80 mg REF: [61 62]		157 REF: [58]			>70 % REF: [59]	1.2 % REF: [58]	70 % REF: [60]
Cerivastatin **Lipobay* [a]	*Lipophilic*	*Fully synthetic*	*Active drug*	*CYP2C8 and CYP3A4*	Mean [b] 2.96 Range [c] 2.2 – 4.0 Dose [d]: 0.2, 0.3 mg REF: [63 66 67]	13 REF: [63]					24 - 30 % REF: [64 65]	70 % REF: [64 65]
Fluvastatin Lescol [a]	Lipophilic	Natural statin	Active drug	CYP2C9 some CYP2C8	Mean [b] 1.9 Range [c] 1.5 – 2.4 Dose [d]: 40, 80 mg REF: [73-76]	68 REF: [68]	120 – 180 REF: [69 70]	69 REF: [71]		73 % REF: [72]	6 % REF: [68]	93 % REF: [68]
Lovastatin ***Mevacor* [a]	*Lipophilic*	*Natural statin*	*Pro-drug*	*CYP3A4*	Mean [b] 2.7 Range [c] 2.6 – 2.8 Dose [d]: 20, 40 mg REF: [76 78 80]	18-75 REF: [71]	175 -351 REF: [69 77 78]			69 % REF: [79]	9.6 % REF: [71]	83.2 % REF: [71]
Pitavastatin Livalo [a]	Lipophilic	Fully synthetic	Active drug	Partially: CYP2C8 and CYP2C9	Mean [b] 10.7 Range [c] 6.9 – 13.1 Dose [d]: 1, 2, 4 mg REF: [81-84 86 87]		16 – 26 REF: [81 82 83 84]				15 % REF: [85]	79 % REF: [85]
Rosuvastatin Crestor [a]	Hydrophilic	Fully synthetic	Active drug	Partially: CYP2CP and CYPC19	Mean [b] 14.2 Range [c] 10.1 – 24.4 Dose [d]: 5, 10, 20, 40 mg REF: [89 92-96]	49 REF: [88]	273 – 281 REF: [89]	82 REF: [90]	12 REF: [88]	63 % REF: [88]	5 – 10 % REF: [88 91]	90 % REF: [91]

Pravastatin Pravachol [a]	Hydro phillic	Semi-synthetic	Active drug	None	Mean [b] 2.17 Range [c] 1.6 – 2.6 Dose [d]: 10, 20, 40 mg REF: [80 97 100-103]	57 REF: [97]			24 – 97 REF: [97 98 99]	46 – 66 % REF: [59]	20 % REF: [97]	71 % REF: [97]
Simvastatin Zocor [a]	Lipo phillic	Semi-synthetic	Pro-drug	CYP3A4	Mean [b] 4.6 Range [c] 1.6 – 7.9 Dose [d]: 20, 40, 60 mg REF: [95 105 106 109-115]	32 REF: [104]	2000 – 3100 REF: [105 106 107]			> 79 % REF: [79]	13 % REF: [104 108]	58 % REF: [104 108]

Table 1. List of statins as a single-ingredient product licensed by the FDA and EMA.

*withdrawn from the market due to rhabdomyolysis in 2001
**not commonly prescribed anymore and not licensed in Great Britain and Switzerland
[a] Common brand name
[b] Mean calculated as average of the means of the cited references
[c] Range of the means from the cited references
[d] Half-life reported from indicated doses from the cited references

1 **Figure 1.** A schematic process of the systemic review.

2 **Supplement 1.** PubMed search strategy.

3 **Supplement 2.** PRISMA-P guidelines.

4 **Supplement 3.** Oxford quality scoring system (Jadad scale).

5 **Supplement 4.** Cochrane Risk of Bias Tool.

REFERENCES

1. Endo A. A historical perspective on the discovery of statins. *Proceedings of the Japan Academy, Ser B,Physics and Biological Science* 2010;86(5):484-93. doi: 10.2183/pjab.86.484
2. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;366(9493):1267-78. doi: 10.1016/S0140-6736(05)67394-1
3. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 2001;292(5519):1160-4. doi: 10.1126/science.1059344
4. Stancu C, Sima A. Statins: mechanism of action and effects. *Journal of cellular and molecular medicine* 2001;5(4):378-87. doi: 10.1111/j.1582-4934.2001.tb00172.x
5. Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 1996;65:241-69. doi: 10.1146/annurev.bi.65.070196.001325
6. Kavalipati N, Shah J, Ramakrishan A, et al. Pleiotropic effects of statins. *Indian J Endocrinol Metab* 2015;19(5):554-62. doi: 10.4103/2230-8210.163106
7. Liao JK, Laufs U. Pleiotropic effects of statins. *Annual review of pharmacology and toxicology* 2005;45:89-118. doi: 10.1146/annurev.pharmtox.45.120403.095748
8. Blanco-Colio LM, Tunon J, Martin-Ventura JL, et al. Anti-inflammatory and immunomodulatory effects of statins. *Kidney Int* 2003;63(1):12-23. doi: 10.1046/j.1523-1755.2003.00744.x
9. Tran KT, McMenamin UC, Coleman HG, et al. Statin use and risk of liver cancer: Evidence from two population-based studies. *International journal of cancer* 2019 doi: 10.1002/ijc.32426
10. Lu Y, Chang R, Yao J, et al. Effectiveness of long-term using statins in COPD - a network meta-analysis. *Respir Res* 2019;20(1):17. doi: 10.1186/s12931-019-0984-3
11. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundamental & clinical pharmacology* 2005;19(1):117-25. doi: 10.1111/j.1472-8206.2004.00299.x
12. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990;265(3):621-36. doi: 10.1042/bj2650621
13. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol* 2018;9:754. doi: 10.3389/fimmu.2018.00754
14. Albert MA, Danielson E, Rifai N, et al. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *Jama* 2001;286(1):64-70.
15. Schaefer EJ, McNamara JR, Asztalos BF, et al. Effects of atorvastatin versus other statins on fasting and postprandial C-reactive protein and lipoprotein-associated phospholipase A2 in patients with coronary heart disease versus control subjects. *Am J Cardiol* 2005;95(9):1025-32. doi: 10.1016/j.amjcard.2005.01.023
16. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med* 2005;352(1):20-8. doi: 10.1056/NEJMoa042378
17. Yang J, Liu C, Zhang L, et al. Intensive Atorvastatin Therapy Attenuates the Inflammatory Responses in Monocytes of Patients with Unstable Angina Undergoing Percutaneous Coronary Intervention via Peroxisome Proliferator-Activated Receptor gamma Activation. *Inflammation* 2015;38(4):1415-23. doi: 10.1007/s10753-015-0116-2

18. Toribio M, Fitch KV, Sanchez L, et al. Effects of pitavastatin and pravastatin on markers of immune activation and arterial inflammation in HIV. *AIDS* 2017;31(6):797-806. doi: 10.1097/QAD.0000000000001427
19. Waehre T, Damas JK, Gullestad L, et al. Hydroxymethylglutaryl coenzyme a reductase inhibitors down-regulate chemokines and chemokine receptors in patients with coronary artery disease. *J Am Coll Cardiol* 2003;41(9):1460-7. doi: 10.1016/s0735-1097(03)00263-8
20. McKenney JM. Pharmacologic characteristics of statins. *Clin Cardiol* 2003;26(4 Suppl 3):III32-8. doi: 10.1002/clc.4960261507
21. Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* 2009;158(3):693-705. doi: 10.1111/j.1476-5381.2009.00430.x
22. Grube M, Kock K, Oswald S, et al. Organic anion transporting polypeptide 2B1 is a high-affinity transporter for atorvastatin and is expressed in the human heart. *Clin Pharmacol Ther* 2006;80(6):607-20. doi: 10.1016/j.clpt.2006.09.010
23. Greupink R, Dillen L, Monshouwer M, et al. Interaction of fluvastatin with the liver-specific Na+ -dependent taurocholate cotransporting polypeptide (NTCP). *Eur J Pharm Sci* 2011;44(4):487-96. doi: 10.1016/j.ejps.2011.09.009
24. Jaipersad AS, Shantsila E, Blann A, et al. The effect of statin therapy withdrawal on monocyte subsets. *Eur J Clin Invest* 2013;43(12):1307-13. doi: 10.1111/eci.12183
25. Yilmaz A, Reiss C, Weng A, et al. Differential effects of statins on relevant functions of human monocyte-derived dendritic cells. *J Leukoc Biol* 2006;79(3):529-38. doi: 10.1189/jlb.0205064
26. Veillard NR, Braunersreuther V, Arnaud C, et al. Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. *Atherosclerosis* 2006;188(1):51-8. doi: 10.1016/j.atherosclerosis.2005.10.015
27. Schramm R, Menger MD, Harder Y, et al. Statins inhibit lymphocyte homing to peripheral lymph nodes. *Immunology* 2007;120(3):315-24. doi: 10.1111/j.1365-2567.2006.02505.x
28. Fraemohs L, Koenen RR, Ostermann G, et al. The functional interaction of the beta 2 integrin lymphocyte function-associated antigen-1 with junctional adhesion molecule-A is mediated by the I domain. *J Immunol* 2004;173(10):6259-64. doi: 10.4049/jimmunol.173.10.6259
29. Romano M, Diomede L, Sironi M, et al. Inhibition of monocyte chemotactic protein-1 synthesis by statins. *Lab Invest* 2000;80(7):1095-100.
30. Lazzerini PE, Lorenzini S, Selvi E, et al. Simvastatin inhibits cytokine production and nuclear factor-kB activation in interleukin 1beta-stimulated synoviocytes from rheumatoid arthritis patients. *Clin Exp Rheumatol* 2007;25(5):696-700.
31. Hatanaka T. Clinical pharmacokinetics of pravastatin: mechanisms of pharmacokinetic events. *Clin Pharmacokinet* 2000;39(6):397-412. doi: 10.2165/00003088-200039060-00002
32. Martin PD, Warwick MJ, Dane AL, et al. Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. *Clin Ther* 2003;25(11):2822-35. doi: 10.1016/s0149-2918(03)80336-3
33. Natanzon SS, Matetzky S, Beigel R, et al. Statin therapy among chronic kidney disease patients presenting with acute coronary syndrome. *Atherosclerosis* 2019;286:14-19. doi: 10.1016/j.atherosclerosis.2019.05.002

34. Hung TH, Tsai CC, Lee HF. Statin use in cirrhotic patients with infectious diseases: A population-based study. *PLoS One* 2019;14(4):e0215839. doi: 10.1371/journal.pone.0215839

35. Lanzillo R, Moccia M, Russo CV, et al. Therapeutic lag in reducing disability progression in relapsing-remitting multiple sclerosis: 8-year follow-up of two randomized add-on trials with atorvastatin. *Mult Scler Relat Disord* 2019;28:193-96. doi: 10.1016/j.msard.2018.12.042

36. Birnbaum G, Cree B, Altafullah I, et al. Combining beta interferon and atorvastatin may increase disease activity in multiple sclerosis. *Neurology* 2008;71:1390–95.

37. Borkar DS, Tham VM, Shen E, et al. Association between statin use and uveitis: results from the Pacific Ocular Inflammation study. *Am J Ophthalmol* 2015;159(4):707-13. doi: 10.1016/j.ajo.2015.01.009

38. Tian J, Hou X, Hu L, et al. Efficacy comparison of atorvastatin versus rosuvastatin on blood lipid and microinflammatory state in maintenance hemodialysis patients. *Ren Fail* 2017;39(1):153-58. doi: 10.1080/0886022X.2016.1256309

39. Ota Y, Kitamura M, Muta K, et al. Effect of statin on life prognosis in Japanese patients undergoing hemodialysis. *PLoS One* 2019;14(10):e0224111. doi: 10.1371/journal.pone.0224111

40. Palmer SC, Navaneethan SD, Craig JC, et al. HMG CoA reductase inhibitors (statins) for dialysis patients. *Cochrane Database Syst Rev* 2013(9):CD004289. doi: 10.1002/14651858.CD004289.pub5

41. Neukamm A, Hoiseth AD, Einvik G, et al. Rosuvastatin treatment in stable chronic obstructive pulmonary disease (RODEO): a randomized controlled trial. *J Intern Med* 2015;278(1):59-67. doi: 10.1111/joim.12337

42. Mandal P, Chalmers JD, Graham C, et al. Atorvastatin as a stable treatment in bronchiectasis: a randomised controlled trial. *Lancet Respir Med* 2014;2(6):455-63. doi: 10.1016/S2213-2600(14)70050-5

43. Allott EH, Howard LE, Vidal AC, et al. Statin Use, Serum Lipids, and Prostate Inflammation in Men with a Negative Prostate Biopsy: Results from the REDUCE Trial. *Cancer prevention research* 2017;10(6):319-26. doi: 10.1158/1940-6207.CAPR-17-0019

44. Lin HW, Lin LF, Chen HC, et al. Chronic obstructive pulmonary disease with short-acting inhaled pharmacotherapy increases the risk of prostate cancer: A two-stage database approach. *PLoS One* 2018;13(9):e0203377. doi: 10.1371/journal.pone.0203377

45. Emilsson L, Garcia-Albeniz X, Logan RW, et al. Examining Bias in Studies of Statin Treatment and Survival in Patients With Cancer. *JAMA Oncol* 2018;4(1):63-70. doi: 10.1001/jamaoncol.2017.2752

46. Willey JZ, Elkind MS. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in the treatment of central nervous system diseases. *Arch Neurol* 2010;67(9):1062-7. doi: 10.1001/archneurol.2010.199

47. Feldman HH, Doody RS, Kivipelto M, et al. Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease: LEADe. *Neurology* 2010;74(12):956-64. doi: 10.1212/WNL.0b013e3181d6476a

48. Sano M, Bell KL, Galasko D, et al. A randomized, double-blind, placebo-controlled trial of simvastatin to treat Alzheimer disease. *Neurology* 2011;77(6):556-63. doi: 10.1212/WNL.0b013e318228bf11

49. Abdelmaksoud AA, Girerd PH, Garcia EM, et al. Association between statin use, the vaginal microbiome, and Gardnerella vaginalis vaginolysin-mediated cytotoxicity. *PLoS One* 2017;12(8):e0183765. doi: 10.1371/journal.pone.0183765

50. Pouwels KB, Widyakusuma NN, Bos JH, et al. Association between statins and infections among patients with diabetes: a cohort and prescription sequence symmetry analysis. *Pharmacoepidemiol Drug Saf* 2016;25(10):1124-30. doi: 10.1002/pds.4052

51. Yeh L-T, Tang C-Y, Yang S-F, et al. Association between Statin Use and Sepsis Risk in Patients with Dementia: A Retrospective Cohort Study. *International Journal of Environmental Research and Public Health* 2019;16(9):1626. doi: 10.3390/ijerph16091626

52. Yeh JJ, Lin CL, Hsu CY, et al. Statin for Tuberculosis and Pneumonia in Patients with Asthma(-)Chronic Pulmonary Disease Overlap Syndrome: A Time-Dependent Population-Based Cohort Study. *Journal of clinical medicine* 2018;7(11) doi: 10.3390/jcm7110381

53. Su VY, Su WJ, Yen YF, et al. Statin Use Is Associated With a Lower Risk of TB. *Chest* 2017;152(3):598-606. doi: 10.1016/j.chest.2017.04.170

54. Lai CC, Lee MT, Lee SH, et al. Statin treatment is associated with a decreased risk of active tuberculosis: an analysis of a nationally representative cohort. *Thorax* 2016;71(7):646-51. doi: 10.1136/thoraxjnl-2015-207052

55. Parihar SP, Guler R, Khutlang R, et al. Statin therapy reduces the mycobacterium tuberculosis burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. *The Journal of infectious diseases* 2014;209(5):754-63. doi: 10.1093/infdis/jit550

56. Parihar SP, Guler R, Brombacher F. Statins: a viable candidate for host-directed therapy against infectious diseases. *Nature Reviews Immunology* 2019;19(2):104-17. doi: 10.1038/s41577-018-0094-3

57. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996;17(1):1-12. doi: 10.1016/0197-2456(95)00134-4

58. Stern RH, Yang BB, Horton M, et al. Renal dysfunction does not alter the pharmacokinetics or LDL-cholesterol reduction of atorvastatin. *J Clin Pharmacol* 1997;37(9):816-9. doi: 10.1002/j.1552-4604.1997.tb05629.x

59. Corsini A, Bellosta S, Baetta R, et al. New insights into the pharmacodynamic and pharmacokinetic properties of statins. *Pharmacol Ther* 1999;84(3):413-28. doi: 10.1016/s0163-7258(99)00045-5

60. Davidson MH. Controversy surrounding the safety of cerivastatin. *Expert Opin Drug Saf* 2002;1(3):207-12. doi: 10.1517/14740338.1.3.207

61. Gibson DM, Bron NJ, Richens A, et al. Effect of age and gender on pharmacokinetics of atorvastatin in humans. *J Clin Pharmacol* 1996;36(3):242-6. doi: 10.1002/j.1552-4604.1996.tb04194.x

62. Cilla DD, Jr., Whitfield LR, Gibson DM, et al. Multiple-dose pharmacokinetics, pharmacodynamics, and safety of atorvastatin, an inhibitor of HMG-CoA reductase, in healthy subjects. *Clin Pharmacol Ther* 1996;60(6):687-95. doi: 10.1016/S0009-9236(96)90218-0

63. Muck W, Ritter W, Ochmann K, et al. Absolute and relative bioavailability of the HMG-CoA reductase inhibitor cerivastatin. *Int J Clin Pharmacol Ther* 1997;35(6):255-60.

64. Bayer. BAYCOL® (cerivastatin sodium tablets).

65. Muck W, Unger S, Kawano K, et al. Inter-ethnic comparisons of the pharmacokinetics of the HMG-CoA reductase inhibitor cerivastatin. *Br J Clin Pharmacol* 1998;45(6):583-90. doi: 10.1046/j.1365-2125.1998.00717.x

66. Schall R, Muller FO, Hundt HK, et al. No pharmacokinetic or pharmacodynamic interaction between rivastatin and warfarin. *J Clin Pharmacol* 1995;35(3):306-13. doi: 10.1002/j.1552-4604.1995.tb04065.x
67. Kantola T, Kivisto KT, Neuvonen PJ. Effect of itraconazole on cerivastatin pharmacokinetics. *Eur J Clin Pharmacol* 1999;54(11):851-5. doi: 10.1007/s002280050566
68. Tse FL, Jaffe JM, Troendle A. Pharmacokinetics of fluvastatin after single and multiple doses in normal volunteers. *J Clin Pharmacol* 1992;32(7):630-8. doi: 10.1002/j.1552-4604.1992.tb05773.x
69. Desager JP, Horsmans Y. Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. *Clin Pharmacokinet* 1996;31(5):348-71. doi: 10.2165/00003088-199631050-00003
70. Smith HT, Jokubaitis LA, Troendle AJ, et al. Pharmacokinetics of fluvastatin and specific drug interactions. *Am J Hypertens* 1993;6(11 Pt 2):375S-82S. doi: 10.1093/ajh/6.11.375s
71. Lennernas H, Fager G. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences. *Clin Pharmacokinet* 1997;32(5):403-25. doi: 10.2165/00003088-199732050-00005
72. Lindahl A, Sandstrom R, Ungell AL, et al. Jejunal permeability and hepatic extraction of fluvastatin in humans. *Clin Pharmacol Ther* 1996;60(5):493-503. doi: 10.1016/S0009-9236(96)90145-9
73. Kantola T, Backman JT, Niemi M, et al. Effect of fluconazole on plasma fluvastatin and pravastatin concentrations. *Eur J Clin Pharmacol* 2000;56(3):225-9. doi: 10.1007/s002280000127
74. Siekmeier R, Lattke P, Mix C, et al. Dose dependency of fluvastatin pharmacokinetics in serum determined by reversed phase HPLC. *J Cardiovasc Pharmacol Ther* 2001;6(2):137-45. doi: 10.1177/107424840100600205
75. Smit JW, Wijnne HJ, Schobben F, et al. Effects of alcohol consumption on pharmacokinetics, efficacy, and safety of fluvastatin. *Am J Cardiol* 1995;76(2):89A-96A. doi: 10.1016/s0002-9149(05)80026-8
76. Kivisto KT, Kantola T, Neuvonen PJ. Different effects of itraconazole on the pharmacokinetics of fluvastatin and lovastatin. *Br J Clin Pharmacol* 1998;46(1):49-53. doi: 10.1046/j.1365-2125.1998.00034.x
77. Pentikainen PJ, Saraheimo M, Schwartz JI, et al. Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. *J Clin Pharmacol* 1992;32(2):136-40. doi: 10.1002/j.1552-4604.1992.tb03818.x
78. Pan HY, Triscari J, DeVault AR, et al. Pharmacokinetic interaction between propranolol and the HMG-CoA reductase inhibitors pravastatin and lovastatin. *Br J Clin Pharmacol* 1991;31(6):665-70. doi: 10.1111/j.1365-2125.1991.tb05590.x
79. Blum CB. Comparison of properties of four inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Am J Cardiol* 1994;73(14):3D-11D. doi: 10.1016/0002-9149(94)90626-2
80. Pan HY, DeVault AR, Wang-Iverson D, et al. Comparative pharmacokinetics and pharmacodynamics of pravastatin and lovastatin. *J Clin Pharmacol* 1990;30(12):1128-35. doi: 10.1002/j.1552-4604.1990.tb01856.x
81. Qi X, Ding L, Wen A, et al. Simple LC-MS/MS methods for simultaneous determination of pitavastatin and its lactone metabolite in human plasma and urine involving a procedure for inhibiting the conversion of pitavastatin lactone to pitavastatin in plasma and its application to a pharmacokinetic study. *J Pharm Biomed Anal* 2013;72:8-15. doi: 10.1016/j.jpba.2012.09.026
82. Wen J, Xiong Y. OATP1B1 388A>G polymorphism and pharmacokinetics of pitavastatin in Chinese healthy volunteers. *J Clin Pharm Ther* 2010;35(1):99-104. doi: 10.1111/j.1365-2710.2009.01071.x

83. Chen Y, Zhang W, Huang WH, et al. Effect of a single-dose rifampin on the pharmacokinetics of pitavastatin in healthy volunteers. *Eur J Clin Pharmacol* 2013;69(11):1933-8. doi: 10.1007/s00228-013-1554-0

84. Shang D, Deng S, Yao Z, et al. The effect of food on the pharmacokinetic properties and bioequivalence of two formulations of pitavastatin calcium in healthy Chinese male subjects. *Xenobiotica* 2016;46(1):34-9. doi: 10.3109/00498254.2015.1046153

85. Kowa. LIVALO® (pitavastatin) Tablet, 2016.

86. Luo Z, Zhang Y, Gu J, et al. Pharmacokinetic Properties of Single- and Multiple-Dose Pitavastatin Calcium Tablets in Healthy Chinese Volunteers. *Curr Ther Res Clin Exp* 2015;77:52-7. doi: 10.1016/j.curtheres.2015.02.001

87. Ando H, Tsuruoka S, Yanagihara H, et al. Effects of grapefruit juice on the pharmacokinetics of pitavastatin and atorvastatin. *Br J Clin Pharmacol* 2005;60(5):494-7. doi: 10.1111/j.1365-2125.2005.02462.x

88. Martin PD, Warwick MJ, Dane AL, et al. Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers. *Clin Ther* 2003;25(10):2553-63. doi: 10.1016/s0149-2918(03)80316-8

89. Wu HF, Hristeva N, Chang J, et al. Rosuvastatin Pharmacokinetics in Asian and White Subjects Wild Type for Both OATP1B1 and BCRP Under Control and Inhibited Conditions. *J Pharm Sci* 2017;106(9):2751-57. doi: 10.1016/j.xphs.2017.03.027

90. Bergman E, Forsell P, Tevell A, et al. Biliary secretion of rosuvastatin and bile acids in humans during the absorption phase. *Eur J Pharm Sci* 2006;29(3-4):205-14. doi: 10.1016/j.ejps.2006.04.015

91. McTaggart F, Buckett L, Davidson R, et al. Preclinical and clinical pharmacology of Rosuvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. *Am J Cardiol* 2001;87(5A):28B-32B. doi: 10.1016/s0002-9149(01)01454-0

92. Lee E, Ryan S, Birmingham B, et al. Rosuvastatin pharmacokinetics and pharmacogenetics in white and Asian subjects residing in the same environment. *Clin Pharmacol Ther* 2005;78(4):330-41. doi: 10.1016/j.clpt.2005.06.013

93. Zhang W, Yu BN, He YJ, et al. Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. *Clin Chim Acta* 2006;373(1-2):99-103. doi: 10.1016/j.cca.2006.05.010

94. Li Y, Jiang X, Lan K, et al. Pharmacokinetic properties of rosuvastatin after single-dose, oral administration in Chinese volunteers: a randomized, open-label, three-way crossover study. *Clin Ther* 2007;29(10):2194-203. doi: 10.1016/j.clinthera.2007.10.005

95. Birmingham BK, Bujac SR, Elsby R, et al. Impact of ABCG2 and SLCO1B1 polymorphisms on pharmacokinetics of rosuvastatin, atorvastatin and simvastatin acid in Caucasian and Asian subjects: a class effect? *Eur J Clin Pharmacol* 2015;71(3):341-55. doi: 10.1007/s00228-014-1801-z

96. Birmingham BK, Bujac SR, Elsby R, et al. Rosuvastatin pharmacokinetics and pharmacogenetics in Caucasian and Asian subjects residing in the United States. *Eur J Clin Pharmacol* 2015;71(3):329-40. doi: 10.1007/s00228-014-1800-0

97. Singhvi SM, Pan HY, Morrison RA, et al. Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. *Br J Clin Pharmacol* 1990;29(2):239-43. doi: 10.1111/j.1365-2125.1990.tb03626.x

98. Kyrklund C, Backman JT, Neuvonen M, et al. Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. *Clin Pharmacol Ther* 2003;73(6):538-44. doi: 10.1016/S0009-9236(03)00052-3

99. Kyrklund C, Backman JT, Neuvonen M, et al. Effect of rifampicin on pravastatin pharmacokinetics in healthy subjects. *Br J Clin Pharmacol* 2004;57(2):181-7. doi: 10.1046/j.1365-2125.2003.01972.x

100. Almeida S, Filipe A, Almeida A, et al. Comparative study on the bioequivalence of two formulations of pravastatin. Data from a crossover, randomised, open-label bioequivalence study in healthy volunteers. *Arzneimittelforschung* 2006;56(2):70-5. doi: 10.1055/s-0031-1296704

101. Ogawa K, Hasegawa S, Udaka Y, et al. Individual difference in the pharmacokinetics of a drug, pravastatin, in healthy subjects. *J Clin Pharmacol* 2003;43(11):1268-73. doi: 10.1177/0091270003257232

102. Pan WJ, Gustavson LE, Achari R, et al. Lack of a clinically significant pharmacokinetic interaction between fenofibrate and pravastatin in healthy volunteers. *J Clin Pharmacol* 2000;40(3):316-23. doi: 10.1177/00912700022008874

103. Escobar Y, Venturelli CR, Hoyo-Vadillo C. Pharmacokinetic properties of pravastatin in Mexicans: An open-label study in healthy adult volunteers. *Curr Ther Res Clin Exp* 2005;66(3):238-46. doi: 10.1016/j.curtheres.2005.06.001

104. Mauro VF. Clinical pharmacokinetics and practical applications of simvastatin. *Clin Pharmacokinet* 1993;24(3):195-202. doi: 10.2165/00003088-199324030-00002

105. Sunkara G, Reynolds CV, Pommier F, et al. Evaluation of a pharmacokinetic interaction between valsartan and simvastatin in healthy subjects. *Curr Med Res Opin* 2007;23(3):631-40. doi: 10.1185/030079906X167471

106. Park SJ, Yeo CW, Shim EJ, et al. Pomegranate juice does not affect the disposition of simvastatin in healthy subjects. *Eur J Drug Metab Pharmacokinet* 2016;41(4):339-44. doi: 10.1007/s13318-015-0263-8

107. O'Brien SG, Meinhardt P, Bond E, et al. Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. *Br J Cancer* 2003;89(10):1855-9. doi: 10.1038/sj.bjc.6601152

108. Merck. ZOCOR (simvastatin) Tablets. 1991

109. Kyrklund C, Backman JT, Kivisto KT, et al. Rifampin greatly reduces plasma simvastatin and simvastatin acid concentrations. *Clin Pharmacol Ther* 2000;68(6):592-7. doi: 10.1067/mcp.2000.111414

110. Ucar M, Neuvonen M, Luurila H, et al. Carbamazepine markedly reduces serum concentrations of simvastatin and simvastatin acid. *Eur J Clin Pharmacol* 2004;59(12):879-82. doi: 10.1007/s00228-003-0700-5

111. Neuvonen PJ, Kantola T, Kivisto KT. Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. *Clin Pharmacol Ther* 1998;63(3):332-41. doi: 10.1016/S0009-9236(98)90165-5

112. Mousa O, Brater DC, Sunblad KJ, et al. The interaction of diltiazem with simvastatin. *Clin Pharmacol Ther* 2000;67(3):267-74. doi: 10.1067/mcp.2000.104609

113. Lilja JJ, Kivisto KT, Neuvonen PJ. Grapefruit juice-simvastatin interaction: effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther* 1998;64(5):477-83. doi: 10.1016/S0009-9236(98)90130-8

114. Backman JT, Kyrklund C, Kivisto KT, et al. Plasma concentrations of active simvastatin acid are increased by gemfibrozil. *Clin Pharmacol Ther* 2000;68(2):122-9. doi: 10.1067/mcp.2000.108507

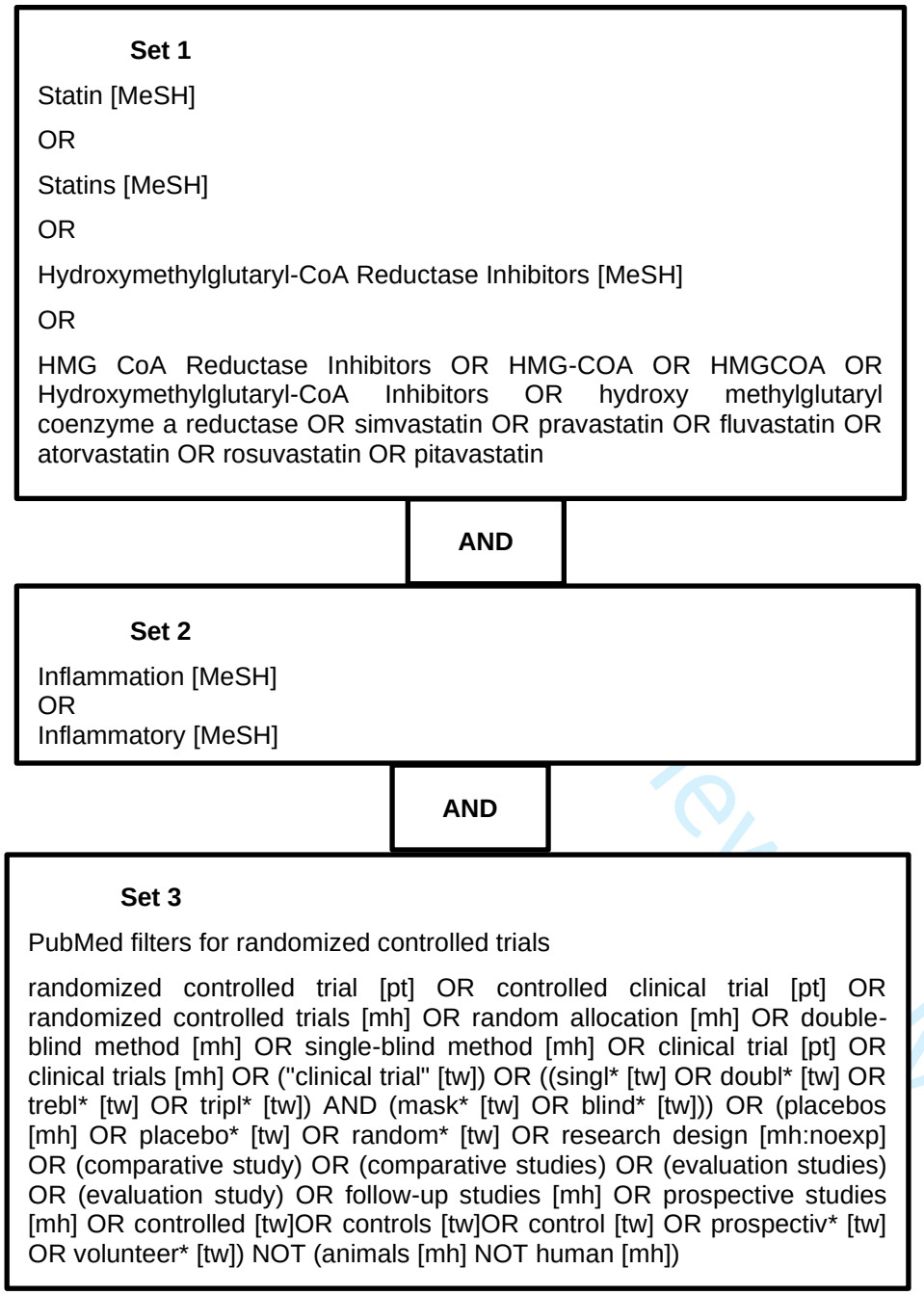
115. Tubic-Grozdanis M, Hilfinger JM, Amidon GL, et al. Pharmacokinetics of the CYP 3A substrate simvastatin following administration of delayed versus immediate release oral dosage forms. *Pharm Res* 2008;25(7):1591-600. doi: 10.1007/s11095-007-9519-6

1. Initial Medline and Scopus search will be performed to identify article on statins and inflammation.
2. A key word list will be generated by analysing text words in each article.

1. Using these identified key words a complete search will be conducted.
2. Medline, Scopus, Web of Science, and Cochrane Library of Systematic Reviews Databases will be searched.

1. The reference lists of key articles will be used to search for additional studies.
2. Selection of studies will follow PRISMA-P guidelines.
3. RCT studies in humans for ≥ 12 weeks intervention duration will be included.
4. English-language restriction will be applied.
5. Studies will be searched that are published from 1999 to 2019 inclusive.
6. Studies with effects of statins on lipid profile LDL-C and inflammation markers hsCRP, CRP, TNF-α, IL-1β, IL-6, IL-8, sCD14, or sCD16
7. All selected RCT will be judged for their quality based on the Jadad scale ≥3.

Supplement 1: Search strategy in PubMed



Supplement 2: PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	(Page No.#)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	10
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	10
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	7-8
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	7
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	9
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8
Data	11c	Describe planned method of extracting data from reports (such as piloting forms,	8

collection process		done independently, in duplicate), any processes for obtaining and confirming data from investigators	
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	9
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9-10
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	10

Supplement 3: Quality assessment according to the Jadad scale.

Quality assessment questionnaire for RCTs	**Evaluation**
Was the study described as randomized (this includes the use of words such as randomly, random, and randomization)?	□ Yes (1 Point) □ No
Was the method to generate the sequence of randomization described and was it appropriate (table of random numbers, computer generated, etc.).	□ Yes (1 Point) □ No
Was the study described as double blind?	□ Yes (1 Point) □ No
The method of double blinding was described, and it was appropriate (identical placebo, active placebo, dummy, etc.)	□ Yes (1 Point) □ No
Was there a description of withdrawals and dropouts?	□ Yes (1 Point) □ No
Assessment	□ <3 → Low Quality □ ≥3 → High Quality

Supplement 4: Cochrane Risk of Bias Tool

Domain	Description	High Risk of Bias	Low Risk of Bias	Unclear Risk of Bias	Reviewer Assessment
Selection bias **Random sequence generation**	Described the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups	Selection bias (biased allocation to interventions) due to inadequate generation of a randomized sequence	Random sequence generation method should produce comparable groups	Not described in sufficient detail	**High Low Unclear**
Selection bias **Allocation concealment**	Described the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrollment	Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment	Intervention allocations likely could not have been foreseen in before or during enrollment	Not described in sufficient detail	**High Low Unclear**
Reporting bias **Selective reporting**	Stated how the possibility of selective outcome reporting was examined by the authors and what was found	Reporting bias due to selective outcome reporting	Selective outcome reporting bias not detected	Insufficient information to permit judgment†	**High Low Unclear**
Other bias **Other sources of bias**	Any important concerns about bias not addressed above*	Bias due to problems not covered elsewhere in the table	No other bias detected	There may be a risk of bias, but there is either insufficient information to assess whether an important risk of bias exists or insufficient rationale or evidence that an identified problem will introduce bias	**High Low Unclear**
Performance bias **Blinding (participants and personnel)**	Described all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provided any information relating to whether the intended blinding was	Performance bias due to knowledge of the allocated interventions by participants and personnel during the study.	Blinding was likely effective.	Not described in sufficient detail	**High Low Unclear**

	effective.				
Detection bias **Blinding (outcome assessment)**	Described all measures used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Provided any information relating to whether the intended blinding was effective.	Detection bias due to knowledge of the allocated interventions by outcome assessors.	Blinding was likely effective.	Not described in sufficient detail	**High** **Low** **Unclear**
Attrition bias **Incomplete outcome data**	Described the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. Stated whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported.	Attrition bias due to amount, nature or handling of incomplete outcome data.	Handling of incomplete outcome data was complete and unlikely to have produced bias	Insufficient reporting of attrition/exclusions to permit judgment (e.g., number randomized not stated, no reasons for missing data provided)	**High** **Low** **Unclear**