Cochrane Library

Cochrane Database of Systematic Reviews

Statins for Smith-Lemli-Opitz syndrome (Review)

Ballout RA, Livinski A, Fu YP, Steiner RD, Remaley AT

Ballout RA, Livinski A, Fu Y-P, Steiner RD, Remaley AT.
Statins for Smith-Lemli-Opitz syndrome.
Cochrane Database of Systematic Reviews 2022, Issue 11. Art. No.: CD013521.
DOI: 10.1002/14651858.CD013521.pub2.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Review]

Statins for Smith-Lemli-Opitz syndrome

Rami A Ballout[1,2], Alicia Livinski[3], Yi-Ping Fu[4], Robert D Steiner[5], Alan T Remaley[1]

[1]Lipoprotein Metabolism Section, Translational Vascular Medicine Branch, National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD, USA. [2]Department of Pediatrics, University of Texas Southwestern (UTSW) Medical Center and Children's Health, Dallas, TX, USA. [3]Division of Library Services, National Institutes of Health Library, Office of Research Services, Bethesda, Maryland, USA. [4]Office of Biostatistics Research, National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD, USA. [5]Marshfield Clinic Research Foundation, Marshfield, Wisconsin, USA

Contact: Rami A Ballout, rami-ballout@hotmail.com.

Editorial group: Cochrane Cystic Fibrosis and Genetic Disorders Group.
Publication status and date: New, published in Issue 11, 2022.

Citation: Ballout RA, Livinski A, Fu Y-P, Steiner RD, Remaley AT. Statins for Smith-Lemli-Opitz syndrome. *Cochrane Database of Systematic Reviews* 2022, Issue 11. Art. No.: CD013521. DOI: 10.1002/14651858.CD013521.pub2.

ABSTRACT

Background

Smith-Lemli-Opitz syndrome (SLOS) is a multiple congenital malformations syndrome caused by defective cholesterol biosynthesis. Affected individuals show cholesterol deficiency and accumulation of various precursor molecules, mainly 7-dehydrocholesterol and 8-dehydrocholesterol. There is currently no cure for SLOS, with cholesterol supplementation being primarily a biochemical therapy of limited evidence. However, several anecdotal reports and preclinical studies have highlighted statins as a potential therapy for SLOS.

Objectives

To evaluate the effects of statins, either alone or in combination with other non-statin therapies (e.g. cholesterol, bile acid, or vitamin co-supplementation), compared to cholesterol supplementation alone or in combination with other non-statin therapies (e.g. bile acid or vitamin supplementation) on several important outcomes including overall survival, neurobehavioral features, and adverse effects in individuals with SLOS.

Search methods

We searched CENTRAL, MEDLINE, Embase, five other databases and three trials registers on 15 February 2022, together with reference checking, citation searching and contact with study authors to identify additional studies.

Selection criteria

Randomized controlled trials (RCTs) and quasi-RCTs with parallel or cross-over designs, and non-randomized studies of interventions (NRSIs) including non-randomized trials, cohort studies, and controlled before-and-after studies, were eligible for inclusion in this review if they met our prespecified inclusion criteria, i.e. involved human participants with biochemically or genetically diagnosed SLOS receiving statin therapy or cholesterol supplementation, or both.

Data collection and analysis

Two authors screened titles and abstracts and subsequently full-texts for all potentially-relevant references. Both authors independently extracted relevant data from included studies and assessed the risks of bias. We analyzed the data extracted from the included NRSIs and cohort studies separately from the data extracted from the single included RCT. We used a random-effects model to account for the inherent heterogeneity and methodological variation between these different study designs. We used GRADE to assess the certainty of evidence.

Main results

We included six studies (61 participants with SLOS); one RCT (N = 18), three prospective NRSIs (N = 20), and two retrospective NRSIs (N = 22). Five studies included only children, and two limited their participant inclusion by disease severity. Overall, there were nearly twice as many males as females. All six studies compared add-on statin therapy to cholesterol supplementation alone. However, the dosages, formulations, and durations of treatment were highly variable across studies.

We judged the RCT as having a high risk of bias due to missing data and selective reporting. All included NRSIs had a serious or critical overall risk of bias assessed by the Risk Of Bias In Non-randomized Studies of Interventions tool (ROBINS-I).

None of the included studies evaluated survival or reported quality of life (QoL). Only the included RCT formally assessed changes in the neurobehavioral manifestations of SLOS, and we are uncertain whether statin therapy improves this outcome (very low-certainty evidence). We are also uncertain whether the adverse events reported in the RCT were statin-related (very low-certainty evidence). In contrast, the adverse events reported in the NRSIs seem to be possibly due to statin therapy (risk ratio 13.00, 95% confidence interval 1.85 to 91.49; P = 0.01; low-certainty evidence), with only one of the NRSIs retrospectively mentioning changes in the irritability of two of their participants. We are uncertain whether statins affect growth based on the RCT or NRSI results (very low-certainty evidence). The RCT showed that statins may make little or no difference to plasma biomarker levels (low-certainty evidence), while we are uncertain of their effects on such parameters in the NRSIs (very low-certainty evidence).

Authors' conclusions

Currently, there is no evidence on the potential effects of statin therapy in people with SLOS regarding survival or QoL, and very limited evidence on the effects on neurobehavioral manifestations. Likewise, current evidence is insufficient and of very low certainty regarding the effects of statins on growth parameters in children with SLOS and plasma or cerebrospinal fluid (CSF) levels of various disease biomarkers. Despite these limitations, current evidence seemingly suggests that statins may increase the risk of adverse reactions in individuals with SLOS receiving statins compared to those who are not. Given the insufficient evidence on potential benefits of statins in individuals with SLOS, and their potential for causing adverse reactions, anyone considering this therapy should take these findings into consideration. Future studies should address the highlighted gaps in evidence on the use of statins in individuals with SLOS by collecting prospective data on survival and performing serial standardized assessments of neurobehavioral features, QoL, anthropometric measures, and plasma and CSF biomarker levels after statin introduction. Future studies should also attempt to use consistent dosages, formulations and durations of cholesterol and statin therapy.

PLAIN LANGUAGE SUMMARY

Statins for Smith-Lemli-Opitz syndrome

Review question

Is statin therapy, either alone or combined with cholesterol therapy, linked to better outcomes (e.g. survival, quality of life, severity or frequency of neurobehavioral abnormalities, changes in growth parameters or biomarker levels) compared to cholesterol therapy alone for people with Smith-Lemli-Opitz syndrome (SLOS), and what are the risks of harmful effects for either option?

Background

Smith-Lemli-Opitz syndrome is a genetic malformation syndrome, which occurs when cholesterol is not able to be produced by the body and there is a build up of several toxic molecules that would, under normal circumstances, go on to become cholesterol, such as 7DHC and 8DHC. This means that people with SLOS may fail to achieve normal physical growth, show various degrees of intellectual disability or developmental delay (or both), and show a range of possible behavioral abnormalities (e.g. irritability, aggressiveness, anxiety, attention-deficit hyperactivity disorder (ADHD), impulsivity and sleep disturbances, among others). It is also notable that SLOS may cause various possible physical abnormalities and disability. There is currently no cure for SLOS, but cholesterol supplementation remains common practice among clinicians caring for people with SLOS. This is based solely on our current understanding of the underlying biochemistry of the disease itself. However, there has been evidence from studies in the laboratory and in single individuals suggesting that statins may be helpful for treating people with SLOS.

Search date

We conducted our latest search on 15 February 2022.

Study characteristics

In this review, we included six studies of different designs that included a total of 61 people with SLOS, mostly males. Five of the studies only included children (18 years old or younger). All studies compared a combination of statin and cholesterol therapy to cholesterol supplementation alone. However, the studies used different doses and formulations of statin or cholesterol therapy (or both), as well as different durations of treatment.

Key results

We found no evidence for the effects of combined statin and cholesterol supplementation, compared to cholesterol supplementation alone, on survival or quality of life in people with SLOS. We are also not sure if combined statin and cholesterol therapy has positive effects on neurobehavioral manifestations or growth in individuals with SLOS compared with cholesterol therapy alone, as we have low confidence in the evidence. We think people with SLOS receiving statin therapy in addition to cholesterol supplementation are more likely to experience adverse events that are usually associated with using statins, compared to those who are only receiving cholesterol supplementation. Finally, we are uncertain about the effects of statin therapy on the levels of various biomarkers usually measured in the blood of people with SLOS.

SUMMARY OF FINDINGS

Summary of findings 1. Statins with cholesterol supplementation versus cholesterol supplementation only

Statins with cholesterol supplementation versus cholesterol supplementation only

Patient or population: children and adults with Smith-Lemli-Opitz syndrome

Settings: outpatients

Intervention: statin therapy in addition to cholesterol supplementation

Comparison: cholesterol supplementation alone

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	No statins	Statins				
Overall survival	This outcome was not reported in any of the included studies.					
Change in neurobehavioral manifestations Follow-up: 12 months (RCT)	There was a positive effect, i.e. a reduction in severity of irritability measured by the irritability subscale of the ABC-C in children with SLOS receiving simvastatin and cholesterol supplementation, compared to those receiving cholesterol supplementation alone (P = 0.017).			14 participants (1 RCT)	⊕⊖⊖⊖ Very low[a,b]	While worsening of self-injurious behaviors was not recorded during the study, the authors note that such an observation was made for one participant during the open-label extension of the study.
Follow-up: N/A (NRSI)	None of the NRSIs formally assessed this outcome; one study retrospectively noted a reduction in severity of self-injurious behavior in the immediate period after statin therapy in one participant, but a worsening of the same behaviors in another participant.					
Statin-related adverse events Follow-up: 12 months (RCT)	56 per 1000	18 per 1000 (0 to 430)	RR 0.33 (0.01 to 7.68)	18 participants (1 RCT)	⊕⊖⊖⊖ Very low [c]	

Follow-up: 10 months to 36 months (NRSIs)	Individuals receiving statin therapy were at an increased risk for experiencing adverse reactions, compared to those receiving cholesterol supplementation only (RR 13.00, 95% CI 1.85 to 91.49; P = 0.010; I^2 = 0%; 2 retrospective NRSIs, 22 participants). No adverse reactions were reported after statin use in the prospective NRSI.	N/A	25 participants in total 3 participants (1 prospective NRSI) 22 (N = 9 + 13) participants (2 retrospective NRSIs)	⊕⊕⊖⊖ Low [d]	Risk of bias for NRSIs was rated using ROBINS-I
Changes in growth (number of children showing change) Follow-up: 24 months (RCT)	There was no change in weight for the children treated with statins compared to control (N = 18 out of 22; P = 0.76) or height (N = 16 out of 22; P = 0.42) in the included RCT.	N/A	18 participants assessed for weight 16 participants assessed for height (1 RCT)	⊕⊖⊖⊖ Very low [b,e]	No numerical data were made available to us by the authors of the trial, and they only reported their results narratively.
Follow-up: 36 months (NRSI)	Three out of 13 participants showed a change in weight, but in different directions. One participant showed a significant increase in weight, while the remaining two participants showed a decrease in weight. Moreover, two out of 13 participants showed a decrease in linear growth (i.e. a slowing in rate of gain in height).	N/A	13 participants (1 retrospective NRSI)	⊕⊖⊖⊖ Very low [b,f]	Risk of bias for NRSIs was rated using ROBINS-I.
Changes in biochemical markers plasma dehydrocholesterol levels (mM); plasma cholesterol levels (mM); plasma 7DHC levels (mM); plasma CoQ levels (uM); CSF cholesterol levels (ug/mL); CSF dehydrocholesterol levels (ug/mL) Follow-up: 12 months (RCT)	Changes in plasma dehydrocholesterol levels slightly favored treatment with statins, while changes in plasma cholesterol levels slightly favored the no statin treatment in the included trial. There was no difference between treatment groups for CSF cholesterol levels or CSF dehydrocholesterol levels.	N/A	18 participants (1 RCT)	⊕⊕⊖⊖ Low [b,g]	None of the RCT analyses showed any significant difference between groups for any of the biochemical markers.

Follow-up: 10 months to 36 months (NRSIs)	No difference was seen between treatment groups for changes in plasma dehydrocholesterol levels, although two studies slightly favored treatment with statins. There was no difference in changes to total plasma cholesterol between treatment groups.	N/A	18 participants receiving statins with cholesterol supplementation and 20 participants receiving cholesterol supplementation only (3 prospective NRSIs) 13 participants (1 retrospective NRSI)	⊕⊖⊖⊖ Very low [b,h]	Risk of bias for NRSIs was rated using ROBINS-I.
QoL	This outcome was not reported in any of the included studies				

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
ABC-C: Aberrant Behaviour Checklist in children; CI: confidence interval; CSF: cerebrospinal fluid; N/A: not applicable; NRSI: non-randomized study of intervention; QoL: quality of life; RCT: randomized controlled trial; ROBINS-I: Risk Of Bias In Non-randomized Studies of Interventions; RR: risk ratio; SLOS: Smith-Lemli-Opitz syndrome

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded twice due to high risk of bias in the single RCT caused by strong suspicion for selective outcome reporting and incomplete outcome data. The protocol did not prespecify that the study would only be looking at the irritability component of the ABC-C tool and does not provide justifications as to why the authors did not report data on any other components of the tool.
[b]Downgraded once due to inconsistency arising from heterogeneity in the direction of the results within the study.
[c]Downgraded three times: twice due to high risk of bias in the single RCT caused by high suspicion for selective outcome reporting and incomplete outcome data caused by the exclusion of 4 participants from the final analysis (3 were noncompliant (1 in first phase from placebo group; 2 in second phase from simvastatin group) and 1 participant in placebo group in first phase who developed myopathy); downgraded a further level due to imprecision (few participants, few events and wide CIs). Additionally, in the open-label part of the trial, three of the participants receiving statins developed abdominal pain, photosensitivity, and elevations in bilirubin levels, all of which fit our prespecified definition of possible statin-related adverse reactions (Methods). However, no further data were provided about these participants, such as whether their symptoms subsided when statin therapy was discontinued, despite our multiple reasonable attempts of contacting the study authors.
[d]Downgraded twice due to serious risk of bias across the three studies included in this outcome caused by confounding, with two of these studies being at serious risk of bias in terms of their selection of participants.
[e]Downgraded twice due to high risk of bias in the single RCT caused by selective reporting and incomplete outcome data. This outcome was not prespecified in the protocol. The growth measurements were only reported in the second arm of the trial, i.e. after the participants had crossed over to the second arm, and the measurements of weight or height were not performed on an equal number of participants.
[f]Downgraded twice due to serious overall risk of bias in four of the seven domains, and critical risk of bias in one of them due to deviations from the intended interventions.

[g]Downgraded once due to high risk of bias in the single RCT caused by strong suspicion for selective outcome reporting and incomplete outcome data, with CSF sterol levels being specified as an exploratory rather than primary outcome of the study.

[h]Downgraded twice due to overall critical risk of bias across all four studies included in this outcome, mainly caused by confounding where some of the included participants had already been receiving cholesterol supplementation only, or with add-on statin therapy, prior to inclusion in the studies.

Note: we decided not to downgrade the certainty of evidence in any of the included studies due to imprecision caused by including a small number of participants, owing to the rare nature of the studied disease, which would otherwise be an unreasonable and discriminatory criteria for all reviews addressing rare diseases where participant recruitment remains a major limitation.

BACKGROUND

Description of the condition

Smith-Lemli-Opitz syndrome (SLOS) is a rare syndrome of congenital malformation and intellectual disability, which is inherited in an autosomal recessive manner and has an estimated incidence of one out of every 40,000 live births, seemingly more prevalent in individuals of northern European ancestry (Ballout 2021; Kelley 2000). The condition is known under the OMIM number 'OMIM # 270400'; an OMIM number is like an ID tag assigned to each gene and its corresponding diseases, such that different genetic diseases have different OMIM numbers. Specifically, SLOS arises from biallelic mutations in the *DHCR7* gene, which encodes 7-dehydrocholesterol reductase (DHCR7), the enzyme that catalyzes the conversion of 7-dehydrocholesterol (7DHC) into cholesterol, in the final step of the cholesterol biosynthesis pathway (Fitzky 1998; Honda 1995).

As a result, SLOS is characterized by two key biochemical abnormalities: cholesterol deficiency, and the accumulation of several of its precursor sterol molecules, most importantly 7DHC and its isomer, 8-dehydrocholesterol (8DHC) (Honda 1995; Tint 1994).

Clinically, SLOS generally manifests as a multiple congenital malformation syndrome that is associated with a spectrum of neurobehavioral abnormalities and variable degrees of intellectual disability (Nowaczyk 1998a; Nowaczyk 1998b). These clinical features of SLOS are speculated to result from its characteristic biochemical abnormalities, i.e. cholesterol deficiency and the accumulation of potentially toxic sterol precursor molecules (7DHC and 8DHC), which together, are thought to interfere with normal organogenesis and central nervous system (CNS) development in a fetus affected by SLOS (Kelley 2000; Nowaczyk 1998b).

The individual clinical manifestations of SLOS are highly variable, but generally include prenatal or postnatal growth retardation (or a combination of both), variable degrees of intellectual disability or neurodevelopmental delays (or both), and a spectrum of possible neurobehavioral abnormalities such as irritability, aggressiveness, self-injurious behaviors, anxiety, attention-deficit hyperactivity disorder (ADHD), emotional lability, impulsivity, sleep disturbances, social and communication deficits, sensory hyperreactivity, and autism spectrum disorders (ASD) (Diaz-Stransky 2012; Sikora 2006; Tierney 2001).

In addition to these developmental, neurocognitive, and behavioral abnormalities seen in SLOS, affected individuals also have concomitant multiple organ malformations and physical manifestations, which contribute to the severity and morbidity of the syndrome (Ballout 2021; Kelley 2000). These include microcephaly, congenital cataracts, optic atrophy, cleft lip or cleft palate (or both), gingival abnormalities, hypospadias or ambiguous genitalia (especially in males), and various brain anomalies, such as ventriculomegaly, corpus callosum thinning, holoprosencephaly, or myelination defects. Moreover, people with SLOS often also have limb and digital anomalies such as phocomelia, post-axial polydactyly, ectrodactyly, or 2,3-toe syndactyly, with the latter being the most consistent feature of the syndrome (Ballout 2021). Other major organ malformations include renal cysts, pyloric stenosis, aganglionic megacolon (i.e. Hirschsprung disease), cholestatic liver disease, and cardiac malformations, namely total anomalous pulmonary venous return and atrioventricular canal defects. People with SLOS have also been reported to have photosensitivity, peripheral neuropathy, and a multitude of potential facial dysmorphic features, such as bitemporal narrowing, ptosis, shortened nose with anteverted nares, and micrognathia (Ballout 2021; Kelley 2000; Nowaczyk 1998b).

Description of the intervention

No curative therapies exist to date for SLOS. However, cholesterol supplementation is commonly recommended for, and employed in, individuals with SLOS in an attempt to correct one of the key biochemical abnormalities of the syndrome (i.e. cholesterol deficiency), despite its demonstrated limited clinical benefits (Ballout 2021; Nowaczyk 1998b; Svoboda 2012). In addition, cholesterol supplementation helps reduce cholesterol precursor levels, such as those of 7DHC and 8DHC (Linck 2000). One likely explanation for the feeble clinical efficacy of cholesterol supplementation in SLOS is the fact that cholesterol cannot traverse the blood-brain barrier to reach the CNS (Dietschy 2001; van Rooij 1997), which is the prime-affected organ system that is responsible for most neurocognitive and neurobehavioral manifestations of the syndrome. Moreover, the ability of oral cholesterol supplementation to increase circulating levels of cholesterol is rather limited, due to the intrinsically limited absorption capacity of the intestines for cholesterol (Grundy 1983; Svoboda 2012), as well as the inhibitory role of dietary or biliary phospholipids, or both, on intestinal absorption of cholesterol (Cohn 2010).

Nonetheless, there have been several reports to date on various beneficial effects seen in children with SLOS who were supplemented with cholesterol, such as improvements in growth parameters, gastrointestinal manifestations, infection tolerance, and nerve function (Elias 1997; Nwokoro 1997; Starck 2002a). Additionally, cholesterol supplementation has been shown to reduce the ultraviolet-A (UV-A) photosensitivity of individuals with SLOS (Azurdia 2001). However, cholesterol supplementation failed to demonstrate any benefits in correcting the growth parameters or ameliorating the neurobehavioral abnormalities of the disorder (Sikora 2004; Tierney 2010), though large randomized and rigorous controlled clinical trials that investigate the effects of cholesterol supplementation in individuals with SLOS are still needed (Tierney 2010). This warrants the need for a continued search for therapies capable of targeting and alleviating the neurobehavioral manifestations of SLOS.

Based on the known biochemical abnormalities of SLOS, an ‘ideal’ treatment would be one that can hypothetically correct these biochemical abnormalities very early on, perhaps during the development of an affected fetus (Svoboda 2012). Specifically, such a prenatal approach to treatment would need to generate two effects for it to be likely beneficial: to increase the levels of total cholesterol in the plasma of the developing fetus, while simultaneously decreasing the levels of its toxic precursors, such as 7DHC and 8DHC (Ballout 2021). Such in utero interventions would be hypothetically expected to attenuate or prevent the development of the neurobehavioral abnormalities or cognitive deficits (or both) characteristic of the condition, especially since total cholesterol deficit and an excess of 7DHC and 8DHC are speculated to be the likely drivers of the multisystemic manifestations of SLOS (Blassberg 2016; Svoboda 2012). Unfortunately, however, such a prenatal intervention

approach is currently unavailable for SLOS, making early postnatal intervention the next best therapeutic option that is feasible at this time.

Since neurobehavioral development is a continuous process that persists beyond the prenatal period and is influenced by various environmental factors, especially during the first five years of life (Gogtay 2004; Tierney 2009; van Dyck 2017), postnatal therapies that can increase plasma cholesterol levels or decrease corresponding 7DHC and 8DHC levels (or both), may prove nearly as beneficial in attenuating or preventing the development of several of the neurobehavioral manifestations of SLOS. Nonetheless, while such postnatal intervention(s) may prove helpful for the neurobehavioral and cognitive aspects of the syndrome, their corresponding effect(s) on the physical manifestations of the syndrome, such as congenital malformations, are expected to be limited or very small, since organogenesis largely occurs very early on during fetal development.

One group of drugs capable of producing such effects, which have been gaining increasing interest in the field over the past several years, are statins. These are pharmacological inhibitors of HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in the cholesterol biosynthesis pathway, used routinely in the treatment of hypercholesterolemia (Stancu 2001). As such, one might get the impression, at first, that this is a counter-intuitive and rather paradoxical approach for treating SLOS, a condition notable for its cholesterol deficit. However, a closer look at the speculated beneficial mechanism of action of statins in the setting of SLOS, corroborated by available empirical data, changes that impression.

Specifically, through inhibiting HMG-CoA reductase, statins inhibit the synthesis of mevalonate, an early precursor of 7DHC and 8DHC, which are implicated in SLOS pathogenesis (Luo 2020; Svoboda 2012). This reduces the availability of mevalonate for subsequent conversion to 7DHC and 8DHC, thereby preventing further accumulation of these neurotoxic sterols (Kelley 2000). Statins have also been shown to simultaneously induce both, increased expression and activity of DHCR7, the enzyme defective in SLOS (Svoboda 2012; Wassif 2005), especially in the setting of hypomorphic *DHCR7* mutations (i.e. in individuals with residual enzymatic activity) (Wassif 2005). These findings have been replicated in several animal studies and anecdotal reports in humans, in which statin therapy resulted in a reduction in the levels of 7DHC and 8DHC, often with an associated paradoxical increase in circulating levels of cholesterol (Haas 2007; Jira 2000; Svoboda 2012).

How the intervention might work

As mentioned previously, it is believed that the cognitive and neurobehavioral abnormalities seen in SLOS are the result of the combined effects of a total cholesterol deficit and a concomitant accumulation of 7DHC and 8DHC within the CNS of a developing fetus while in utero (Kelley 2000).

This is because cholesterol is essential for fetal development and growth, especially after the first trimester, when most of the cholesterol needed for fetal development must be endogenously synthesized in the fetus, as opposed to being maternally acquired via the premature and 'leaky' placenta, which is the process that occurs during the first trimester (Baardman 2013; Lin 1977). Besides its function as an integral structural and stabilizing component of cellular membranes, cholesterol is a precursor for a variety of key bioactive molecules, such as steroid hormones, vitamin D, and bile acids, while also being an essential cofactor for the post-translational modification of hedgehog signaling proteins, primarily Sonic hedgehog (SHH), which is a master regulator of fetal organogenesis (Bikle 2017; Kelley 2000; Porter 1996; Russell 1992).

Furthermore, as argued previously, the ideal treatment approach in SLOS would be to supplement the fetus with the deficient cholesterol, while helping clear or reduce its circulating levels of 7DHC and 8DHC. However, because this approach is not yet available and neurodevelopment continues to occur in the postnatal period, therapies that can help increase cholesterol levels while simultaneously reducing 7DHC levels comprise a potentially promising postnatal therapeutic approach in SLOS.

In that regard, statins have been proposed as one such therapy that can exert both effects: reducing the levels of 7DHC and 8DHC by way of inhibiting HMG-CoA reductase and, therefore, reducing the formation of mevalonate, an upstream precursor of 7DHC and 8DHC (Fitzky 2001; Svoboda 2012), and simultaneously increasing the conversion of 7DHC and 8DHC into cholesterol, by inducing the expression or activity (or both) of partially active DHCR7 (Wassif 2005).

Why it is important to do this review

While most congenital malformations and physical abnormalities associated with SLOS can be surgically corrected or clinically managed (or both), most individuals with SLOS continue to exhibit variable types and degrees of neurobehavioral abnormalities, which preclude their optimal development or functioning (or both) (Kelley 2000; Nowaczyk 2012). However, unlike the visceral malformations and physical features of SLOS, nearly all of which develop during fetal development in utero (Ballout 2021; Kelley 2000; Lazarin 2017), neurodevelopment and behavioral modulation persist well beyond the fetal life, throughout early childhood, extending even into early adulthood (e.g. myelination) (Gogtay 2004; Tierney 2009; van Dyck 2017). Consequently, the early institution of treatments capable of increasing cholesterol levels or reducing the levels of 7DHC and 8DHC (or both) during the postnatal period through early childhood, a period characterized by marked neuroplasticity and modifiable neurodevelopment, may prevent the development of, or attenuate the severity of, some of the syndrome's neurobehavioral abnormalities (Jira 2000; Kelley 2000).

Thus, given the lack of a clinical consensus surrounding the use of statins in people with SLOS, we conducted this review to systematically assess the available evidence on the efficacy of statins on survival, in reducing the severity and frequency of neurobehavioral abnormalities and on adverse events in individuals with SLOS (primary outcomes), as well as the effects of such an intervention on other key outcomes in SLOS. Since life expectancy is poorly characterized in individuals with SLOS (Kelley 2000), survival remains a clinically superior outcome, even when treatments fail to show improvement on other outcomes of interest.

OBJECTIVES

To evaluate the effects of statins, either alone or in combination with other non-statin therapies (e.g. cholesterol, bile

acid, or vitamin co-supplementation), compared to cholesterol supplementation alone or in combination with other non-statin therapies (e.g. bile acid or vitamin supplementation) on several important outcomes including overall survival, neurobehavioral features, and adverse effects in individuals with SLOS.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials (RCTs) or quasi-RCTs with parallel or cross-over designs were eligible for inclusion in this review.

Non-randomized studies of interventions (NRSIs), including non-randomized clinical trials, retrospective* and prospective cohort studies, controlled before-and-after (pre-post) studies, and studies employing an interrupted-time-series (ITS) design, were also considered eligible for inclusion if they met the inclusion criteria that were set a priori in our published protocol (Ballout 2020).

We decided a priori not to pool any included RCTs and NRSIs in the analysis (EPOC 2017). Instead, we presented studies employing different designs separately for meta-analysis (Ballout 2020).

* While we had planned in our protocol to include only cohort studies with a prospective design (Ballout 2020), we have included retrospective cohort studies in this full review. Please see Differences between protocol and review.

Types of participants

Adults or children diagnosed biochemically (i.e. through detecting elevated plasma levels of 7DHC and 8DHC, or diminished DHCR7 activity in patient fibroblasts, provided they are not taking any drugs known to inhibit DHCR7 activity such as haloperidol) or genetically (i.e. through detecting biallelic pathogenic mutations in *DHCR7* on molecular genetic testing) with SLOS, irrespective of age, sex, or disease severity, who received any statin of any dosage or duration (Nowaczyk 2013; Shefer 1997).

As outlined in our protocol, we also excluded studies of individuals with SLOS who had other congenital or chronic comorbid conditions (e.g. neurometabolic diseases, chronic kidney diseases, or other genetic disorders), given that such conditions themselves can interfere with cognitive and behavioral development (i.e. confounders) (Ballout 2020).

Types of interventions

We sought to compare the following interventions:

1. any statin (of any dosage or duration) alone compared with cholesterol supplementation only;
2. any statin (of any dosage or duration) combined with cholesterol supplementation compared with cholesterol supplementation only;
3. any statin (of any dosage or duration) with or without bile acid supplementation compared with cholesterol supplementation alone or in combination with bile acid supplementation (Starck 2002a);
4. any statin (of any dosage or duration) with or without coenzyme Q10 (CoQ10), or vitamins E, A, or C, or other antioxidants (e.g. selenium), or any combination of the latter, compared with cholesterol supplementation alone or in combination with any of the aforementioned vitamins and antioxidants (Fliesler 2018; Korade 2014); and
5. lipophilic statins (e.g. simvastatin, lovastatin, pitavastatin, or atorvastatin) compared with hydrophilic statins (e.g. pravastatin or rosuvastatin) (Ballout 2020).

We excluded any studies co-administering two or more statins simultaneously, i.e. combined statin therapy, due to the inability to attribute the observed effects to a particular statin, especially when using a lipophilic and hydrophilic statin combination, for instance. However, as stated a priori, we did include studies with individuals who sequentially received more than one statin agent, provided that the administration of the different statin agents was interspersed by an adequate washout interval of at least four weeks.

Types of outcome measures

Primary outcomes

1. Survival including:
   a. overall survival, encompassing SLOS-related (see below) and SLOS-unrelated deaths (e.g. death due to accidents, prematurity, sudden infant death syndrome (SIDS), other genetic or metabolic conditions, etc.); and
   b. SLOS-related deaths specifically, which include all deaths deemed by the investigators of the individual studies to have been most likely a direct result of the individual having SLOS (e.g. due to overwhelming infections without an underlying immunodeficiency, severe feeding problems and malnutrition due to gastrointestinal abnormalities related to SLOS, or death from severe visceral or brain malformations associated with SLOS).
2. Changes in severity or frequency (or both) of the neurobehavioral manifestations associated with SLOS, assessed by comparison with each individual's corresponding baseline, i.e. at the time of initial enrolment in the study:
   a. anxiety (evaluated using, e.g. the Pediatric Anxiety Rating Scale (PARS)) (PARS 2002);
   b. ADHD (evaluated using, e.g. the Conner's Continuous Performance Test (CCPT) or the Test of Variables of Attention (TOVA)) (Edwards 2007);
   c. pro-active aggression against others or self (i.e. self-mutilation) (assessed using, e.g. the Buss-Perry Aggression Questionnaire (BPAQ) and the Functional Assessment of Self-Mutilation (FASM) tools) (Buss 1992; Lloyd 1997);
   d. emotional lability (e.g. tantrums or aggressive outbursts to obtain tangible objects, i.e. reactive aggression), or agitation, or irritability (assessed using, e.g. the Emotion Regulation Checklist (ERC) or the irritability subscale of the Aberrant Behavior Checklist-Community (ABC-C) tools) (Aman 1995; Shields 1997);
   e. sleep disturbances (evaluated using, e.g. the Pediatric Sleep Questionnaire (PSQ) or the Children's Sleep Habits Questionnaire tools) (Chervin 2000; Owens 2000); and
   f. ASD (evaluated using, e.g. the Autism Diagnostic Observational Schedule (ADOS)) (Lord 2001).
3. Statin-related adverse reactions:
   a. liver-related: hepatotoxicity assessed by hepatic injury biomarkers and defined as having serum glutamic-

oxaloacetatic transaminase (SGOT; also known as aspartate aminotransferase (AST)) or serum glutamic-pyruvic transaminase (SGPT; also known as alanine aminotransferase (ALT)) levels reaching or exceeding three times the upper limit of normal (ULN) (Rosenson 2019a);

b. muscle-related: myalgias (self-reported by participants or their caregivers or both), myopathy (defined as elevations in the levels of creatine phosphokinase (CPK) often to 10 times the upper limit of normal (ULN) (Starck 2002a), or aldolase, or lactate dehydrogenase (LDH), or any combination of these parameters), or rhabdomyolysis (Rosenson 2019b);

c. skin-related: increased or worsening photosensitivity following statin initiation (measured quantitatively as UV-A tolerance in joules/cm^2) (Starck 2002a); and

d. others: depletion of or reduction in CoQ10 levels (Qu 2018; Rundek 2004), or developing one of the rare statin-related adverse reactions such as cognitive dysfunction, sleep disturbances, abdominal pain, diarrhea or neuropathy (Haas 2007; Rosenson 2019a).

Secondary outcomes

1. Changes in the anthropometric and growth parameters of children receiving statins, during or following the statin treatment (for at least two years in adolescents, i.e. males and females who attained puberty, and up to 10 years in prepubertal children and those under the age of 10 years), including:
   a. height;
   b. weight;
   c. head circumference;
   d. body mass index (BMI); and
   e. Tanner staging.
2. Changes in the biochemical markers of the disorder:
   a. plasma lipid levels (total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apoA-I, and apoB);
   b. vitamin D levels (25-hydroxy and 1, 25-dihydroxy forms);
   c. plasma or cerebrospinal fluid (CSF) levels of oxysterols;
   d. plasma or cerebral spinal fluid (CSF) levels of 7DHC or 8DHC, or other dehydrocholesterols (reported preferably as ratios of total sterols); and
   e. any other markers such as plasma levels of CoQ10, vitamin E, plant sterols, etc. (Haas 2008; Kelley 2000; Korade 2014; Oláh 2013a).
3. Quality of life (QoL) (measured by, e.g. validated instruments or scales or health outcome rating scales, or self-reported satisfaction or dissatisfaction), including feeding behavior or tolerance.

Search methods for identification of studies

We searched for all relevant published and unpublished studies, without restrictions on language, year of publication, or publication status.

Electronic searches

The Cochrane Cystic Fibrosis and Genetic Disorders Group's Information Specialist conducted a search of the Group's Cystic Fibrosis Trials Register for relevant trials using the following terms: Smith-Lemli-Opitz:kw.

The Inborn Errors of Metabolism Trials Register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated with each new issue of the *Cochrane Library*), weekly searches of MEDLINE and the prospective handsearching of one journal - *Journal of Inherited Metabolic Disease*. Unpublished work was to be identified by searching through the abstract books of the Society for the Study of Inborn Errors of Metabolism (SSIEM) conference and the SHS Inborn Error Review Series. For full details of all searching activities for the register, please see the relevant section of the Cochrane Cystic Fibrosis and Genetic Disorders Group's website.

Date of most recent search: 15 February 2022.

We searched the following databases:

1. Cochrane Central Register of Controlled Trials (CENTRAL; 2021, Issue 11) in the *Cochrane Library* (www.cochranelibrary.com/; searched 19 November 2021);
2. PubMed (www.ncbi.nlm.nih.gov/pubmed; 1946 to 19 November 2021);
3. Embase.com (1982 to 19 November 2021);
4. Web of Science (WoS) Core Collection (covering Science Citation Index Expanded (1900 to present), Social Sciences Citation Index (1900 to present), Conference Proceedings Citation Index-Science (1990 to present), Book Citation Index-Science (2005 to present), Emerging Sources Citation Index (2005 to present), SciELO Citation Index (2002 to present); searched 19 November 2021);
5. Scopus (1823 to 19 November 2021);
6. LILACS (Latin American and Caribbean Health Science Information Database) (lilacs.bvsalud.org/en/; 1982 to 19 November 2021);
7. CRD (Centre for Reviews and Dissemination) Database (www.crd.york.ac.uk/CRDWeb/; searched 19 November 2021);
8. PROSPERO (International Prospective Register of Systematic Reviews) (www.crd.york.ac.uk/PROSPERO/; searched 19 November 2021);
9. NARCIS (National Academic Research and Collaborations Information system) (www.narcis.nl/; searched 19 November 2021); and
10. OpenGrey (www.opengrey.eu/; searched 19 November 2021).

Additionally, we searched the following trial registers:

1. US National Institutes of Health Ongoing Trials Register Clinicaltrials.gov (www.clinicaltrials.gov; searched 19 November 2021);
2. World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (apps.who.int/trialsearch; searched 19 November 2021); and
3. EU Clinical Trials Register (www.clinicaltrialsregister.eu; searched 19 November 2021).

For details of our search strategies, please refer to the appendices (Appendix 1).

Searching other resources

We handsearched the abstracts and conference proceedings of the Society for Inherited Metabolic Disorders (SIMD), which are available online as supplements to the Molecular Genetics and

Metabolism journal (MGM) (1998 to present), and the abstracts and conference proceedings of the SSIEM available online in the Journal of Inherited Metabolic Disease (JIMD) (1978 to present).

We also searched the Sterol and Isoprenoid Research Consortium (STAIR) of the National Institutes of Health's Rare Disease Clinical Research Network (NIH RDCRN) for any ongoing work in SLOS relevant to our review (www.rarediseasesnetwork.org/cms/stair).

We examined the reference lists of all included studies, as well as any studies deemed potentially eligible for inclusion at the title and abstract screening stage, to identify any additional studies not found through electronic searching.

Data collection and analysis

We used the standard Cochrane systematic review methods, outlined in the *Cochrane Handbook for Systematic Reviews of Interventions,* when conducting our study selection and data extraction and analysis (Higgins 2022a).

Selection of studies

Prior to commencing the title and abstract screening stage, the two review authors involved in the subsequent study screening (RB and AL) underwent a calibration exercise to check for their starting inter-reviewer agreement and, subsequently, work on improving it to ensure maximum consistency of inter-reviewer screening of studies. The generated kappa statistic at the end of this calibration exercise was 0.96, indicating high inter-reviewer screening consistency and negating the need for subsequent screening optimization exercises among the two review authors (Higgins 2022b).

Then, both review authors (RB and AL), independently and in duplicate, performed the title and abstract screening for all references retrieved by the search strategy. The same review authors subsequently retrieved and screened, in duplicate and independently, the full texts of all references deemed potentially relevant for the review by either of them during the previous title and abstract screening stage. Whenever they encountered disagreements in screening and selecting studies, they discussed them in the first instance to reach a resolution. If consensus could not be reached through discussion, they consulted with a third review author on the team for resolution.

Data extraction and management

Two review authors (RB and AL) independently and in duplicate extracted relevant data from the included studies, utilizing the data extraction tool within the Review Manager software (Review Manager 2020). We extracted data on the following.

1. Study characteristics (i.e. year of publication, country of publication, study design, duration of the study, and the disclosure of funding or conflicts of interest (or both)).
2. Participant characteristics (i.e. age, sex, and disease severity of the participants included in the study, how these individuals were selected or randomized into the study, and the number of participants enrolled in each arm/group, with their attrition rates whenever available).
3. Numbers of participants enrolled in each arm, with their attrition rates (where applicable).
4. Details of the intervention(s) and their control(s) or comparator(s) (i.e. dosage, formulation, route of administration, and duration of therapy of statins or cholesterol supplementation (or both)).
5. Outcomes reported by each study (e.g. changes in neurobehavioral assessments, plasma levels of 7DHC, 8DHC, total cholesterol, or statin-related adverse reactions, etc.).
6. Notes on any special considerations that should be taken into account with regard to a particular study, including how potential confounders (e.g. ethnicity or race (Benjamin 2018), diet, diease severity, age, and sex) were handled in NRSIs.

For our first primary outcome (survival), we had planned to present data at 6, 12, 24, and 36 months, and annually thereafter, if and wherever applicable. For all other outcomes, we planned to group their data into those measured at 2, 6 and 12 months and annually thereafter. However, if investigators recorded relevant outcome data at other time periods, we considered presenting these as well. However, none of the included studies had evaluated survival, and the times of reporting for all other relevant outcomes were highly variable across the studies. As a result, we have reported the data collected by the individual studies, irrespective of their time points for collecting such data. Nonetheless, we relied on our clinical knowledge and experience for interpreting such data recorded at different time points by the included studies, while taking into consideration the quantitative nature of the majority of outcomes of interest, such as growth parameters and plasma biomarker levels, to ensure cautious interpretations of the reported findings,

The two authors (RB and AL) resolved any disagreements encountered during data extraction through discussion and without the need to consult a third review author. Wherever we encountered missing, unclear, or incomplete data, we made multiple and reasonable attempts to contact the first authors and corresponding author(s) of the studies in question, for further clarification. When more than a single report was published for the same study, we ensured that all data across all of the reports were included in our review.

The lead author (RB) entered all extracted data into Review Manager software (Review Manager 2020), which was subsequently reviewed by the team's biostatistician (YF) for confirmation.

Assessment of risk of bias in included studies

Two review authors (RB and AL), independently and in duplicate, conducted the overall risk of bias assessment for each of the included studies, using the relevant risk of bias assessment tool outlined below. We resolved any disagreement(s) by discussion, and did not need to consult with a third review author.

RCTs

For the single included RCT, we used the risk of bias assessment tool outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2017). We summarized our overall risk of bias assessment for that study in the Review Manager software (Review Manager 2020).

We presented our risk of bias assessments for included RCT in the risk of bias table, which appraises the following domains.

1. Random sequence generation (selection bias)

2. Allocation concealment (selection bias)
3. Blinding of participants and personnel (performance bias)
4. Blinding of outcome assessment (detection bias)
5. Incomplete or missing outcome data (i.e. high rates of attrition, or improper handling or reporting (or both) of incomplete outcome data) (attrition bias)
6. Selective outcome reporting (i.e. non-adherence to preset protocol) (reporting bias)
7. Other biases possibly arising from issues not covered or addressed by the above domains

We judged each domain as having a 'high risk', 'low risk' or 'unclear risk' of bias, using a supporting quotation or statement from the study together with a narrative statement, to justify our judgment for each domain within the study.

NRSIs, cohort or ITS studies

For all NRSIs included in our review, we used the ROBINS-I tool (Risk Of Bias In Non-randomized Studies of Interventions) to evaluate the risks of bias (Sterne 2016). This tool uses 'signaling questions' to rate the risk of bias judgment as 'low', 'moderate', 'serious', or 'critical', or alternatively, 'no information' when insufficient information is present for a particular domain. The tool evaluates the risk of bias in seven distinct domains of possible methodological issues in NRSIs, which are listed below.

1. Bias due to confounding (e.g. different disease severities between interventions, receiving any intervention prior to starting the study without adequate washout, etc.)
2. Bias in the selection of participants into the study
3. Bias in classification of interventions
4. Bias due to deviations from intended interventions (with intention-to-treat (ITT) analyses being preferred, wherever reported; as discussed in more detail in later sections)
5. Bias due to missing data
6. Bias in measurement of outcomes
7. Bias in the selection of the reported result

We used this tool to assess the risk of bias in the included NRSIs for two key outcomes in our review (the only outcomes with quantitative data available from included studies): the incidence of statin-related adverse reactions and changes in various plasma or CSF biomarker levels.

The ROBINS-I tool adequately covered all aspects of our risk of bias assessment of all the NRSIs, cohort, and before-and-after studies included in our review. In future updates of this review, if ROBINS-I fails to adequately cover all relevant aspects of new cohort studies or ITS studies eligible for inclusion, we will use the criteria outlined below in the Newcastle-Ottowa Scale (NOS) (NOS 2019) to assess the risk of bias in cohort studies, and the criteria listed thereafter by Cochrane Effective Practice and Organisation of Care (EPOC) reviews (EPOC 2017) for assessing the risk of bias in ITS studies.

Cohort studies (using the NOS)

1. Was the selected cohort representative of the target population?
2. Was selection of exposed and non-exposed cohorts drawn from the same population?
3. Can we be confident in the assessment (i.e. ascertainment) of exposure?
4. Can we be confident that the outcome of interest was not present at start of study?
5. Did the study match exposed and unexposed for all variables that are associated with the outcome of interest (i.e. comparability of the different cohorts) or did the statistical analysis adjust for these prognostic variables?
6. Can we be confident in the assessment of outcome?
7. Was the follow-up long enough?
8. Was there adequate follow up of cohorts to minimize attrition rates?

ITS studies (using EPOC criteria)

1. Was the intervention independent of other changes?
2. Was the shape of the intervention effect prespecified?
3. Was the intervention unlikely to affect data collection?
4. Was knowledge of the allocated interventions adequately prevented during the study?
5. Was incomplete outcome data adequately addressed?
6. Was the study free from selective outcome reporting?
7. Was the study free from other risks of bias?

Measures of treatment effect

We used Review Manager software to conduct the meta-analytical part of this review (Review Manager 2020).

1. RCTs

As planned a priori, we reported the data from the single included RCT separately and not in combination with data obtained from the included NRSIs or cohort studies. As such, we have narratively reported the RCT data within our review. However, if new RCTs are conducted and included in future updates of this review, we will handle the pooled analysis of their extracted data as outlined below.

For dichotomous data (e.g. adverse drug reactions (ADRs)), we reported the number of participants experiencing the event relative to the total number of participants evaluated for that outcome, thereby reporting risk ratios (RRs) (preferably) or risk differences (RDs) (less preferred) as effect measures with their corresponding 95% confidence intervals (95% CIs), depending on the data reported by each included study.

For continuous data (e.g. plasma cholesterol levels), we reported the mean differences (MDs) or standardized mean differences (SMDs) as effect measures with their corresponding 95% CIs. We used MDs when all relevant trials measured the same outcome of interest using a comparable or identical scale or standard, and planned to use SMDs when relevant trials measured the same outcome of interest using different or incomparable instruments or scales. If not directly reported, we planned to use any of the available reported data to derive the required SMD (wherever applicable), using ITT analysis with imputation. If SMDs had been generated, we planned to interpret them using the rules of thumb established by researchers in the social sciences, such that an SMD of 0.2 represents a small effect, 0.5 a moderate effect, and 0.8 a large effect (Cohen 1988). Variations still exist, however (e.g. < 0.40 = small, 0.40 to 0.70 = moderate, > 0.70 = large). As such, some methodologists believe that such interpretations are problematic because patient importance of a finding is context-dependent

and not amenable to generic statements. A transformation of an SMD to a log of odds ratios can be pursued in that instance, based on the assumption that an underlying continuous variable has a logistic distribution with equal standard deviation in the two intervention groups (Chinn 2000; Furukawa 1999). Such an assumption is unlikely to hold precisely, so the generated results must be regarded only as an approximation. The log odds ratio can be estimated as: lnOR = 1.81 × SMD, approximately.

2. NRSIs

As planned a priori, we analyzed dichotomous outcomes, such as ADRs (e.g. hepatotoxicity), by reporting the number of participants experiencing that event (i.e. marked elevation in AST or ALT levels, or both) relative to the total number of participants evaluated for that outcome (i.e. at-risk individuals who received statins). We then reported an RR and a corresponding 95% CI as the effect measure for such outcomes.

For continuous data, we analyzed outcomes such as disease biomarkers (e.g. plasma levels of cholesterol or 7DHC) by reporting the absolute change in the outcome measures (e.g. the absolute mean plasma levels of cholesterol post-intervention (i.e. after statin therapy) compared with their respective absolute mean level pre-intervention (i.e. before statin therapy)). It is also worth noting here that, since the different studies included in our review had reported plasma levels of cholesterol or 7DHC using different units (e.g. mmol/L versus mg/dL), we first had to adjust these differences, through converting all reported values to the same unit, before analyzing the data.

Finally, for all other outcomes with insufficient data to perform a quantitative analysis, and those outcomes in which an analysis was not possible (e.g. anthropometric and growth parameter measures), we simply summarized the relevant data narratively in our review.

For future updates of this review, if we include time-to-event data from newly-identified relevant ITS studies (i.e. studies accounting for the number and timing of events e.g. overall survival at different years), we plan to summarize and analyze such data using hazard ratios (HRs) and their corresponding 95% CIs (Higgins 2011).

Likewise, in future updates of this review, if we are to identify and include new before-and-after studies that do not report change-from-baseline data, i.e. those presenting only absolute post-treatment data without baseline data, so it is not possible to calculate change data, we will consider reporting the absolute post-treatment data instead of change from baseline if we are unable to obtain the missing data from the study authors. However, in such cases, we will separate these studies from ones that contain change data.

Unit of analysis issues

Since we included RCTs, cohort studies, and before-and-after studies in this review, we potentially may have encountered unit of analysis issues pertaining to:

1. groups of individuals who were randomized together to the same intervention (i.e. cluster-randomization);
2. individuals who underwent more than one intervention (e.g. in a cross-over trial) (Wassif 2017); and
3. multiple observations for the same outcome (e.g. repeated measurements, recurring events, etc.).

Of the above listed issues, we only encountered the second, i.e. cross-over, in the single included RCT in our review (Wassif 2017). For that study, in which participants were randomized to two treatment arms in a different order, we made multiple reasonable attempts to contact the first and corresponding authors of the study to obtain the data associated with their respective initial arm prior to crossing over; however we have received no reply. We are therefore unable to treat this as a parallel trial for certain outcomes, such as changes in anthropometric measures. We opted to narratively include the data available from the published report of that trial, because we deemed that the risk for a carry-over effect in that study was minimal as the trial had employed a two-month washout period; we deemed this adequate to negate any carry-over effects of statin therapy, as prespecified in our protocol (Ballout 2020).

If we encounter the other issues in future updates of this review, we will treat them according to the guidance given in the *Cochrane Handbook for Systematic Reviews of Interventions,* which is summarized below (Higgins 2022c).

1. For cluster designs, we will extract results adjusted for clustering, and if the reported analyses have not been already adjusted for clustering, we will reanalyze the data taking clustering into account, whenever such analysis is possible. If adjustment is not possible, we will present the data in a table.
2. For studies with multiple treatment groups, we will include subgroups that are considered relevant to the analysis and clinically reasonable choices. When appropriate, we will combine groups to create a single pair-wise comparison. If this is not possible, we will select the most appropriate pair of interventions and exclude the others (Higgins 2022c).
3. Any other unit of analysis issues arising from the inclusion of ITS studies will be dealt with according to the EPOC recommendations (EPOC 2017).
4. For cross-over studies, we will restrict the analysis to only the instances where a carry-over effect of the intervention is known to be minimal or negligible across the different time periods (Elbourne 2002), such as the case with the currently included trial (Wassif 2017). As set a priori in our protocol (Ballout 2020), studies with cross-over designs have to employ a washout period of at least four weeks (Henriques-Forsythe 2015), and preferably six or more weeks (McGowan 2004), between statin therapy discontinuation and the start of another therapy, to ensure that the physiological effects of statins have completely subsided prior to instituting another intervention. In such cases, we will use a paired analysis for analyzing data from cross-over studies, if at least one of the following conditions is met:
   a. individual participant data (IPD) from the paper or by correspondence with the study author (i.e. investigator) are available;
   b. the mean and standard deviation (SD) (or standard error (SE)) of the participant-specific differences between experimental intervention (E) and control intervention (C) measurements are available;
   c. the MD and one of the following are available:
      i. a t-statistic from a paired t-test;
      ii. a P value from a paired t-test; or

iii. a CI from a paired analysis.

d. a graph of measurements on experimental intervention (E) and control intervention (C) from which individual data values can be extracted is available, provided that matched measurements for each individual can be identified as well.

5. For studies with multiple observations per participant, we will attempt to use the following strategies to conduct their analyses:
   a. obtain IPD and perform an analysis (such as time-to-event analysis) that uses the whole follow-up duration for each participant;
   b. compute an effect measure for each individual participant which incorporates all time points, such as total number of events, an overall mean, or a trend over time; or
   c. select a single time point and analyze only data at that point for studies with such data.

Dealing with missing data

We made multiple attempts to obtain any missing, unclear, or incomplete data for any of the included studies through contacting the corresponding and lead authors of these studies. However, for the studies for which we were unable to obtain the required or clear information, we have simply reported the readily available data, while including a statement to clarify which data assessed by that study could not be obtained.

Assessment of heterogeneity

We assessed the methodological variability (i.e. statistical heterogeneity) between the included studies by visually assessing the degree of overlap between the CIs of the different studies included in the forest plot, as well as by calculating a formal I^2 statistic. The latter test estimates the percentage of total variation observed across the included studies as a result of methodological variability, rather than sampling (i.e. random) errors (Higgins 2003). We also evaluated the potential for important clinical and design differences among the included studies, as part of our overall assessment of the 'similarity' of such studies and subsequently, our ability to meaningfully combine them in a pooled analysis.

We graded the degree of heterogeneity using the generated I^2 statistic, into 'no heterogeneity' when I^2 was 0% to 24%, 'low-degree heterogeneity' when I^2 was 25% to 49%, 'moderate-degree heterogeneity' when I^2 was 50% to 74%, and 'high-degree heterogeneity' when I^2 was greater than or equal to 75% (Higgins 2003).

For studies where effect estimates were not directly reported, we analyzed their corresponding extracted data using an ITT analysis.

Due to the limited number of eligible studies identified for our review, we were unable to conduct a subgroup analysis for outcomes with a moderate or high degree of statistical heterogeneity. However, in future updates of this review, if more studies become available and they yield such levels of heterogeneity, we will attempt to identify the source(s) of such heterogeneity by conducting relevant subgroup analyses to identify possible sources of bias or methodological differences between these studies.

Assessment of reporting biases

Since we only found a single relevant RCT for inclusion in our review, we were unable to examine funnel plots to identify any potential reporting biases, such as publication bias.

In future updates of this review, however, we will examine funnel plots to identify any reporting biases, such as publication, language, time-lag, or location biases, if we identify at least five relevant trials published in the literature. The reason for selecting five studies instead of the conventional 10 as a 'cut-off' for when to analyze publication or reporting bias is the rarity of the condition per se and paucity of centers involved in studying it. In such cases, the funnel plot would show asymmetry if reporting biases exist, for which we shall probe any discernible underlying causes. A number of causes can lead to an asymmetrical funnel plot, such as the inherently different methodological qualities of small studies compared to larger ones (small-study effects), the presence of true heterogeneity, remarkable time-lapse between the conduction of small trials and larger ones (i.e. time-lag bias), language bias, or simply chance (Sterne 2022).

Data synthesis

We conducted the statistical analysis of our extracted data using RevMan 5.4 (Review Manager 2020).

Since we only had one relevant RCT included in this review, we could not perform a meta-analysis for randomized studies, as outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Deeks 2021).

In future updates of this review, if we identify more eligible RCTs for inclusion and if a meta-analysis of these RCTs is possible, we will follow guidance in the *Cochrane Handbook for Systematic Reviews of Interventions* for conducting meta-analyses (Deeks 2021). We will also be using RevMan version 5.4 to conduct the statistical analyses of our extracted data (Review Manager 2020); and will use a random-effects model, due to the likelihood of the studies addressing our question being heterogeneous owing to the condition's rarity, and consequently, the anticipated paucity of relevant studies and their reporting of variable effects (such as differences in response to treatment) among their respectively different enrolled participants (due to age, sex, and disease severity) (Riley 2011).

We do not plan to perform any meta-analyses for studies with an insufficient similarity of populations (e.g. a study of adult participants versus a study with children only), interventions (e.g. a study of combined statin and cholesterol therapy compared to cholesterol monotherapy versus a study of statin monotherapy compared to cholesterol monotherapy), or methods, or those studies where we cannot explain substantial heterogeneity either through subgroup analyses or by individually assessing the methodologies employed by those studies. In such cases, we will report the data narratively.

In contrast, as we identified more than one eligible NRSI or cohort study and deemed these studies to be sufficiently similar, we were able to perform a meta-analysis for some of the outcomes of interest of our review. However, prior to this, we assessed whether these studies were sufficiently homogeneous to allow for the pooling and combination of their data (Taggart 2001). We analyzed the data extracted from these NRSIs and cohort

studies separately from the included RCT (Reeves 2011), and used a random-effects model to account for the heterogeneity and methodological variation across these studies (Riley 2011).

In future updates of this review, however, if included NRSIs are not deemed to be sufficiently homogeneous to combine in a meta-analysis, we will display their results in a forest plot in which we will suppress the pooled estimate, or include them in additional tables with a systematic format.

Subgroup analysis and investigation of heterogeneity

In this review, we were unable to perform any of the previously specified subgroup analyses in our protocol (Ballout 2020), because the data available for each of them were insufficient. However, in future updates of the review, when there are sufficient data in the included studies, we will attempt to address any heterogeneity that emerges for a specific outcome, among any pooled group of included studies (RCTs and NRSIs to be analyzed separately), by conducting the following subgroup analyses:

1. the effects of disease severity on survival (e.g. by comparing survival rates in those with 'mild' versus 'classical' versus 'severe' SLOS)*;
2. the effects of low (reduction in LDL-C by < 30%) versus moderate (reduction in LDL-C by < 50% and > 30%) versus high (reduction in LDL-C by > 50%; primarily atorvastatin or rosuvastatin) intensity statin therapy (ACC/AHA 2014; Stone 2014);
3. the effects of age (pediatric (1 to 18 years of age) versus adults (over 18 years of age)) and sex (males versus females);
4. the effects of the chemical nature of the statin used, i.e. lipophilic (simvastatin, lovastatin, pitavastatin, or atorvastatin) versus hydrophilic (pravastatin or rosuvastatin) statins (Schachter 2004);
5. the effects of different formulations, dosages, and durations of cholesterol or bile acid supplementation alone, or in combination with statins;
6. the effects of markedly diminished residual *DHCR7* enzymatic activity (i.e. < 5%; usually in the severe forms of the syndrome) versus moderately reduced residual enzymatic activity (i.e. ≥ 5% the activity of normal; usually in the mild and moderate forms of the syndrome).

*defined based on the severity of their physical manifestations into 'mild' (a score of less than 20), 'moderate' or 'classic' (a score of 20 to 50), or 'severe' (a score of more than 50) (Bialer 1987; Kelley 2000).

Sensitivity analysis

We had planned in our protocol to conduct a sensitivity analysis to test for the robustness of the generated data, when at least five studies are included in the review, by excluding studies with an overall high risk of bias to see how that affects the overall pooled effect estimates for each outcome of interest (Ballout 2020).

However, because we deemed all included NRSIs to be at a serious or critical overall risk of bias (as assessed by ROBINS-I), we were unable to conduct a sensitivity analysis for any of our outcomes of interest at this time.

Summary of findings and assessment of the certainty of the evidence

In our protocol, we planned for two review authors to assess the overall certainty of the evidence for the six outcomes listed below independently and in duplicate, using the GRADE approach outlined in the *GRADE Handbook* (Schünemann 2013).

1. Overall survival at 6, 12, 24, and 36 months
2. Changes (improvement or exacerbations) in any of the neurobehavioral manifestations during the study period
3. Statin-related adverse reactions (hepatotoxicity, myalgias or myopathy, and any others)
4. Negative changes in the growth parameters of children receiving a statin (e.g. falling off their baseline growth curve for height or weight)
5. Changes in the biochemical markers of the disorder during treatment
6. QoL

For RCTs

We started with the certainty of evidence being 'high certainty', and then downgraded it by one or two levels for serious and very serious limitations respectively, based on: the individual quality of each study (i.e. risk of bias), the degree of consistency across studies, the extent of directness of the reported evidence, the precision of estimates, and the presence of publication bias.

For NRSIs

Likewise, because we evaluated the quality of evidence in all NRSIs included in this review using ROBINS-I, we started with their certainty of evidence being set as 'high certainty', and then downgraded it by one or two levels, with appropriate justifications, in the presence of serious methodological concerns and limitations such as indirectness of evidence, heterogeneity, imprecision, or publication bias (Schünemann 2019).

However, due to the paucity of included studies, and absence of studies addressing some of these outcomes (e.g. QoL or survival), we were only able to assess the overall certainty of evidence for two outcomes that were sufficiently addressed by the NRSIs included in our review. These included the improvements in the biochemical markers of the disorder and statin-related ADRs.

We then used the Guideline Development Tool to create a summary of findings table that reports an overall certainty of the evidence available for each outcome of interest (GRADEpro 2011). We used footnotes to justify our decisions to downgrade or upgrade the certainty of evidence for each outcome, and included additional comments in the table, where necessary.

The GRADE approach assigns the certainty of a body of evidence to one of four grades, defined below.

1. High: we are very confident that the true effect lies close to the generated effect estimate.
2. Moderate: we are moderately confident in the effect estimate such that the true effect is likely to be close to the generated effect estimate but may be substantially different.

3. Low: we have limited confidence in the effect estimate such that the true effect may be substantially different from the generated effect estimate.
4. Very low: we have very little confidence in the effect estimate such that the true effect is likely to be substantially different from the generated effect estimate.

Note that, contrary to our initial intent to create separate summary of findings tables for RCTs and NRSIs, we deemed it acceptable and more reasonable to present all study types together in a single summary of findings table, given that there was only one RCT included in this review at this time, and to summarize all currently available data by outcome rather than study design, making it easier for readers to see the findings of this review. Should we include further RCTs in future updates of this review and if we deem that keeping both study types in the same summary of findings table makes the interpretation of the review data too complicated, we may consider generating separate summary of findings tables for RCTs and NRSIs as originally planned in our protocol (Ballout 2020).

RESULTS

Description of studies

We present all information about the included studies in the characteristics tables and an additional table (Characteristics of included studies; Table 1); we present information about excluded studies in a separate table (Characteristics of excluded studies).

Results of the search

Using the search strategy outlined in our protocol, we initially identified 2664 records from the electronic database search and an additional four records from searching other sources. After removing duplicate references, we were left with a total of 1494 records to screen, of which we excluded 1461 during the title and abstract screening stage. This left us with 33 records for full-text screening (Figure 1). Of these 33 references, we eventually found only six studies (eight references) to be eligible for inclusion in our review (Chan 2009; Haas 2007; Haas 2008; Oláh 2013a; Roullet 2012; Wassif 2017). We excluded the remaining 24 studies (25 references) for various reasons, summarized below (Excluded studies).

Figure 1. PRISMA Flow Diagram

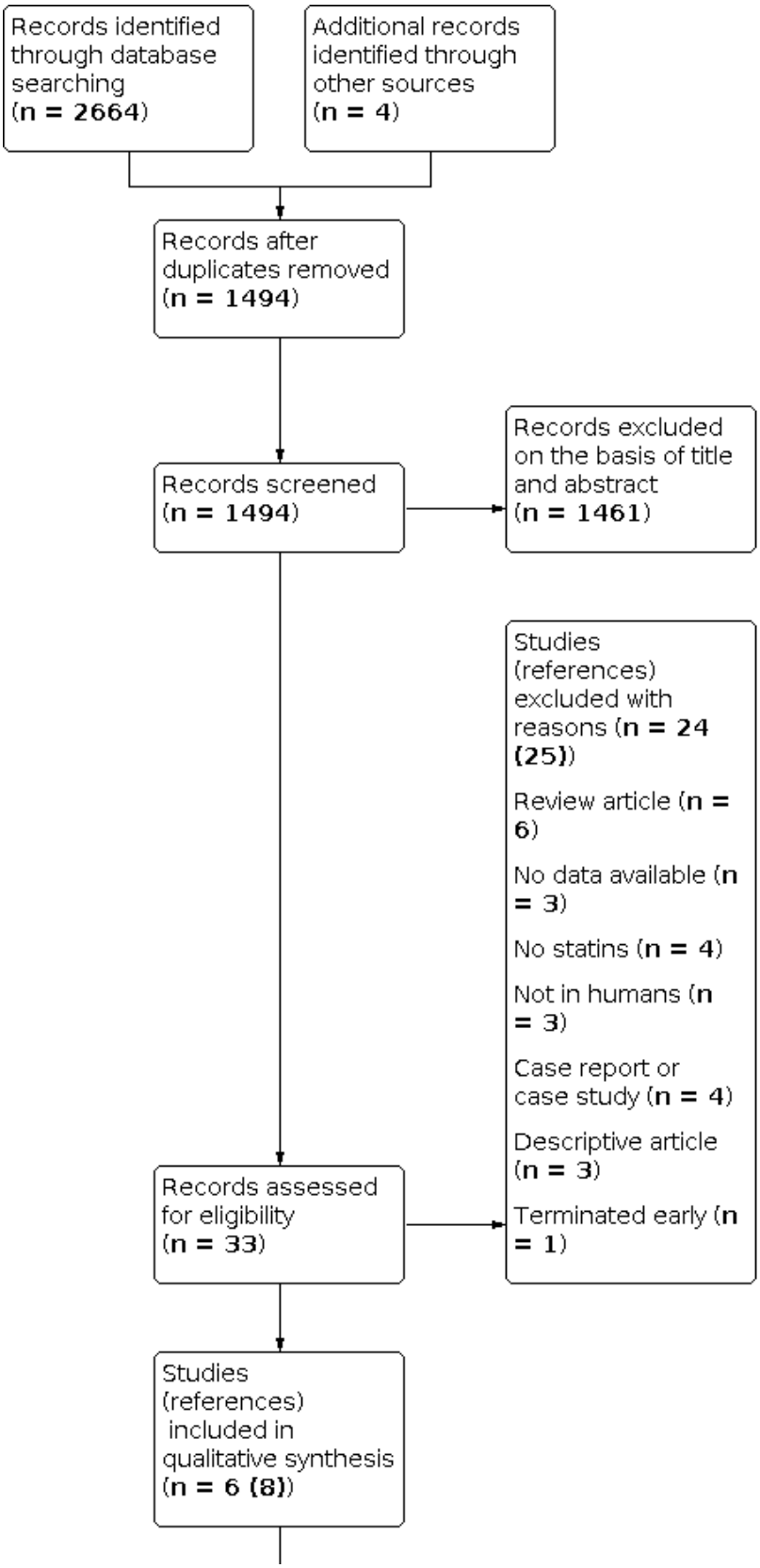
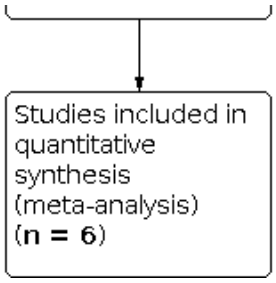


Figure 1. (Continued)



Included studies

Study design and sample size

Only one of the six studies included in our review was an RCT, with a cross-over design (N = 18) (Wassif 2017). The remaining five studies were NRSIs (N = 42); of these, three were prospective cohort studies (N = 20) (Chan 2009; Haas 2008; Roullet 2012), and two were retrospective cohort studies (N = 22) (Haas 2007; Oláh 2013a).

Setting

Three of the included studies were conducted in the USA (Chan 2009; Roullet 2012; Wassif 2017), two in Germany (Haas 2007; Haas 2008), and one in Hungary (Oláh 2013a).

Participants

Only one of the included studies in our review had a mixed participant population, i.e. included adults and children together, with no separate reporting of their data (Haas 2007). All participants enrolled in the five other studies were children (i.e. 18 years of age or younger).

Only two of the included studies limited their enrollment to individuals with mild to moderate SLOS severity (Roullet 2012; Wassif 2017). The remaining four studies included individuals with SLOS of any severity (Chan 2009; Haas 2007; Haas 2008; Oláh 2013a).

Moreover, two included studies enrolled participants with a biochemical diagnosis of SLOS only, i.e. elevated plasma 7DHC levels or elevated plasma 7DHC/total sterol ratio (Oláh 2013a; Wassif 2017). The remaining four studies enrolled participants with either a biochemical or a genetic diagnosis of SLOS, or a biochemical diagnosis of SLOS that was subsequently confirmed with genetic testing (Chan 2009; Haas 2007; Haas 2008; Roullet 2012).

Overall, there were more male participants than female participants within the included studies (32 males versus 20 females in total), although one study did not mention its enrolled number of males or females (Haas 2008).

See the additional table for more details (Table 1).

Interventions

All included studies designated statin therapy in addition to cholesterol supplementation as their intervention arm, with cholesterol supplementation alone serving as the corresponding control/comparator arm (Chan 2009; Haas 2007; Haas 2008; Oláh 2013a; Roullet 2012; Wassif 2017).

With the exception of one study, which used simvastatin or atorvastatin (Oláh 2013a), all other studies used simvastatin as their particular choice of statin (Chan 2009; Haas 2007; Haas 2008; Roullet 2012; Wassif 2017).

The dosages, durations and formulations of statin therapy and cholesterol supplementation varied considerably across the included studies.

See the additional table for more details (Table 1).

Outcomes

An additional table shows a tabular representation of the outcomes assessed by each study included in this review (Table 2).

Only the included RCT had planned to assess changes in the neurobehavioral manifestations of participants with SLOS following add-on statin therapy (Wassif 2017). Specifically, it evaluated changes in irritability after receiving statin therapy for 12 months using the irritability subscale of the Aberrant Behavior Checklist in children (ABC-C).

Despite not clearly stating their intention to assess neurobehavioral outcomes in their participants, the authors of a further study reported in their discussion that they found contradictory behavioral outcomes in two participants following treatment with simvastatin and cholesterol supplementation (Haas 2007). Specifically, they noted worsening in self-injurious behaviors in one of their participants, with a paradoxical reduction in self-injurious behaviors in another participant after statin introduction (Haas 2007).

Four studies included in this review assessed statin-related adverse reactions (Chan 2009; Haas 2007; Oláh 2013a; Wassif 2017).

All included studies, except for one (Oláh 2013a), evaluated changes in plasma biomarker levels of SLOS (i.e. total cholesterol, 7DHC, 8DHC, etc.).

Only one of the included studies reported actual data on changes in the anthropometric parameters of participants following statin therapy (Haas 2007). In contrast, the included RCT only assessed this outcome anecdotally and stated finding "no significant changes in the anthropometric parameters" of their participants before and after receiving statin therapy, without including the corresponding numerical data of the outcome (Wassif 2017). To overcome this, we have made multiple reasonable attempts to contact the study authors in order to obtain these data, but to date we have not received a response.

None of the included studies assessed survival or QoL in individuals with SLOS receiving statin therapy (Table 2).

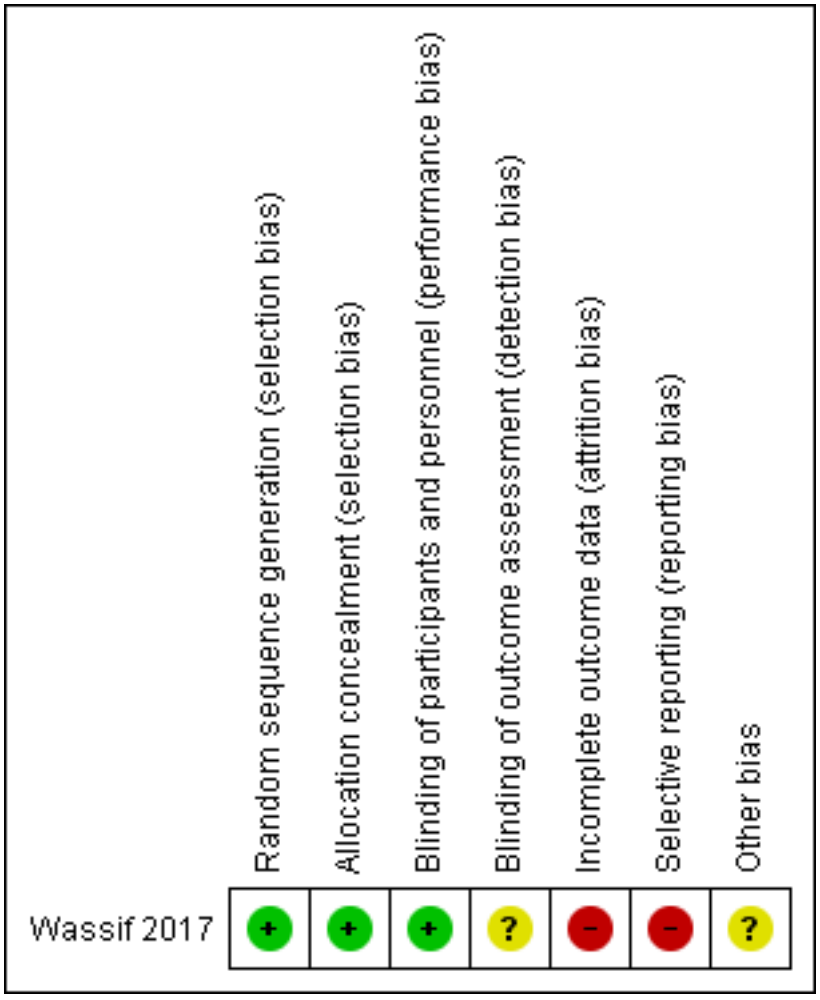
Excluded studies

After title and abstract screening, we excluded 24 studies (25 references) for various reasons. One study would have been eligible for inclusion in the review, but was terminated early (NCT01434745), and a further three studies had no data available as confirmed by the investigators (Palm 2019; Pappu 2011; Tavori 2015). We excluded seven references to six studies as they were narrative review articles (i.e. not original studies) (Aneja 2008; Correa-Cerro 2005; Irons 2004; Jira 1997b; Kelley 2000; Tint 1997); three studies were descriptive studies only (i.e. no intervention) (Haas 2005; Oláh 2018; Scalco 2006); and four were case studies (Jira 1997a; Jira 2000; Jira 2005; Starck 2002b). We excluded four studies as statins were not the intervention (Kilic 2011; Linck 1999; Sikora 2004; Ullrich 1996); and three studies which were not performed in humans (Prabhu 2016; Suzuki 2020; Wright 2002).

Risk of bias in included studies

We conducted a risk of bias (RoB) assessment for the RCT included in our review using the Cochrane RoB tool for RCTs (Wassif 2017). We present details in an additional table (Table 3), summarizing the overall RoB assessment (Figure 2).

Figure 2. Risk of bias summary for the single included RCT



We also conducted a separate RoB for each of the included NRSIs using the ROBINS-I tool for two key outcomes of this review: statin-related adverse reactions and changes in the levels of various plasma or CSF biomarkers (or both), including our detailed assessments for each domain within each study, per respective outcome in Table 4 and Table 5, respectively. The corresponding overall RoB summaries are presented in Figure 3 and Figure 4, respectively.

Figure 3. ROBINS-I for outcome 1: Statin-related ADRs

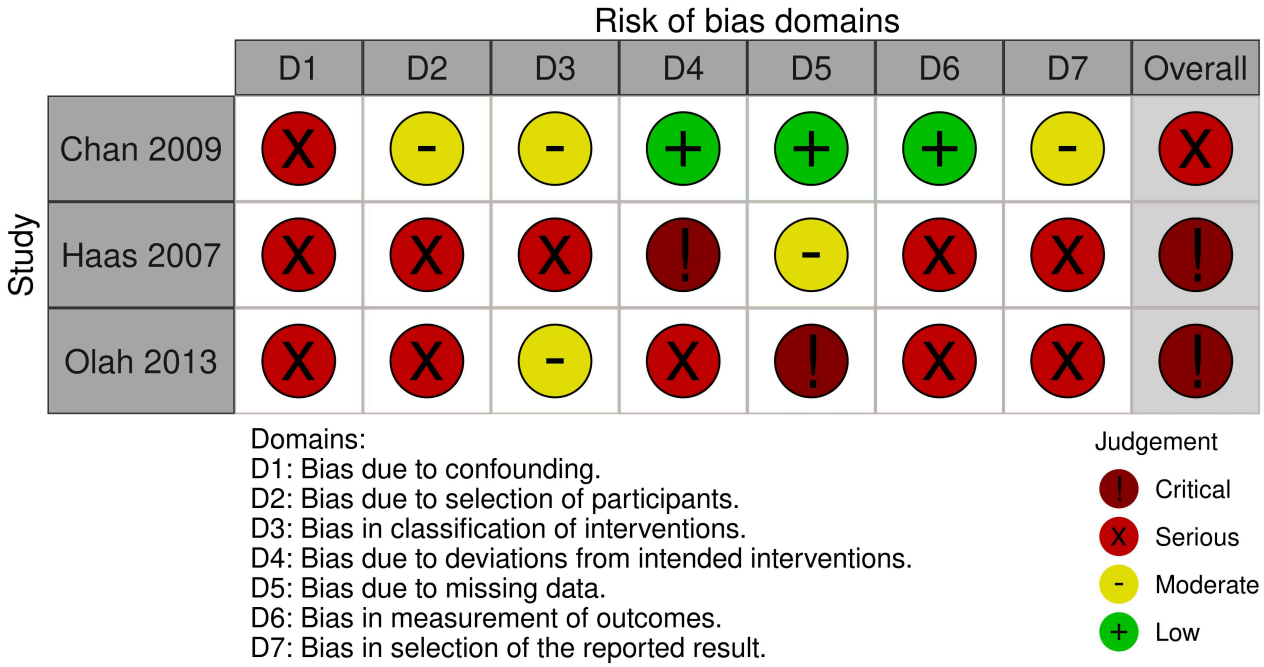
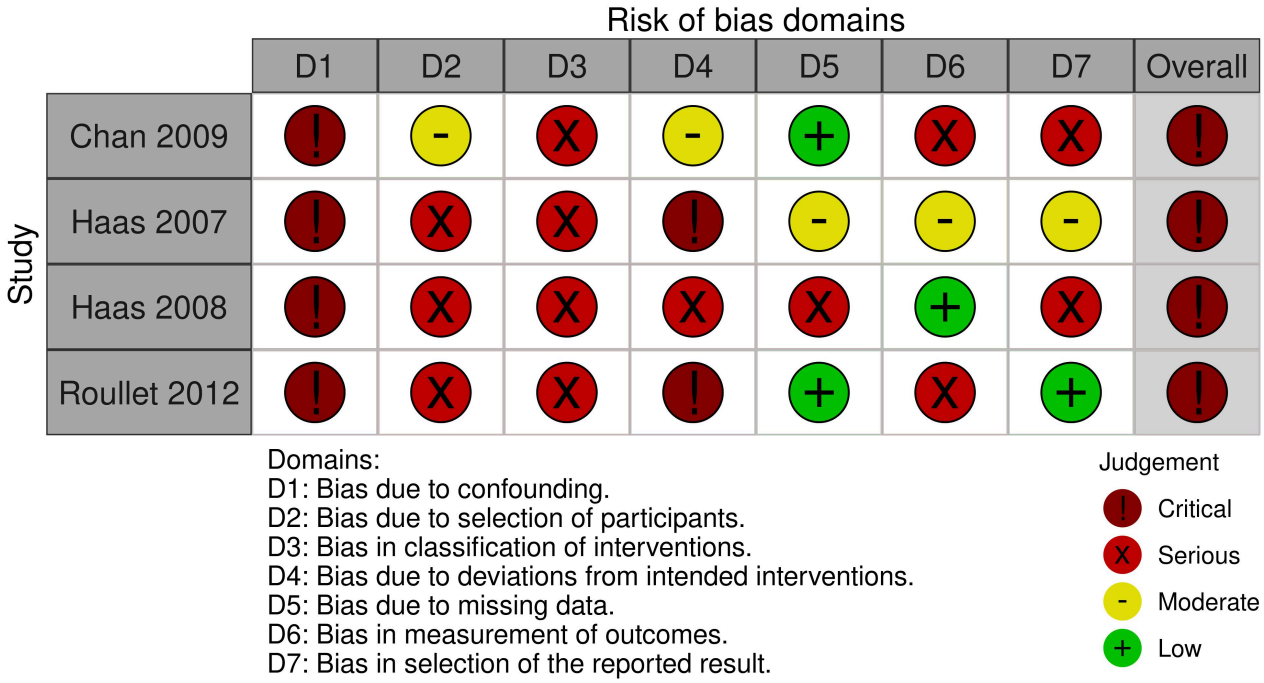


Figure 4. ROBINS-I for outcome 2: Changes in plasma and/or CSF biomarker levels



Below, we provide a narrative summary of the domains used for RoB assessment of each of the included studies, starting first with the included RCT alone (Wassif 2017), followed by a section on the RoB assessment of the included NRSIs studies (Chan 2009; Haas 2007; Haas 2008; Oláh 2013a; Roullet 2012).

For RCTs

Owing to its randomized nature, the single trial included in this review had a low risk of bias in three of the seven domains assessed, yet a high risk of bias in two key domains as discussed below (Wassif 2017).

1. Sequence generation

Wassif 2017 reported adequate sequence generation using blocked randomization for enrolled participants, which was performed by the pharmacy (low risk of bias).

2. Allocation concealment

Wassif 2017 also employed proper allocation concealment by having the pharmacy development services, and not the investigators themselves, perform the allocation via randomization in blocks of four (low risk of bias).

3. Blinding of participants and personnel (performance bias)

Wassif 2017 also had a low risk of bias with regard to blinding of participants and personnel by employing a placebo that was "indistinguishable" from the active product (i.e. statin) in appearance and taste, ensuring adequate blinding of participants and personnel (low risk of bias).

4. Blinding of outcome assessment (detection bias)

The risk of detection bias in the Wassif 2017 RCT was not clear, because the authors did not specify whether outcome assessors were blinded to the allocation or the intervention received by each participant. It is likely that there is a risk of bias for this domain since the trial was described as "double-blinded" and not "triple-blinded", which suggests that data analysts may not have been blinded with regard to the allocation of each participant (unclear risk of bias).

5. Incomplete outcome data (attrition bias)

Wassif 2017 excluded four out of 22 enrolled participants from the analysis. Three participants were excluded due to noncompliance (i.e. deviation from intended therapy), two of whom were receiving the intervention (simvastatin); and one was excluded after experiencing an ADR. Such an approach deviates from an ITT analysis and implies the use of a per-protocol analysis with a failure to impute missing data. Moreover, the RCT authors reported that three participants experienced various manifestations consistent with statin-related ADRs (e.g. abdominal pain, photosensitivity) during the open-label extension of the RCT, without providing further details on the eventual outcomes of these participants. We were not able to obtain this information, despite having contacted the investigators multiple times (high risk of attrition bias).

6. Selective reporting (reporting bias)

We also deemed the RCT to have a high risk of bias with regard to selective outcome reporting, because the investigators have clearly deviated from their registered protocol by collecting and reporting data on changes in the anthropometric measures of their enrolled participants, an outcome not prespecified in their published protocol. Additionally, they did not report several of the other outcomes they had prespecified in their protocol (e.g. activity levels as measured by an activity monitor, corticotropin-releasing hormone (CRH) stimulation test result, vitamin D levels, etc.) (NCT00064792). Additionally, while the authors had mentioned in their protocol that they would collect data on the effects of statin therapy on “behavioral problems” in participants, it is not clear why they specifically measured and only reported changes in irritability, while failing to report the data on other behavioral manifestations that can be seen in SLOS, such as hyperactivity and auto-aggression, which are covered by the tool they used for such measurements (i.e. ABC-C). This poses a high risk of bias with regard to selective outcome reporting because it gives the impression that the authors may have selected to report only favorable outcomes, i.e. those that seem to have attained significance only (high risk of bias).

7. Other potential sources of bias

Since the RCT has a cross-over design, it is inherently at risk for a carry-over effect of treatment (Wassif 2017). The investigators implemented an adequate two-month washout period before switching arms, which fits with the prespecified minimum washout period of four weeks outlined in our protocol (Ballout 2020); as such, we judged there to be a low risk of bias from carry-over effects.

The questionnaire (ABC-C) was completed by the caregivers and parents of the recruited participants, and it is unclear whether they all received adequate training for standardization purposes to complete the checklist, leading to an unclear risk of bias.

For NRSIs

All included NRSIs had an overall serious or critical risk of bias for the two outcomes assessed (Table 4; Table 5). We assessed the specific risk of bias assigned to each domain per outcome for each of the included studies, and discuss these risks below.

A. Outcome 1: statin-related ADRs

Only three of the NRSIs included in our review reported data on statin-related ADRs (Chan 2009; Haas 2007; Oláh 2013a). We used ROBINS-I tool to assess the risk of bias in each of these three studies evaluating this outcome (Figure 3; Table 4).

1. Bias due to confounding

All three NRSIs had a serious risk of bias for this domain (Chan 2009; Haas 2007; Oláh 2013a). The main reasons for confounding in these studies were either the inclusion of participants of different disease severities (Chan 2009; Oláh 2013a), or the inclusion of participants already receiving pharmacological interventions (e.g. cholesterol supplementation with or without statin therapy) or dietary interventions, or a combination of both, with direct effects on this outcome prior to starting the study (Haas 2007).

2. Bias in the selection of participants into the study

Two of the NRSIs had a serious risk of bias for this domain (Haas 2007; Oláh 2013a), while the third NRSI had a moderate risk of bias (Chan 2009). In the two studies with a serious risk of bias, participants were already receiving cholesterol supplementation or statin therapy, or a combination of both, prior to study initiation, raising concern for prevalent user bias (Haas 2007; Oláh 2013a). In

contrast, it was unclear in the Chan study whether participants had been followed up for a long period of time prior to study initiation or were recently diagnosed cases (Chan 2009).

3. Bias in classification of interventions

Two of the NRSIs assessing this outcome had a moderate risk of bias with regard to their classification of intervention (Chan 2009; Oláh 2013a). In Oláh 2013a, this was due to the retrospective nature of the study in which participants were receiving cholesterol supplementation for different durations prior to inclusion in the study, while in Chan 2009, participants received statins whose individual dosages were continually adjusted throughout the study. In contrast, the remaining study had a serious risk of bias for this category due to its retrospective nature and selective administration of the intervention to individuals with mild disease severity only, which constitutes a deviation from usual practice (Haas 2007).

4. Bias due to deviations from intended interventions

The Oláh 2013a study had a serious risk of bias resulting from deviations from the intended intervention due to different durations of statin therapy or cholesterol supplementation (or both) among participants, as well as inconsistent dosing for the interventions across participants, posing a serious risk for unbalanced interventions.

The Haas 2007 study had a critical risk of bias for this domain due to administering different dosages and formulations of cholesterol therapy to participants, as well as employing different follow-up times and multiple interruptions in intervention among participants.

Conversely, we deemed Chan 2009 to be at low risk of bias for this domain due to having seemingly balanced and consistent interventions.

5. Bias due to missing data

The Chan 2009 study had a low risk of bias due to missing data, while the Haas 2007 study had a moderate risk of bias for this domain due to a combination of noncompliance in two of their participants, as well as lack of data on the period preceding study initiation where participants were also receiving intervention.

In contrast, we regarded the Oláh 2013a study to have a critical risk of bias arising from the considerable loss of follow-up and missing data from participants.

6. Bias in measurement of outcomes

Of the three studies addressing this outcome, only Chan 2009 had a low risk of bias in terms of outcome measurement; largely resulting from the quantitative and objective nature of this outcome, where the study authors measured plasma levels of liver injury biomarkers (AST or ALT) and CK. It is unlikely that prior knowledge of intervention assignment would have had a significant impact on actual outcome measures (i.e. negligible assessor judgment bias). Conversely, each of the two other studies had a serious risk of bias with regard to their measurement of relevant outcomes, due to their retrospective nature which precludes the ability of the study authors to ensure consistent and standardized assessments of this outcome among their participants (Haas 2007; Oláh 2013a).

7. Bias in the selection of the reported result

One of the NRSIs had a moderate risk of bias; the reason for this was that the authors did not report the numerical values for the changes detected in plasma 7DHC levels following statin therapy, despite stating that they had found "significant changes" (Chan 2009).

In contrast, two of the three NRSIs assessing this outcome had a serious risk of bias for selective outcome reporting (Haas 2007; Oláh 2013a). Both were retrospective studies, which meant that they did not have a prespecified list of outcomes that they had planned or intended to assess in their respective participants.

B. Outcome 2: changes in plasma biomarker levels

Four NRSIs included in our review reported data on the changes in levels of various plasma biomarkers of SLOS (Chan 2009; Haas 2007; Haas 2008; Roullet 2012). Therefore, we used the ROBINS-I tool to assess the risk of bias in each of these four studies for this outcome (Figure 4 ; Table 5).

1. Bias due to confounding.

All four NRSIs assessing this outcome had a critical risk of bias for potential confounding (Chan 2009; Haas 2007; Haas 2008; Roullet 2012). The main reasons for confounding in these studies were the inclusion of participants of different disease severities together within a group, and inclusion of participants already maintained on dietary or pharmacological interventions (or a combination of both) prior to inclusion in the study (Chan 2009; Haas 2007; Haas 2008; Roullet 2012).

2. Bias in the selection of participants into the study

Three of the NRSIs had a serious risk of bias in their participant selection, mainly because the start of intervention and follow-up did not coincide for most participants and this was not adjusted for in the analysis (Haas 2007; Haas 2008; Oláh 2013a). In contrast, the Chan 2009 study had a moderate risk of bias for this domain because it was unclear whether participants had already been receiving any treatment prior to inclusion in the study as well as employing variable duration of participant follow-up.

3. Bias in classification of interventions

All four NRSIs had serious risk of bias with regard to their classification of intervention (Chan 2009; Haas 2007; Haas 2008; Roullet 2012). This was due to the use of different dosages, formulations and durations of treatment, posing a serious risk for unbalanced interventions.

4. Bias due to deviations from intended interventions

Two studies had a critical risk of bias in terms of deviating from their intended interventions, resulting from inconsistent dosages and durations of cholesterol supplementation prior to initiating add-on statin therapy, as well as interruptions in treatments for variable durations (Haas 2007); or noncompliance with assigned interventions (Roullet 2012).

We deemed the Haas 2008 study to have a serious risk of bias for this domain because the authors of the study limited the administration of statin therapy only to participants with mild SLOS, without implementing the same criterion for participants receiving cholesterol monotherapy.

Conversely, the Chan 2009 study had a moderate risk of bias for this domain, arising from inconsistencies in dosing of supplemental cholesterol and statin therapy in participants, with these dosages fluctuating even within the same participant throughout the study.

5. Bias due to missing data

Two studies had low risk of bias for missing or incomplete data because they seemed to have reasonably reported all intended data for their participants (Chan 2009; Roullet 2012).

In contrast, Haas 2007 had a moderate risk of bias for this domain, resulting from noncompliance in two participants as well as a lack of data on the period preceding study initiation during which the participants were receiving cholesterol or statin therapy, or a combination of both.

Finally, Haas 2008 had a serious risk of bias for this domain due to having missing data for 5/14 participants.

6. Bias in measurement of outcomes

Haas 2008 had a low risk of bias in terms of outcome measurement due to the quantitative and objective nature of the outcome assessed, which consists of objectively measured biochemical parameters (e.g. plasma CoQ10 and cholesterol levels). For this we expect negligible assessor judgment bias, since prior knowledge of intervention assignment would have little if any, influence on such measurements.

Haas 2007 had a moderate risk of bias for this domain caused largely by its retrospective nature, which precludes the blinding of study investigators or data analysts (or both), although the impact of such knowledge remains limited on this objectively assessed outcome.

The two remaining studies had a serious risk of bias in their measurement of relevant outcomes, mainly due to their inconsistent dosages and durations of treatments administered to their participants throughout the study (Chan 2009; Roullet 2012).

7. Bias in the selection of the reported result

Two of the included NRSIs had a serious risk of bias for selective outcome reporting due to not reporting data for several outcomes they assessed following statin therapy (Chan 2009; Haas 2008); the Chan 2009 study did not report 7DHC levels and the Haas 2008 study did not report vitamin E levels.

The Haas 2007 study had a moderate risk of bias for this domain due to its retrospective nature.

We assigned the Roullet 2012 study a low risk of bias for this domain due to their reporting of all intended data on their nine participants, with most of the reported data being negative findings.

Effects of interventions

See: **Summary of findings 1** Statins with cholesterol supplementation versus cholesterol supplementation only

Statins with cholesterol supplementation versus cholesterol supplementation only

Primary outcomes

1. Survival

None of the included studies assessed overall survival, SLOS-related deaths, or SLOS-unrelated deaths. Consequently, there is no evidence to date on whether add-on statin therapy compared to cholesterol monotherapy affects survival in individuals with SLOS (Table 6).

2. Reductions in the severity or frequency (or both) of neurobehavioral manifestations

Only the included RCT assessed the effects of add-on statin therapy compared to cholesterol monotherapy on the neurobehavioral abnormalities of individuals with SLOS (N = 14 out of 22 randomized participants) (Wassif 2017). The trial reported a positive effect with a reduction in severity of irritability measured by the irritability subscale of the ABC-C in children with SLOS receiving simvastatin and cholesterol supplementation compared to those receiving cholesterol supplementation only (P = 0.017) (very low-certainty evidence) (Wassif 2017). Despite this being the first study to assess changes in a neurobehavioral manifestation of SLOS in association with statin therapy, in their published protocol the study authors did not justify their intent to evaluate changes in irritability only, without including the other subscales of ABC-C (i.e. hyperactivity, stereotypy, lethargy, and inappropriate speech). In fact, while the authors had planned to assess changes in hyperactivity, a key neurobehavioral outcome in SLOS, they did not report any data on this outcome (NCT00064792). Additionally, one of their participants had experienced a worsening in self-injurious behaviors during the open-label extension part of the trial, with no clear statement on whether or not the data of that participant was included in the analysis or not (Wassif 2017).

None of the included NRSIs formally assessed changes in any of the neurobehavioral manifestations of individuals with SLOS. However, the authors in one study mentioned in the discussion section that they retrospectively noted a reduction in the severity of self-injurious behaviors in one of their participants following statin treatment, and a paradoxical worsening of such behaviors in another participant following statin therapy in their study (Haas 2007).

3. Statin-related adverse reactions

Four studies reported data on statin-related ADRs, one of which was the RCT (Wassif 2017), while the remaining three were NRSIs (Chan 2009; Haas 2007; Oláh 2013a).

The included RCT regarded any adverse reaction(s) experienced by any participant during the study as a statin-related ADR (Wassif 2017). In that RCT, the authors reported muscle pain and corresponding elevations in creatine phosphokinase (CPK) levels in one participant, who was paradoxically receiving placebo (Wassif 2017). However, upon careful examination of Supplementary Table 2 of the study, we found that one of the trial participants in the intervention arm had experienced sleep disturbances (Wassif 2017). While not being considered a statin-related ADR by the trial authors, it meets the prespecified definition of a statin-related ADR defined in our protocol (Ballout 2020). Therefore, in future updates of this review, if new RCTs become available and are eligible for

inclusion and pooling of their data with the currently eligible trial (Wassif 2017), we will count the recorded sleep disturbance in that specific participant as a statin-related ADR.

In contrast, all three NRSIs assessing statin-related ADRs only considered hepatotoxicity or myotoxicity (or both) as statin-related ADRs (Chan 2009; Haas 2007; Oláh 2013a). However, after careful consideration and thorough discussion with the group's editorial board, we decided not to combine the data extracted from the prospective study (Chan 2009) with that of the two other retrospective NRSIs (Haas 2007; Oláh 2013a).

While the prospective study reported no ADRs in association with statin use (effect not estimable) (low-certainty evidence) (Chan 2009), each of the two retrospective cohort studies assessing this outcome independently reported six seemingly statin-related ADRs (Haas 2007; Oláh 2013a). Upon pooling their data, we found that individuals with SLOS receiving statin therapy potentially have a higher risk of experiencing ADRs compared to those receiving cholesterol supplementation only (RR 13.00, 95% CI 1.85 to 91.48; P = 0.01; I^2 = 0%; low-certainty evidence; Analysis 1.1).

Secondary outcomes

1. Changes in growth parameters

Only the included RCT and one retrospective NRSI assessed changes in the growth parameters of children with SLOS receiving statin therapy compared to those receiving cholesterol only (Haas 2007; Wassif 2017).

The authors of the included RCT (22 participants) reported finding no significant changes in the weight (N = 18; P = 0.76) or height (N = 16; P = 0.42) of children with SLOS receiving simvastatin compared to placebo (very low-certainty evidence) (Wassif 2017). However, the authors did not prespecify in their protocol the intention of assessing this outcome, raising suspicion of selective outcome reporting and protocol deviation (NCT00064792). Moreover, the authors reported measuring changes in growth parameters only during the second phase of the RCT, i.e. after participants were crossed-over to the opposite arm, with the weight and height of each participant at the end of the first phase serving as their respective comparator (i.e. control) (Wassif 2017). This also poses a high risk of bias in outcome measurement, since the recruited participants had already been receiving simvastatin therapy for 12 months prior to the start of data collection for this outcome. Specifically, we are unable to rule out a profound risk for a carry-over effect of treatment from the first phase of the RCT onto the subsequent one. To overcome this limitation, we made multiple reasonable attempts to contact the investigators for further clarification regarding whether baseline growth parameters were available for use as comparators, and if not, if they had performed any special censoring for the available data when handling its analysis. However, we have not received a response to date.

One of the included NRSIs reported changes for three out of 20 participants monitored for weight (Haas 2007). The authors reported finding a significant increase in the weight SD score of one participant, yet a significant decrease in the weight SD scores for two other participants, after receiving combined therapy (very low-certainty evidence) (Haas 2007). The authors also reported a significant decrease in the linear growth SD score (i.e. a slowing in rate of gain in height) in two out of 14 participants monitored for height after receiving combination therapy (very low-certainty evidence) (Haas 2007). However, the study found no significant changes in head circumference (Haas 2007). The study investigators only present changes in growth parameters of the included participants graphically and not numerically, and we were unable to retrieve the actual values from the authors despite multiple reasonable attempts to contact them.

2. Changes in plasma or CSF biomarker levels (or both)

We present the results for this outcome in the analysis sections (Analysis 1.2; Analysis 1.3; Analysis 1.4; Analysis 1.5; Analysis 1.6; Analysis 1.7; Analysis 1.8; Analysis 1.9).

With the exception of one study (Oláh 2013a), all included studies evaluated changes in the plasma or CSF levels (or both) of various biomarkers of SLOS in participants receiving statin therapy and cholesterol supplementation compared to those receiving cholesterol supplementation only. However, the specific biochemical markers measured by each of these studies were highly variable (Table 6; Table 7). Only the included RCT measured changes in the levels of total cholesterol, dehydrocholesterols, and dehydrocholesterol-to-total sterol ratio in CSF samples of participants (Wassif 2017) (Table 2).

a. Dehydrocholesterol levels

Only the included RCT and one of the NRSI assessed changes in total plasma dehydrocholesterol levels (i.e. 7DHC plus 8DHC) in their participants following statin therapy (Haas 2007; Wassif 2017); both studies reported lower plasma levels of dehydrocholesterol in the statins group, but when analysed the RCT showed no difference between groups (MD -0.10, 95% CI -0.23 to 0.03; Analysis 1.2). A third study measured the changes in plasma levels of 7DHC and 8DHC separately (Roullet 2012; Table 2). In contrast, Chan 2009 measured only the changes in plasma 7DHC levels and not 8DHC levels, but did not report the actual means and SDs precluding any meaningful interpretations. We were unable to generate a pooled estimate of the change in total plasma dehydrocholesterol levels in participants receiving statin therapy due to the different study designs (Haas 2007; Wassif 2017), missing relevant numerical data (Chan 2009), or the reporting of individual components of this composite measure with no clear way of combining them (Roullet 2012). We contacted the authors of two studies for the missing or required data, but were informed that they no longer have access to such data (Chan 2009; Roullet 2012).

b. Total cholesterol levels

Five included studies (one RCT and four NRSIs) assessed changes in plasma levels of total cholesterol following statin therapy (Chan 2009; Haas 2007; Haas 2008; Roullet 2012; Wassif 2017). Of these, only the RCT reported finding statistically significant reductions when participants were analyzed as a group: "When the subjects were evaluated as a group, we observed a significant decrease (P < 0.005, paired t-test) in mean plasma cholesterol concentrations while taking simvastatin (105 ± 16 mg/dl) compared with placebo (120 ± 31 mg/dl)" (Wassif 2017); however, when analysed in our review, the results only showed a borderline difference, MD -0.39 (95% CI -0.81 to 0.03) (Analysis 1.3). In contrast, when combining the data reported by three of the four NRSIs which had a similar prospective design (Chan 2009; Haas 2008; Roullet 2012), we found that statin therapy in individuals with SLOS is clearly not associated with statistically significant changes in total plasma cholesterol

levels (RR - 0.02, 95% CI - 0.31 to 0.27; P = 0.89; I^2 = 0; very low-certainty evidence; Analysis 1.4).

c. Dehydrocholesterol-to-total sterol ratio

Only the RCT (Wassif 2017) and two NRSIs (Haas 2007; Roullet 2012) measured the changes in plasma dehydrocholesterol-to-total sterol ratio in individuals with SLOS following statin therapy, with none of these studies finding statistically significant differences (i.e. reductions) in this ratio among participants receiving statin therapy compared to those who did not, with low-certainty evidence for the RCT and very low-certainty evidence for the two NRSIs (Summary of findings 1).

d. CoQ10 levels

Only one NRSI measured changes in plasma CoQ10 levels in individuals with SLOS (Haas 2008), finding no statistically significant changes before and after statin treatment (Table 2).

3. Quality of life

None of the included studies assessed QoL; as a result, there is no evidence to date on whether add-on statin therapy compared to cholesterol monotherapy impacts the QoL of individuals with SLOS (Table 6).

DISCUSSION

Summary of main results

To date, there is no evidence on the effects of statin therapy on survival or QoL in individuals with SLOS. Only the included RCT assessed the effects of add-on statin therapy compared to cholesterol monotherapy on neurobehavioral manifestations, but due to the very low-certainty evidence, we are not sure if the reduction in irritability in participants receiving statins combined with cholesterol supplementation reported by the investigators of that study, compared to cholesterol supplementation alone, was due to the intervention or not.

Four of the included studies reported on statin-related ADRs. The included RCT (Wassif 2017) and one of the prospective NRSIs included in this review (Chan 2009) found no differences between add-on statin therapy compared to cholesterol supplementation only. In contrast, evidence from the two retrospective NRSIs included in the review suggested that add-on statin therapy potentially carries a higher risk of adverse reactions (i.e. harm) in individuals with SLOS, compared to cholesterol supplementation alone (Haas 2007; Oláh 2013a). Most reported cases of statin-related ADRs rapidly improved following statin discontinuation or dosage reduction in both of these studies.

Only two studies reported on the effects of statin therapy on growth parameters in children with SLOS and these employed different designs and outcome measurements (Haas 2007; Wassif 2017). One cross-over RCT reported no changes in the weight or height of children with SLOS receiving simvastatin compared to placebo (very low-certainty evidence) (Wassif 2017). The retrospective NRSI reported changes in weight SD scores and linear growth SD score after receiving combination therapy for a very small subset of their participants with the results being highly inconsistent (very low-certainty evidence) (Haas 2007).

Current evidence on the effects of statin therapy on plasma levels of total cholesterol, 7DHC or 8DHC or both (i.e. total dehydrocholesterol levels) in individuals with SLOS is very limited and unclear (low-certainty evidence in the included RCT and very low-certainty evidence in the included NRSIs). We found inconsistencies in the dosages of cholesterol or statins, or both, used in individuals within the same study, different durations or formulations of treatments among the different studies, as well as inconsistencies in measuring or reporting the levels of these plasma biomarkers across the different studies. These, altogether, precluded our ability to meaningfully pool the relevant data reported for this outcome. However, the included RCT and two of the NRSIs showed similar trends of lower dehydrocholesterol levels with add-on statin therapy compared to cholesterol monotherapy. It should be noted that while some of these biomarkers have been shown to correlate with disease severity, correlation does not imply causation and using statins to reduce or normalize their levels would not necessarily translate into positive clinical outcomes.

Given the overall limited and low-certainty evidence on the benefits or harms of using statin therapy in individuals with SLOS, further studies, ideally large-size RCTs, remain essential to discern whether statin therapy holds any potential benefits for individuals with SLOS.

Overall completeness and applicability of evidence

Only one of the six outcomes in our review (statin-related ADRs) was relatively adequately addressed (i.e. had the most information reported about it) by our included studies. Nonetheless, the currently available evidence for this outcome is of low-certainty, pointing only to a potential increased risk of statin-related ADRs (harms) when using statin therapy in individuals with SLOS.

In contrast, for the remaining outcomes of this review we identified either no evidence at all (i.e. survival and QoL), or few data with low- or very low-certainty evidence (i.e. neurobehavioral manifestations of SLOS, growth parameters, and disease biomarker levels other than plasma cholesterol (e.g. plasma 7DHC levels)). Future studies, therefore, should aim to address the current gaps in the available evidence on the use of statins in individuals with SLOS.

Thus, there is currently only limited evidence regarding the role of statin therapy in individuals with SLOS, with a potentially increased risk of ADRs related to statin use in these individuals.

Quality of the evidence

Overall, we identified six studies eligible for inclusion in our review, only one of which is an RCT (Wassif 2017) with the remaining five being NRSIs (Chan 2009; Haas 2007; Haas 2008; Oláh 2013a; Roullet 2012). Only the included RCT reported on changes in the neurobehavioral manifestations of 14 individuals with SLOS (Wassif 2017); we downgraded the overall certainty of this limited evidence to very low owing to the high risk of bias found for selective outcome reporting and incomplete outcome data (Table 3; Summary of findings 1). Specifically, the study exclusively assessed changes in irritability, only one of the multiple possible neurobehavioral manifestations of individuals with SLOS, without justification for this choice. Furthermore, the authors did not report on changes in hyperactivity despite listing this outcome in the protocol (Wassif 2017). We further downgraded the evidence as it was unclear whether the neurobehavioral

assessments performed in the study were completed by the same healthcare professional or caregiver for each participant or not, raising concerns about inconsistency (i.e. inter-reviewer variability) in outcome assessment.

The rates of statin-related ADRs were assessed by the included RCT (N = 18) (Wassif 2017) and three NRSIs (N = 25) (Chan 2009; Haas 2007; Oláh 2013a). We were unable to pool the reported data in a single meta-analysis, due to their different study designs (interventional versus observational) and forms of data collection (retrospective versus prospective). However, we were able to combine data from the two retrospective NRSIs (N = 22) (Haas 2007; Oláh 2013a), and graded the evidence as low certainty due to the overall serious risk of bias in both studies (Table 4). Our analysis of data from the RCT also suggested that statin therapy in individuals with SLOS may lead to increased rates of ADRs compared to cholesterol supplementation alone (Wassif 2017). We deemed the corresponding evidence to be of very low certainty due to the high risk of bias for selective outcome reporting and incomplete outcome data caused by the exclusion of non-compliant participants from the final analysis (Table 3) and due to imprecision (few participants, few events and wide CIs) (Summary of findings 1). Additionally, in the open-label part of the trial, three participants receiving statins developed ADRs which fit our prespecified definition of possible statin-related ADRs; however, no further data were provided about these participants, such as whether their symptoms subsided when statin therapy was discontinued.

Two studies assessed changes in the growth parameters of children with SLOS receiving add-on statin therapy compared to those receiving cholesterol supplementation only; one of these was the only RCT (N = 16 for height and N = 18 for weight) (Wassif 2017) and the second was a retrospective NRSI (N = 13) (Haas 2007). We deemed the certainty of the evidence generated by each of these studies for this outcome to be very low (Summary of findings 1). We downgraded the certainty of the evidence due to heterogeneity (i.e. inconsistency) in the direction of the results within each of the two studies, as well as their use of different 'controls' against which the changes in growth parameters were compared (e.g. Haas 2007 used the German growth chart as their standard, while Wassif 2017 used the baseline growth parameters of each participant as their respective comparator). Moreover, the number of measures obtained for each parameter were variable among the participants of the same study, as well as across the two studies. Finally, the authors of the RCT had not prespecified in their protocol their plan to collect data on anthropometric measures in their included participants (Wassif 2017).

With the exception of one study (Oláh 2013a), all studies assessed changes in the plasma levels of various disease biomarkers. For the RCT (N = 18) we judged the overall certainty of the evidence for this outcome as low. We downgraded the certainty of the evidence twice: once due to the high risk of bias for selective outcome reporting and incomplete outcome data in the trial; and again due to the inconsistency arising from heterogeneity in the direction of the results within the study (Summary of findings 1). In contrast, we judged the certainty of the evidence from the four NRSIs assessing this outcome as very low (Chan 2009; Haas 2007; Haas 2008; Roullet 2012). We downgraded the evidence contributed by these studies three times: once due to inconsistency arising from heterogeneity in the direction of the results; and twice due to the overall critical risk of bias, mainly caused by confounding following the inclusion of participants already receiving treatment(s) prior to study initiation (Summary of findings 1). It is also worth mentioning that, if all studies (including the RCT) were to be pooled together, results would be remarkably heterogenous since the specific biomarkers assessed by each of these studies were highly variable (e.g. some studies measured 7DHC alone, while others measured 7DHC and 8DHC together) (Table 3), and they were measured by different laboratory techniques using different units. Such inconsistency in the definition and measurement of outcomes would preclude our ability to pool data from these studies together in a rigorous meta-analysis, warranting the consideration of such an issue in the design of future relevant studies.

Potential biases in the review process

It is very unlikely that we have missed any studies on statin therapy in people with SLOS, given the extensive search strategies designed for and run on multiple electronic databases and clinical trial registries (Appendix 1). We also conducted a thorough screening of the reference lists of all included studies, handsearched the conference abstracts and proceedings of the Society for Inherited Metabolic Disorders, and conducted an extensive gray literature search, all of which allowed us to maximize our search coverage for potentially relevant studies.

By including multiple gray literature resources in our search strategy, we are confident that we have successfully accounted for any publication bias that may otherwise exist. In fact, we retrieved three unpublished studies (Palm 2019; Pappu 2011; Tavori 2015); however, none of these turned out to be eligible for inclusion in our review. As a result, given the limited number of unpublished studies retrieved by our gray literature search (less than five studies, which does not meet our minimum set number in our protocol (Ballout 2020)), we were unable to construct a funnel plot to check for publication bias. This is not unusual in a rare disease like SLOS, where the number of studies and clinicians and researchers investigating the disease worldwide is limited.

Agreements and disagreements with other studies or reviews

As stated in our protocol and review, we conducted this review to systematically evaluate the current evidence available on the use of statins in individuals with SLOS. We found that all current studies evaluating the effects of statin therapy on various outcomes in individuals with SLOS are limited by number and quality (i.e. internal validity), with the majority of available studies being primarily anecdotal in nature.

One of the three unpublished studies we identified was only available in an abstract format, with insufficient details on study design and no intention to pursue full-text publication, as disclosed to us by the authors. However, based on the available abstract, the authors found statistically significant results that favor the addition of statin therapy to cholesterol supplementation in individuals with SLOS for lowering plasma levels of 7DHC (decreased by 25.4%; P = 0.011) and increasing their total plasma cholesterol level (increased by 17.6%; P = 0.048) (Tavori 2015).

The few observational studies that are available on this topic, and which have been included in this review, had multiple limitations in terms of their design and execution. To the best of our

knowledge, there are no other published or ongoing studies or reviews addressing our research questions. This fact stresses the importance of conducting this review to highlight and cover the current gaps in evidence for the topic.

AUTHORS' CONCLUSIONS

Implications for practice

In the current systematic review, we found no evidence assessing the effects of statin therapy on survival or quality of life (QoL) in individuals with Smith-Lemli-Opitz syndrome (SLOS). In addition, we found limited and insufficient evidence regarding the effects of statin therapy on neurobehavioral manifestations, growth parameters and biomarker levels in plasma or cerebral spinal fluid (CSF) in individuals with SLOS, apart from plasma cholesterol levels for which the current evidence shows no effect of statin therapy based on two retrospective non-randomized studies of interventions (NRSIs).

More evidence was available for statin-related adverse reactions, and we found that statin therapy combined with cholesterol supplementation may be associated with an almost 13-times higher risk of statin-related adverse reactions (i.e. hepatotoxicity, myopathy, or sleep disturbances) compared to cholesterol supplementation alone, but the certainty of the evidence ranged from low to very low (Summary of findings 1).

The current lack of evidence on any beneficial effects of statin therapy in individuals with SLOS, and the simultaneously limited and very low- to low-certainty evidence suggesting a potentially increased risk of adverse reactions, should be taken into account when considering the routine use of statins in individuals with SLOS.

Until further, well-designed studies that carefully evaluate the potential benefits of statin therapy in individuals with SLOS become available, we propose a cautious and judicious use of statins in individuals with SLOS.

For any individuals with SLOS who are already taking statins as part of an ongoing treatment, or are expected to begin statin therapy as part of new research, their clinicians should be aware of the potential for statin-related adverse drug reactions and diligently watch out for these.

Implications for research

There is a complete lack of evidence evaluating the possible effects of statin therapy on survival or QoL in individuals with SLOS. Along with the limited and inconsistent nature of evidence currently available on the effects of statin therapy on neurobehavioral abnormalities, anthropometric parameters and biomarker levels, future research efforts should attempt to address these evidence gaps (Table 6). In particular, overall survival and QoL are important, both from the clinical and patient-care perspectives. To clarify, if, for instance, statins are found to improve overall survival or reduce SLOS-related deaths in individuals with SLOS then, irrespective of their effects on other outcomes, they should be incorporated as part of the standard of care for individuals with SLOS, since survival remains a superior and clinically desirable outcome. The same is true for QoL, since QoL remains a key priority, especially for families affected by rare diseases. Nonetheless, further studies should also consider addressing other clinically important outcomes mentioned above.

It would be ideal to investigate the potential benefits of statin therapy in individuals with SLOS in a randomized controlled trial (RCT) with a well-defined and a priori listed set of clinically meaningful outcomes (e.g. survival). Such trials should attempt to build upon the shortcomings and limitations previously discussed for the single RCT currently available on this topic (Wassif 2017), by ensuring adequate blinding of data analysis, adopting an intention-to-treat analysis, attempting to maintain individual participant data for easier dissemination and sharing, and strictly adhering to their protocols.

As RCTs are known to be costly, time-consuming, and rather difficult to conduct for rare diseases such as SLOS, we also propose performing properly-designed prospective cohort studies as a feasible and realistic alternative. However, such studies should also have a clearly predetermined set of clinically relevant outcomes.

In either scenario, future studies should also aim to use consistent preset dosages (i.e. per kg per day) of cholesterol supplementation or statin therapy (or both) for all of their enrolled participants, providing adequate justifications when deviations from such dosages are made. This is important to overcome the current inconsistencies seen, both within and across the available studies with regard to the dosages used for either treatment. Likewise, future studies should also attempt to compare different formulations (e.g. dietary versus crystalline) and durations of cholesterol supplementation, as well as different statins (e.g. lipophilic versus hydrophilic). Finally, future studies should consider collecting separate data on adults versus children with SLOS, while accounting, in the process, for various possible effect modifiers such as sex, disease severity, or the nature of statin agent used (i.e. lipophilic versus hydrophilic) on their assessed outcomes.

ACKNOWLEDGEMENTS

We would like to thank Tracey Remmington, the Managing Editor of the Cochrane Cystic Fibrosis and Genetic Disorders Group, for her continuous support and guidance throughout the entire preparation, conduction and drafting of this review.

REFERENCES

References to studies included in this review

Chan 2009 *{published data only}*

Chan YM, Merkens LS, Connor WE, Roullet JB, Penfield JA, Jordan JM, et al. Effects of dietary cholesterol and simvastatin on cholesterol synthesis in Smith-Lemli-Opitz syndrome (SLOS). *Pediatric Research* 2009;**65**(6):681-5. [PMID: 19430384]

Haas 2007 *{published data only}*

Haas D, Garbade SF, Vohwinkel C, Muschol N, Trefz FK, Penzien JM, et al. Effects of cholesterol and simvastatin treatment in patients with Smith-Lemli-Opitz syndrome (SLOS). *Journal of Inherited Metabolic Disease* 2007;**30**(3):375-87.

Haas 2008 *{published data only}*

Haas D, Niklowitz P, Hoffmann GF, Andler W, Menke T. Plasma and thrombocyte levels of coenzyme Q10 in children with Smith-Lemli-Opitz syndrome (SLOS) and the influence of HMG-CoA reductase inhibitors. *Biofactors* 2008;**32**(1-4):191-7. [PMID: 19096116]

Oláh 2013a *{published data only}*

Oláh AV, Szabó GP, Varga J, Balogh L, Csábi G, Csákváry V, et al. Relation between biomarkers and clinical severity in patients with Smith-Lemli-Opitz syndrome. *European Journal of Pediatrics* 2013;**172**(5):623-30. [PMID: 23319240]

Roullet 2012 *{published data only}*

Roullet JB, Merkens LS, Pappu AS, Jacobs MD, Winter R, Connor WE, et al. No evidence for mevalonate shunting in moderately affected children with Smith-Lemli-Opitz syndrome. *Journal of Inherited Metabolic Disease* 2012;**35**(5):859-69.

Wassif 2017 *{published data only}*

NCT00064792. Simvastatin therapy in smith-lemli-opitz syndrome. clinicaltrials.gov/show/NCT00064792 (first posted 14 July 2003).

Schreiber JM, Lanham DC, Trescher WH, Sparks SE, Wassif CA, Caffo BS, et al. Variations in EEG discharges predict ADHD severity within individual Smith-Lemli-Opitz patients. *Neurology* 2014;**83**(2):151-9.

Wassif CA, Kratz L, Sparks SE, Wheeler C, Bianconi S, Gropman A, et al. A placebo-controlled trial of simvastatin therapy in Smith-Lemli-Opitz syndrome. *Genetics in Medicine* 2017;**19**(3):297-305. [PMID: 27513191]

References to studies excluded from this review

Aneja 2008 *{published data only}*

* Aneja A, Tierney E. Autism: the role of cholesterol in treatment. *International Review of Psychiatry* 2008;**20**(2):165-70. [DOI: 10.1080/09540260801889062] [PMID: 18386207]

Aneja A, Tierney E. Cholesterol deficit in autism: insights from Smith–Lemli–Opitz Syndrome. In: Zimmerman AW, editors(s). Autism. Current Theories and Evidence (Current Clinical Neurology collection). Humana Totowa, NJ, 2008. [DOI: 10.1007/978-1-60327-489-0_3]

Correa-Cerro 2005 *{published data only}*

Correa-Cerro LS, Forbes D, Porter FD. 3beta-hydroxysterol Delta7-reductase and the Smith-Lemli-Opitz syndrome. *Molecular Genetics and Metabolism* 2005;**84**(2):112-26. [DOI: 10.1016/j.ymgme.2004.09.017] [PMID: 15670717]

Haas 2005 *{published data only}*

Haas D, Armbrust S, Haas JP, Zschocke J, Muhlmann K, Fusch C, et al. Smith–Lemli–Opitz syndrome with a classical phenotype, oesophageal achalasia and borderline plasma sterol concentrations. *Journal of Inherited Metabolic Disease* 2005;**28**(6):1191-6. [DOI: 10.1007/s10545-005-0168-9]

Irons 2004 *{published data only}*

Irons MB. Cholesterol in childhood: friend or foe? *Pediatric Research* 2004;**56**(5):679-81. [DOI: 10.1203/01.PDR.0000146398.61649.74]

Jira 1997a *{published data only}*

Jira P, Wevers R, de Jong J, Rubio-Gozalbo E, Smeitink J. New treatment strategy for Smith-Lemli-Opitz syndrome. *Lancet* 1997;**349**(9060):1222.

Jira 1997b *{published data only}*

Jira PE, de Jong JG, de Metz M, Aalfs CM, Hennekam RC, Wanders RJ, et al. Smith-Lemli-Opitz syndrome: a disorder in cholesterol biosynthesis [Het Smith-Lemli-Opitz syndroom: een stoornis in de cholesterol biosynthese]. *Nederlands Tijdschrift voor Klinische Chemie* 1997;**22**(4):175-9.

Jira 2000 *{published data only}*

Jira PE, Wevers RA, de Jong J, Rubio-Gozalbo E, Janssen-Zijlstra FS, van Heyst AF, et al. Simvastatin. A new therapeutic approach for Smith-Lemli-Opitz syndrome. *Journal of Lipid Research* 2000;**41**(8):1339-46.

Jira 2005 *{published data only}*

Jira P. The Smith-Lemli-Opitz syndrome: a multiple malformation syndrome due to a defect in cholesterol biosynthesis [PhD Thesis]. Nijmegen, NL: Radboud Repository of the Radboud University, 2005.

Kelley 2000 *{published data only}*

Kelley RI, Hennekam RC. The Smith-Lemli-Opitz syndrome. *Journal of Medical Genetics* 2000;**37**:321-35.

Kilic 2011 *{published data only}*

Kilic M, Tokatli A, Alanay Y, Kilic E, Kalkanoglu-Sivri HS, Dursun A, et al. Phenotypical properties and response to cholesterol therapy of Smith-Lemli-Opitz syndrome cases. *Journal of Inherited Metabolic Disease* 2011;**34**(Suppl 3):S49–S286. [DOI: 10.1007/s10545-011-9371-z]

Linck 1999 *{published data only}*

Linck LM, Lin D, Connor WE, Steiner RD. Will dietary cholesterol decrease plasma cholesterol and decrease abnormal 7-

dehydrocholesterol levels in Smith-Lemli-Opitz syndrome? *Journal of Investigative Medicine* 1999;**47**(Western Regional Meeting):54A.

NCT01434745 *{published data only}*

NCT01434745. SLOS: the effect of simvastatin in patients receiving cholesterol supplementation. clinicaltrials.gov/show/NCT01434745 (first posted 15 September 2011).

Oláh 2018 *{published data only}*

Oláh AV, Szabó GP, Balogh I. Clinical significance of low cholesterol and high 7-dehydrocholesterol in inherited metabolic disorders. *Clinical Chemistry and Laboratory Medicine* 2018;**56**(9):eA121–69. [DOI: 10.1515/cclm-2018-0718] [PMID: 33593042]

Palm 2019 *{published data only}*

Palm K, Kovács T, Empting S, Mohnike K. Longitudinal cholesterol metabolite profiles in 20 patients with Smith-Lemli-Opitz-syndrome. *Monatsschrift fur Kinderheilkunde* 2019;**167**(4):371. [DOI: 10.1007/ s00112- 019- 0665-9]

Pappu 2011 *{published data only}*

Pappu AS, DeBarber AD, Winter RF, Merkens LS, Roullet JB, Steiner RD. Trienol a potential new marker of oxidative stress in Smith–Lemli–Opitz syndrome (SLOS). *Molecular Genetics and Metabolism* 2011;**102**(3):307-8.

Prabhu 2016 *{published data only}*

Prabhu AV, Luu W, Sharpe LJ, Brown AJ. Cholesterol-mediated degradation of 7-dehydrocholesterol reductase switches the balance from cholesterol to vitamin D synthesis. *Journal of Biological Chemistry* 2016;**291**(16):8363-73. [DOI: 10.1074/jbc.M115.699546] [PMID: 26887953]

Scalco 2006 *{published data only}*

Scalco FB, Otto PA, Brunetti IL, Cruzes VM, Moretti-Ferreira D. Smith-Lemli-Opitz syndrome: clinical and biochemical findings in Brazilian patients. *Genetics and Molecular Biology* 2006;**29**(3):429-36. [DOI: 10.1590/S1415-47572006000300003]

Sikora 2004 *{published data only}*

Sikora DM, Ruggiero M, Petit-Kekel K, Merkens LS, Connor WE, Steiner RD. Cholesterol supplementation does not improve developmental progress in Smith-Lemli-Opitz syndrome. *Journal of Pediatrics* 2004;**144**(6):783-91.

Starck 2002b *{published data only}*

Starck L, Lövgren-Sandblom A, Björkhem I. Simvastatin treatment in the SLO syndrome: a safe approach? *American Journal of Medical Genetics* 2002;**113**(2):183-9. [PMID: 12407710]

Suzuki 2020 *{published data only}*

Suzuki A, Ogata K, Yoshioka H, Shim J, Wassif CA, Porter FD, et al. Disruption of *Dhcr7* and *Insig1/2* in cholesterol metabolism causes defects in bone formation and homeostasis through primary cilium formation. *Bone Research* 2020;**8**(1). [DOI: 10.1038/s41413-019-0078-3] [PMID: 31934493]

Tavori 2015 *{published data only}*

Tavori H, Roullet JB, Minnier J, Pappu A, Steiner RD, Fazio S. Plasma lipids and proprotein convertase subtilisin/kexin type 9 (PCSK9) in patients with Smith-Lemli-Opitz syndrome (SLOS). *Journal of Inherited Metabolic Disease* 2015;**38**(1):S234. [DOI: 10.1007/s10545-015-9877-x]

Tint 1997 *{published data only}*

Tint GS, Batta AK, Xu G, Shefer S, Honda A, Irons M, et al. The Smith-Lemli-Opitz syndrome: a potentially fatal birth defect caused by a block in the last enzymatic step in cholesterol biosynthesis. *Sub-cellular Biochemistry* 1997;**28**:117-44. [DOI: 10.1007/978-1-4615-5901-6_5] [PMID: 9090293]

Ullrich 1996 *{published data only}*

Ullrich K, Koch HG, Meschede D, Flotmann U, Seedorf U. Smith-Lemli-Opitz syndrome: treatment with cholesterol and bile acids. *Neuropediatrics* 1996;**27**(2):111-2. [DOI: 10.1055/s-2007-973760] [PMID: 8737829]

Wright 2002 *{published data only}*

Wright BS, Wassif CA, Porter FD. Investigation of simvastatin therapy in Smith-Lemli-Opitz syndrome. *Pediatric Research* 2002;**51**(4):229A.

Additional references

ACC/AHA 2014

ACC/AHA. Practice guidelines: ACC/AHA release updated guideline on the treatment of blood cholesterol to reduce ASCVD risk. *American Family Physician* 2014;**90**(4):260-5.

Aman 1995

Aman MG, Burrow WH, Wolford PL. The aberrant behavior checklist-community: factor validity and effect of subject variables for adults in group homes. *American Journal of Mental Retardation* 1995;**100**(3):283-92. [PMID: 8554775]

Azurdia 2001

Azurdia RM, Anstey AV, Rhodes LE. Cholesterol supplementation objectively reduces photosensitivity in the Smith-Lemli-Opitz syndrome. *British Journal of Dermatology* 2001;**144**(1):143-5. [PMID: 11167696]

Baardman 2013

Baardman ME, Kerstjens-Frederikse WS, Berger RM, Bakker MK, Hofstra RM, Plösch T. The role of maternal-fetal cholesterol transport in early fetal life: current insights. *Biology of Reproduction* 2013;**88**(1):24.

Ballout 2021

Ballout RA. Smith-Lemli-Opitz Syndrome (SLOS). In: Rezaei N, editors(s). Genetic Syndromes. Basel, Switzerland: Springer, Cham, 2021:1-10. [DOI: 10.1007/978-3-319-66816-1_501-1]

Bialer 1987

Bialer MG, Penchaszadeh VB, Kahn E, Libes R, Krigsman G, Lesser ML. Female external genitalia and müllerian duct derivatives in a 46, XY infant with the Smith-Lemli-

Opitz syndrome. *American Journal of Medical Genetics* 1987;**28**(3):723-31. [PMID: 3322011]

Bikle 2017

Bikle D. Vitamin D: Production, Metabolism, and Mechanisms of Action. www.ncbi.nlm.nih.gov/books/NBK278935/ (accessed 25 January 2021).

Blassberg 2016

Blassberg R, Macrae JI, Briscoe J, Jacob J. Reduced cholesterol levels impair smoothened activation in Smith-Lemli-Opitz syndrome. *Human Molecular Genetics* 2016;**25**(4):693-705. [PMID: 26685159]

Buss 1992

Buss AH, Perry M. The aggression questionnaire. *Journal of Personality and Social Psychology* 1992;**63**(3):452-9. [PMID: 1403624]

Chervin 2000

Chervin RD, Hedger K, Dillon JE, Pituch KJ. Pediatric sleep questionnaire (PSQ): validity and reliability of scales for sleep-disordered breathing, snoring, sleepiness, and behavioral problems. *Sleep Medicine* 2000;**1**(1):21-32. [PMID: 10733617]

Chinn 2000

Chinn S. A simple method for converting an odds ratio to effect size for use in meta-analysis. *Statistics in Medicine* 2000;**19**(22):3127-31.

Cohen 1988

Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd edition. HIllsdale (NJ): Lawrence Erlbaum Associates, Inc, 1988.

Cohn 2010

Cohn JS, Kamili A, Wat E, Chung RW, Tandy S. Dietary phospholipids and intestinal cholesterol absorption. *Nutrients* 2010;**2**(2):116-27.

Deeks 2021

Deeks JJ, Higgins JP, Altman DG, on behalf of the Cochrane Statistical Methods Group. Chapter 10: Analysing data and undertaking meta-analyses. In Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook of Systematic Reviews of Interventions Version 6.2 (updated February 2021). Cochrane, 2021. Available from training.cochrane.org/handbook.

Diaz-Stransky 2012

Diaz-Stransky A, Tierney E. Cognitive and behavioral aspects of Smith-Lemli-Opitz syndrome. *Medical Journal of Medical Genetics* 2012;**160C**(4):295-300.

Dietschy 2001

Dietschy JM, Turley SD. Cholesterol metabolism in the brain. *Current Opinion in Lipidology* 2001;**12**(2):105-12.

Edwards 2007

Edwards MC, Gardner ES, Chelonis JJ, Schulz EG, Flake RA, Diaz PF. Estimates of the validity and utility of the Conners' Continuous Performance Test in the assessment of inattentive and/or hyperactive-impulsive behaviors in children. *Journal of Abnormal Child Psychology* 2007;**35**(3):393-404. [PMID: 17295064]

Elbourne 2002

Elbourne DR, Altman DG, Higgins JP, Curtin F, Worthington HV, Vail A. Meta-analyses involving cross-over trials: methodological issues. *International Journal of Epidemiology* 2002;**31**(1):140-9.

Elias 1997

Elias R, Irons EM, Hurley A, Tint G, Salen G. Clinical effects of cholesterol supplementation in six patients with Smith-Lemli-Opitz syndrome (SLOS). *American Journal of Medical Genetics* 1997;**68**(3):305-10. [PMID: 9024564]

EPOC 2017

Cochrane Effective Practice and Organisation of Care (EPOC). EPOC resources for review authors; 2017. epoc.cochrane.org/epoc-resources-review-authors.

Fitzky 1998

Fitzky BU, Witsch-Baumgartner M, Erdel M, Lee JN, Paik YK, Glossmann H, et al. Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome. *Proceedings of the National Academy of Sciences of the United States of America* 1998;**95**(14):8181-6. [DOI: 10.1073/pnas.95.14.8181]

Fitzky 2001

Fitzky BU, Moebius FF, Asaoka H, Waage-Baudet H, Xu L, Xu G, et al. 7-Dehydrocholesterol-dependent proteolysis of HMG-CoA reductase suppresses sterol biosynthesis in a mouse model of Smith-Lemli-Opitz/RSH syndrome. *Journal of Clinical Investigation* 2001;**108**(6):905-15.

Fliesler 2018

Fliesler SJ, Peachey NS, Herron J, Hines KM, Weinstock NI, Rao SR, et al. Prevention of retinal degeneration in a rat model of Smith-Lemli-Opitz Syndrome. *Scientific Reports* 2018;**8**(1):1286. [DOI: 10.1038/s41598-018-19592-8] [PMID: 29352199]

Furukawa 1999

Furukawa TA. From effect size into number needed to treat. *Lancet* 1999;**353**(9165):1680.

Gogtay 2004

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Science of the United States of America* 2004;**101**(21):8174-9.

Grundy 1983

Grundy SM. Absorption and metabolism of dietary cholesterol. *Annual Review of Nutrition* 1983;**3**:71-96.

Henriques-Forsythe 2015

Henriques-Forsythe M. Wanna be statin something? *Science Translational Medicine* 2015;**7**(315):315ec201.

Higgins 2017

Higgins JP, Savović J, Page MJ, Elbers RG, Sterne JA. Chapter 8: Assessing risk of bias in a randomized trial. In: Higgins JP, Churchill R, Chandler J, Cumpston MS, editor(s), Cochrane Handbook for Systematic Reviews of Interventions version 5.2.0 (updated June 2017), Cochrane, 2017. Available from training.cochrane.org/handbook/archive/v5.2.

Higgins 2022a

Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Cochrane, 2022. Available from training.cochrane.org/handbook.

Higgins 2022b

Lefebvre C, Glanville J, Briscoe S, Featherstone R, Littlewood A, Marshall C, et al. Chapter 4: Searching for and selecting studies. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane, 2022. Available from www.training.cochrane.org/handbook.

Higgins 2022c

Higgins JP, Li T, Deeks JJ, editor(s). Chapter 6: Choosing effect measures and computing estimates of effect. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane, 2022. www.training.cochrane.org/handbook.

Honda 1995

Honda A, Tint GS, Salen G, Batta AK, Chen TS, Shefer S. Defective conversion of 7-dehydrocholesterol to cholesterol in cultured skin fibroblasts from Smith-Lemli-Opitz syndrome homozygotes. *Journal of Lipid Research* 1995;**36**(7):1595-601.

Korade 2014

Korade Z, Xu L, Harrison FE, Ahsen R, Hart SE, Folkes OM, et al. Antioxidant supplementation ameliorates molecular deficits in Smith-Lemli-Opitz Syndrome (SLOS). *Biological Psychiatry* 2014;**75**(3):215-22. [DOI: 10.1016/j.biopsych.2013.06.013] [PMID: 23896203]

Lazarin 2017

Lazarin GA, Haque IS, Evans EA, Goldberg JD. Smith-Lemli-Opitz syndrome carrier frequency and estimates of in utero mortality rates. *Prenatal Diagnosis* 2017;**37**(4):350-5.

Lin 1977

Lin DS, Pitkin RM, Connor WE. Placental transfer of cholesterol into the human fetus. *American Journal of Obstetrics and Gynecology* 1977;**128**(7):735-9.

Linck 2000

Linck LM, Lin DS, Flavell D, Connor WE, Steiner RD. Cholesterol supplementation with egg yolk increases plasma cholesterol and decreases plasma 7-dehydrocholesterol in Smith-Lemli-Opitz syndrome. *American Journal of Medical Genetics* 2000;**93**(5):360-5. [DOI: 10.1002/1096-8628(20000828)93:5<360::aid-ajmg4>3.0.co;2-p] [PMID: 10951458]

Lloyd 1997

Lloyd EE. Self-mutilation in a community sample of adolescents. digitalcommons.lsu.edu/cgi/viewcontent.cgi?article=7545&context=gradschool_disstheses (accessed 6 November 2019).

Lord 2001

Lord C, Leventhal BL, Cook EH Jr. Quantifying the phenotype in autism spectrum disorders. *American Journal of Medical Genetics* 2001;**105**(1):36-8. [PMID: 11424991]

Luo 2020

Luo J, Yang H, Song B-L. Mechanisms and regulation of cholesterol homeostasis. *Nature Reviews: Molecular Cell Biology* 2020;**21**(4):225-45.

McGowan 2004

McGowan MP, Treating to New Target (TNT) Study Group. There is no evidence for an increase in acute coronary syndromes after short-term abrupt discontinuation of statins in stable cardiac patients. *Circulation* 2004;**110**(16):2333-5.

NCT00064792

NCT00064792. Simvastatin therapy in Smith-Lemli-Opitz syndrome. clinicaltrials.gov/show/NCT00064792 (first posted 14 July 2003).

NOS 2019

Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. Newcastle - Ottawa Quality Assessment Scale Case Control Studies. www.ohri.ca/programs/clinical_epidemiology/nosgen.pdf (accessed 18 April 2019).

Nowaczyk 1998a

Nowaczyk MJ, Whelan DT, Hill RE. Smith-Lemli-Opitz syndrome: phenotypic extreme with minimal clinical findings. *American Journal of Medical Genetics* 1998;**78**(5):419-23.

Nowaczyk 1998b

Nowaczyk MJ, Wassif CA. Smith-Lemli-Opitz Syndrome. www.ncbi.nlm.nih.gov/books/NBK1143/ (accessed 25 Janury 2021).

Nowaczyk 2012

Nowaczyk MJ, Irons MB. Smith-Lemli-Opitz syndrome: phenotype, natural history, and epidemiology. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics* 2012;**160C**(4):250-62.

Nowaczyk 2013

Nowaczyk MJ. GeneReviews®. Smith-Lemli-Opitz Syndrome. www.ncbi.nlm.nih.gov/books/NBK1116/ (accessed 06 November 2019). [PMID: 20301322]

Nwokoro 1997

Nwokoro NA, Mulvihill JJ. Cholesterol and bile acid replacement therapy in children and adults with Smith-Lemli-Opitz (SLO/RSH) syndrome. *American Journal of Medical Genetics* 1997;**68**(3):315-21. [PMID: 9024566]

Owens 2000

Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. *Sleep* 2000;**23**(8):1043-51. [PMID: 11145319]

PARS 2002

The Research Units on Pediatric Psychopharmacology Anxiety Study Group. The Pediatric Anxiety Rating Scale (PARS): development and psychometric properties. *Journal of the American Academy of Child & Adolescent Psychiatry* 2002;**41**(9):1061-9. [PMID: 12218427]

Porter 1996

Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. *Science* 1996;**274**(5285):255-9. [PMID: 8824192]

Qu 2018

Qu H, Guo M, Chai H, Wang WT, Gao ZY, Shi DZ. Effects of coenzyme Q10 on statin-induced myopathy: an updated meta-analysis of randomized controlled trials. *Journal of the American Heart Association* 2018;**7**(19):e009835. [PMID: 30371340]

Reeves 2011

Reeves BC, Deeks JJ, Higgins JP, Shea B, Tugwell P, Wells GA, on behalf of the Cochrane Non-Randomized Studies of Interventions Methods Group. Chapter 24: Including non-randomized studies on intervention effects. In Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.2 (updated February 2021). Cochrane, 2021. Available from training.cochrane.org/handbook.

Review Manager 2020 [Computer program]

Review Manager 5 (RevMan 5). Version 5.4. Copenhagen: The Cochrane Collaboration, 2020.

Riley 2011

Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. *BMJ* 2011;**342**:d549.

Rosenson 2019a

Rosenson RS. Statins: actions, side effects, and administration. www.uptodate.com/contents/statins-actions-side-effects-and-administration (accessed 6 November 2019).

Rosenson 2019b

Rosenson RS, Baker SK. Statin muscle-related adverse events. www.uptodate.com/contents/statin-muscle-related-adverse-events (accessed 6 November 2019).

Rundek 2004

Rundek T, Naini A, Sacco R, Coates K, DiMauro S. Atorvastatin decreases the coenzyme Q10 level in the blood of patients at risk for cardiovascular disease and stroke. *Archives of Neurology* 2004;**61**(6):889-92. [PMID: 15210526]

Russell 1992

Russell DW, Setchell KD. Bile acid biosynthesis. *Biochemistry* 1992;**31**(20):4737-49.

Schachter 2004

Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundamental and Clinical Pharmacology* 2004;**19**(1):117-25. [DOI: 10.1111/j.1472-8206.2004.00299.x] [PMID: 15660968]

Schünemann 2013

Schünemann H, Brożek J, Guyatt G, Oxman A, editor(s). Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach (updated 2013). GRADE Working Group, 2013. gdt.gradepro.org/app/handbook/handbook.html.

Schünemann 2019

Schünemann HJ, Cuello C, Akl EA, Mustafa RA, Meerpohl JJ, Thayer K, et al. GRADE guidelines: 18. How ROBINS-I and other tools to assess risk of bias in nonrandomized studies should be used to rate the certainty of a body of evidence. *Journal of Clinical Epidemiology* 2019;**111**:105-14. [PMID: 29432858]

Shefer 1997

Shefer S, Salen G, Honda A, Batta A, Hauser S, Tint GS, et al. Rapid identification of Smith-Lemli-Opitz syndrome homozygotes and heterozygotes (carriers) by measurement of deficient 7-dehydrocholesterol-delta 7-reductase activity in fibroblasts. *Metabolism* 1997;**46**(7):844-50. [PMID: 9225842]

Shields 1997

Shields A, Cicchetti D. Emotion regulation among school-age children: the development and validation of a new criterion Q-sort scale. *Developmental Psychology* 1997;**33**(6):906-16. [PMID: 9383613]

Sikora 2006

Sikora DM, Pettit-Kekel K, Penfield J, Merkens LS, Steiner RD. The near universal presence of autism spectrum disorders in children with Smith-Lemli-Opitz syndrome. *American Journal of Medical Genetics Part A* 2006;**140**(14):1511-8. [DOI: 10.1002/ajmg.a.31294] [PMID: 16761297]

Stancu 2001

Stancu C, Sima A. Statins: mechanism of action and effects. *Journal of Cellular and Molecular Medicine* 2001;**5**(4):378-87.

Starck 2002a

Starck L, Lövgren-Sandblom A, Björkhem I. Cholesterol treatment forever? The first Scandinavian trial of cholesterol supplementation in the cholesterol-synthesis defect Smith-Lemli-Opitz syndrome. *Journal of Internal Medicine* 2002;**252**(4):314-21. [PMID: 12366604]

Sterne 2016

Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016;**355**:i4919.

Sterne 2022

Page MJ, Higgins JP, Sterne JA. Chapter 13: Assessing risk of bias due to missing results in a synthesis. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s).

Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane, 2022. Available from www.training.cochrane.org/handbook.

Stone 2014

Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology* 2014;**63**(25 Pt B):2889-934.

Svoboda 2012

Svoboda MD, Christie JM, Eroglu Y, Freeman KA, Steiner RD. Treatment of Smith-Lemli-Opitz syndrome and other sterol disorders. *American Journal of Medical Genetics* 2012;**160C**(4):285-94.

Tierney 2001

Tierney E, Nwokoro NA, Porter FD, Freund LS, Ghuman JK, Kelley RI. Behavior phenotype in the RSH/Smith-Lemli-Opitz syndrome. *American Journal of Medical Genetics* 2001;**98**(2):191-200. [PMID: 11223857]

Tierney 2009

Tierney AL, Nelson CA 3rd. Brain development and the role of experience in the early years. *Zero Three* 2009;**30**(2):9-13. [PMID: 23894221]

Tierney 2010

Tierney E, Conley SK, Goodwin H, Porter FD. Analysis of short-term behavioral effects of dietary cholesterol supplementation in Smith-Lemli-Opitz syndrome. *American Journal of Medical Genetics* 2010;**152A**(1):91-5. [PMID: 20014133]

Tint 1994

Tint GS, Irons M, Elias ER, Batta AK, Frieden R, Chen TS, et al. Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. *New England Journal of Medicine* 1994;**330**(2):107-13.

van Dyck 2017

van Dyck LI, Morrow EM. Genetic control of postnatal human brain growth. *Current Opinion in Neurology* 2017;**30**(1):114-24.

van Rooij 1997

van Rooij A, Nijenhuis AA, Wijburg FA, Schutgens RB. Highly increased CSF concentrations of cholesterol precursors in Smith-Lemli-Opitz syndrome. *Journal of Inherited Metabolic Disease* 1997;**20**(4):578-80.

Wassif 2005

Wassif CA, Krakowiak PA, Wright BS, Gewandter JS, Sterner AL, Javitt N, et al. Residual cholesterol synthesis and simvastatin induction of cholesterol synthesis in Smith-Lemli-Opitz syndrome fibroblasts. *Molecular Genetics and Metabolism* 2005;**85**(2):96-107.

References to other published versions of this review

Ballout 2020

Ballout RA, Bianconi S, Livinski A, Fu YP, Remaley AT, Porter FD. Statins for Smith-Lemli-Opitz syndrome. *Cochrane Database of Systematic Reviews* 2020, Issue 1. Art. No: CD013521. [DOI: 10.1002/14651858.CD013521] [PMID: 32132878]

* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Chan 2009

Study characteristics	
Methods	Prospective cohort study consisting of an initial 3-year period of high-dose cholesterol supplementation, followed by an additional 0.8 years of simvastatin + high-dose cholesterol supplementation.
Participants	12 children (5 males and 7 females), ages 1.1 to 15.7 years, with biochemically and/or genetically diagnosed SLOS, without a preset requirement of a specific SLOS severity score, were included in the study.
Interventions	**Intervention** 9/12 enrolled participants were treated with high-dose oral cholesterol supplementation, either in a food-based form (at a mean concentration of 34.5 mg/kg/d) or crystalline form (suspended in ORA-Plus, at a mean concentration of 47 mg/kg/d) for a period of 3 years. 3/9 participants initially maintained on high-dose dietary cholesterol supplementation (2 males and 1 female) were subsequently treated for an additional 0.8 years, with a daily oral combination therapy of high-dose cholesterol supplementation and simvastatin (administered at a dose of 0.2 mg/kg to 0.4 mg/kg, depending on participant tolerance and plasma sterol profiles).

Chan 2009 *(Continued)*

	Comparator The 3 remaining participants of the initial 12 participants enrolled in the study were maintained on a low-cholesterol diet (defined as oral cholesterol intake of 0.5 to 5 mg/kg/day) for 4 weeks and served as the control group against which the fractional cholesterol synthesis rate of the other 9 participants maintained on a high cholesterol diet was compared to. However, for the sake of our review, we will only report the data for the 3 participants who were treated with high-dose cholesterol supplementation + simvastatin for 0.8 years and were then compared to their own selves during their previous enrollment in the high-dose cholesterol supplementation arm only, for 3 years.
Outcomes	Plasma cholesterol levels Statin-related adverse reactions
Notes	**Country**: Canada & USA **Funding**: • National Institutes of Health (NIH) [R01 HL073980] • The Oregon Clinical and Translational Research Institute (OCTRI) • Grant number UL1 RR024140 from the National Center for Research Resources (NCRR) **COI**: not reported.

Haas 2007

Study characteristics	
Methods	Retrospective cohort study with pre-post data comparison.
Participants	39 adults and children (22 males and 17 females), ages 3.03 to 265 months, with biochemically diagnosed SLOS via GC-MS (defined as detecting plasma 7DHC > 0.11mg/dL or 7DHC/cholesterol > 0.002), with or without subsequent diagnostic confirmation with genetic testing, but without a preset requirement of a specific SLOS severity score, were enrolled in the study. 2 of these participants (Participants 23 and 24) were subsequently excluded due to noncompliance for receiving simvastatin only, without simultaneous cholesterol supplementation.
Interventions	**Intervention** 35 out of the 37 included participants were treated with high-dose oral cholesterol supplementation, either in a food-based form (i.e. egg-yolk in 7 participants, at a mean concentration of 40 mg/kg/d) or crystalline form (28 participants, at a mean concentration of 100 mg/kg/d in children, and 40 mg/kg/d in adults) for a median follow-up period of 52 months (range: 6 to 131 months). Among the latter participants, only 13 with mild SLOS, and normal transaminase and CK levels were subsequently co-administered simvastatin (at a dose of 0.5 mg/kg/d for the first 4 weeks of the study, followed by 1.0 mg/kg/d for the remainder of the study). **Comparator** For the sake of our review, we will only include the data of the 13 participants (4 females and 9 males) who had been receiving cholesterol supplementation and subsequently switched to cholesterol and simvastatin combination therapy, comparing their data before and after the introduction of simvastatin therapy.
Outcomes	Plasma dehydrocholesterol-to-cholesterol ratio

Haas 2007 *(Continued)*

	Plasma 7+8DHC levels Plasma cholesterol levels Statin-related adverse reactions Anthropometric measures (weight and height)
Notes	**Country**: Germany. **Funding**: not reported. **COI**: none.

Haas 2008

Study characteristics	
Methods	Prospective cohort study (conducted between 2005 and 2007).
Participants	19 children (11 males and 8 females), ages 0.3 to 13 years, with biochemically diagnosed SLOS via GC-MS (defined as detecting plasma 7DHC > 0.11mg/dL or 7DHC/Chol > 0.002), were included in the study, with subsequent diagnostic confirmation via genetic testing in 17 of these participants. Among the included participants, 5 had both their plasma and platelet CoQ10 levels measured, while the remaining 9 had only their plasma CoQ10 levels assayed.
Interventions	**Intervention** 8 of the 14 participants whose plasma CoQ10 levels were assayed had received combination therapy with cholesterol supplementation (at a mean dose of 107 mg/kg/d) and simvastatin treatment (at a dose of 1.0 mg/kg/d). **Comparator** The remaining 6 participants had received cholesterol supplementation only (at a mean dose of 107 mg/kg/d).
Outcomes	Plasma CoQ10 levels Plasma cholesterol levels
Notes	**Country**: Germany. **Funding**: not reported. **COI**: not reported.

Oláh 2013a

Study characteristics	
Methods	Retrospective cohort study.
Participants	15 children (8 males and 7 females), ages 0.1 to 18 years, with biochemically diagnosed SLOS via ultraviolet spectrophotometric assay of plasma 7DHC levels, without a preset requirement of a specific SLOS severity score, were included in the study.

Oláh 2013a *(Continued)*

Interventions	**Intervention** 9 of the 15 included participants (6 males and 3 females) were treated with a high-dose oral cholesterol and statin (simvastatin or atorvastatin) combination therapy, with the cholesterol being administered in a crystalline form (at a mean dose range of 50 mg/kg/d to 250 mg/kg/d), and statin given at a mean dose range of 0.2 mg/kg/d to 0.4 mg/kg/d. However, statin therapy was discontinued in 5 of these 9 participants after they experienced statin-related adverse reactions. Therefore, for the sake of our review, we will only report the data for the 4 participants who received high-dose cholesterol supplementation + statin combination therapy, comparing them to their own selves during their previous enrollment in the high-dose cholesterol supplementation stage only. **Comparator** 10 out of the 15 included participants were treated with high-dose oral cholesterol supplementation in a crystalline form (at a mean dose range of 50 mg/kg/d to 250 mg/kg/d). However, only 9 of those went on to receive add-on statin therapy, of which 5 discontinued the latter therapy due to side effects.
Outcomes	Statin-related adverse reactions (AST/ALT levels, LDH levels, CK levels)
Notes	**Country**: Hungary **Funding**: the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 project **COI**: none

Roullet 2012

Study characteristics	
Methods	Prospective cohort study with pre-post data comparison
Participants	19 children (9 males and 10 females), ages to years (mean 6.1 +/- 1.5 years), with biochemically diagnosed and genetically confirmed diagnosis of mild or moderate SLOS, all maintained on high-dose cholesterol supplementation, were included in the study.
Interventions	**Intervention** 9/19 included participants (6 males and 3 females), with a mean age of 5.4 +/- 2.0 years, agreed to take simvastatin (mean dosage of 0.23 mg/kg/d) in addition to high-dose cholesterol supplementation (mean dosage of 444 +/- 55 mg/d), for 1.2 +/- 0.2 years. For the sake of our review, only these 9 participants are relevant for inclusion. **Comparator** The data for each participant prior to statin introduction, when taking only high-dose cholesterol supplementation (mean dosage of 389 +/- 44 mg/d), will be used as the comparator.
Outcomes	Plasma dehydrocholesterol-to-cholesterol ratio Plasma 7DHC levels Plasma 8DHC levels Plasma cholesterol levels
Notes	**Country**: USA **Funding**:

Roullet 2012 *(Continued)*

- National Institutes for Health (NIH) [R01 HL073980]
- The Oregon Clinical and Translational Research Institute (OCTRI)
- Grant number UL1 RR024140 from the National Center for Research Resources (NCRR)

COI: not reported.

Wassif 2017

Study characteristics	
Methods	Randomized controlled cross-over trial consisting of two 12-month periods separated by a 2-month washout period.
Participants	22 children (13 males and 9 females), ages 4.0 to 17.5 years (mean age = 8.2 years), with biochemically diagnosed mild to moderate SLOS (i.e. SLOS severity score < or = 30), having demonstrated residual DHCR7 function (defined as having residual fibroblast cholesterol synthesis that exceeds 10% that of healthy controls) were included in the study. However, only 18 of them were included in the final analysis.
Interventions	**Intervention** Simvastatin (administered orally in a 1:4 solution of cherry flavor in ORA-Plus,) given at 0.5 mg/kg/d for the first 6 weeks of the trial, and then at 1.0 mg/kg dose for the remainder of the trial) plus dietary cholesterol supplementation at a 150 mg/kg/d dose. **Comparator** Placebo consisting of the same 1:4 solution of cherry flavor in ORA-Plus, but without simvastatin plus daily oral cholesterol supplementation at a 150 mg/kg dose.
Outcomes	Plasma dehydrocholesterol-to-total sterol ratio Plasma 7DHC levels Plasma cholesterol levels CSF 7-DHC levels Aberrant Behavior Checklist-C (including irritability subscale) Statin-related adverse reactions Anthropometric measures
Notes	**Country**: USA **Funding**: • The intramural research program of the Eunice Kennedy Shriver NICHD • Autism Speaks foundation • The Johns Hopkins Institute for Clinical and Translational Research (ICTR) **COI**: none

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**

Wassif 2017 *(Continued)*

Random sequence generation (selection bias)	Low risk	"Randomization was performed by the Pharmacy Development Service in blocks of four." (i.e. blocked randomization method)
Allocation concealment (selection bias)	Low risk	The Pharmacy Development Service handled randomization and not the investigators, and the placebo used was "indistinguishable from the active product in appearance and taste."
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Neither the participants nor the evaluating physicians knew the assignments."
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	The study fails to mention blinding of outcome assessors and/or data analysts and was clearly stated as being double blinded only. "The study was designed as a randomized, double-blind, placebo-controlled, crossover trial..."
Incomplete outcome data (attrition bias) All outcomes	High risk	The authors did not employ an intention-to-treat analysis, but instead a per-protocol (i.e. as-treated) analysis in which they excluded 3 noncompliant participants (1 in first phase from placebo group; 2 in second phase from simvastatin group) and 1 who developed myopathy (in placebo group in first phase) from their final analysis (S04, S07, S18 and S22), with a failure to impute the missing data.
Selective reporting (reporting bias)	High risk	The authors had not prespecified their intent to collect data on changes in liver transaminase levels, assessment of aggression or irritability or self-injurious behaviors, or anthropomorphic measures under the primary or secondary outcomes outlined in their protocol (NCT 00064792) but reported such data in the subsequent manuscript of their study.
Other bias	Unclear risk	A cross-over design inherently poses a risk of treatment carry-over effect(s). However, the authors implemented a two-month washout period, which exceeds the 6-week acceptable washout period prespecified in our protocol. However, the ABC-C questionnaire was filled by the parents or caregivers, who likely come from different socioeconomic status, education background etc. The authors had not specified whether training on the use of the questionnaire was performed or not, before starting the trial.

ALT: alanine transaminase
AST: aspartate aminotransferase
CK: creatine kinase
COI: conflict of interest
DHC: dehydrocholesterol
GC-MS: gas chromatography–mass spectrometry
HC: head circumference
LDH: lactate dehydrogenase
SLOS: Smith-Lemli-Opitz syndrome
vs: versus

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Aneja 2008	Narrative review article

Study	Reason for exclusion
Correa-Cerro 2005	Narrative review article
Haas 2005	Descriptive study only (i.e. no intervention)
Irons 2004	Narrative review article
Jira 1997a	Case study
Jira 1997b	Narrative review article
Jira 2000	Case study
Jira 2005	Case study
Kelley 2000	Narrative review article
Kilic 2011	Statins not used as an intervention
Linck 1999	Statins not used as an intervention
NCT01434745	This trial would have been eligible for inclusion in our review, but it was terminated early due to poor participant enrollment, as stated on its corresponding clinicaltrials.gov page and also informed by the lead investigator of that trial.
Oláh 2018	Descriptive study only (i.e. no intervention)
Palm 2019	We contacted the study authors in an attempt to obtain relevant unpublished data for inclusion in our review. However, they independently informed us that they no longer had access to such data and are unable to retrieve them.
Pappu 2011	We contacted the study authors in an attempt to obtain relevant unpublished data for inclusion in our review. However, they independently informed us that they no longer had access to such data and are unable to retrieve them.
Prabhu 2016	Not performed in humans
Scalco 2006	Descriptive study only (i.e. no intervention)
Sikora 2004	Statins not used as an intervention
Starck 2002b	Case study
Suzuki 2020	Not performed in humans
Tavori 2015	We contacted the study authors in an attempt to obtain relevant unpublished data for inclusion in our review. However, they independently informed us that they no longer had access to such data and are unable to retrieve them.
Tint 1997	Narrative review article
Ullrich 1996	Statins not used as an intervention
Wright 2002	Not performed in humans

DATA AND ANALYSES

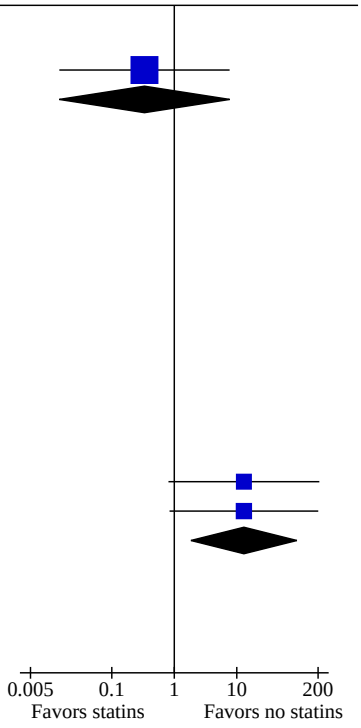
Comparison 1. Statins versus no statins

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Statin-related adverse reactions	4		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.1.1 RCTs	1	36	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.01, 7.68]
1.1.2 Prospective cohort	1	6	Risk Ratio (M-H, Random, 95% CI)	Not estimable
1.1.3 Retrospective cohort	2	44	Risk Ratio (M-H, Random, 95% CI)	13.00 [1.85, 91.48]
1.2 Plasma total dehydrocholesterol (7DHC+8DHC) levels (in mM)	2		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.2.1 RCTs	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.2.2 Retrospective cohort	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.3 Plasma 7DHC levels (in mM)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
1.3.1 Prospective cohort	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
1.4 Plasma total cholesterol levels (in mM)	5		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.4.1 RCTs	1	36	Mean Difference (IV, Random, 95% CI)	-0.39 [-0.81, 0.03]
1.4.2 Prospective cohort	3	38	Mean Difference (IV, Random, 95% CI)	-0.02 [-0.31, 0.27]
1.4.3 Retrospective cohort	1	26	Mean Difference (IV, Random, 95% CI)	Not estimable
1.5 Plasma dehydrocholesterol-to-total cholesterol ratio (in %)	3		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.5.1 RCTs	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.5.2 Prospective cohort	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.5.3 Retrospective cohort	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.6 Plasma CoQ10 levels (in uM)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.6.1 Prospective cohort	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.7 CSF total cholesterol levels (in mM)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.7.1 RCTs	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.8 CSF total dehydrocholesterol (7DHC+8DHC) levels (in mM)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.8.1 RCTs	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.9 CSF dehydrocholesterol-to-total cholesterol ratio (in %)	1		Mean Difference (IV, Random, 95% CI)	Totals not selected
1.9.1 RCTs	1		Mean Difference (IV, Random, 95% CI)	Totals not selected

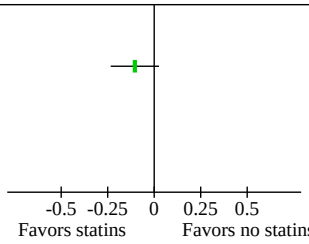
Analysis 1.1. Comparison 1: Statins versus no statins, Outcome 1: Statin-related adverse reactions

Study or Subgroup	Statins Events	Statins Total	No statins Events	No statins Total	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 RCTs						
Wassif 2017	0	18	1	18	100.0%	0.33 [0.01 , 7.68]
Subtotal (95% CI)		**18**		**18**	**100.0%**	**0.33 [0.01 , 7.68]**
Total events:	0		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.69 (P = 0.49)						
1.1.2 Prospective cohort						
Chan 2009	0	3	0	3		Not estimable
Subtotal (95% CI)		**3**		**3**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
1.1.3 Retrospective cohort						
Haas 2007	6	13	0	13	49.3%	13.00 [0.81 , 209.42]
Oláh 2013a	6	9	0	9	50.7%	13.00 [0.84 , 201.26]
Subtotal (95% CI)		**22**		**22**	**100.0%**	**13.00 [1.85 , 91.48]**
Total events:	12		0			
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 1.00); I² = 0%						
Test for overall effect: Z = 2.58 (P = 0.010)						



Analysis 1.2. Comparison 1: Statins versus no statins, Outcome 2: Plasma total dehydrocholesterol (7DHC+8DHC) levels (in mM)

Study or Subgroup	Statins Mean	Statins SD	Statins Total	No statins Mean	No statins SD	No statins Total	Mean Difference IV, Random, 95% CI
1.2.1 RCTs							
Wassif 2017	0.172	0.151	18	0.276	0.237	18	-0.10 [-0.23 , 0.03]
1.2.2 Retrospective cohort							
Haas 2007 (1)	0.161	0	13	0.536	0	13	Not estimable

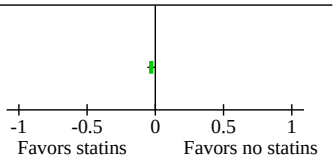


Footnotes

(1) Investigators reported means only (no SDs), but we present the means on this analysis for comparison with the RCT and will include the SDs in future if we ar

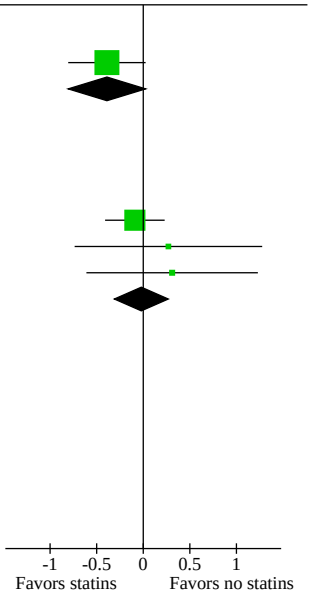
Analysis 1.3. Comparison 1: Statins versus no statins, Outcome 3: Plasma 7DHC levels (in mM)

Study or Subgroup	Statins Mean	Statins SD	Statins Total	No statins Mean	No statins SD	No statins Total	Mean Difference IV, Fixed, 95% CI
1.3.1 Prospective cohort							
Roullet 2012	0.13	0.03	9	0.16	0.03	9	-0.03 [-0.06 , -0.00]



Analysis 1.4. Comparison 1: Statins versus no statins, Outcome 4: Plasma total cholesterol levels (in mM)

Study or Subgroup	Statins Mean	Statins SD	Statins Total	No statins Mean	No statins SD	No statins Total	Weight	Mean Difference IV, Random, 95% CI
1.4.1 RCTs								
Wassif 2017	2.72	0.41	18	3.11	0.8	18	100.0%	-0.39 [-0.81 , 0.03]
Subtotal (95% CI)			**18**			**18**	**100.0%**	**-0.39 [-0.81 , 0.03]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.84 (P = 0.07)								
1.4.2 Prospective cohort								
Roullet 2012	2.95	0.37	9	3.04	0.32	9	81.9%	-0.09 [-0.41 , 0.23]
Haas 2008	2.94	0.85	8	2.67	1.02	6	8.3%	0.27 [-0.74 , 1.28]
Chan 2009	2.77	0.43	3	2.46	0.69	3	9.9%	0.31 [-0.61 , 1.23]
Subtotal (95% CI)			**20**			**18**	**100.0%**	**-0.02 [-0.31 , 0.27]**
Heterogeneity: Tau² = 0.00; Chi² = 1.00, df = 2 (P = 0.61); I² = 0%								
Test for overall effect: Z = 0.14 (P = 0.89)								
1.4.3 Retrospective cohort								
Haas 2007 (1)	2.18	0	13	2.85	0	13		Not estimable
Subtotal (95% CI)			**13**			**13**		**Not estimable**
Heterogeneity: Not applicable								
Test for overall effect: Not applicable								
Test for subgroup differences: Chi² = 2.05, df = 1 (P = 0.15), I² = 51.1%								

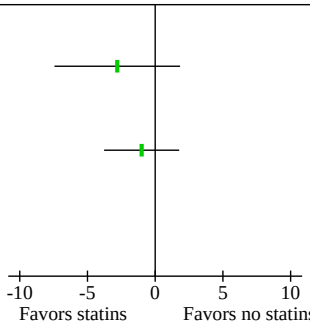


Footnotes

(1) Investigators reported means only (no SDs), but we present the means on this analysis for comparison with the other studies and will include the SDs in future if we are a

Analysis 1.5. Comparison 1: Statins versus no statins, Outcome 5: Plasma dehydrocholesterol-to-total cholesterol ratio (in %)

Study or Subgroup	Statins Mean	Statins SD	Statins Total	No statins Mean	No statins SD	No statins Total	Mean Difference IV, Random, 95% CI
1.5.1 RCTs							
Wassif 2017	6.1	5.5	18	8.9	8.4	18	-2.80 [-7.44 , 1.84]
1.5.2 Prospective cohort							
Roullet 2012	11	3	9	12	3	9	-1.00 [-3.77 , 1.77]
1.5.3 Retrospective cohort							
Haas 2007 (1)	7	0	13	19	0	13	Not estimable

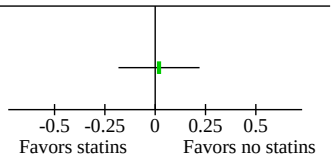


Footnotes

(1) Investigators reported means only (no SDs), but we present the means on this analysis for comparison with the other studies and will include the SDs in future

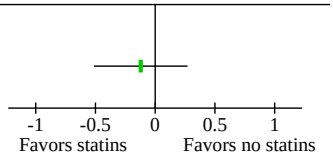
Analysis 1.6. Comparison 1: Statins versus no statins, Outcome 6: Plasma CoQ10 levels (in uM)

Study or Subgroup	Statins Mean	Statins SD	Statins Total	No statins Mean	No statins SD	No statins Total	Mean Difference IV, Random, 95% CI
1.6.1 Prospective cohort							
Haas 2008	0.641	0.21	8	0.622	0.174	6	0.02 [-0.18 , 0.22]



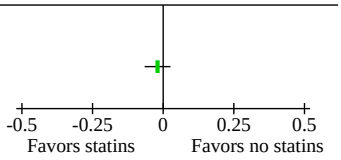
Analysis 1.7. Comparison 1: Statins versus no statins, Outcome 7: CSF total cholesterol levels (in mM)

Study or Subgroup	Statins Mean	Statins SD	Statins Total	No statins Mean	No statins SD	No statins Total	Mean Difference IV, Random, 95% CI
1.7.1 RCTs							
Wassif 2017	2.074	0.502	18	2.195	0.683	18	-0.12 [-0.51 , 0.27]

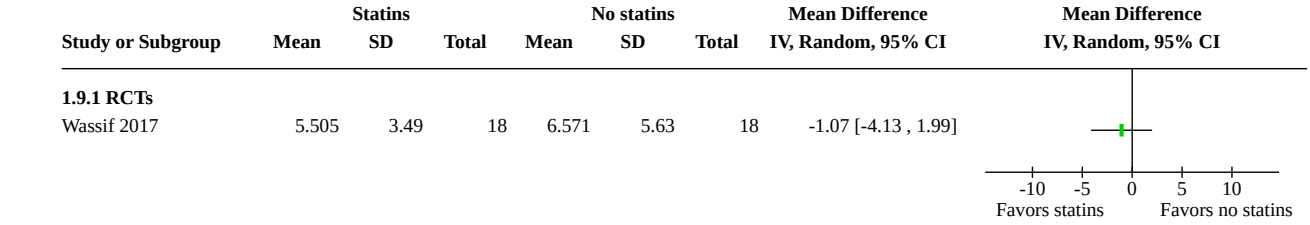


Analysis 1.8. Comparison 1: Statins versus no statins, Outcome 8: CSF total dehydrocholesterol (7DHC+8DHC) levels (in mM)

Study or Subgroup	Statins Mean	Statins SD	Statins Total	No statins Mean	No statins SD	No statins Total	Mean Difference IV, Random, 95% CI
1.8.1 RCTs							
Wassif 2017	0.103	0.0574	18	0.123	0.0805	18	-0.02 [-0.07 , 0.03]



Analysis 1.9. Comparison 1: Statins versus no statins, Outcome 9: CSF dehydrocholesterol-to-total cholesterol ratio (in %)



ADDITIONAL TABLES

Table 1. Characteristics of included studies

Study	Design	Participants/Population	Intervention	Comparator/Control	Outcomes Assessed
Wassif 2017 Country: USA Funding: - The intramural research program of the Eunice Kennedy Shriver NICHD - Autism Speaks foundation - The Johns Hopkins Institute for Clinical and Translational Research (ICTR) COI: none	Randomized controlled cross-over trial consisting of two 12-month periods separated by a 2-month washout period	22 children (13 males and 9 females), ages 4.0 to 17.5 years (mean age = 8.2 years), with biochemically diagnosed mild to moderate SLOS (i.e. SLOS severity score < or = 30), having demonstrated residual DHCR7 function (defined as having residual fibroblast cholesterol synthesis that exceeds 10% that of healthy controls) were included in the study. However, only 18 of them were included in the final analysis.	Simvastatin (administered orally in a 1:4 solution of cherry flavor in ORA-Plus,) given at 0.5 mg/kg/d for the first 6 weeks of the trial, and then at 1.0 mg/kg dose for the remainder of the trial) + dietary cholesterol supplementation at a 150 mg/kg/d dose.	Placebo consisting of the same 1:4 solution of cherry flavor in ORA-Plus, but without simvastatin, + daily oral cholesterol supplementation at a 150 mg/kg dose.	Plasma dehydrocholesterol-to-total sterol ratio: decreased from 8.9 +/- 8.4% on placebo to 6.1 +/- 5.5% on simvastatin (P < 0.005). Plasma 7DHC levels: decreased from 106 +/- 91 ug/ml on placebo to 66 +/- 58 ug/ml on simvastatin (P < 0.001). Plasma cholesterol levels: decreased from 120 +/- 31 mg/dl on placebo to 105 +/-16 mg/dl on simvastatin (P < 0.005). CSF 7-DHC levels: a trend of decrease after simvastatin treatment (P = 0.07). Reduction in the severity of the irritability subscale of the Aberrant Behavior Checklist-C (P = 0.017). Statin-related adverse reactions: none reported. No significant changes in anthropometric measures (P = 0.76 for weight; P = 0.42 for height).
Chan 2009 Country: Canada & USA Funding:	Prospective cohort study consisting of an initial 3-year period of high-	12 children (5 males and 7 females), ages 1.1 to 15.7 years, with biochemically and/or genetically	Nine out of the 12 enrolled participants were treated with high-dose oral cholesterol supplementation, either	The 3 remaining participants of the initial 12 participants enrolled in the study were	Plasma cholesterol levels unchanged (2.46 +/- 0.69 mM vs 2.77 +/- 0.43 mM in the high-dose cholesterol supplementation vs high-dose cholesterol supplementation and

Table 1. Characteristics of included studies *(Continued)*

- NIH [R01 HL073980] - The Oregon Clinical and Translational Research Institute (OCTRI) - Grant number UL1 RR024140 from the National Center for Research Resources (NCRR) COI: not reported	dose cholesterol supplementation, followed by an additional 0.8 years of simvastatin + high-dose cholesterol supplementation	diagnosed SLOS, without a preset requirement of a specific SLOS severity score, were included in the study.	in a food-based form (at a mean concentration of 34.5 mg/kg/d) or crystalline form (suspended in ORA-Plus, at a mean concentration of 47 mg/kg/d) for a period of 3 years. 3 of the 9 participants initially maintained on high-dose dietary cholesterol supplementation (2 males and 1 female) were subsequently treated for an additional 0.8 years, with a daily oral combination therapy of high-dose cholesterol supplementation and simvastatin (administered at a dose of 0.2 mg/kg to 0.4 mg/kg, depending on participant tolerance and plasma sterol profiles).	maintained on a low cholesterol diet (defined as oral cholesterol intake of 0.5 to 5 mg/kg/day) for 4 weeks and served as the control group against which the fractional cholesterol synthesis rate of the other 9 participants maintained on a high cholesterol diet was compared to. However, for the sake of our review, we will only report the data for the 3 participants who were treated with high-dose cholesterol supplementation + simvastatin for 0.8 years and were then compared to their own selves during their previous enrollment in the high-dose cholesterol supplementation arm only, for 3 years.	simvastatin groups, respectively; P = 0.5). Statin-related adverse reactions: none reported.
Haas 2007 Country: Germany Funding: not reported COI: none	Retrospective cohort study with pre-post data comparison	39 adults and children (22 males and 17 females), ages 3.03 to 265 months, with biochemically diagnosed SLOS via GC-MS (defined as detecting plasma 7DHC > 0.11mg/dL or 7DHC/Chol > 0.002), with or without subsequent diagnostic confirmation	35 out of the 37 included participants were treated with high-dose oral cholesterol supplementation, either in a food-based form (i.e. egg-yolk in 7 participants, at a mean concentration of 40 mg/kg/d) or crystalline form (28 participants, at a mean concentration of 100 mg/kg/d in children, and 40 mg/kg/d	For the sake of our review, we will only include the data of the 13 participants (4 females and 9 males) who had a been receiving cholesterol supplementation and subsequently switched to cholesterol and	Plasma dehydrocholesterol-to-cholesterol ratio: decreased from 0.19 on cholesterol only, to 0.07 on cholesterol + simvastatin (P = 0.005). Plasma 7+8DHC levels: decreased from 20.6 mg/dL on cholesterol only, to 6.2 mg/dL on cholesterol + simvastatin (P < 0.001). Plasma cholesterol levels: decreased from 110 mg/dL

Table 1. Characteristics of included studies *(Continued)*

		with genetic testing, but without a preset requirement of a specific SLOS severity score, were enrolled in the study. 2 of these participants were subsequently excluded due to noncompliance for receiving simvastatin only, without simultaneous cholesterol supplementation.	in adults) for a median follow-up period of 52 months (range: 6 to 131 months). Among the latter participants, only 13 of them with mild SLOS, and normal transaminase and CK levels were subsequently co-administered simvastatin (at a dose of 0.5 mg/kg/d for the first 4 weeks of the study, followed by 1.0 mg/kg/d for the remainder of the study).	simvastatin combination therapy, comparing their data before and after the introduction of simvastatin therapy.	on cholesterol only, to 84 mg/dL on cholesterol + simvastatin (P = 0.008). Statin-related adverse reactions: none of the 24 participants receiving cholesterol only were reported to have side effects, vs 6/13 participants receiving cholesterol supplementation with simvastatin experienced statin-related adverse reactions: 1 participant (participant 10) had AST elevations to 200 U/L, 3 (participants 19, 64, 69) developed sleep disturbances (with participant 19 also developing anxiety that resolved on statin discontinuation), and 2 (participants 31 and 65) had worsening of self-mutilating behaviors and auto-aggression. No significant changes in anthropometric measures (P = 0.44 for weight, 0.52 for height, and 0.2 for head circumference).
Haas 2008 Country: Germany Funding: not reported COI: not reported	Prospective cohort study (conducted between 2005 and 2007)	19 children (11 males and 8 females), ages 0.3 to 13 years, with biochemically diagnosed SLOS via GC-MS (defined as detecting plasma 7DHC > 0.11mg/dL or 7DHC/Chol > 0.002), were included in the study, with subsequent diagnostic confirmation via genetic testing in 17 of these participants. Among the included participants, 5 had both their plasma and platelet CoQ10 levels measured, while the remaining 9 had only their plasma CoQ10 levels assayed.	8 of the 14 participants whose plasma CoQ10 levels were assayed had received combination therapy with cholesterol supplementation (at a mean dose of 107 mg/kg/d) and simvastatin treatment (at a dose of 1.0 mg/kg/d).	The remaining 6 participants had received cholesterol supplementation only (at a mean dose of 107 mg/kg/d).	No significant change in plasma CoQ10 levels: 0.622 +/- 0.174 umol/L in the cholesterol-only group vs 0.641 +/- 0.210 umol/L in the combined cholesterol + simvastatin group (P = 0.9). No significant change in plasma cholesterol levels: 2.67 +/- 1.02 mmol/L in the cholesterol only group vs 2.94 +/- 0.85 mmol/L in the combined cholesterol + simvastatin group (P = 0.6).

Table 1. Characteristics of included studies *(Continued)*

Oláh 2013a Country: Hungary Funding: the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 project COI: none	Retrospective cohort study	15 children (8 males and 7 females), ages 0.1 to 18 years, with biochemically diagnosed SLOS via UV spectrophotometric assay of plasma 7DHC levels, without a preset requirement of a specific SLOS severity score, were included in the study.	9 of the 15 included participants (6 males and 3 females) were treated with a high-dose oral cholesterol and statin (simvastatin or atorvastatin) combination therapy, with the cholesterol being administered in a crystalline form (at a mean dose range of 50 mg/kg/d to 250 mg/kg/d), and statin given at a mean dose range of 0.2 mg/kg/d to 0.4 mg/kg/d. However, statin therapy was discontinued in 5 of these 9 participants after they experienced statin-related adverse reactions. Therefore, for the sake of our review, we will only report the data for the 4 participants who received high-dose cholesterol supplementation + statin combination therapy, comparing them to their own selves during their previous enrollment in the high-dose cholesterol supplementation stage only.	10 out of the 15 included participants were treated with high-dose oral cholesterol supplementation in a crystalline form (at a mean dose range of 50 mg/kg/d to 250 mg/kg/d). However, only 9 of those went on to receive add-on statin therapy, of which 5 discontinued the latter therapy due to side effects.	Statin-related adverse reactions: 5 out of the 9 participants treated with statins; 3 had remarkable increases in AST/ALT levels, one had an additional remarkable increase in LDH levels, while the remaining participant had a remarkable increase in CK levels. No data collected sequentially on changes in plasma cholesterol and/or 7DHC levels (only baseline/pre-treatment levels were measured).
Roullet 2012 Country: USA Funding: - NIH [R01 HL073980] - The Oregon Clinical and Translational Research Institute (OCTRI) - Grant number UL1 RR024140 from the National Center for Research	Prospective cohort study with pre-post data comparison	19 children (9 males and 10 females), ages to years (mean 6.1 +/- 1.5 years), with biochemically diagnosed and genetically confirmed diagnosis of mild or moderate SLOS, all maintained on high-dose cholesterol supplementation, were included in the study.	9 of the 19 included participants (6 males and 3 females), with a mean age of 5.4 +/- 2.0 years, agreed to take simvastatin (mean dosage of 0.23 mg/kg/d) in addition to high-dose cholesterol supplementation (mean dosage of 444 +/- 55 mg/d), for 1.2 +/- 0.2 years. For the sake of our review, only these 9 participants are relevant for inclusion.	The data for each participant prior to statin introduction, when taking only high-dose cholesterol supplementation (mean dosage of 389 +/- 44 mg/d), will be used as the comparator.	Plasma dehydrocholesterol-to-cholesterol ratio: decreased from 0.12 +/- 0.03 in the high-dose cholesterol supplementation group to 0.11 +/- 0.03 in the combination therapy (cholesterol + simvastatin) group (P > 0.05). Plasma 7DHC levels: decreased from 0.16 +/- 0.03 mM in the high-dose cholesterol supplementation group to 0.13 +/- 0.03 mM in the combination therapy (cholesterol + simvastatin) group (P > 0.05). Plasma 8DHC levels: decreased from 0.15 +/- 0.03 mM in the high-dose cholesterol supplementation group

Table 1. Characteristics of included studies *(Continued)*

	Resources (NCRR) COI: not reported		to 0.12 +/- 0.02 mM in the combination therapy (cholesterol + simvastatin) group (P > 0.05) Plasma cholesterol levels decreased from 3.04 +/- 0.32 mM in the high-dose cholesterol supplementation group to 2.95 +/- 0.37 mM in the combination therapy (cholesterol + simvastatin) group (P > 0.05).

7DHC: 7-dehydrocholesterol; 8DHC: 8-dehydrocholesterol; ALT: alanine transaminase; AST: aspartate aminotransferase; COI: conflicts of interest; CK: creatine kinase; CoQ10: coenzyme Q10; CSF: cerebral spinal fluid; GC-MS: gas chromatography–mass spectrometry; LDH: lactate dehydrogenase; SLOS: Smith-Lemli-Opitz syndrome; UV: ultraviolet; vs: versus.

Table 2. All data extracted from included studies

Outcome		Wassif 2017	Chan 2009	Haas 2007	Haas 2008	Oláh 2013a	Roullet 2012
Changes in neurobehavioral manifestations		"Significant reduction in the irritability subscale of ABC-C"					
Statin-related adverse reactions		1/22 in placebo group	None	6/13 (46 %)		5/9 (56 %)	
Changes in anthropometric measures		"No significant changes"		"No significant changes"			
Plasma cholesterol (mM) mean (SD)	Control	3.11 (0.8)	2.46 (0.69)	2.85	2.67 (1.02)		3.04 (0.32)
	Statins	2.72 (0.41)	2.77 (0.43)	2.18	2.94 (0.85)		2.95 (0.37)
Plasma 7DHC + 8DHC (mM) mean (SD)	Control	0.276 (0.237)		0.536			
	Statins	0.172 (0.151)		0.161			
Plasma 7DHC (mM) mean (SD)	Control		0.23 (0.089)				0.16 (0.03)
	Statins		Not reported				0.13 (0.03)
Plasma 8DHC (mM) mean (SD)	Control						0.15 (0.03)
	Statins						0.12 (0.02)
Plasma dehydrocholesterol/sterol ratio (%) mean (SD)	Control	8.9 (8.4)		19			12 (3)
	Statins	6.1 (5.5)		7			11 (3)
CSF Cholesterol	Control	"No significant changes"					
	Statins						
CSF 7DHC	Control	"Trend of decrease"					
	Statins						
CSF dehydrocholesterol/sterol ratio	Control	"No significant changes"					

Table 2. All data extracted from included studies *(Continued)*

	Statins	
CoQ10 levels (uM) mean (SD)	Control	0.622 (0.174)
	Statins	0.641 (0.21)

7DHC: 7-dehydrocholesterol; 8DHC: 8-dehydrocholesterol; ABC-C: Aberrant Behavior Checklist-Community; CSF: cerebral spinal fluid; SD: standard deviation

Table 3. Risk of bias in RCTs

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete/Missing outcome data	Selective Reporting	Other biases
Wassif 2017	**Low risk** "Randomization was performed by the Pharmacy Development Service in blocks of four." (i.e. blocked randomization method)	**Low risk** The Pharmacy Development Service handled randomization and not the investigators, and the placebo used was "indistinguishable from the active product in appearance and taste."	**Low risk** "Neither the participants nor the evaluating physicians knew the assignments."	**Unclear risk** The study fails to mention blinding of outcome assessors or data analysts and was clearly stated as being double blinded only. "The study was designed as a randomized, double-blind, placebo-controlled, crossover trial..."	**High risk** The authors did not employ an intention-to-treat analysis, but instead a per-protocol (i.e. as-treated) analysis in which they excluded 3 noncompliant participants and one who developed myopathy from their final analysis (S04, S07, S18 and S22), with a failure to impute the missing data.	**High risk** The authors had not prespecified their intent to collect data on changes in liver transaminase levels, assessment of aggression or irritability or self-injurious behaviors, or anthropomorphic measures under the primary or secondary outcomes outlined in their protocol (NCT00064792), but reported such data in the subsequent manuscript of their study.	**Unclear risk** A cross-over design inherently poses a risk of treatment carry-over effect(s). However, the authors implemented a 2-month washout period, which exceeds the 6-week acceptable washout period prespecified in our protocol. The ABC-C questionnaire was filled by the parents or caregivers, who likely come from different socioeconomic status, education background etc. The authors had not specified whether training on the use of the questionnaire was performed or not, before the trial.

ABC-C: Aberrant Behavior Checklist-Community

Table 4. ROBINS-I for statin-related adverse reactions

Study	Pre-intervention		At or during intervention	Post-intervention			
	Confounding bias	**Selection bias**	**Bias in classification of intervention**	**Deviations from intended intervention(s)**	**Bias in outcome measurement**	**Incomplete or missing outcome data**	**Selective Reporting (i.e. Reporting Bias)**
Chan 2009	**Serious risk** The 3 participants receiving statin therapy had different disease severity at baseline (S06 had mild SLOS, vs S07 and S08 who had moderate SLOS), raising concern for baseline confounding. However, due to the dichotomous nature of this outcome and its specific association with statin therapy only, and not cholesterol supplementation alone, we do not expect remarkable time-varying confounding bias for this outcome.	**Moderate risk** The authors do not specify whether individuals recruited into the study were recently diagnosed cases or instead, were participants who had been followed up for a long period of time prior to enrollment in the study. Moreover, the follow-up durations of the 3 participants included in the study and receiving simvastatin were inconsistent.	**Moderate risk** The doses of simvastatin given to the 3 participants were adjusted throughout the study, depending on their corresponding plasma sterol levels. However, due to the prospective follow-up conducted on these participants after statin initiation, along with the dichotomous and largely idiosyncratic nature of this outcome, we do not expect a significant degree of bias in classification of intervention for this outcome.	**Low risk** Cholesterol supplementation, a key co-intervention in the study, was balanced in the 3 participants receiving simvastatin. Moreover, despite having different follow-up times post-statin initiation, it is not suggested in the study that the 3 participants had any deviations or interruptions in their intended treatment.	**Low risk** Participants, outcome assessors, and study investigators were all aware of the intervention assignment of the included participants (i.e. no blinding). However, because of the objective measurement nature for this outcome, via measuring plasma levels of liver enzymes and CK, it is unlikely for this prior knowledge of intervention assignment to have had a significant impact on this outcome (i.e. negligible assessor judgement bias).	**Low risk** Data were reasonably complete for the 3 relevant participants.	**Moderate risk** The authors narratively reported that liver transaminase and CK levels remained normal in all 3 participants receiving statin therapy. However, the corresponding numerical data were not included in the study report and the authors could not retrieve this data upon our request. Moreover, the study did not have a prepublished protocol or plan available for inspection.
Haas 2007	**Serious risk**	**Serious risk**	**Serious risk**	**Critical risk**	**Serious risk**	**Moderate risk**	**Serious risk**

Table 4. ROBINS-I for statin-related adverse reactions *(Continued)*

	The study employs a retrospective design which minimizes the ability to control for various important baseline confounders (age, disease severity, distribution of intervention, etc.).	3 participants were already receiving a combination of cholesterol supplementation and statin therapy prior to enrollment in the study and were subsequently chosen to continue with that intervention, raising strong suspicion for prevalent user bias. The authors did not employ a sensitivity analysis to test the effect that excluding such participants from the analysis would have on the assessed outcomes.	The study has a retrospective design and the intervention was only given to participants with a mild biochemical phenotype (defined as a dehydrocholesterol-to-cholesterol ratio < 0.5), with cholesterol supplementation being started for a variable period in each participant before add-on statin therapy. "They received cholesterol only until their (7+8–DHC)/cholesterol ratio fell below 0.5. Simvastatin was then started with a dosage of 0.5 mg/kg per day and increased to 1.0 mg/kg per day after 4 weeks when transaminases and CK remained normal."	The dosages (range 40 mg/kg/d to 180 mg/kg/d) and formulations of cholesterol (egg yolk versus crystalline cholesterol) administered to participants were inconsistent. 3 participants were already receiving combination therapy for an unspecified duration before inclusion in the study. 2 participants were excluded from the analysis because of noncompliance by receiving only statin therapy, without cholesterol supplementation. Moreover, 6 participants receiving combination therapy discontinued their statin treatment shortly after initiation due to side effects, with resumption at different intervals, keeping cholesterol supplementation as the only 'intervention' in the interim.	Given its retrospective nature, the investigators of the study were not blinded regarding the interventions received by each participant. Moreover, the assessors of this outcome (the parents of participants) were also aware of the interventions received by their children. Additionally, since data collection occurred in the past, it is very likely that outcome measurement was not standardized between participants.	3 of the 13 participants receiving combination therapy were receiving cholesterol plus simvastatin prior to inclusion in the study (participants 14, 62 and 63), without data on this outcome during that interval, posing a high risk of carry-over effects that are unaccounted for in the study. Additionally, 2 participants were excluded from the analysis because of noncompliance.	While the authors of this study reported all relevant outcomes in their published report, the retrospective nature of the study means that outcomes and analyses were not prespecified or precontemplated. As such, we cannot exclude the possibility of selective data analysis and outcome reporting in this study especially pertaining to the likelihood of over-reporting statin-related adverse reactions since treatment status of participants is already known.
Oláh 2013a	**Serious risk** The 9 participants receiving statin therapy in	**Serious risk** The participants were all receiving cho-	**Moderate risk** The study is of a retrospective design and all	**Serious risk** The dosages (range 50 mg/kg/d to 250 mg/kg/d) of supplemental cholesterol	**Serious risk** Given its retrospective nature, the investigators of the	**Critical risk** The PI of the study informed us that an im-	**Serious risk** While the authors of this study reported all rele-

Table 4. ROBINS-I for statin-related adverse reactions *(Continued)*

	this study had different disease severities (4 had mild SLOS and 5 had moderate SLOS) and were all receiving cholesterol supplementation for different durations (longer in those with moderate SLOS) prior to statin initiation. The participants with moderate SLOS were generally younger than those with mild disease (due to earlier diagnosis), further raising concern for baseline confounding. However, due to the dichotomous nature of this outcome and its specific association with statin therapy only, and not cholesterol supplementation, we do not expect remarkable time-varying confounding bias.	lesterol supplementation prior to enrollment in the study, raising strong suspicion for prevalent user bias. Moreover, the follow-up durations of participants were highly inconsistent, with those having higher disease severity being followed up for longer durations. "The duration of therapy was longer (2.9 ± 2.6 years) in the moderate group compared to the mild SLOS (0.8 ± 1 year)"	participants were receiving cholesterol supplementation for different durations prior to add-on statin therapy.	administered to participants were inconsistent. All 9 participants were already receiving cholesterol supplementation for variable durations prior to inclusion in the study. During the study, 5 out of 9 participants (i.e. > 50%) discontinued statin therapy after experiencing significant side effects. Moreover, the PI of the study informed us that all participants eventually discontinued statin therapy due to liver impairment, posing a serious risk of bias in terms of assessing adherence to statin therapy compared to cholesterol supplementation only. Finally, the duration of statin therapy was longer in participants with moderate disease compared to those with mild disease, introducing an imbalance within the treatment arm.	study were not blinded regarding the interventions received by each participant. However, because of the objective nature for this outcome (measuring plasma levels of liver enzymes, LDH and CK, it is unlikely that such prior knowledge of intervention assignment to have a significant impact on outcome measurement (i.e. negligible assessor judgement bias). Nonetheless, since people with moderate SLOS were treated with statins for a longer duration than those with mild SLOS, it is likely that the significantly higher levels of liver enzymes reported by the study for people with moderate SLOS, compared to mild SLOS, was related to this longer duration of treatment, apart from the worse disease severity.	portant duration of follow-up is missing from the analysis, because many of the included participants relocated or were lost to follow-up. As a result, neither plasma cholesterol and 7DHC levels, nor individual clinical status could be monitored over time following study initiation. The nature of such missing data means that no meaningful comparison can be made between the levels of key biomarkers of the disease before and after statin therapy. Moreover, study participants were all receiving cholesterol supplementation prior to enrollment in the study without data on this outcome during that interval, posing a high risk of carry-over effects that are unaccounted for in the study.	vant data in their published report for this outcome, the retrospective nature of the study means that outcomes and analyses were not prespecified or pre-contemplated. As such, we cannot exclude the possibility of selective data analysis and outcome reporting in this study especially pertaining to the likelihood of over-reporting statin-related adverse reactions since treatment status of participants is already known.

Table 4. ROBINS-I for statin-related adverse reactions *(Continued)*

					Finally, several outcomes planned to be assessed by the study were incompletely or not measured at all.		

7DHC: 7-dehydrocholesterol; CK: creatine kinase; LDH: lactate dehydrogenase; PI: principal investigator; ROBINS-I: Risk Of Bias In Non-randomized Studies of Interventions; SLOS: Smith-Lemli-Opitz syndrome

Table 5. ROBINS-I for changes in plasma or CSF biomarker levels (or both)

Study	Pre-intervention		At or during intervention	Post-intervention			
	Confounding bias	**Selection bias**	**Bias in classification of intervention**	**Deviation from intended intervention(s)**	**Bias in outcome measurement**	**Incomplete or missing outcome data**	**Selective Reporting (i.e. reporting bias)**
Chan 2009	**Critical risk** The 3 participants receiving statin therapy had different disease severity at baseline (S06 mild vs S07 and S08 had moderate SLOS). They had also been maintained on a high cholesterol diet for 3 years prior to initiating statin therapy, posing a risk for a time-varying confounding bias, along with baseline confounding caused by the different disease	**Moderate risk** The authors do not specify whether participants were recently diagnosed or had been followed up for a long period of time prior to enrollment in the study. Moreover, the follow-up durations of the 3 participants receiving simvastatin were inconsistent.	**Serious risk** "The dose of simvastatin was gradually increased from 0.2 mg/kg to a maximum of 0.4 mg/kg, based on effects of the medication on plasma sterols in patients with SLOS." This raises a high likelihood of having unbalanced interventions with direct effects on this outcome across the 3 participants.	**Moderate risk** The dosage of cholesterol supplementation, an important co-intervention in the study that can markedly impact this outcome, was unbalanced among the 3 participants who received simvastatin (cholesterol doses ranged from 27.7 mg/kg/d to 37.4 mg/kg/d). Likewise, the dosages of simvastatin fluctuated throughout the study depending on plasma sterol levels of the participants, constituting an unusual deviation from practice.	**Serious risk** Since the doses of cholesterol supplementation were inconsistent across the 3 participants, and their corresponding simvastatin dosages were regularly adjusted during the study based on their plasma sterol levels, there is a high likelihood of errors in outcome measurement directly related to the intervention status (e.g. statin dosage).	**Low risk** Data were reasonably complete for the 3 relevant participants.	**Serious risk** While study authors assessed changes in the plasma sterol levels altogether, they only reported the mean and SD values for total cholesterol in pre- vs post-statin treatment. The authors did not report the mean and SD values of plasma 7-

Table 5. ROBINS-I for changes in plasma or CSF biomarker levels (or both) *(Continued)*

	severity of participants.						DHC levels, as planned; instead, they narratively stated "a significant decrease in plasma 7DHC concentrations in subjects receiving simvastatin".
Haas 2007	**Critical risk** 3 out of 13 participants treated with cholesterol and simvastatin combination therapy (participants 14, 62 and 63) were receiving cholesterol plus simvastatin prior to enrollment, posing a high risk of residual confounding and carry-over effects that were unaccounted for in the study. In addition, the study employs a retrospective design which minimizes the ability to control for various baseline confounders (age, disease severity, distribution of intervention, etc.).	**Serious risk** 3 participants were already receiving a combination of cholesterol supplementation and statin therapy prior to enrollment in the study and were subsequently chosen to continue with that intervention, raising strong suspicion for prevalent user bias. The authors did not employ a sensitivity analysis to test the effect that excluding such participants from the analysis could have	**Serious risk** The study has a retrospective design and the intervention was only given to participants with a mild biochemical phenotype (defined as a dehydrocholesterol-to-cholesterol ratio < 0.5), with cholesterol supplementation being started for a variable period in each participant, before add-on statin therapy. "They received cholesterol only until their (7+8–DHC)/cholesterol ratio fell below 0.5. Simvastatin was then started with a dosage of 0.5 mg/kg per day and increased to 1.0 mg/kg per day after 4 weeks when transaminases and	**Critical risk** The dosages (range 40 mg/kg/d to 180 mg/kg/d) and formulations of cholesterol (egg yolk versus crystalline cholesterol) administered to participants were inconsistent. 3 participants were already receiving combination therapy for an unspecified duration before inclusion in the study. 2 participants were excluded from the analysis because of noncompliance by receiving only statin therapy, without cholesterol supplementation. Moreover, 6 participants receiving combination therapy discontinued their statin treatment shortly after initiation due to side effects, with resumption at different intervals and cholesterol supplementation as the only 'intervention' in the interim.	**Moderate risk** Given its retrospective nature, the investigators of the study were not blinded regarding the interventions received by each participant. However, since this outcome entails objectively measured biochemical parameters (e.g. plasma 7DHC and cholesterol levels), we expect negligible assessor judgement bias for this specific outcome, since prior knowledge of intervention assignment has minimal if any influence on this outcome.	**Moderate risk** 3 of the 13 participants receiving combination therapy were receiving cholesterol plus simvastatin prior to inclusion in the study (participants 14, 62 and 63), without data on this outcome during that interval, posing a high risk of carry-over effects that are unaccounted for in the study. Additionally, 2 participants were excluded from the analysis because of noncompliance.	**Moderate risk** While the study authors reported all relevant outcomes in their published report, the retrospective nature of the study means that outcomes and analyses were not prespecified or precontemplated. As such, we cannot exclude the possibility of selective data analysis and outcome reporting in this study.

Table 5. ROBINS-I for changes in plasma or CSF biomarker levels (or both) *(Continued)*

		had on this outcome.	CK remained normal."				
Haas 2008	**Critical risk** The PI confirmed to us that participants receiving statin therapy all had mild phenotype (defined as a low DHC/cholesterol ratio), which was not a preset criteria for those receiving cholesterol supplementation. This raises strong suspicion for baseline confounding with regards to including participants with different disease severities among those receiving cholesterol supplementation only. Moreover, some participants were already receiving cholesterol supplementation or combination therapy prior to inclusion in the study, posing a high likelihood of time-varying confounding bias.	**Serious risk** The authors do not provide sufficient information regarding participant recruitment process, which as per the PI, likely included a combination of recently-diagnosed and followed-up participants. The PI of the study also informed us that some participants were already receiving cholesterol supplementation or simvastatin (or a combination of both) prior to enrollment in the study, posing a risk of prevalent user bias.	**Serious risk** Data were collected prospectively for all participants, i.e. following individual enrollment in the study. However, because the formulation and dosages of cholesterol used in the study were not clearly defined in the report and likely varied between included participants, yielding a vaguely defined "intervention" status in the included participants.	**Serious risk** The dosages and formulations of cholesterol administered to the study participants were not clearly defined and appeared to be inconsistent. "Ten of these patients received cholesterol supplementation only (mean dosage 107 mg/kg/d)". Moreover, all participants receiving statins necessarily had mild SLOS (defined as a low DHC/cholesterol ratio), while those receiving cholesterol only likely included participants of different disease severities, which constitutes a key variable between the intervention groups that is known to independently associate with this specific outcome.	**Low risk** Most participants were maintained on the same therapy they had been receiving prior to study enrollment, making it very likely that study investigators and outcome assessors were aware of the intervention status of each participant. Nonetheless, because this specific outcome includes objectively measured biochemical parameters (e.g. plasma CoQ10 and cholesterol levels), we expect negligible assessor judgement bias for this specific outcome, since prior knowledge of intervention assignment has minimal, if any, influence on this outcome.	**Serious risk** Study included 19 participants; 10 were allocated to cholesterol supplementation only, and the remaining 9 were allocated to combination therapy (cholesterol and simvastatin). Data were only reported for 8 of the 10 participants receiving cholesterol supplementation and 6 of 9 participants receiving combination therapy. No justification was given for excluding data from 5 participants.	**Serious risk** The authors did not report any data pertaining to vitamin A levels, an outcome stated to have been assessed in the methods section. In addition, the authors reported mean plasma cholesterol and CoQ10 levels for the cholesterol only versus combination therapy group, without reporting their respective data on plasma 7DHC and vitamin E levels, which they also planned to measure. Moreover, the study did not have a prepublished protocol or plan available for inspection.
Roullet 2012	**Critical risk**	**Serious risk**	**Serious risk**	**Critical risk**	**Serious risk**	**Low risk**	**Low risk**

Table 5. ROBINS-I for changes in plasma or CSF biomarker levels (or both) *(Continued)*

	The included participants had different disease severities at baseline, ranging from mild to moderate SLOS, and had been all maintained on a high-cholesterol diet for variable durations prior to initiating add-on statin therapy. This poses a risk for time-varying confounding bias, as well as baseline confounding arising from the lumping of participants with different disease severities together. The study also included negative controls, further raising suspicion for uncontrolled confounding.	Participants had all been maintained on high-cholesterol diets for variable durations prior to inclusion in the study, posing a risk of prevalent user bias. Moreover, the follow-up durations of the 9 participants receiving simvastatin were inconsistent.	Data were collected prospectively for all participants, i.e. following individual enrollment in the study. However, because the dosage of cholesterol supplemented after statin introduction was higher than that given during cholesterol monotherapy only (with the individual dosages also being highly variable), the interventions were unbalanced and there was only an overall vague definition of their "intervention" status. "The Cholesterol intake was 444±55 mg/day and 389±44 mg/day for the 'simvastatin' and 'No simvastatin' groups respectively."	The dosage of cholesterol administered to the participants receiving combination therapy was higher than that given to them during cholesterol monotherapy. The authors did not justify the reasons for such an imbalance in cholesterol supplementation before vs after statin introduction. Additionally, some participants did not undergo the 4-week washout period of a low-cholesterol diet prior to beginning combined therapy. This raises strong suspicion for unbalanced co-interventions between study participants, which are likely to impact this specific outcome. Moreover, all 9 participants were receiving cholesterol supplementation, of variable dosages and durations, prior to inclusion in the study.	Despite its prospective nature, the study investigators were aware of the intervention assignment of each participant (i.e. no blinding) since consent had to be obtained prior to adding a statin to the participant's current cholesterol supplementation. However, because this outcome is an objectively measured biochemical parameter (e.g. plasma 7DHC and cholesterol levels), it is unlikely that such prior knowledge of intervention assignment to significantly impact the measured parameters. Nonetheless, because the dosage and durations of cholesterol supplementation pre- and post-statin introduction were inconsistent, there is a high likelihood of systemic errors in outcome measurement (e.g. overestimation of plasma cholesterol level since a higher mean dose of cholesterol was given after statin introduction).	Data were reasonably complete for the 9 participants.	The authors reported all relevant data for the 9 participants, most of which were actually negative findings (i.e. non-significant).

7-DHC: 7-dehydrocholesterol; CoQ10: CoQ: coenzyme Q10; PI: principal investigator; ROBINS-I: Risk Of Bias In Non-randomized Studies of Interventions; SD: standard deviation; SLOS: Smith-Lemli-Opitz syndrome; vs: versus

Table 6. Current gaps in evidence

Study design (no. of studies)	Primary outcomes of interest			Secondary outcomes of interest		
	Overall survival	Changes in the severity and/or frequency of neurobehavioral abnormalities	Statin-related adverse reactions	Changes in growth parameters (e.g. height, weight)	Changes in biochemical markers (e.g. sterols, CoQ10)	Quality of life
RCT (n = 1)	-	Wassif 2017	Wassif 2017	[a]	Wassif 2017	-
Prospective cohort (n = 3)	-	-	Chan 2009	-	Chan 2009	-
					Haas 2008	
					Roullet 2012	
Retrospective cohort (n = 2)	-	-	Haas 2007	Haas 2007	Haas 2007	-
			Oláh 2013a	-	-	

[a]The authors of Wassif 2017 narratively stated that no significant changes in anthropometric measures were noted. However, they did not provide actual data for it.

CoQ10: coenzyme Q10; RCT: randomized controlled trial

Table 7. Tabulated summary of included studies

Study	Outcomes Assessed	Timepoint(s)	N (intervention/control)	Overall risk of bias	Direction favored
Wassif 2017	Neurobehavioral outcomes (irritability)	24 months	18/18	High	Statins
	Growth parameters				None
	Statin-related adverse reactions				Statins
	Plasma cholesterol levels				No statins
	Plasma dehydrocholesterol levels				Statins
	CSF cholesterol levels				None
	CSF dehydrocholesterol levels				None
Chan 2009	Statin-related adverse reactions	10 months	3/3	Serious	None
	Plasma cholesterol levels			Critical	None
	Plasma 7DHC levels			Critical	Statins
Haas 2007	Growth parameters	36 months (median)	13/13	Not assessed	None
	Statin-related adverse reactions			Critical	No statins

Table 7. Tabulated summary of included studies *(Continued)*

	Plasma cholesterol levels			Critical	No statins
	Plasma dehydrocholesterol levels			Critical	Statins
Haas 2008	Plasma cholesterol levels	24 months	8/6	Critical	None
	Plasma CoQ levels		8/6	Critical	None
Oláh 2013a	Statin-related adverse reactions	22 months	9/9	Critical	No statins
Roullet 2012	Statin-related adverse reactions	26 months	9/9	Not assessed	None
	Plasma cholesterol levels			Critical	None
	Plasma 7DHC levels			Critical	None

7DHC: 7-dehydrocholesterol; CoQ: coenzyme Q; CSF: cerebral spinal fluid.

APPENDICES

Appendix 1. Search Methods - electronic searching

Database/Resource	Strategy
CENTRAL in the Cochrane Library	#1 MeSH descriptor: [Smith-Lemli-Opitz Syndrome] explode all trees #2 lemli OR opitz OR SLO OR SLOS OR “cholesterol deficiency” OR “cholesterol deficient” OR “cholesterol deficiencies” OR RSH OR “Lethal Multiple Congenital Anomaly Syndrome” OR “Lethal Acrodysgenital” OR “7 Dehydrocholesterol Reductase” OR DHCR7 OR “7 dehydrocholesterol reductase” OR "7 dehydrocholesterol delta 7 reductase" OR "3beta hydroxysterol delta 7 reductase" OR “NADPH sterol delta 7 reductase” #3 #1 OR #2 #4 MeSH descriptor: [Hydroxymethylglutaryl-CoA Reductase Inhibitors] explode all trees #5 MeSH descriptor: [Hydroxymethylglutaryl CoA Reductases] explode all trees #6 MeSH descriptor: [Atorvastatin] explode all trees #7 MeSH descriptor: [Fluvastatin] explode all trees #8 MeSH descriptor: [Lovastatin] explode all trees #9 MeSH descriptor: [Rosuvastatin] explode all trees #10 MeSH descriptor: [Meglutol] explode all trees #11 MeSH descriptor: [Pravastatin] explode all trees #12 MeSH descriptor: [Simvastatin] explode all trees #13 Statin OR statins OR Atorvastatin* OR Liptonorm OR Lipitor OR atorlip OR aplactin OR atovarol OR glustar OR lowlipen OR sortis OR storvas OR tahor OR torvast OR zarator OR bervastatin OR cerivastatin OR crilvastatin OR dalvastatin OR fluvastatin* OR Fluvastatinum OR fluindostatin OR lescol OR canef OR cranoc OR lochol OR locol OR vastin OR glenvastatin OR lovastatin OR monacolin OR Mevinolin* OR Mevacor OR altocor OR altoprev OR artein OR belvas OR cholestra OR lipdip OR

(Continued)

	lipivas OR lostatin OR lovalip OR lovalord OR lovasterol OR lovastin OR lozutin OR mevinacor OR nergadan OR rodatin OR rovacor OR taucor OR meglutol OR mevastatin OR compactin OR mevastin OR medostatin OR Pitavastatin OR nisvastatin OR itavastatin OR Itavastin OR alipza OR livalo OR livazo OR pitava OR vezepra OR pravastatin OR eptastatin* OR epatostantin OR epistatin OR fluindostatin OR vasten OR lipemol OR liplast OR prareduct OR mevalotin OR pravachol OR pralidon OR elisor OR selektine OR pravacol OR lipostat OR baycol OR bristacol OR astin OR epatostantin OR eptastatine OR lipidal OR liprevil OR prastan OR pravaselect OR pravasin OR pravator OR sanaprav OR selipran OR Rosuvastatin OR crestor OR rosuvas OR Simvastatin OR Synvinolin OR Zocor OR cholestat OR colastatina OR covastin OR denan OR epistatin OR eucor OR ifistatin OR klonastin OR kolestevan OR lipex OR lipinorm OR lipovas OR lodales OR medipo OR rechol OR simcard OR simovil OR simvacor OR simvatin OR simvor OR simvotin OR sinvacor OR sinvastatin OR sivastin OR valemia OR Velastatin OR vasilip OR vasotenal OR zocord OR zovast OR tenivastatin OR "Hydroxymethylglutaryl CoA Reductase Inhibitor" OR "HydroxymethylglutarylCoA Reductase Inhibitors" OR "Hydroxymethylglutaryl coenzyme A Reductase Inhibitor" OR "Hydroxymethylglutaryl coenzyme A Reductase Inhibitors" OR “HMG CoA” OR HMGcoA OR “HMG co a” OR “beta Hydroxy beta Methylglutarate” OR “3 Hydroxy 3 methylglutaric Acid” OR “HMGcoenzyme A” OR “3hydroxy3methylglutaryl-CoA” OR “3hydroxy3methylglutarylcoenzyme A” OR “HMG CoA reductase inhibitor” OR “HMG CoA reductase inhibitors” OR “hmg coenzyme a reductase inhibitor” OR “hmg coenzyme a reductase inhibitors” OR "Anticholesteremic Agent" OR "Anticholesteremic Agents" OR "Hydroxymethylglutaryl CoA Reductases" #14 #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 #15 #3 AND #14
PubMed	("Smith-Lemli-Opitz Syndrome"[Mesh] OR lemli[tw] OR opitz[tw] OR SLO[tw] OR SLOS[tw] OR “cholesterol deficiency”[tw] OR “cholesterol deficient”[tw] OR “cholesterol deficiencies” OR RSH[tw] OR “Lethal Multiple Congenital Anomaly Syndrome”[tw] OR “Lethal Acrodysgenital”[tw] OR “7-Dehydrocholesterol Reductase”[tw] OR DHCR7[tw] OR 7-dehydrocholesterol reductase[Supplementary Concept] OR "7 dehydrocholesterol delta 7 reductase"[tw] OR "3beta hydroxysterol delta 7 reductase"[tw] OR “NADPH-sterol delta 7-reductase”[tw]) AND (Statin[tw] OR statins[tw] OR Atorvastatin*[tw] OR Liptonorm[tw] OR Lipitor[tw] OR atorlip[tw] OR aplactin[tw] OR atovarol[tw] OR glustar[tw] OR lowlipen[tw] OR sortis[tw] OR storvas[tw] OR tahor[tw] OR torvast[tw] OR zarator[tw] OR bervastatin[tw] OR cerivastatin[tw] OR crilvastatin[tw] OR dalvastatin[tw] OR fluvastatin*[tw] OR Fluvastatinum[tw] OR fluindostatin[tw] OR lescol[tw] OR canef[tw] OR cranoc[tw] OR lochol[tw] OR locol[tw] OR vastin[tw] OR glenvastatin[tw] OR lovastatin[tw] OR monacolin[tw] OR Mevinolin*[tw] OR Mevacor[tw] OR altocor[tw] OR altoprev[tw] OR artein[tw] OR belvas[tw] OR cholestra[tw] OR lipdip[tw] OR lipivas[tw] OR lostatin[tw] OR lovalip[tw] OR lovalord[tw] OR lovasterol[tw] OR lovastin[tw] OR lozutin[tw] OR mevinacor[tw] OR nergadan[tw] OR rodatin[tw] OR rovacor[tw] OR taucor[tw] OR meglutol[tw] OR mevastatin[tw] OR compactin[tw] OR mevastin[tw] OR medostatin[tw] OR Pitavastatin[tw] OR nisvastatin[tw] OR itavastatin[tw] OR Itavastin[tw] OR alipza[tw] OR livalo[tw] OR livazo[tw] OR pitava[tw] OR vezepra[tw] OR pravastatin[tw] OR eptastatin*[tw] OR epatostantin[tw] OR epistatin[tw] OR fluindostatin[tw] OR vasten[tw] OR lipemol[tw] OR liplast[tw] OR prareduct[tw] OR mevalotin[tw] OR pravachol[tw] OR pralidon[tw] OR elisor[tw] OR selektine[tw] OR pravacol[tw] OR lipostat[tw] OR baycol[tw] OR bristacol[tw] OR astin[tw] OR epatostantin[tw] OR eptastatine[tw] OR lipidal[tw] OR liprevil[tw] OR prastan[tw] OR pravaselect[tw] OR pravasin[tw] OR pravator[tw] OR sanaprav[tw] OR selipran[tw] OR Rosuvastatin[tw] OR crestor[tw] OR rosuvas[tw] OR Simvastatin[tw] OR Synvinolin[tw] OR Zocor[tw] OR cholestat[tw] OR colastatina[tw] OR covastin[tw] OR denan[tw] OR epistatin[tw] OR eucor[tw] OR ifistatin[tw] OR klonastin[tw] OR kolestevan[tw] OR lipex[tw] OR lipinorm[tw] OR lipovas[tw] OR lodales[tw] OR medipo[tw] OR rechol[tw] OR simcard[tw] OR simovil[tw] OR simvacor[tw] OR simvatin[tw] OR simvor[tw] OR simvotin[tw] OR sinvacor[tw] OR sinvastatin[tw] OR sivastin[tw] OR valemia[tw] OR Velastatin[tw] OR vasilip[tw] OR vasotenal[tw] OR zocord[tw] OR zovast[tw] OR tenivastatin[tw] OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors"[tw] OR "Hydroxymethylglutaryl coenzyme a Reductase Inhibitor"[tw] OR "Hydroxymethylglutaryl coenzyme a Reductase Inhibitors"[tw] OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors"[tw] OR “HMG CoA”[tw] OR HMGcoA[tw] OR “HMG-coA”[tw] OR “HMG-co-A”[tw] OR “beta Hydroxy beta Methylglutarate”[tw] OR “3 Hydroxy 3 methylglutaric Acid”[tw] OR HMG-CoA[tw] OR “HMG-coenzyme A”[tw] OR “3-hydroxy-3-methylglutaryl-CoA”[tw] OR “3- hydroxy-3-methylglutaryl-coenzyme A”[tw] OR “HMG CoA reductase inhibitor”[tw] OR “HMG CoA reductase inhibitors”[tw] OR “hmg coenzyme a reductase inhibitor”[tw] OR "Atorvastatin"[Mesh] OR "Fluvastatin"[Mesh] OR "Lovastatin"[Mesh] OR "Rosuvastatin Cal-

(Continued)

	cium"[Mesh] OR "Meglutol"[Mesh] OR "Pravastatin"[Mesh] OR "Simvastatin"[Mesh] OR atorvastatin[Substance Name] OR fluvastatin[Substance Name] OR pitavastatin[Supplementary Concept] OR "Anticholesteremic Agents"[Pharmacological Action] OR "Hydroxymethylglutaryl CoA Reductases"[Mesh] OR "Hydroxymethylglutaryl- CoA Reductase Inhibitors" [Pharmacological Action] OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors"[Mesh] OR "3-hydroxy-3-methylglutaryl-coenzyme A"[Supplementary Concept])
Embase.com	('Smith Lemli Opitz syndrome'/exp OR '7 dehydrocholesterol reductase'/exp OR lemli:ab,ti OR opitz:ab,ti OR SLO:ab,ti OR SLOS:ab,ti OR “cholesterol deficiency”:ab,ti OR “cholesterol deficiencies”:ab,ti OR “cholesterol deficient”:ab,ti OR RSH:ab,ti OR “Lethal Multiple Congenital Anomaly Syndrome”:ab,ti OR “Lethal Acrodysgenital”:ab,ti OR “7-Dehydrocholesterol Reductase”:ab,ti OR DHCR7:ab,ti OR "7 dehydrocholesterol delta 7 reductase":ab,ti OR "3beta hydroxysterol delta 7 reductase":ab,ti OR “NADPH-sterol delta 7-reductase”:ab,ti) AND ('atorvastatin'/exp OR 'bervastatin'/exp OR 'cerivastatin'/exp OR 'compactin'/exp OR 'crilvastatin'/exp OR 'dalvastatin'/exp OR 'fluindostatin'/exp OR 'glenvastatin'/exp OR 'mevinolin'/exp OR 'pitavastatin'/exp OR 'pravastatin'/exp OR 'rosuvastatin'/exp OR 'simvastatin'/exp OR 'tenivastatin'/exp OR Statin:ab,ti OR statins:ab,ti OR vastatin:ab,ti OR Atorvastatin*:ab,ti OR Liptonorm:ab,ti OR Lipitor:ab,ti OR atorlip:ab,ti OR aplactin:ab,ti OR atovarol:ab,ti OR glustar:ab,ti OR lowlipen:ab,ti OR sortis:ab,ti OR storvas:ab,ti OR tahor:ab,ti OR torvast:ab,ti OR zarator:ab,ti OR bervastatin:ab,ti OR cerivastatin:ab,ti OR crilvastatin:ab,ti OR dalvastatin:ab,ti OR fluvastatin*:ab,ti OR Fluvastatinum:ab,ti OR fluindostatin:ab,ti OR lescol:ab,ti OR canef:ab,ti OR cranoc:ab,ti OR lochol:ab,ti OR locol:ab,ti OR vastin:ab,ti OR glenvastatin:ab,ti OR lovastatin:ab,ti OR monacolin:ab,ti OR Mevinolin*:ab,ti OR Mevacor:ab,ti OR altocor:ab,ti OR altoprev:ab,ti OR artein:ab,ti OR belvas:ab,ti OR cholestra:ab,ti OR lipdip:ab,ti OR lipivas:ab,ti OR lostatin:ab,ti OR lovalip:ab,ti OR lovalord:ab,ti OR lovasterol:ab,ti OR lovastin:ab,ti OR lozutin:ab,ti OR mevinacor:ab,ti OR nergadan:ab,ti OR rodatin:ab,ti OR rovacor:ab,ti OR taucor:ab,ti OR meglutol:ab,ti OR mevastatin:ab,ti OR compactin:ab,ti OR mevastin:ab,ti OR medostatin:ab,ti OR Pitavastatin:ab,ti OR nisvastatin:ab,ti OR itavastatin:ab,ti OR Itavastin:ab,ti OR alipza:ab,ti OR livalo:ab,ti OR livazo:ab,ti OR pitava:ab,ti OR vezepra:ab, ti OR pravastatin:ab,ti OR eptastatin*:ab,ti OR epatostantin:ab,ti OR epistatin:ab,ti OR fluindostatin:ab,ti OR vasten:ab,ti OR lipemol:ab,ti OR liplast:ab,ti OR prareduct:ab,ti OR mevalotin:ab,ti OR pravachol:ab,ti OR pralidon:ab,ti OR elisor:ab,ti OR selektine:ab,ti OR pravacol:ab,ti OR lipostat:ab,ti OR baycol:ab,ti OR bristacol:ab,ti OR astin:ab,ti OR epatostantin:ab,ti OR eptastatine:ab,ti OR lipidal:ab,ti OR liprevil:ab,ti OR prastan:ab,ti OR pravaselect:ab,ti OR pravasin:ab,ti OR pravator:ab,ti OR sanaprav:ab,ti OR selipran:ab,ti OR Rosuvastatin:ab,ti OR crestor:ab,ti OR rosuvas:ab,ti OR Simvastatin:ab,ti OR Synvinolin:ab,ti OR Zocor:ab,ti OR cholestat:ab,ti OR colastatina:ab,ti OR covastin:ab,ti OR denan:ab,ti OR epistatin:ab,ti OR eucor:ab,ti OR ifistatin:ab,ti OR klonastin:ab,ti OR kolestevan:ab,ti OR lipex:ab,ti OR lipinorm:ab,ti OR lipovas:ab,ti OR lodales:ab,ti OR medipo:ab,ti OR rechol:ab,ti OR simcard:ab,ti OR simovil:ab,ti OR simvacor:ab,ti OR simvatin:ab,ti OR simvor:ab,ti OR simvotin:ab,ti OR sinvacor:ab,ti OR sinvastatin:ab,ti OR sivastin:ab,ti OR valemia:ab,ti OR Velastatin:ab,ti OR vasilip:ab,ti OR vasotenal:ab,ti OR zocord:ab,ti OR zovast:ab,ti OR tenivastatin:ab,ti OR 'hydroxymethylglutaryl coenzyme A reductase inhibitor'/exp OR “HMG CoA reductase inhibitor”:ab,ti OR “HMG CoA reductase inhibitors”:ab,ti OR “hmg coenzyme a reductase inhibitor”:ab,ti OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors":ab,ti OR "Hydroxymethylglutaryl coenzyme a Reductase Inhibitor":ab,ti OR "Hydroxymethylglutaryl coenzyme a Reductase Inhibitors":ab,ti OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors":ab,ti OR “HMG CoA”:ab,ti OR HMGcoA:ab,ti OR “HMG-coA”:ab,ti OR “HMG-co-A”:ab,ti OR “beta Hydroxy beta Methylglutarate”:ab,ti OR “3 Hydroxy 3 methylglutaric Acid”:ab,ti OR HMG-CoA:ab,ti OR “HMG-coenzyme A”:ab,ti OR “3-hydroxy-3-methylglutaryl-CoA”:ab,ti OR “3- hydroxy-3-methylglutaryl-coenzyme A”:ab,ti OR "Anticholesteremic Agents":ab,ti OR "Hydroxymethylglutaryl CoA Reductases":ab,ti OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors":ab,ti OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors":ab,ti OR "3- hydroxy-3-methylglutaryl-coenzyme A":ab,ti OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors":ab,ti)
Web of Science Core Collection (Science Citation Index Expanded, Social Sciences Citation Index, Conference	TS=(("Smith-Lemli-Opitz Syndrome" OR lemli OR opitz OR SLO OR SLOS OR “cholesterol deficiency” OR “cholesterol deficiencies” OR “cholesterol deficient” OR “RSH-SLO” OR "rsh slos" OR "rsh smith lemli opitz" OR "slo syndrome" OR RSH OR “Lethal Multiple Congenital Anomaly Syndrome” OR “Lethal Acrodysgenital” OR “7-Dehydrocholesterol Reductase” OR DHCR7 OR "7 dehydrocholes-

(Continued)

Proceedings Citation Index-Science, Book Citation Index-Science, Emerging Sources Citation Index, SciELO Citation Index)	terol delta 7 reductase" OR "3beta hydroxysterol delta 7 reductase" OR "NADPH-sterol delta 7-reductase") AND (Statin OR statins OR Atorvastatin* OR Liptonorm OR Lipitor OR atorlip OR aplactin OR atovarol OR glustar OR lowlipen OR sortis OR storvas OR tahor OR torvast OR zarator OR bervastatin OR cerivastatin OR crilvastatin OR dalvastatin OR fluvastatin* OR Fluvastatinum OR fluindostatin OR lescol OR canef OR cranoc OR lochol OR locol OR vastin OR glenvastatin OR lovastatin OR monacolin OR Mevinolin* OR Mevacor OR altocor OR altoprev OR artein OR belvas OR cholestra OR lipdip OR lipivas OR lostatin OR lovalip OR lovalord OR lovasterol OR lovastin OR lozutin OR mevinacor OR nergadan OR rodatin OR rovacor OR taucor OR meglutol OR mevastatin OR compactin OR mevastin OR medostatin OR Pitavastatin OR nisvastatin OR itavastatin OR Itavastin OR alipza OR livalo OR livazo OR pitava OR vezepra OR pravastatin OR eptastatin* OR epatostantin OR epistatin OR fluindostatin OR vasten OR lipemol OR liplast OR prareduct OR mevalotin OR pravachol OR pralidon OR elisor OR selektine OR pravacol OR lipostat OR baycol OR bristacol OR astin OR epatostantin OR eptastatine OR lipidal OR liprevil OR prastan OR pravaselect OR pravasin OR pravator OR sanaprav OR selipran OR Rosuvastatin OR crestor OR rosuvas OR Simvastatin OR Synvinolin OR Zocor OR cholestat OR colastatina OR covastin OR denan OR epistatin OR eucor OR ifistatin OR klonastin OR kolestevan OR lipex OR lipinorm OR lipovas OR lodales OR medipo OR rechol OR simcard OR simovil OR simvacor OR simvatin OR simvor OR simvotin OR sinvacor OR sinvastatin OR sivastin OR valemia OR Velastatin OR vasilip OR vasotenal OR zocord OR zovast OR tenivastatin OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors" OR "Hydroxymethylglutaryl coenzyme a Reductase Inhibitor" OR "Hydroxymethylglutaryl coenzyme a Reductase Inhibitors" OR “HMG CoA” OR HMGcoA OR “HMG-coA” OR “HMG-co-A” OR “beta Hydroxy beta Methylglutarate” OR “3 Hydroxy 3 methylglutaric Acid” OR “HMG-CoA” OR “HMG-coenzyme A” OR “3-hydroxy-3-methylglutaryl-CoA” OR “3- hydroxy-3-methylglutaryl-coenzyme A” OR “HMG CoA reductase inhibitor” OR “HMG CoA reductase inhibitors” OR “hmg coenzyme a reductase inhibitor” OR "Anticholesteremic Agents" OR "Hydroxymethylglutaryl CoA Reductases" OR "3-hydroxy-3-methylglutaryl- coenzyme A"))
Scopus	Title-Abstract-Keywords((lemli OR opitz OR SLO OR SLOS OR {cholesterol deficiency} OR {cholesterol deficient} OR {cholesterol deficiencies} OR RSH OR {Lethal Multiple Congenital Anomaly Syndrome} OR {Lethal Acrodysgenital} OR {7-Dehydrocholesterol Reductase} OR DHCR7 OR {7 dehydrocholesterol delta 7 reductase} OR {3beta hydroxysterol delta 7 reductase} OR {NADPH-sterol delta 7-reductase}) AND (Statin OR statins OR Atorvastatin* OR Liptonorm OR Lipitor OR atorlip OR aplactin OR atovarol OR glustar OR lowlipen OR sortis OR storvas OR tahor OR torvast OR zarator OR bervastatin OR cerivastatin OR crilvastatin OR dalvastatin OR fluvastatin* OR Fluvastatinum OR fluindostatin OR lescol OR canef OR cranoc OR lochol OR locol OR vastin OR glenvastatin OR lovastatin OR monacolin OR Mevinolin* OR Mevacor OR altocor OR altoprev OR artein OR belvas OR cholestra OR lipdip OR lipivas OR lostatin OR lovalip OR lovalord OR lovasterol OR lovastin OR lozutin OR mevinacor OR nergadan OR rodatin OR rovacor OR taucor OR meglutol OR mevastatin OR compactin OR mevastin OR medostatin OR Pitavastatin OR nisvastatin OR itavastatin OR Itavastin OR alipza OR livalo OR livazo OR pitava OR vezepra OR pravastatin OR eptastatin* OR epatostantin OR epistatin OR fluindostatin OR vasten OR lipemol OR liplast OR prareduct OR mevalotin OR pravachol OR pralidon OR elisor OR selektine OR pravacol OR lipostat OR baycol OR bristacol OR astin OR epatostantin OR eptastatine OR lipidal OR liprevil OR prastan OR pravaselect OR pravasin OR pravator OR sanaprav OR selipran OR Rosuvastatin OR crestor OR rosuvas OR Simvastatin OR Synvinolin OR Zocor OR cholestat OR colastatina OR covastin OR denan OR epistatin OR eucor OR ifistatin OR klonastin OR kolestevan OR lipex OR lipinorm OR lipovas OR lodales OR medipo OR rechol OR simcard OR simovil OR simvacor OR simvatin OR simvor OR simvotin OR sinvacor OR sinvastatin OR sivastin OR valemia OR Velastatin OR vasilip OR vasotenal OR zocord OR zovast OR tenivastatin OR {Hydroxymethylglutaryl-CoA Reductase Inhibitors} OR {Hydroxymethylglutaryl coenzyme a Reductase Inhibitor} OR {Hydroxymethylglutaryl coenzyme a Reductase Inhibitors} OR {HMG CoA} OR HMGcoA OR {HMG-coA} OR {HMG-co-A} OR {beta Hydroxy beta Methylglutarate} OR {3 Hydroxy 3 methylglutaric Acid} OR {HMG-CoA} OR {HMG- coenzyme A} OR {3-hydroxy-3-methylglutaryl-CoA} OR {3-hydroxy-3-methylglutaryl- coenzyme A} OR {HMG CoA reductase inhibitor} OR {HMG CoA reductase inhibitors} OR {hmg coenzyme a reductase inhibitor} OR {Anticholesteremic Agents} OR {Hydroxymethylglutaryl CoA Reductases} OR {3-hydroxy-3-methylglutaryl-coenzyme A}))
LILACS	[iAH Advanced Form] Words: Lemli OR

(Continued)

	Words: Opitz
CRD Database	Any Field: Lemli OR Opitz OR SLOS OR SLO OR RSH OR “cholesterol deficiency” OR “cholesterol deficient” OR "cholesterol deficiencies" OR “Lethal Multiple Congenital Anomaly Syndrome” OR “Lethal Acrodysgenital” OR "7 Dehydrocholesterol reductase" OR DHCR7 OR "7 dehydrocholesterol delta 7 reductase" OR "3beta hydroxysterol delta 7 reductase" OR “NADPH sterol delta 7 reductase”
Prospero	Lemli OR Opitz OR SLOS OR SLO OR RSH OR cholesterol deficiency OR cholesterol deficient OR cholesterol deficiencies OR Lethal Multiple Congenital Anomaly Syndrome OR Lethal Acrodysgenital OR 7 Dehydrocholesterol reductase OR DHCR7 OR 7 dehydrocholesterol delta 7 reductase OR 3beta hydroxysterol delta 7 reductase OR NADPH sterol delta 7 reductase
NARCIS	All Sources: Lemli OR Opitz OR SLOS OR SLO OR RSH OR “cholesterol deficiency” OR “cholesterol deficient” OR "cholesterol deficiencies" OR “Lethal Multiple Congenital Anomaly Syndrome” OR “Lethal Acrodysgenital” OR "7 Dehydrocholesterol reductase" OR DHCR7 OR "7 dehydrocholesterol delta 7 reductase" OR "3beta hydroxysterol delta 7 reductase" OR “NADPH sterol delta 7 reductase”
OpenGrey	Lemli OR Opitz OR SLOS OR SLO OR RSH OR “cholesterol deficiency” OR “cholesterol deficient” OR "cholesterol deficiencies" OR “Lethal Multiple Congenital Anomaly Syndrome” OR “Lethal Acrodysgenital” OR "7 Dehydrocholesterol reductase" OR DHCR7 OR "7 dehydrocholesterol delta 7 reductase" OR "3beta hydroxysterol delta 7 reductase" OR “NADPH sterol delta 7 reductase”
ClinicalTrials.gov	SEARCH 1 Status: All studies Condition or disease: Lemli OR Opitz OR SLOS OR SLO OR RSH OR "cholesterol deficiency" OR "cholesterol deficient" OR "cholesterol deficiencies" OR "Lethal Multiple Congenital Anomaly Syndrome" OR “Lethal Acrodysgenital” SEARCH 2 Status: All studies Condition OR disease: "7 Dehydrocholesterol reductase" OR DHCR7 OR "7 dehydrocholesterol delta 7 reductase" OR "3beta hydroxysterol delta 7 reductase" OR "NADPH sterol delta 7 reductase"
WHO ICTRP	[Basic Search] Lemli OR Opitz OR SLOS OR SLO OR RSH OR cholesterol deficiency OR cholesterol deficient OR cholesterol deficiencies OR Lethal Multiple Congenital Anomaly Syndrome OR Lethal Acrodysgenital OR 7 Dehydrocholesterol reductase OR DHCR7 OR 7 dehydrocholesterol delta 7 reductase OR 3beta hydroxysterol delta 7 reductase OR NADPH sterol delta 7 reductase
EU Clinical Trials Register	[Basic Search] Lemli OR Opitz OR SLOS OR SLO OR RSH OR "cholesterol deficiency" OR "cholesterol deficient" OR "cholesterol deficiencies" OR "Lethal Multiple Congenital Anomaly Syndrome" OR “Lethal Acrodysgenital” OR "7 Dehydrocholesterol reductase" OR DHCR7 OR "7 dehydrocholesterol delta 7 reductase" OR "3beta hydroxysterol delta 7 reductase" OR "NADPH sterol delta 7 reductase"

HISTORY

Protocol first published: Issue 1, 2020

CONTRIBUTIONS OF AUTHORS

- Conceiving the review: RAB
- Designing the review: RAB
- Coordinating the review: RAB
- Data collection for the review: RAB, AL
- Designing search strategies: RAB, AL
- Undertaking searches: RAB, AL
- Screening search results: RAB, AL
- Organizing retrieval of papers: RAB, AL
- Screening retrieved papers against eligibility criteria: RAB, AL
- Appraising quality of papers: RAB, AL
- Extracting data from papers: RAB, AL
- Writing to authors of papers for additional information: RAB
- Providing additional data about papers: RAB
- Obtaining and screening data on unpublished studies: RAB, AL
- Data management for the review: RAB
- Entering data into RevMan: RAB
- Analysis of data: RAB, YF
- Interpretation of data: RAB
- Providing a methodological perspective: RAB
- Providing a clinical perspective: RAB, AR, RS
- Providing a policy perspective: RAB
- Writing the protocol: RAB, YF
- Performing previous work that was the foundation of the current review: RAB, RS

DECLARATIONS OF INTEREST

RAB declares no known conflicts of interest to disclose.

AL declares no known conflicts of interest to disclose.

YPF declares no known conflicts of interest to disclose.

RDS declares he has acted as a consultant for Acer Therapeutics (a pharmaceutical company who may have interest in developing treatment for Smith-Lemli-Opitz syndrome (SLOS)) and also Travere Therapeutics, Inc. (a pharmaceutical company, one of whose drugs is currently used off label for SLOS). RDS is part of a research team receiving research grant funding at the Smith Lemli Opitz Syndrome Foundation and he holds a part-time position as Chief Medical Officer at PreventionGenetics, a subsidiary of Exact Sciences. RDS holds several patents in the area of newborn screening for sterol and bile acid disorders; however, none of these are licensed and he receives no revenue from them.
ATR declares no known conflicts of interest to disclose; he is employed as senior investigator by the National Institutes for Health.

SOURCES OF SUPPORT

Internal sources

- No sources of support provided

External sources

- National Institute for Health Research, UK

  This systematic review was supported by the National Institute for Health Research, via Cochrane Infrastructure funding to the Cochrane Cystic Fibrosis and Genetic Disorders Group.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We made our best attempt to adhere to the preset criteria in our protocol (Ballout 2020) regarding the type studies eligible for inclusion in our review, to ensure maximum transparency and minimize any reporting bias. However, while we specified in our protocol that we would only include NRSIs with prospective designs (e.g. non-randomized trials, prospective cohort studies, and controlled before-and-

after studies), we were obliged to reconsider including observational studies employing a retrospective design (Haas 2007; Oláh 2013a), in light of the limited number of studies that would otherwise be eligible for inclusion in our review. Nonetheless, after careful consideration and discussion with the group's editorial team, we opted not to pool any of the data extracted from these retrospective studies with those coming from the included prospective studies.

While we had planned in our protocol to include only cohort studies with a prospective design (Ballout 2020), we have included retrospective cohort studies in this full review for two reasons. Firstly, due to the very limited number of relevant studies available on this topic; as well as secondly our inability to pool the data of the included prospective cohort studies due to their marked heterogeneity and inconsistency in outcome measures (see Risk of bias in included studies and Effects of interventions). We felt it important to include such studies in the review after carefully contemplating the fact that rare diseases such as SLOS already suffer from a scarcity of relevant studies (especially prospective or randomized interventional studies) due to their rare nature, their variable and often unpredictable clinical course (due to lack of sufficient natural history data), and limits on the funding needed to orchestrate large multicenter studies. Nonetheless, after thorough discussion among the review authors and the Cochrane Cystic Fibrosis and Genetic Disorders Group's methodological experts, we opted not to pool the data from retrospective cohort studies with prospective cohort studies. Briefly, the main reasons for not pooling data from prospective and retrospective cohort studies include: the differences in degree of ascertainment of exposure (i.e. statin treatment) between participants recruited prospectively and those analyzed retrospectively; differences in degree of controlling the starting time as well as the duration of follow-up of included participants across the two designs; and the inability to account for confounding factors or interfering incident events, or both, that may have occurred throughout a retrospective cohort study compared with a prospective one.

INDEX TERMS

Medical Subject Headings (MeSH)

Bile Acids and Salts; Cholesterol; Cross-Over Studies; *Hydroxymethylglutaryl-CoA Reductase Inhibitors [adverse effects]; Randomized Controlled Trials as Topic; *Smith-Lemli-Opitz Syndrome [drug therapy]; Vitamins

MeSH check words

Child; Female; Humans; Male