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Choline supplementation does not have an impact on blood lipids

A systematic review on the effects of oral supplemental choline on blood lipid profile

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Sammanfattning

Titel	Kosttillskott av kolin har ingen effekt på blodlipider – En systematisk översikt om effekten av oralt kolintillskott på blodlipid-profil
Författare	Angelica Skaldeman och Rebecka Stridsberg
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Nyckelord:	Kolin, kosttillskott, lipider, kolesterol, LDL-kolesterol, HDL-kolesterol, totalkolesterol.

Syfte:	Syftet med projektet är att systematiskt sammanfatta och granska evidens från randomiserade kontrollerade studier om den potentiella effekten av kolintillskott på blodfetterna low-density lipoprotein (LDL), high-density lipoprotein (HDL) och totalkolesterol (TC).
Metod:	Litteratursökningen genomfördes i databaserna PubMed och Scopus i januari 2023. Utfallsmåtten var LDL, HDL och TC. Deltagarna i interventionsgruppen tilldelades kosttillskott med kolin och deltagarna i kontrollgruppen tilldelades placebo. Inklusionskriterierna var att deltagarna skulle vara vuxna över 18 år och att kolin i de inkluderade studierna endast fick administreras oralt. Exklusionskriterierna var personer under 18 år, studier som inte var RCT, studier som använde annan form av kontroll än placebo och studier skrivna på andra språk än svenska eller engelska. Studier som uppfyllde dessa kriterier kvalitetsgranskades enligt SBU:s mall “bedömning av randomiserade studier (effekt av att tilldelas en intervention (ITT))” och evidensgraderades enligt GRADE.
Resultat:	Litteratursökningen resulterade i 266 träffar. Fjorton artiklar lästes i fulltext, varav två inkluderades i denna systematiska översikt. Dessa utgjorde en studiepopulation på totalt 83 personer, pågick i åtta till tolv veckor, och bedömdes ha låg samt måttlig risk för bias. Ingen statistiskt signifikant effekt observerades för utfallsmåtten LDL, HDL, eller TC. Resultaten visade konsekvent att oralt tillskott av kolin inte har någon effekt på samtliga utfallsmått hos vuxna över 18 år.
Slutsats:	Det finns en hög tillförlitlighet (++++) till att oralt tillskott av kolin inte har någon effekt på LDL, HDL eller TC.

Abstract

Title:	Choline supplementation does not have an impact on blood lipids- A systematic review on the effects of oral supplemental choline on blood lipid profile
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Examiner:	Linnea Bärebring
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Date:	2024-03-19
Key words:	Choline, supplementation, lipids, cholesterol, LDL cholesterol, HDL cholesterol, total cholesterol.

Aim: The aim of this project is to systematically summarize and review evidence from randomized controlled trials on the potential effect of choline supplementation on blood lipids low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol (TC).

Methods: The literature search was conducted in the PubMed and Scopus databases in January 2023. The outcome measures were LDL, HDL, and TC. The intervention group was assigned dietary supplements with choline and the control group was assigned a placebo. The criteria for inclusion were individuals aged 18 and above, and in the studies considered, choline was solely administered orally. The criteria for exclusion comprised individuals under the age of 18, studies that were not RCTs, studies employing a control method other than a placebo, and studies written in languages other than Swedish or English. Studies that met the selected criteria were quality-reviewed according to the SBU's template "assessment of randomized studies (effect of being assigned an intervention (ITT))" and evidence-graded according to GRADE.

Results: The literature search resulted in 266 matches. Fourteen articles were read in full text, out of which two were included in this systematic review. The two included studies constituted a study population of a total of 83 people and were assessed to have a low as well as moderate risk of bias. The interventions spanned a duration of eight to twelve weeks. No statistically significant effects were observed for the outcome measures LDL, HDL, nor TC. The result consistently showed that oral supplementation of choline has no effect on these outcome measures in adults over 18 years.

Conclusion: There is high certainty (++++) evidence to suggest that oral supplementation of choline has no effect on LDL, HDL, or TC.

Abbreviations

ASCVD: Atherosclerotic cardiovascular disease.
ASDR: Age-standardized death rate.
BMI: Body mass index.
CVD: Cardiovascular disease.
DALYs: Disability adjusted life years.
GRADE: Grading of recommendations, assessment, development and evaluation. A way to assess reliability for different outcome measures.
HDL: High-density lipoprotein.
ITT: Intention to treat.
LDL: Low-density lipoprotein.
PICO: Population, intervention, comparison, outcome. A framework to generate clear and precise research questions.
PC: Phosphatidylcholine.
RCT: Randomized controlled trial.
SBU: Swedish Agency for Health Technology Assessment and Assessment of Social Services (Statens beredning för medicinsk och social utvärdering).
TC: Total cholesterol.
TMAO: Trimethylamine-n-oxide.
T2DM: Type 2 Diabetes Mellitus.
VLDL: Very-low-density lipoprotein.
WHO: World Health Organization.
NNR: Nordic Nutrition Recommendations.

Glossary

Apoptosis: Programmed cell death.
Chylomicrons: Lipoprotein particles that transport dietary lipids from the intestines to other parts of the body.
De Novo Biosynthesis: The synthesis of complex molecules from simple molecules such as sugars or amino acids, as opposed to recycling after partial degradation.
Endogenous: Originating from within an organism, tissue, or cell.
Exogenous: Originating from outside an organism, tissue, or cell.
Foam Cells: Lipid-laden macrophages seen in atherosclerosis.
Heterogeneity: The quality of being diverse or different.
Hydrophobic: Having a tendency to repel or fail to mix with water.
Hypercholesterolemia: An excess of cholesterol in the bloodstream.
Hyperlipidemia: An abnormally high concentration of fats or lipids in the blood.
Ischemic Stroke: A type of stroke that occurs when the flow of blood to the brain is blocked.
Leukocytes: White blood cells.
Myocardial Infarction: A medical term for a heart attack, caused by a blockage in the heart's arteries.
Neurotransmitter Synthesis: The process of producing neurotransmitters within neurons.
Normolipidemics: Individuals with a normal amount of lipid in the blood.
Occlusion: Something that blocks a tube or opening in the body.
Placebo: A substance or treatment that resembles the intervention in appearance and administration but lacks any pharmacological effect.

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Introduction

Cardiovascular disease

Cardiovascular disease (CVD) and its associated clinical outcomes, including myocardial infarction (MI) and ischemic stroke, stand as the predominant contributors to global morbidity and mortality (1). Atherosclerotic cardiovascular disease (ASCVD) is the most prevalent form of CVD (2). ASCVD begins when cholesterol-rich lipoproteins accumulate within the intima, the innermost layer of the arterial wall. These particles, including low-density lipoprotein (LDL), efficiently enter and exit the intima. At low blood LDL levels, the risk of pathological accumulation within the arterial wall is minimal. However, when LDL concentration rises, the likelihood of pathological retention within the arterial wall increases, leading to the initiation of atherosclerosis (3). Macrophages, drawn to the site, engulf these lipoproteins, subsequently transforming into foam cells. These cells display a foamy appearance, laden with lipid droplets. As active participants in the inflammatory response, the foam cells release cytokines and chemokines, perpetuating inflammation. Simultaneously, oxidative stress intensifies, inflicting further damage upon the arterial wall. The foam cells gather within the vessel wall, forming what is known as fatty streaks. As more particles congregate, these streaks progress into plaques. The emergence of these plaques leads to a constriction of the vessels, potentially resulting in a decrease in blood flow and subsequent vessel occlusion. The plaques can also rupture and migrate via the bloodstream to lodge in a remote part of the body, thereby causing a clot. These events can lead to oxygen deficiency which can result in serious conditions such as MI or stroke (4, 5).

How blood lipids affect cardiovascular disease

Various factors such as blood lipid profile, age, sex, systolic blood pressure, and smoking status have been correlated with the risk of cardiovascular events.

Lipids, such as triglycerides and cholesterol, are naturally hydrophobic and require transportation in the bloodstream via specialized proteins called lipoproteins. These particles consist of various types that all originate from the liver. There are four types of lipoproteins classified based on density and function, namely chylomicrons, very-low-density lipoprotein (VLDL), LDL, and high-density lipoprotein (HDL). These lipoproteins possess a lipid-soluble core and a water-soluble exterior, allowing them to transport hydrophobic particles in the bloodstream. The LDL and HDL particles are recognized as the most significant in the matter of CVD (6). The most thoroughly investigated risk factor for CVD is, however, by a considerable margin the LDL particle. It is responsible for transporting cholesterol to peripheral tissues (7), and has been demonstrated to play a direct role in the progression of ASCVD (8, 9). HDL, on the other hand, improves cardiovascular health by facilitating the removal of excess lipids from the arteries and transporting it back to the liver where it is metabolized and then eliminated from the body via the bile and through the feces. This mechanism is known as "reverse cholesterol transport" (6, 10). Research suggests that for each unit increase in HDL levels, the risk of CVD decreases by two to three percent (11). The link between low levels of HDL and elevated CVD risk has been firmly established across multiple large population-based cohorts, with notable findings originating from the Framingham Heart Study (12-14).

Total cholesterol (TC) refers to the collective assessment of triglycerides, LDL and HDL in the blood stream. As elevations in TC levels may escalate the narrowing and obstruction of heart vessels, it is strongly associated with an elevated risk of CVD (15). It is therefore one of the primary screening lipid parameters in many CVD risk assessment models (16). Although

both TC and LDL levels are used to calculate total CVD risk, a reduction in LDL levels remains the principal focus of lifestyle adjustments and pharmacological treatments aimed at preventing CVD (9).

Choline and its function in the body

Choline is an essential nutrient with a multifaceted role in the human body. It is a necessary component of phospholipids as a significant contributor to the mediation of cell membrane signaling, it participates in neurotransmitter synthesis (particularly acetylcholine), it facilitates lipid transport via lipoproteins, and it contributes to methyl-group metabolism. Choline also plays a crucial role in fetal development by impacting stem cell proliferation and apoptosis, consequently influencing the structure and function of the brain (17, 18). Importantly, it also serves as a precursor for the production of phosphatidylcholine (PC), the most prevalent type of phospholipid in the body (19). This phospholipid is a vital component of all cell membranes and can be obtained from dietary sources or synthesized through de novo biosynthesis via the methylation process of phosphatidylethanolamine (19, 20). However, endogenous production of choline is not sufficient to meet nutritional requirements, necessitating exogenous sources (20). In foods, choline is available in both water-soluble and lipid-soluble forms (21). Different types of choline undergo distinct processes of absorption and metabolism; water-soluble forms of choline travel to the liver through portal circulation after absorption, while lipid-soluble forms are incorporated into chylomicrons and transported via the lymphatic system (22). Some of the richest dietary sources of choline include liver, eggs, beef, fish, pork, and chicken, although it is also found in plant-based foods (21). The Institute of Medicine officially acknowledged choline as an essential nutrient in 1998, and recently updated reference values were included in the Nordic Nutrition Recommendations (NNR) 2023 (23). The reference values for choline are set as an adequate intake level at 400 mg/day for both males and females (20).

Consequences of a choline deficient diet

Due to its diverse function in human metabolism, choline deficiency is currently believed to influence a range of conditions such as nonalcoholic fatty liver disease, atherosclerosis, and potentially neurological disorders (24).

In the liver, PC is essential for the packaging and transportation of triglycerides within the VLDL particles (25). Insufficient access to PC can hinder the export of excess triglycerides from the liver, leading to a potentially pathological accumulation (26, 27). This could, in turn, result in the development of a fatty liver condition (28, 29). A choline deficient diet can also have other serious health implications. Studies have shown that within just three weeks of limiting dietary choline intake, both liver and muscle damage can be observed in otherwise healthy men (30, 31). The observed tissue damage could be ascribed to changes in structural integrity and increased permeability of cellular membranes, stemming from a reduced ratio of PC to phosphatidylethanolamine (32-35).

Other CVD risk biomarkers in relation to choline

Choline's role as a potential treatment or risk factor for CVD is currently under investigation, yielding conflicting results. While some studies propose potential benefits, others indicate correlations with increased risk (36-41). Since choline affects other CVD risk biomarkers, there are multiple factors that need to be taken into consideration when assessing the effect of dietary choline on CVD. Trimethylamine-n-oxide (TMAO) is a metabolite associated with atherosclerosis. It is generated through a multi-step process involving dietary precursors, gut microbiota, and liver enzymes. Notably, choline serves as a significant dietary precursor in this process, thereby linking dietary choline intake to increased TMAO levels in the body (42-

45). Homocysteine, on the other hand, is an amino acid that plays a crucial role in the metabolism of B vitamins, including B_9, B_6, and B_{12}. While its function is vital, elevated levels of homocysteine are also associated with an increased risk of CVD (46, 47). Consumption of choline has been correlated with reduced homocysteine concentrations, whether derived from dietary sources or from supplements (48). This homocysteine-lowering effect may have a potential to be helpful in the treatment of CVD (47).

Research problem

CVD is the world's leading cause of morbidity and mortality and there is a great need to discover suitable and effective treatments. Dietary choline is an essential nutrient that is involved in lipid metabolism and transportation in the body. It is being investigated as a potential treatment for CVD, but at the same time, also as a potential risk factor. Blood lipid levels of LDL, HDL and TC have a direct effect on CVD, yet there is very little research conducted on the effect of choline on blood lipids. The purpose of this systematic review is to further shed light on the effect of oral choline supplementation on blood lipids LDL, HDL and TC.

Aim

The aim of this project is to systematically summarize and review evidence from randomized controlled trials on the potential effect of choline supplementation on blood lipids LDL, HDL, and TC.

Research question

How might the consumption of choline supplementation impact blood LDL, HDL and TC?

Methods

Criteria for included articles

PICO

To construct a well-built research strategy and inclusion criteria, a Population, Intervention, Control, Study design (PICO) was formed prior to the literature search. The PICO was constructed to include a design of randomized controlled trials (RCT), and a population of adults over 18 years. It also outlined the inclusion of choline supplementation as intervention compared to a placebo control on the outcomes of LDL, HDL and TC. Table 1 displays the PICO formulated for this review.

Table 1. PICO (Population, Intervention, Control, Study design).

Population	Intervention	Control	Outcome	Design
Adults, over the age of 18	Choline supplementation	Placebo	Blood lipids (LDL, HDL, and TC)	Randomized controlled trials

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol.

Inclusion and exclusion criteria

In addition to the inclusion criteria outlined in the PICO (Table 1) this review only included human subjects. The articles included had to be written in English or Swedish, and the choline supplementation was only to be administered orally. All trials including parenteral nutrition or other ways of administration were excluded. The articles had to present outcome data on

LDL, HDL, and TC derived from blood samples. Studies only presenting two or less of these outcomes were excluded. Likewise, articles presenting results from a placebo that may potentially alter blood lipids (i.e. eggs, edible oils) were rejected. Articles were also rejected if the placebo also contained choline, even in small amounts. Trials including combined interventions, or where participants received other lipid-lowering treatments at the same time were also excluded.

Data collection

The two databases PubMed and Scopus were used to conduct this literature search. Both searches were completed by the two authors at the same time. The PubMed search was completed on 2024-01-26, and the Scopus search on 2024-01-29. Search blocks were in categories of choline supplementation (i.e. *phosphatidylcholines, lecithin*), lipids (i.e. *lipid, lipids*) and RCTs (i.e. *randomised intervention studies, RCT*). The advanced search application was used through both databases in order to identify titles and abstracts relevant to the review. Medical Subject Headings (MeSH) terms were used while using the database PubMed. The same words were used to search in the Article title/Abstract/Keyword section in Scopus. The boolean operator OR was used within the blocks to expand the search, whilst AND was used between the blocks to narrow the searches toward relevant content. Some keywords were truncated with an asterisk (*) to include other inflexions. Due to the risks of not capturing articles that had not yet been marked or tagged by the database, no filters or other restrictive settings were used in the searches. The EndNote software was used to identify and remove doubles. Upon identifying potentially relevant articles through the selection of title and abstract together, the authors screened articles in full individually. The reference lists of the articles found were examined to find further relevant articles. The selection process was completed using the PICO as well as the inclusion and exclusion criteria previously defined. Once assessment had been completed by the authors individually the results were compared and discussed, and the authors reached consensus to allow for a final few to be included in this review. Appendix 1 outlines the details of the search blocks used. An additional search was conducted on 2024-03-04 and at this time, no new relevant articles could be identified.

Data analysis

To calculate the intervention effect, the mean difference from the control category was subtracted from the mean difference of the intervention category. A p-value of < 0.05 indicated statistical significance and was recorded as reported in the articles. To be able to compare the articles' results, values in mg/dl were converted to mmol/l. Hence, values in mg/dL were divided by 38.6 (49).

Review of risk of bias

The risk of bias was reviewed by the authors independently using the bias tool for RCTs "*Bedömning av randomiserad studie (effekt av att tilldelas en intervention)"* published by the Swedish Agency for Health Technology Assessment and Assessment of Social Services (SBU; 50). SBU's manuals "*Utvärdering av insatser i hälso- och sjukvården och socialtjänsten*" (51) and "*Manual till mallarna för randomiserade och icke randomiserade interventionsstudier*" (52) was used for clarification and guidance in the assessment process. The five domains "*risk of bias arising from the randomization process*", "*risk of bias due to deviations from intended intervention*", "*risk of bias due to missing outcome data*", "*risk of bias in measurement of the outcome*", "*risk of bias in selection of the reported result*", and "*risk of bias due to conflict of interes*t" shaped the foundation of the assessment. Upon

assessing the risk of bias on a scale of low, moderate or high for each domain, a combined assessment of the overall risk of systematic distortion was established. The authors compared and discussed the individual results and reached consensus and a final decision.

Review of certainty of evidence

A combined assessment of the articles' certainty of evidence was conducted according to the principles of Grading of Recommendations, Assessment, Development and Evaluation (GRADE; 53). A review template developed by the University of Gothenburg (54) was used as a guide in this process. The domains assessed included "*study limitations (risk of bias)*", "*inconsistency of results*", "*imprecision*", "*indirectness of evidence*", and "*publication bias*". The overall certainty of evidence was graded as high certainty (++++), moderate certainty (+++), low certainty (++), or very low certainty (+). The baseline for RCT studies is high certainty (++++) and based on the quality of evidence in the different domains, the grading may decrease by one (+) or two (++) points. The authors completed individual assessments of certainty of evidence. Consensus of the GRADE assessment was then reached upon comparison and discussion of results.

Results

A total of two articles met the inclusion and exclusion criteria outlined for this systematic review. These articles were reviewed and assessed for risk of bias as well as certainty of evidence.

Results following searching and selection of articles

Figure 1 summarizes the results and process in identifying relevant RCTs for this systematic review. A total of 266 objects were found through systematic searching of PubMed (n = 78) and Scopus (n = 188). Following the removal of doubles, a total of 246 unique articles remained. Reference lists of relevant articles revealed another five objects which were screened along with the 246 objects found through the databases. Following screening of 251 titles and abstracts, 237 were excluded as they did not meet inclusion criteria. Fourteen articles remained to be read in full. Twelve were excluded due to various reasons. Five (55-59) were excluded as they were using oil as intervention and control. It is well established that oils and fats can have a significant impact on blood lipids (60), and these trials were therefore excluded. Four (61-64) did not investigate relevant outcomes, one (65) used smaller amounts of choline as control, one (66) used choline as control and not intervention, and one (67) used eggs as control. Empirical research has established that eggs can have a significant impact on blood lipids, and this study was therefore also excluded (68). Consequently, two articles remained to be included in this systematic review. No previous systematic review on the topic was identified upon searching the databases. Appendix 2 outlines reasons for exclusion in detail.

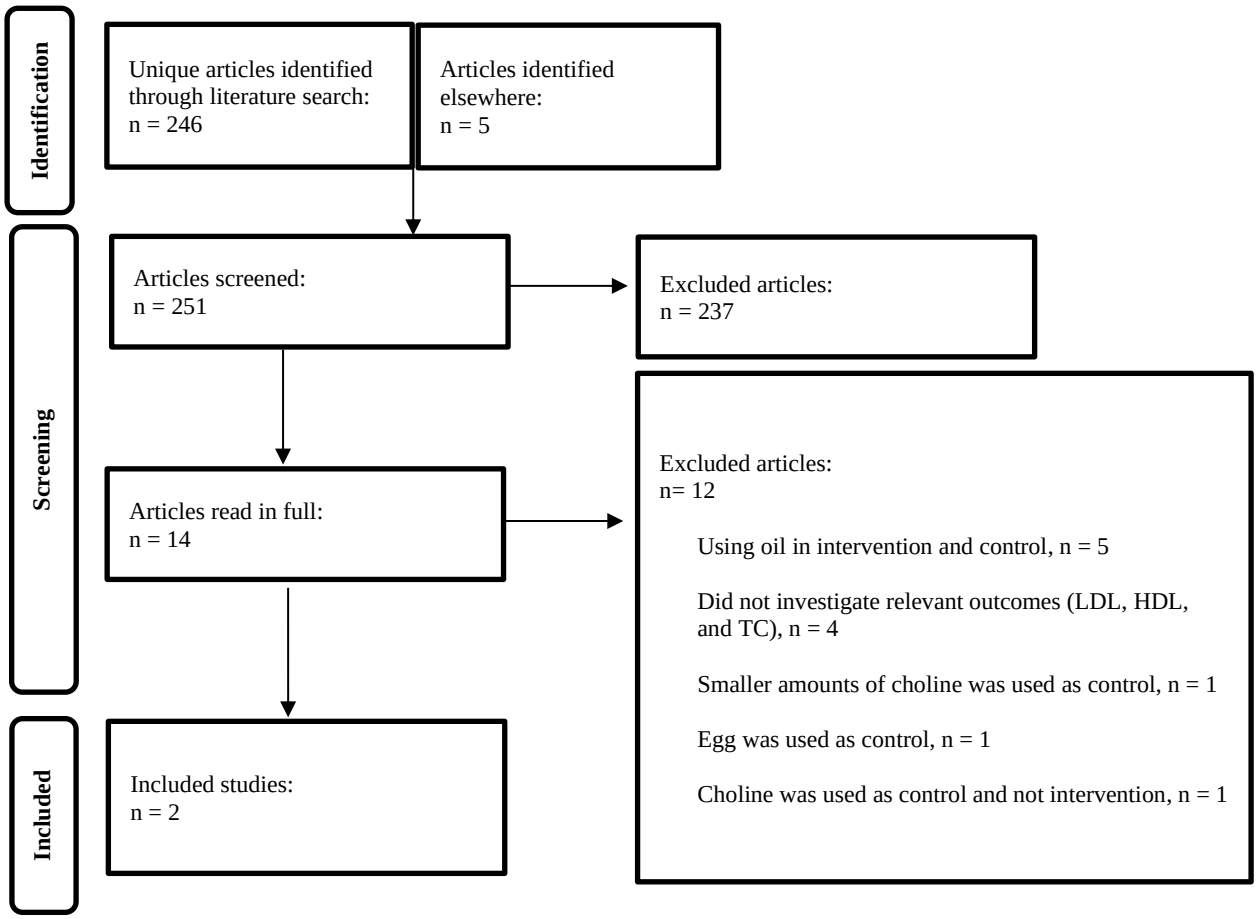

Figure 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram of papers selection for inclusion in the review. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol

Included articles

A total of 15 authors have been involved in the publication of the two articles included in this review. One article (69) has 26 citations, and the other (70) has 14 citations on Scopus. A summary of included articles and their details are displayed in table 2. Both studies are RCTs, use choline as intervention, and adopt placebo controls with no known effect on blood lipid profile.

Table 2. Included articles following the search and review of papers investigating the effect of choline supplementation on blood lipids.

First author, year, country	**Study design**	**Study duration**	**Population**	**Participants** N randomized, % dropout rate	**Intervention**	**Control**
Rashvand, 2020, Iran (70)	Parallel double-blinded RCT	8 weeks	Patients aged 30-60 years with T2DM and BMI 25–35 kg/m2	N=37 23% dropout rate	1000 mg choline bitartrate + 500 mg starch	Placebo, 1500 mg starch
Wallace, 2012, Ireland (69)	Parallel double-blinded RCT	12 weeks	Independently mobile, postmenopausal women aged 49–71 years	N=46 9% dropout rate	2.4 g choline bitartrate (1 g choline) per day	Placebo, 2.4 g tartaric acid per day

RCT, Randomized Controlled Trial; T2DM, Type 2 Diabetes Mellitus

Rashvand et al., 2020. Iran (70)

Study design

This randomized, double-blinded, placebo-controlled, parallel clinical trial included 96 individuals in a two-month study investigating the effects of oral magnesium and choline supplementation on coagulation parameters and lipid profile in patients with Type 2 Diabetes Mellitus (T2DM). Participants had to present with a body mass index (BMI) = 25-35kg/m^2, be 30-60 years of age, and were excluded if they were medicated with insulin, corticosteroids, or non-steroidal anti-inflammatory drugs, or if they used supplements of vitamins/minerals. They were also excluded if they were diagnosed with inflammatory or autoimmune disorders, chronic renal or liver disease, malignancies or gastrointestinal dysfunction. Smokers, pregnant or breastfeeding individuals were also excluded. Participants were stratified by age and sex and randomly assigned to choline-magnesium (1000 mg choline bitartrate + 500 mg magnesium oxide, n = 24), magnesium (500 mg magnesium oxide + 1000 mg placebo, n = 24), choline (1000 mg choline bitartrate + 500 mg placebo, n = 24), or placebo (1500 mg starch, n = 24). Subjects were requested to consume three capsules daily with main meals and to follow their regular diet and physical activity during the time of the trial. Capsules and bottles were designed to be similar in form, size, and color. Subjects returned their bottles so that the remaining capsules could be counted, and adherence recorded. Assessment data of anthropometric measurements, dietary intake, physical activity and fasting venous blood samples was obtained at baseline. Follow-up assessment of blood samples was completed after one and then two months. Neither staff, investigators, nor participants were aware of the capsule content until analysis was completed. There had been an error in reporting cholesterol values in mg/dl as mmol/l in this article.

Results

Thirty-seven individuals (17 women, 20 men) completed the study. The choline group included 18 and the placebo group included 19 individuals. No statistically significant changes were observed in the choline nor placebo groups on either of the outcome measures. Twenty-five percent in the choline group and 21% in the placebo group dropped out or were excluded due to noncompliance or for personal reasons. The remaining subjects consumed > 90% of the capsules throughout the intervention. Table 3-5 summarize the results from this clinical trial.

Side effects

No side effects were reported by the subjects following the intervention.

Risk of bias

The overall assessment of risk of bias was marked as moderate as all domains were assessed as low, except one that was assessed as moderate. The group allocation was randomized and could not be predicted nor calculated. Baseline values were even, and imbalances suggesting deficiencies in the randomization process could not be identified. The "*risk of bias arising from the randomization process*" domain was therefore marked as low. Neither the participants nor the practitioners were aware of the group allocation and the "*risk of bias due to deviations from intended intervention*" domain was therefore also marked as low. An appropriate analysis method within the framework of Intention To Treat (ITT) evaluation was however not used. Instead, since the authors excluded participants due to "non-compliance" and "personal reasons". This design could instead be classified as a per-protocol analysis. The dropout rate was evenly distributed across all groups and was therefore unlikely to be related to the outcome measures. The authors did not analyze the dropouts at all. However, the risk of the outcome being significantly influenced by participants not being analyzed for exclusion or dropout was assessed as low. Nonetheless, the results for those with high compliance who did complete the study did not indicate any changes, and the small changes observed were statistically insignificant. It was assessed highly unlikely that those who did not take choline would have provided blood samples demonstrating an effect. In contrast, it is not known whether the individuals that dropped out may have had higher or lower blood lipid levels than those included in the analysis, nor if they have had a different intake of choline or other nutrients relevant to blood lipids. Therefore, the "*risk of bias due to missing outcome data*" domain was marked as moderate. The data collection did not differ between the groups since all participants had their blood tested, regardless of intervention. According to the authors, neither participants, practitioners, nor any other personnel were aware of the contents of the bottles until the end of the analysis and the "packet method" was used to administer the supplements to the participants. Therefore, "*the risk of bias in measurement of the outcome*" domain was marked as low. The authors declared a protocol published, but it is unavailable. However, there are no other ways of measuring blood lipids than blood tests, all samples were taken at the same time, and all outcomes were reported in the same unit. It was also assessed that comprehensive statistical analyses were completed to generate a result. The "*risk of bias in selection of the reported result*" domain was therefore assessed as low. There was no mention of conflicts of interest, nor any funding or sponsors that could pose a risk of bias declared. However, because the paper delivers no statistically significant results, the "*risk of bias due to conflict of interest*" section was deemed low. Table 6 presents the assessment of risk of bias for all domains regarding this paper.

Wallace et al., 2012. Ireland (69)

Study design

This randomized, double-blinded, placebo-controlled trial investigated the effects of oral choline supplementation on plasma concentrations of betaine, free choline, tHcy, B-vitamin status, and lipid profiles in postmenopausal women aged 49–71 years. Subjects were included if they were independently mobile and had not had menstrual bleeding for more than twelve months. Exclusion criteria included BMI $\geq$ 35 kg/m^2, smoking, presence of hepatic, gastrointestinal or renal disease, and metabolic or endocrine abnormalities. Subjects were also excluded if they were diagnosed with haematological disorders, consumed drugs or dietary supplements influencing choline or B-vitamin status or metabolism, if serum vitamin B_{12} concentration < 111 pmol/l, or serum creatinine concentration $\geq$ 130mmol/l. Forty-two participants were stratified by use of hormone replacement therapy and fasting tHcy, and then

randomized to consume 2.4 g tartaric acid per day as placebo or 2.4 g choline bitartrate containing 1 g choline per day for twelve weeks. The placebo and intervention capsules were both identical in taste and appearance. Pillboxes were collected so that any remaining capsules could be counted and adherence recorded. The subjects were asked to maintain their regular diet and lifestyle throughout the study and not to take any B-vitamin or choline-containing supplements besides the ones provided. Assessment data of anthropometric measurements, dietary intake, physical activity and fasting venous blood samples was obtained at baseline. Follow-up assessment of blood samples was completed at week six and again at week twelve.

Results

Out of the 46 individuals deemed eligible for the study, four subjects (9%) did not complete the trial. The authors have given no reason for the dropout, nor declared any distribution between groups. At the end of the trial, the choline group included 19 and the placebo group 23 subjects. No statistically significant changes were observed in the choline nor placebo groups on either of the outcome measures. The remaining subjects consumed > 95% of capsules throughout the intervention. Table 3-5 summarize the results from this clinical trial.

Side effects

No side effects were reported by the subjects following the intervention.

Risk of bias

The overall assessment of risk of bias was marked as low as all domains were also marked as low. The study design was double-blinded, and it was described that stratification and randomization was used. It was therefore concluded that group allocation could not be predicted and remained unknown throughout the study. This can also be inferred from the data presented, showing that there are no statistically significant differences between groups at baseline. The "*risk of bias arising from the randomization process*" domain was therefore marked as low. It was declared that both the intervention and the placebo pills were identical in taste and appearance and the "*risk of bias due to deviations from intended intervention*" domain was therefore marked as low. An appropriate analysis method within the framework of ITT evaluation was not used as the authors excluded "non-completers", which would be more in line with a per-protocol design. The authors did not declare any dropout distribution between groups. It was only declared that four individuals "did not complete." It is, however, unclear from which group. Regardless, the risk of the results being significantly affected by the dropouts or excluded participants not being analyzed was assessed as unlikely. The results for those with high adherence who completed the study did not indicate any changes, and the miniscule changes observed were statistically insignificant. It was considered highly unlikely that those who did not take choline would have provided blood samples demonstrating an effect. Therefore, the "*risk of bias due to missing outcome data*" domain was also marked as low. All participants had blood samples taken regardless of allocation. It was not explicitly stated that those measuring the outcomes were unaware of any allocation, but it was deemed likely as neither participants nor practitioners were aware. Additionally, the laboratory analyzing the samples was likely a third party fully unaware of group allocation and with no interest in manipulating the results. Therefore, it was determined that the "*risk of bias in measurement of the outcome*" domain was also low. Lastly, the authors declared a protocol published, but it is unavailable. There are however no other ways of measuring blood lipids than blood tests, all samples were collected at the same time, and the outcomes were reported in the same unit. It was also assessed that comprehensive statistical analyses were competed to generate a result. It was therefore determined that the "*risk of bias in selection of the reported result*" domain was low. There were no conflicts of interest declared, and no

financial funding that could pose a risk of bias identified. Choline supplements as well as the placebo tablets were however supplied by a sponsor. Yet, because of the study delivering no statistically significant results, the "*risk of bias due to conflict of interest*" section was deemed low. Table 6 presents the assessment of risk of bias for all domains regarding this paper.

Table 3. Total blood cholesterol in mmol/L (mean) as reported in the articles.

	Effect intervention **Δ TC mmol/L**	**Effect control** **Δ TC mmol/L**	**Intervention effect** **Δ intervention - Δ control TC mmol/L**	**Statistical significance for difference**
Rashvand, 2020 (70)	0.00 ± 1.02	0.04 ± 0.81	-0.04	Not significant, presented in text as "p > 0.05". Exact value not given.
Wallace, 2012 (69)	0.42 ± 1.29	0.13 ± 0.47	0.29	Not significant (as presented in text - value not given).

TC, total cholesterol.

Table 4. Blood LDL in mmol/L (mean) as reported in the articles.

	Effect intervention **Δ LDL mmol/L**	**Effect control** **Δ LDL mmol/L**	**Intervention effect** **Δ intervention - Δ control LDL mmol/L**	**Statistical significance for difference**
Rashvand, 2020 (70)	-0.38 ± 0.87	0.00 ± 0.73	-0.38	Not significant, presented in text as "p > 0.05". Exact value not given.
Wallace, 2012 (69)	0.22 ± 0.92	0.06 ± 0.39	0.6	Not significant (as presented in text - value not given).

LDL, low-density lipoprotein.

Table 5. Blood HDL in mmol/L (mean) as reported in the articles.

	Effect intervention **Δ HDL mmol/L**	**Effect control** **Δ HDL mmol/L**	**Intervention effect** **Δ intervention - Δ control HDL mmol/L**	**Statistical significance for difference**
Rashvand, 2020 (70)	0.06 ± 0.24	0.02 ± 0.12	0.04	Not significant, presented in text as "p > 0.05". Exact value not given.
Wallace, 2012 (69)	0.02 ± 0.43	0.09 ± 0.35	-0.07	Not significant (as presented in text - value not given).

HDL, high-density lipoprotein.

Table 6. Assessment of risk of bias for the included articles

	Domain 1 *Randomization*	**Domain 2** *Deviation from intended intervention*	**Domain 3** *Dropout rate*	**Domain 4** *Data collection*	**Domain 5** *Reporting*	**Bias** *Conflict of interest*	**General**
Rashvand, 2020 (70)	Low	Low	Moderate	Low	Low	No	Moderate
Wallace, 2012 (69)	Low	Low	Low	Low	Low	No	Low

Confidence in the combined results

The evidence certainty according to GRADE was assessed as high certainty (++++) for all outcome measures. The certainty assessment for all outcome measures is presented in table 7.

Study limitations (Risk of Bias)
All domains pertaining to LDL, HDL and TC for one article (69) were considered to have low risk of bias. For the other article (70), the overall assessment of risk of bias was marked as moderate as all domains were assessed as low, except one that was marked moderate. However, no section resulted in a deduction of points as there were some concerns but not enough to warrant any downgrading.

Inconsistency of results
There was no inconsistency of results as both studies yielded identical outcomes, namely, no effect of choline supplementation on blood lipids. No points were removed in this section.

Imprecision
For both articles, no significant differences were observed in LDL, HDL, nor TC. The results are consistent, and the effect size is small. Rashwand et al. (70) had an estimated power of 18 participants per group, and their project met this requirement (18 in the choline group and 19 in the placebo group). Wallace et al. (69) had an estimated power of 19 participants per group, which was also achieved (19 in the choline group and 23 in the placebo group). No lack of precision could be identified. No points were removed in this section.

Indirectness of evidence
The outcome measurements of the studies (blood sample), follow-up period (eight and twelve weeks) are both comparable to studies investigating statin therapy (71). Therefore, to be able to observe changes in blood lipids, this time frame and method was deemed to be reasonable. Rashwand et al. (70) examined patients with T2DM, and Wallace et al. (69) examined post-menopausal women. Both conditions are known to correlate with dyslipidemia, which makes these populations relevant for a cholesterol-lowering intervention study (72, 73). No points were removed in this section.

Publication bias
Two different groups of authors based in different regions of the world were included in this review. The article by Rashvand et al. (70) included the researchers Samaneh Rashvand, Majid Mobasseri, and Ali Tarighat-Esfanjani. These researchers were based in Iran. The article by Wallace et al. (69), in contrast, included the researchers Julie Wallace, Jacqueline McCormack, Helene McNulty, Paula Walsh, Paula Robson, Maxine Bonham, Maresa Duffy,

Mary Ward, Anne Molloy, John Scott, Per Ueland, and J. J. Strain. These were based in Ireland. The latter study was sponsored by Akzo Nobel Functional Chemicals, which contributed both placebo and choline supplements. Both studies showed that choline supplementation had no effect on blood lipids. An argument could be made that this presents a reduced risk of bias towards a singular outcome, unlike if the findings were to favor the efficacy of a particular product. No unpublished studies related to this field were identified on clinicaltrials.gov. No points were removed in this section.

Table 7. Assessment summary of certainty of evidence based on the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) method.

	TC	**LDL**	**HDL**
Number of studies:	2	2	2
Study limitations (Risk of Bias):	No limitations (0)	No limitations (0)	No limitations (0)
Inconsistency of results:	No problems (0)	No problems (0)	No problems (0)
Imprecision:	No problems (0)	No problems (0)	No problems (0)
Indirectness of evidence:	No shortcomings (0)	No shortcomings (0)	No shortcomings (0)
Publication bias:	No problems (0)	No problems (0)	No problems (0)
Overall certainty of evidence:	High certainty (++++)	High certainty (++++)	High certainty (++++)

Discussion

The primary aim of this systematic review was to provide a comprehensive summary of existing RCTs investigating the potential effect of choline supplementation on blood LDL, HDL, and TC. Rigorous methodology was practiced, and the data collection process was thorough. The results demonstrated no statistically significant effects, which suggests that oral choline supplementation is unlikely to significantly impact blood lipid levels in adults over the age of 18. These findings are in line with studies in the current body of literature, which also report statistically insignificant results regarding the impact of choline supplementation on blood lipid levels. However, the existing body of literature on this subject is notably sparse.

While choline is an essential nutrient involved in various physiological processes, including lipid metabolism, its role in modulating blood lipid levels remains unclear. This could be due to the complex nature of lipid metabolism, which is influenced by a multitude of factors including diet, lifestyle, genetics, and other health conditions, in addition to the scarcity of research on the subject.

Results discussion

The results of this review suggest that choline supplementation does not significantly impact blood LDL, HDL, and TC levels. However, it is important to interpret these findings in the context of the study populations included in the review. The reviewed studies were intended to include adults above 18 years of age, but ultimately only incorporated individuals with

T2DM and postmenopausal women, thereby not representing the broader population. It would be particularly valuable to examine the impact on individuals with dyslipidemia, familial hypercholesterolemia, or other conditions influencing blood lipid profiles. The results presented in this review may not be generalizable to these populations.

A positive finding was that adherence in both studies was high, which is an important aspect to consider. This strengthens the potential for the treatment method to be generalizable. High adherence indicates that the participants were able to follow the treatment protocol consistently, which can enhance the validity of the study results and their applicability to a broader population.

In addition to the primary focus of this review, it is noteworthy to mention a correlation between elevated blood triglyceride levels and the intake of oral choline supplements has been observed. This particular association, although not within the scope of the PICO formulated of this review presents an intriguing avenue for further exploration.

Consideration of result bias due to specific populations

One of the challenges encountered during this review was the heterogeneity of the studies included in terms of their study populations and choline supplementation protocols. This made it difficult to draw definitive conclusions and may have contributed to the lack of significant findings. The study by Rashvand et al. (70) was conducted on patients with T2DM and may have been influenced by confounding factors inherent to this specific health condition, such as elevated blood glucose levels. It is well-documented that T2DM increases the risk of CVD, particularly ASCVD, which could have affected the results (73). The study by Wallace et al. (69) was focused on postmenopausal women; a demographic that does not represent the general population. The hormonal changes associated with menopause, particularly the fluctuating levels of estrogen, may have influenced the outcomes (72). This limitation, in the scope of the review, highlights a gap in the current literature and underscores the need for additional high-quality RCTs investigating the impact of choline supplementation on blood lipid levels in a broader population. Future studies should aim to include a more diverse and representative sample of the population to enhance the generalizability of the findings. Moreover, controlling for potential confounding factors, such as co-existing health conditions and hormonal levels, will be crucial in understanding the true impact of choline supplementation on blood LDL, HDL, and TC levels.

Comparison of baseline measurements

It could be hypothesized that the baseline values could potentially influence the outcome. For instance, if the blood lipid levels were initially within the normal range, it is plausible that the potential for further reduction might be limited due to physiological homeostasis. While observing the baseline values in the study conducted by Wallace et al. (69), it can be observed that participants' initial measurements average at 3.66 mmol/L. These levels surpassed the healthy range of < 3.4 mmol/L, indicating elevated LDL levels. Conversely, HDL levels were found to be within the normal range, with a mean value of 1.79 mmol/L, surpassing the threshold of 1.0 mmol/L for men and suggesting favorable cardiovascular health. However, participants demonstrated elevated TC levels, averaging at 5.85 mmol/L, exceeding the normal range of < 5.2 mmol/L. This observation highlights potential cardiovascular risk factors. These baseline measurements indicate that the observed non-significant outcomes cannot be attributed to favorable baseline levels of LDL and TC, as the initial measurements suggest potential for improvement in their blood lipid profiles. In the study conducted by Rashvand et al. (70), the average baseline lipid profiles were as follows: TC was 4.80

mmol/L, LDL cholesterol was 2.92 mmol/L, and HDL cholesterol was 1.14 mmol/L. All values of which fall within the generally accepted healthy ranges. Despite the initial differences in baseline results between the two studies, the post-intervention outcomes were strikingly similar. This suggests that the starting values do not significantly influence the effectiveness of the treatment.

Potential Influences on Study Outcomes

There are several other elements that could have influenced the study outcomes. The question of whether a two to three months intervention duration is sufficient to manifest a tangible effect is worth exploring. The dosage administered in the study is another potential variable as it is possible that a different dosage might have led to more significant results. The potential impact of choline from dietary wholefood sources on the results, as opposed to choline in supplement form, is another factor that could potentially lead to more detectable effects. A recent study by Mohammad Sadegh Pour Abbasi et al. (74) indicates a potential correlation between elevated dietary choline intake and reduced blood LDL concentrations in obese individuals. Another large study by Díez-Ricote et al. (75), the PREDIMED study, found that dietary intake of choline had a significant LDL and TC lowering effect. In this study, the average, adequate, and high intakes were approximately 350 mg, 400-600 mg, and above 600 mg, respectively. The two studies included in this review, however, reported a choline bitartrate dosage of 1000-2400 mg, markedly exceeding the dietary choline intake observed in the PREDIMED study (75). Notably, despite the lower choline intake from whole foods, significant effects on blood lipids were observed, contrasting the lack of effect from higher supplement dosages. These findings justify further exploration of variations in dosage, as well as the impact of food versus supplements, on blood lipid profiles including LDL, HDL, and TC.

Comparing Findings with Existing Literature in the Field

The findings presented in this review are consistent with some of the studies that were read in full but excluded due to using a different control than placebo. These studies also reported no significant effects of choline supplementation on blood lipids. Additionally, several other studies have yielded intriguing results that merit more in-depth investigation. Childs et al. (55) examined the effects of supplementation with soya lecithin, a rich source of dietary choline, on normolipidemic and hypercholesterolemic individuals. The findings revealed that soya lecithin increased HDL and slightly decreased LDL in both groups. Triglycerides decreased in normolipidemics but increased in hypercholesterolemics, who also gained weight. In another study, patients with familial hypercholesterolemia and normal controls were given highly polyunsaturated PC. Despite similar diets, no changes in plasma lipids were observed. However, adding PC to the diet resulted in a significant increase in fecal sterol excretion, indicating a loss of cholesterol and bile acids. Phospholipid and cholesterol content in bile, however, remained unchanged (56). In conclusion, while this systematic review provides valuable insights into the potential effects of choline supplementation on blood lipid levels, it also highlights the need for further research. Future studies should aim to investigate the impact of choline supplementation in both the general population as well as populations with high blood lipids and other diseases that can affect blood lipid profiles. This will help to further understand the role of choline in lipid metabolism and its potential therapeutic applications in the management of ASCVD, dyslipidemia and related conditions.

Methods discussion

The methodology of this review was designed to be comprehensive, incorporating a PICO framework to guide the research strategy and inclusion criteria and a thorough data collection process that adheres to the standards set by SBU for conducting systematic reviews. The literature search was completed using two databases, PubMed and Scopus, following specified guidelines. It is unknown whether additional database searches would have yielded more results, however, given that PubMed and Scopus are extensive medical databases, it is assessed that these would provide relevant data. The initial searches were conducted in several stages with the assistance of an experienced librarian to optimize the search. The construction of search blocks posed a challenge due to the extensive number of search terms and their numerous synonyms. Expert guidance was sought for synonym suggestions and assistance with the search blocks, where it was discovered that one of the search blocks was too restrictive and failed to capture one of the key studies. The number of free keywords and MeSH terms were considered to be relevant and comprehensive. A significant amount of time was dedicated to ensuring a rigorous and comprehensive search to avoid overlooking any relevant articles. The language of included studies has been limited to Swedish and English, which can be considered a weakness. The search as well as literature collection were independently conducted by both authors, which is a strength as it reduces the risk of biased assessment. Another strength of this systematic review is that it only includes double blinded RCTs, which are studies with a very high level of evidence. During the literature search for this systematic review, several studies were encountered where oil was utilized as a control instead of a placebo. Initially, the intention was to include three such studies in the review. However, as oil was deemed inappropriate for control due to its impact on blood lipids, they were excluded. This decision led to the exclusion of a total of four studies. The exclusion of these studies, while necessary to maintain the integrity of the PICO, further narrowed the scope of the review. For the assessment of risk, the SBU's Risk of Bias tool, the GRADE tool, developed by the GRADE Working Group and the SBU's handbook for guidance were utilized. To minimize the risk of bias, the review was independently conducted by both authors. This approach ensured a more objective and reliable review process. This was followed by a discussion among the authors to gain two perspectives on the execution and to reach a unanimous assessment. Both SBU's and GRADE's review templates allow room for subjective interpretations, which could be considered a weakness. However, this was counterbalanced by the opportunity to consult with a supervisor during these moments, which facilitated correcting mistakes. Initially, the intention was to include triglycerides in the outcome measure, but as one of the studies included lacked data on this outcome measure, it had to be excluded. It was decided to include a very broad population in the PICO to have the opportunity to capture as many studies as possible, given the limited amount of literature available.

Human rights and equal health

The World health organization (WHO) defines equity as "*the absence of unfair, avoidable or remediable differences among groups of people, whether those groups are defined socially, economically, demographically, or geographically or by other dimensions of inequality (e.g. sex, gender, ethnicity, disability, or sexual orientation)*". It is stated that health equity is realized when everyone has the opportunity to achieve their utmost health and well-being. The state of health and the attainment of health equity are influenced by various factors such as the environment where individuals are born, raised, reside, work, engage in leisure activities, and age, as well as biological determinants. Structural factors including political, legal, and economic elements, combined with social norms and institutional mechanisms, determine the

allocation of power and resources within these environments (76). In 2015, the United Nations Development Programme developed 17 global goals to attain peace, protect the planet, and to end poverty by 2030. Goal number three "*Good Health And Well-Being*" includes the goal target "*By 2030, reduce by one third premature mortality from non-communicable diseases through prevention and treatment and promote mental health and well-being*" (77).

Data shows that elevated blood lipids significantly contribute to the disease burden experienced worldwide, posing a notable risk for conditions such as ischemic heart disease and stroke in both developed and developing countries (78). However, some differences can be observed between regions, as well as between men and women.

Zheng et al. (79) examined data from the Global Burden of Disease study 2019 concerning worldwide deaths, Summary Exposure Value, and Disability-Adjusted Life Years (DALYs) that was linked to elevated LDL levels between 1990 and 2019. The authors concluded that out of 21 analyzed regions, Eastern Europe represented the highest age-standardized DALYs per 100,000 attributed to high LDL in both 1990 and 2019. Despite experiencing a total decline of –17.6%, this region remained highest of all throughout this time span. In 2019, Central Asia ranked as the second-highest region, experiencing a cumulative increase of 10.0% from 1990 to 2019. North Africa and the Middle East, on the other hand, ranked as the third-highest region in 2019, witnessed a decline of -26.5% over the same period. Oceania, while experiencing a slight increase during the same period, remained among the lower-ranked regions. In 2019, Eastern Sub-Saharan Africa, despite experiencing a 13.1% increase from 1990 to 2019, continued to maintain the lowest status compared to the other regions. The authors also more precisely determined that among the 204 countries and territories globally, the highest Age-Standardized Death Rate (ASDR) per 100,000 individuals attributed to elevated LDL was observed in Uzbekistan, followed by Ukraine, and the Solomon Islands. Conversely, Japan presented the lowest ASDR per 100,000 individuals at, followed by the Republic of Korea, and Rwanda. Not only has it become evident that the human species suffer inequalities on a global scale, where regions carry vastly different numbers of burden of DALY and ASDR, but there are also discrepancies to be observed between the sexes. WHO reported that in 2008, 39% of adults worldwide suffered from hyperlipidemia and that 37% of these were male (78). Interestingly however, although women are more likely to suffer from elevated cholesterol levels, men seem to suffer a greater impact. The rate, number, standardized rate of deaths, age, and DALYs attributed to elevated LDL were lower in females than in males between 1990 and 2019 (79). According to Tsao et al. (80), males globally represented 51.98% of all deaths caused by high LDL in 2020. Studies have also shown that men are more likely to be prescribed, accept and maintain statin therapy (81).

It can be concluded that inequalities can be observed in terms of pathological LDL levels amongst different populations worldwide. Different regions carry different rates of burden of disease, and substantial differences in ASDR between countries can be observed. Furthermore, not only are there differences in terms of death rates and prevalence between the sexes, but also in the likelihood of receiving LDL-lowering therapy. These observations highlight the importance of initiatives such as the United Nations Development Programme in achieving equality and equity between regions, countries, and between men and women.

Sustainability

Studies have shown that the very common lipid lowering medication group statins can cause damage in both vertebrates and arthropods, and disrupt crustacean reproduction and development (82, 83). These medications also need transportation across the globe which adds

to the carbon emissions that cause global warming. In 2007, the US prescription drug sector alone was responsible for 76.44 million metric tons of carbon dioxide equivalents (84). It has also been established that the meat and dairy industry contributes with a significant amount of both greenhouse gases and reactive nitrogen. One paper presents data suggesting that cutting livestock production by 50% could decrease greenhouse gas emissions by 25–40% and reactive nitrogen emissions by about 40% (85). Therefore, by adapting sustainable dietary habits, such as decreasing the consumption of processed and high-fat foods and increasing plant-based food intake, personal health can be optimized, but it also contributes positively to environmental sustainability (86).

Conclusion

No statistically significant effects were observed for the investigated outcomes LDL, HDL, and TC. The collective certainty of evidence was graded as high certainty (++++) for all outcome measures, and there is no scientific evidence to suggest that the consumption of oral choline supplementation has an effect on blood lipids in the post-menopausal and T2DM populations. The results presented in this review may only be transferable to individuals who meet these criteria. The present studies did not investigate the effects of oral choline supplements in individuals with the presence of familial hypercholesterolemia, or other diseases that may affect blood lipid profile. Thus, research on subjects with high blood lipids may yield different results. Future research should examine the impact on individuals with dyslipidemia, familial hypercholesterolemia, or other conditions influencing blood lipid profiles.

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Appendix 1. Search strategy

Search	Database	Search terms	Limitations	Number of articles
Date: 26/1 2024	PubMed	((("Choline"[MeSH Terms] OR "Choline"[Title/Abstract] OR "Choline supplements"[Title/Abstract] OR "Choline Citrate"[Title/Abstract] OR "citrate choline"[Title/Abstract] OR "choline o sulfate"[Title/Abstract] OR "choline o sulfate"[Title/Abstract] OR "Choline Hydroxide"[Title/Abstract] OR "Bursine"[Title/Abstract] OR "Choline Bitartrate"[Title/Abstract] OR "bitartrate choline"[Title/Abstract] OR "Choline Chloride"[Title/Abstract] OR "chloride choline"[Title/Abstract] OR "phosphatidylcholines"[MeSH Terms] OR "Phosphatidylcholine"[Title/Abstract] OR "choline phosphatidyl"[Title/Abstract] OR "Choline Phosphoglycerides"[Title/Abstract] OR "phosphoglycerides choline"[Title/Abstract] OR "Phosphatidylcholine"[Title/Abstract] OR "Phosphatidyl Cholines"[Title/Abstract] OR "cholines phosphatidyl"[Title/Abstract] OR "Choline Glycerophospholipids"[Title/Abstract] OR "glycerophospholipids choline"[Title/Abstract] OR "Lecithins"[Title/Abstract] OR "Lecithin"[Title/Abstract] OR "Lecithins"[MeSH Terms]) AND ("Dietary supplements"[MeSH Terms] OR "Dietary supplements"[Title/Abstract] OR "Dietary Supplement"[Title/Abstract] OR "supplements dietary"[Title/Abstract] OR "Dietary Supplementations"[Title/Abstract] OR "supplementations dietary"[Title/Abstract] OR "Food Supplementations"[Title/Abstract] OR "Food Supplements"[Title/Abstract] OR "Food Supplement"[Title/Abstract] OR "supplement food"[Title/Abstract] OR "supplements food"[Title/Abstract] OR "Nutraceuticals"[Title/Abstract] OR	None	78

		"Nutraceutical"[Title/Abstract] OR "Nutriceuticals"[Title/Abstract] OR "Nutriceutical"[Title/Abstract] OR "Neutraceuticals"[Title/Abstract] OR "Neutraceutical"[Title/Abstract] OR "Herbal Supplements"[Title/Abstract] OR "Herbal Supplement"[Title/Abstract] OR "supplement herbal"[Title/Abstract] OR "supplements herbal"[Title/Abstract] OR "supplement*"[Title/Abstract] OR "oral*"[Title/Abstract] OR "administration, oral"[MeSH Terms] OR "administration oral"[Title/Abstract] OR "drug administration oral"[Title/Abstract] OR "Oral Drug Administration"[Title/Abstract] OR "Oral Administration"[Title/Abstract] OR "administrations oral"[Title/Abstract] OR "Oral Administrations"[Title/Abstract] OR "administration oral drug"[Title/Abstract] OR "Oral Drug Administrations"[Title/Abstract] OR "administration oral"[Title/Abstract]) AND ("randomized controlled trials as topic"[MeSH Terms] OR "Randomized Controlled Trial"[Title/Abstract] OR "controlled trial*"[Title/Abstract] OR "RCT"[Title/Abstract])) OR "randomised intervention studies"[Title/Abstract]) AND ("lipids"[MeSH Terms] OR "lipid"[Title/Abstract])		
Date: 29/1 2024	Scopus	Choline OR "Choline supplements" OR "Choline Citrate" OR "citrate choline" OR "choline o sulfate" OR "choline o sulfate" OR "Choline Hydroxide" OR Bursine OR "Choline Bitartrate" OR "bitartrate choline" OR "Choline Chloride" OR "chloride choline" OR phosphatidylcholines OR Phosphatidylcholine OR "choline phosphatidyl" OR "Choline Phosphoglycerides" OR "phosphoglycerides choline" OR Phosphatidylcholine OR "Phosphatidyl Cholines" OR "cholines phosphatidyl" OR "Choline Glycerophospholipids" OR "glycerophospholipids choline" OR Lecithins OR Lecithin AND "Dietary supplements" OR "Dietary Supplement"	None	188

		OR "supplements dietary" OR "Dietary Supplementations" OR "supplementations dietary" OR "Food Supplementations" OR "Food Supplements" OR "Food Supplement" OR "supplement food" OR "supplements food" OR Nutraceuticals OR Nutraceutical OR Nutriceuticals OR Nutriceutical OR Neutraceuticals OR Neutraceutical OR "Herbal Supplements" OR "Herbal Supplement" OR "supplement herbal" OR "supplements herbal" OR supplement* OR oral* OR "administration oral" OR "drug administration oral" OR "Oral Drug Administration" OR "Oral Administration" OR "administrations oral" OR "Oral Administrations" OR "administration oral drug" OR "Oral Drug Administrations" OR "administration oral" AND "Randomized Controlled Trial" OR "controlled trial*" OR RCT OR "randomised intervention studies" AND lipids OR lipid		
Total number of articles *				266

*including doubles

Appendix 2. Excluded articles read in full

	Reference	Reason for exclusion
1	Olthof MR, van Vliet T, Verhoef P, Zock PL, Katan MB. Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. PLoS Med. 2005 May;2(5):e135.	This study uses oil in both the intervention and placebo group. Although they were both matched and controlled for fatty acid composition, it deems it difficult to compare with other RCTs where fat was not used in either intervention nor placebo.
2	Olthof MR, Brink EJ, Katan MB, Verhoef P. Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men. Am J Clin Nutr. 2005 Jul;82(1):111-7.	It became apparent that this article is one of the 4 studies presented in ”*Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans*” above.
3	Oosthuizen W, Vorster HH, Vermaak W, Smuts CM, Jerling JC, Veldman FJ, et al. Lecithin has no effect on serum lipoprotein, plasma fibrinogen and macro molecular protein complex levels in hyperlipidaemic men in a double-blind controlled study. European journal of clinical nutrition. 1998;52(6):419-24.	Choline was used as intervention, but is mixed with other oils. Both control and intervention contain mixed fatty acids, which makes it difficult to compare the results with studies that do not.
4	Childs MT, Bowlin JA, Ogilvie JT, Hazzard WR, Albers JJ. The contrasting effects of a dietary soya lecithin product and corn oil on lipoprotein lipids in normolipidemic and familial hypercholesterolemic subjects. Atherosclerosis. 1981;38(1):217-28.	Excluded as the study used other oils as a control. There are risks that these oils may affect the result.
5	Greten H, Raetzer H, Stiehl A, Schettler G. The effect of polyunsaturated phosphatidylcholine on plasma lipids and fecal sterol excretion. Atherosclerosis. 1980;36(1):81-8.	Excluded as the study used other oils as a control. There are risks that these oils may affect the result.
6	Bernhard W, Bockmann K, Maas C, Mathes M, Hovelmann J, Shunova A, et al. Combined choline and DHA supplementation: a randomized controlled trial. Eur J Nutr. 2020 Mar;59(2):729-39.	Excluded as the study did not include relevant outcome measures (LDL, HDL, and TC).

7	Hongu N, Sachan DS. Carnitine and choline supplementation with exercise alter carnitine profiles, biochemical markers of fat metabolism and serum leptin concentration in healthy women. J Nutr. 2003 Jan;133(1):84-9.	Excluded as the study did not include relevant outcome measures (LDL, HDL, and TC)
8	Klatt KC, McDougall MQ, Malysheva OV, Brenna JT, Roberson MS, Caudill MA. Reproductive state and choline intake influence enrichment of plasma lysophosphatidylcholine-DHA: a post hoc analysis of a controlled feeding trial. Br J Nutr. 2019 Dec 14;122(11):1221-9.	Excluded as the study did not include relevant outcome measures (LDL, HDL, and TC)
9	Rashvand S, Mobasseri M, Tarighat-Esfanjani A. The Effects of Choline and Magnesium Co-Supplementation on Metabolic Parameters, Inflammation, and Endothelial Dysfunction in Patients With Type 2 Diabetes Mellitus: A Randomized, Double-Blind, Placebo-Controlled Trial. J Am Coll Nutr. 2019 Nov-Dec;38(8):714-21.	Excluded as the study did not include relevant outcome measures (LDL, HDL, and TC)
10	Spilburg CA, Goldberg AC, McGill JB, Stenson WF, Racette SB, Bateman J, et al. Fat-free foods supplemented with soy stanol-lecithin powder reduce cholesterol absorption and LDL cholesterol. J Am Diet Assoc. 2003 May;103(5):577-81.	Choline was used as a control, and not as an intervention.
11	Taesuwan S, McDougall MQ, Malysheva OV, Bender E, Nevins JEH, Devapatla S, et al. Choline metabolome response to prenatal choline supplementation across pregnancy: A randomized controlled trial. FASEB J. 2021 Dec;35(12):e22063.	500 mg/d d0-choline + 50 mg/d methyl-d9-choline was used as intervention, and 25 mg/d d9-choline was used as control. Thus, this study did not use a placebo control but only choline in a smaller amount.
12	DiBella M, Thomas MS, Alyousef H, Millar C, Blesso C, Malysheva O, et al. Choline Intake as Supplement or as a Component of Eggs Increases Plasma Choline and Reduces Interleukin-6 without Modifying Plasma Cholesterol in Participants with Metabolic Syndrome. Nutrients. 2020 Oct 13;12(10).	Excluded as this study does not use placebo as control. They compare choline in eggs with choline supplements.

LDL, low-density lipoprotein; HDL, high-density lipoprotein; TC, total cholesterol