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Lipid-Modifying Effects of Chitosan Supplementation in Humans: A Pooled Analysis with Trial Sequential Analysis

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Scope: We performed a pooled analysis with trial sequential analysis (TSA) to evaluate the efficacy and safety of chitosan supplementation on serum lipids in humans.

Methods and results: Medline, EMBASE, and CENTRAL databases were queried. Impact was expressed as a weighted mean difference (WMD) and 95% confidence interval (CI). Sensitivity analysis was conducted using the leave-one-out method. Statistical heterogeneity, publication bias, TSA, and subgroup analyses were also assessed. Fourteen trials (21 treatment arms) encompassing 1108 participants were suitable for statistical pooling. Chitosan supplementation significantly improved the total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations in all patients. The WMDs were −0.20 mmol L^{-1} (95% CI, −0.35 to −0.05; $p = 0.009$) for TC, and −0.20 mol L^{-1} (95% CI, −0.26 to −0.15; $p = 0.0001$) for LDL-C, respectively. TSA demonstrated that the cumulative *Z*-curve crossed the trial sequential monitoring boundary for benefit providing conclusive evidence for the benefit of chitosan. However, no significant changes were seen with high-density lipoprotein cholesterol (HDL-C) and triglycerides. Our findings were robust after sensitivity analyses, and no serious adverse events were reported with chitosan intake.

Conclusion: Supplementation with chitosan effectively reduces plasma concentrations of TC and LDL-C. Current evidence indicates daily chitosan supplementation as a candidate for therapeutic lipid management strategies.

1. Introduction

Cardiovascular disease (CVD) is the major cause of mortality and disability worldwide. Reports from the World Health Organization show that more than 17.5 million people die of CVD every year, and the number of CVD-related deaths is projected to rise to > 23.6 million by the year 2030.[1] Epidemiological studies have highlighted dyslipidemias as significantly important to the etiology and development of CVD.[2] Those patients with hyperlipidemia have a 3 times risk of heart attack compared with those with normal lipid status.[3]

In the last few decades, several classes of lipid-modifying agents have been recommended as first-line therapy drugs for the pharmacotherapy of dyslipidemia, including statins, ezetimibe, fibrates, bile acid sequestrants (BAS), nicotinic acid (niacin), and omega-3 fatty acids.[4,5] Despite supplementary improvement in patient lipid profiles with these lipid-lowering drugs, their efficacy in reducing CVD outcomes has not been completely established.[6] Statins have long been the first choice in the treatment of dyslipidemia, and have shown not only to significantly lower LDL cholesterol (LDL-C), but also to prevent cardiovascular morbidity and mortality.[7] However, statins' use is associated with muscle-related adverse effects (AEs) and residual risk, including myalgia, muscle weakness, neuropathy, gastrointestinal disturbances, cognitive dysfunction, and rhabdomyolysis (occurs very rarely) in a considerable number of patients.[8,9] Given these drawbacks, statin-related AEs might impair the effectiveness of statin therapy. Accumulating evidence suggests that healthy dietary practices

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presumably play critical roles in CVD prevention and hypolipidemic treatment. Natural products or functional food with cardiovascular protective properties may help patients achieve and maintain cardiovascular health and improve lipid disorders through novel mechanisms.[10–12]

Chitosan, a partially deacetylated polymer of N-acetyl glucosamine derived from the polysaccharide chitin, is a dietary fiber obtained primarily from fungal cell walls and the exoskeletons of various crustaceans such as crab, lobster, and shrimp.[13] The effect of chitosan on fat absorption has been shown to be different than digesting resistant maltodextrin (MD).[14] MD is a nonviscous, soluble, dietary fiber that has been reported to decrease blood lipids by delaying lipid absorption.[15] Chitosan is believed to act as a cationic polysaccharide in the gastrointestinal tract; its chemical structure is similar to that of cellulose and is not cleaved by digestive enzymes in humans.[16] In acidic gastric fluid, chitosan swells and forms a positively charged gel which bonds with strong negative-charged molecules such as fatty and bile acids. Chitosan also interferes with emulsification of neutral lipids like cholesterol and triglycerides (TG) by binding them with hydrophobic bonds.[17,18] Several studies have shown that dietary chitosan may help reduce body weight and was shown to lower blood lipids in both in animal and human trials due to its specific chemical structure.

However, a comprehensive analysis of the safety of chitosan has not previously been completed, and the efficacy of chitosan on plasma lipids outcomes is not uniformly consistent. In the present study, all available randomized controlled trials (RCTs) on chitosan supplementation were searched systematically and assessed to determine the safety and overall efficacy of chitosan on blood lipids in a meta-analysis. To determine whether the currently available evidence was sufficient and conclusive, the trial sequential analysis (TSA) was further applied.

2. Methods

2.1. Data Sources and Searches

The present study was following the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.[19] We identified relevant articles published between January 1965 (index date) and March 2017, through literature searches of PubMed, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL) databases. The following search terms were used: "chitosan" OR "chitin". Our literature search was filtered with human studies and English publications. Furthermore, clinical trials or review articles were also manually searched to identify additional relevant publications. Articles were rejected during initial screening if titles or abstracts were clearly irrelevant.

2.2. Selection of Studies

Only randomized control trials (RCTs) that evaluated the effect of chitosan administration on lipids were included in this study. The primary outcomes were the mean differences in lipid profiles, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and TG. Original studies were selected for analysis if they met the following inclusion criteria: 1) participants were adult male or female (age >18 years); 2) types of interventions or exposure: participants ingested the chitosan interventions (regardless of regimen type applied) for at least 2 weeks; 3) types of studies: studies were human RCT trials with either a parallel or a crossover design; 4) types of outcome measures: studies investigated the impact of chitosan on plasma or serum concentrations of at least one-lipid profiles of interest. Exclusion criteria were as follows: 1) reviews or nonclinical studies; 2) study design was not a RCT or a control group was lacking; 3) outcome measure values were at the end of the trial or changes from baseline were not reported; 4) biomarker concentrations were monitored ≤1 week after acute intervention.

2.3. Quality Assessment

We used the Cochrane Collaboration bias risk analysis tool to assess the quality assessment of included trials.[20] Random sequence generation, allocation concealment, blinding of outcome assessments, blinding of participants and personnel, selective reporting, incomplete outcome data, and other sources of bias were classified as high, low, or unclear for each domain of the included studies.

2.4. Data Extraction

Following assessment of methodological quality, data from each eligible study was abstracted independently by two investigators using a standardized data collection form. When there were disagreements, a third reviewer checked the data. The following items were extracted: general information (first author's name, year of publication, trial name); study characteristics (study design, study location, intervention duration, control group allocation, and the form and amount of chitosan intake); participant characteristics (number of patients involved, age, gender, baseline values for BMI and for the markers of interest, baseline TC, and health status); and outcome measures (definition of outcomes, pre- and post-intervention means and SDs, and sample size of each arm). All continuous variables were captured as means ± standard deviation. Blood lipid levels were collated in mmol L^{-1}, and the extracted data in mg dL^{-1} were converted to using the standard conversion factors.

2.5. Data Synthesis and Analysis

We performed this meta-analysis using the STATA software program (Version 12.0; (StataCorp LP). Treatment effects were expressed as weighed mean difference (WMD) and 95% confidence interval (CI) between the treatment and control groups. We completed I^2 testing to assess heterogeneity between trials, with values> 50% regarded as significant heterogeneity.[21] Results were generated from a fixed-effects model if no significant heterogeneity was shown, otherwise, a random-effects model was used for analysis. To examine the possible influence of covariates

on our primary outcomes, previously defined subgroup analyses were conducted according to the intervention duration, study design, chitosan dose, mean age of the subjects, and baseline TC. The sensitivity analysis was performed using the leave-one-out approach (deleting each study once and then repeating the analysis) as well as removing studies with a higher risk of bias. *p*-Values less than 0.05 were considered to indicate a statistically significant difference for all analyses.

2.6. Publication Bias Assessment

Publication bias was evaluated by the Egger test and visual inspection of the respective funnel plot.[22] If there were any evidence of publication bias, the trim and fill method was used to adjust the effect on chitosan efficacy to mitigate potential publication bias.

2.7. Trial Sequential Analysis

Repeated significance testing of sparse and accumulated data in a traditional meta-analysis may increase the risk of type I errors which may cause false positive or negative results. TSA depends on the quantification of the required information size (the meta-analysis sample size), which was performed to reduce the risk of random errors and false positive results by adapting the monitoring boundaries to evaluate the accumulated evidence and calculating the estimated information size to guide protocols in subsequent trials.[23] To increase the robustness of the meta-analyses, and to determine whether the current sample size is sufficiently enough, we applied TSA using $\alpha = 0.05$ (two sided) and $\beta = 0.20$ (power of 80%). TSA version 0.9 software program was applied to the cumulative meta-analysis for all outcomes.

3. Results

3.1. Identification of Relevant Studies

Supporting Information Figure S1 presents our procedure for literature screening, study selection, and exclusion justification. The original literature search generated 266 potentially relevant records, in which eight additional studies were identified from the reference list of the retrieved papers. After screening of titles and abstracts, 135 studies were excluded either because of duplication or because they were not relevant to our study. Thirty-one studies were considered of interest and the full text articles were retrieved for detailed evaluation. After a closer assessment, 14 RCTs (equivalent to 21 treatment arms) achieved the inclusion criteria and were preferred for the final pooled analysis.[17,24–36]

3.2. Characteristics of Included Studies

A summary of the primary characteristics of the 14 eligible trials and 1108 participants are outlined in **Table 1**. All trials were randomized, placebo-controlled studies published between 1999 and 2016, and were conducted in the USA, Finland, United Kingdom, China, Australia, Canada, Japan, Mexico, Singapore, Korea, and India. The sample size of participants per study ranged from 12 to 250 (total 1108, 63.3% female). The mean age ranged from 35.9 to 62.4 years (median: 48.1). Twelve studies employed a parallel study design, and two trials used a crossover design.[32,34] Eleven studies were randomized double-blind placebo-controlled trials, except three studies, which used a single-blind approach.[17,33,36] Types of chitosan used included: chitosan (11 studies), chitin-glucan (1 study),[28] and microcrystalline chitosan (2 studies).[35,37] Doses ranged from 0.312 to 6.75 g d^{-1} of chitosan. The duration of the chitosan intervention varied from 4 weeks to 24 weeks, with a median of 13.75 weeks. The mean BMI values of patients at baseline ranged from 22.9 to 35.5 kg m^{-2}. Selected studies were performed in subjects who were overweight or had obesity, hypercholesterolemia, and prediabetes. Among the 14 studies included in the meta-analysis, 12 trials were designed as parallel study, and two adopted a crossover study design.[35,37] During the study period, all eligible participants attempted to maintain their usual lifestyles.

3.3. Risk-of-Bias Assessment

The methodological quality of the included studies was variable. Four studies did not provide sufficient data about random sequence generation, and two studies were conducted with only a single-blind approach. The details of the systematic assessment of bias are shown in Supporting Information Table S1.

3.4. Pooled Estimate of the Effects of Chitosan Supplementation on Lipid Profiles

Pooled analysis of data from 20 treatment arms showed significant reductions in TC levels following chitosan administration (WMD: −0.20 mmol L^{-1}; 95% CI: −0.35 to −0.05; $p = 0.009$) (**Figure 1**A). Significant heterogeneity for this outcome was found ($I^2 = 72\%$). TSA showed the required information size (RIS) of 1121 patients was reached and the cumulative *Z*-curve crossed the conventional significance test boundary and RIS-adjusted boundary value. The conclusion for TC outcome is sufficient and no more trials are needed (**Figure 2**A).

The effect of chitosan supplementation on patient LDL-C levels was reported in 21 treatment arms. There was a statistically significant reduction in LDL-C in the chitosan supplemented patient group. The pooled mean difference for LDL-C between chitosan supplemented and the placebo group using fixed-effect analysis was −0.20 mmol L^{-1} (95% CI: −0.26 to −0.15; $p = 0.00001$) (Figure 1B). Heterogeneity was insignificant for this outcome ($I^2 = 0\%$). TSA was conducted and figured out RIS of 561. The cumulative *Z*-curve crossed the conventional significance test boundary and RIS-adjusted boundary value, indicating sufficient power to draw a conclusive conclusion (Figure 2B).

Nineteen treatment arms explored the effect of chitosan supplementation on HDL-C. A pooled effect identified in fixed-effect analysis was found to be nonsignificant (WMD: −0.01 mmol L^{-1}; 95% CI: −0.04 to 0.02; $p = 0.58$; $I^2 = 0\%$; Figure 1C). For TSA, the cumulative *Z*-curve did not cross either the conventional boundary for benefit or the trial sequential monitoring boundary for

Table 1. Characteristics of study populations, type of interventions, and study designs in the included trials.[a)]

Reference	Year	No. of subjects	Inclusion criteria	Sex (M/F)	Location	Mean age[b)]	BMI [kg m^{-2}][b)]	Intervention group	Control group	Duration [weeks]	Study design	Baseline TC [mmol L^{-1}]
Pittler et al.	1999	30	Overweight volunteers	6/24	United Kingdom	44.0	26.6	Chitosan; 1 g d^{-1}	Placebo	4	R, DB, P	5.56
Ho_a et al.	2001	31	Obesity subjects with hypercholesterolemia	0/31	Singapore	43.5	25.1	Chitosan consumption in females, 3.1 g d^{-1}	Placebo	12	R, DB, P	6.29
Ho_b et al.	2001	37	Obesity subjects with hypercholesterolemia	37/0	Singapore	42.4	26.3	Chitosan consumption in males, 3.1 g d^{-1}	Placebo	12	R, DB, P	6.29
Bokura et al.	2003	36	Female volunteers with confirmed mild to moderate hypercholesterolemia	0/36	Japan	56.5	22.9	Chitosan, 1.2 g d^{-1}	Placebo	7	R, DB, P	6.23
Metso et al.	2003	96	Subjects with moderate hypercolesterolemia	39/57	Finland	46.0	26.4	Microcrystalline chitosan, 2.4 g d^{-1}	Placebo	12	R, DB, C	6.00
Mhurchu et al.	2004	250	Overweight and obese adults	45/205	Australia	48.0	35.5	Chitosan, 3 g d^{-1}	Placebo	24	R, DB, P	5.50
Lehtimäki_a et al.	2005	29	Subjects with mild to moderate hypercholesterolemia	16/13	Finland	43.5	25.5	Microcrystalline Chitosan administration in apolipoprotein E e4 carrier persons, 2.4 g d^{-1}	Placebo	12	R, DB, C	5.79
Lehtimäki_b et al.	2005	56	Subjects with mild to moderate hypercholesterolemia	21/35	Finland	44.8	25.4	Microcrystalline Chitosan administration in non-carrier persons persons, 2.4 g d^{-1}	Placebo	12	R, DB, C	5.66
Kaats	2006	88	Overweight adults	14/74	USA	46.3	≥25.0	Chitosan capsules, 3 g d^{-1}	Placebo	8.6	R, DB, P	5.41
Liao_a et al.	2007	40	Patients with hyperlipidemic	12/28	China	62.5	27.3	Water-soluble chitosan, 0.312 g d^{-1}	Placebo	8	R, SB, P	6.18
Liao_b et al.	2007	40	Patients with hyperlipidemic	12/28	China	62.7	27.2	Water-insoluble chitosan, 0.312 g d^{-1}	Placebo	8	R, SB, P	6.47
Jaffer_a et al.	2007	36	Subjects with hypercholesterolemia	22/14	Canada	54.0	29.3	Chitosan, 1.2 g d^{-1}	Placebo	12	R, DB, P,	5.49
Jaffer_b et al.	2007	43	Subjects with hypercholesterolemia	28/15	Canada	53.0	30.4	Chitosan, 1.6 g d^{-1}	Placebo	12	R, DB, P,	5.46
Jaffer_c et al.	2007	42	Subjects with hypercholesterolemia	28/14	Canada	52.5	28.0	Chitosan, 0.8 g TID d^{-1}	Placebo	12	R, DB, P,	5.31
Jaffer_d et al.	2007	41	Subjects with hypercholesterolemia	23/18	Canada	52.0	29.2	Chitosan, 2.4 g d^{-1}	Placebo	12	R, DB, P,	5.39
Tapola_a et al.	2008	29	Subjects with mild hypercholesterolemia	12/17	USA	45.5	25.5	Chitosan tablets, 4.5 g d^{-1}	Placebo	8	R, SB, P	5.80
Tapola_b et al.	2008	26	Subjects with mild hypercholesterolemia	12/14	USA	45.0	26.0	Chitosan tablets, 6.75 g d^{-1}	Placebo	8	R, SB, P	5.80
González et al.	2010	12	Obese adults without diabetes mellitus	6/6	Mexico	42.1	33.5	Chitosan, 1.35 g d^{-1}	Placebo	12	R, DB, P	4.45
Bays_a et al.	2013	68	Subjects with LDL-C ranging from 3.37 to 4.92 mmol l^{-1}	31/37	USA	50.5	28.0	Chitin-glucan, 4.5 g d^{-1}	Placebo	6	R, DB, P	6.03
Bays_b et al.	2013	67	Subjects with LDL-C ranging from 3.37 to 4.92 mmol l^{-1}	27/40	USA	51.4	27.6	Chitin-glucan, 1.5 g d^{-1}	Placebo	6	R, DB, P	5.97
Kim et al.	2014	51	Subjects with prediabetes	36/15	Korea	56.1	24.1	Chitosan, 1.5 mg d^{-1}	Placebo	12	R, DB, P	5.04
Trivedi	2016	86	Overweight and obese subjects	34/52	India	35.9	30.9	Chitosan capsules, 2.5 g d^{-1}	Placebo	12.8	R, SB, P	NA

a) M, male; F, female; BMI, body mass index; R, randomized; DB, double-blind; SB, single blinded; P, parallel; C, crossover; NA, not available. Studies conducted by Ho et al.,[28] Liao et al.,[33] Tapola et al.[17] and Bays et al.[25] were separated into 2 treatment arms, respectively. The work conducted by Jaffer et al.[29] was divided into 4 treatment arms.

b) Values for age, BMI, and baseline TC are expressed as mean unless otherwise stated. To convert values for cholesterol from mg dL^{-1} to mmol L^{-1}, multiply by 0.02586; to convert values for TG from mg dL^{-1} to mmol L^{-1}, multiply by 0.01129.

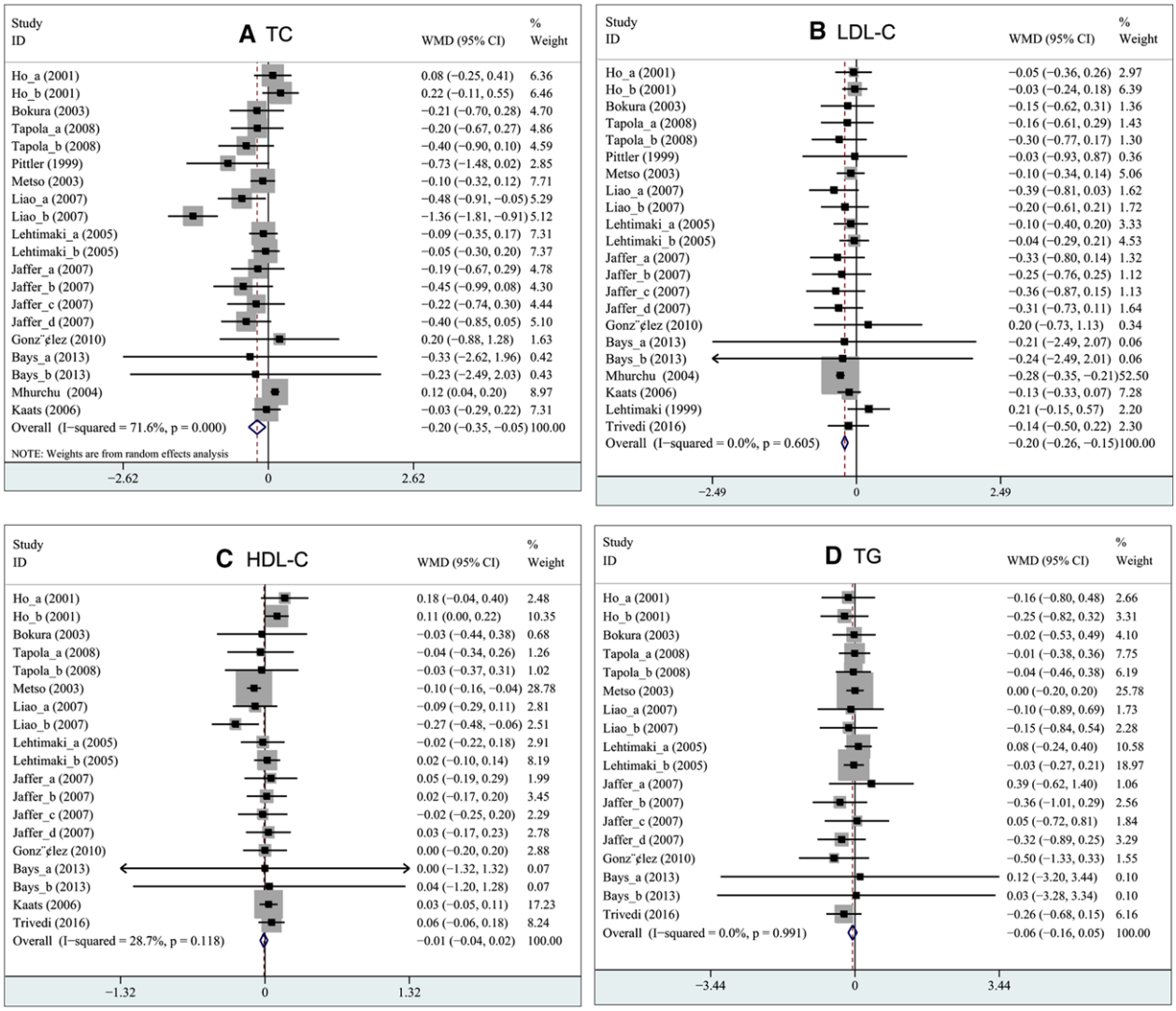

Figure 1. Forest plot detailing weighted mean difference and 95% confidence intervals (CI) for the impact of chitosan supplementation on serum lipids in humans. Horizontal lines of each study correspond to the 95% CI. Areas of shadow rectangles reflect weight.

benefit. Therefore, it established insufficient and inconclusive evidence (Figure 2C).

There were 18 interventions studying the effect of chitosan supplementation on TG. The pooled result show that chitosan intervention did not significantly change the TG levels compared with controls (WMD: −0.06 mmol L^{-1}; 95% CI: −0.16 to 0.05; $p = 0.28$; $I^2 = 0\%$) (Figure 1D). Under TSA, the cumulative *Z*-curve did not cross either the conventional boundary for benefit or the trial sequential monitoring boundary for benefit. Therefore, it established insufficient and inconclusive evidence (Figure 2D).

3.5. Subgroup Analysis

Subgroups analysis according to participant characteristics are summarized in **Table 2**. To explore the dose-effect relation, chitosan doses were divided into 2 categories, ≤2.4 g d^{-1} versus >2.4g d^{-1}. Results indicated that the supplementation of chitosan significantly decreased TC (WMD: −0.34 mmol L^{-1}; 95% CI: −0.58 to −0.10; $p = 0.006$) and LDL-C (WMD: −0.14 mmol L^{-1}; 95% CI: −0.26 to −0.02; $p = 0.02$) in the low-dose chitosan group (≤2.4 g d^{-1}). Moreover, a significant reduction in LDL-C was also found in patients who consumed >2.4 g of chitosan daily. In a subgroup analysis stratified by study design, chitosan intervention significantly reduced TC (WMD: −0.25 mmol L^{-1}; 95% CI: −0.45 to –0.05; $p = 0.01$) and LDL-C (WMD: v0.23 mmol L^{-1}; 95% CI: −0.29 to −0.17; $p = 0.0001$) in studies with parallel design.

In subgroup analysis by intervention duration (<12 weeks or ≥12 weeks), there was a significant reduction of TC (WMD: −0.46 mmol L^{-1}; 95% CI: −0.80 to −0.12; $p = 0.008$) and LDL-C (WMD: −0.18 mmol L^{-1}; 95% CI: −0.32 to v0.04; $p = 0.01$) shown in the shorter-term subgroup. Chitosan also significantly decreased LDL-C in the longer-term subgroup (WMD:

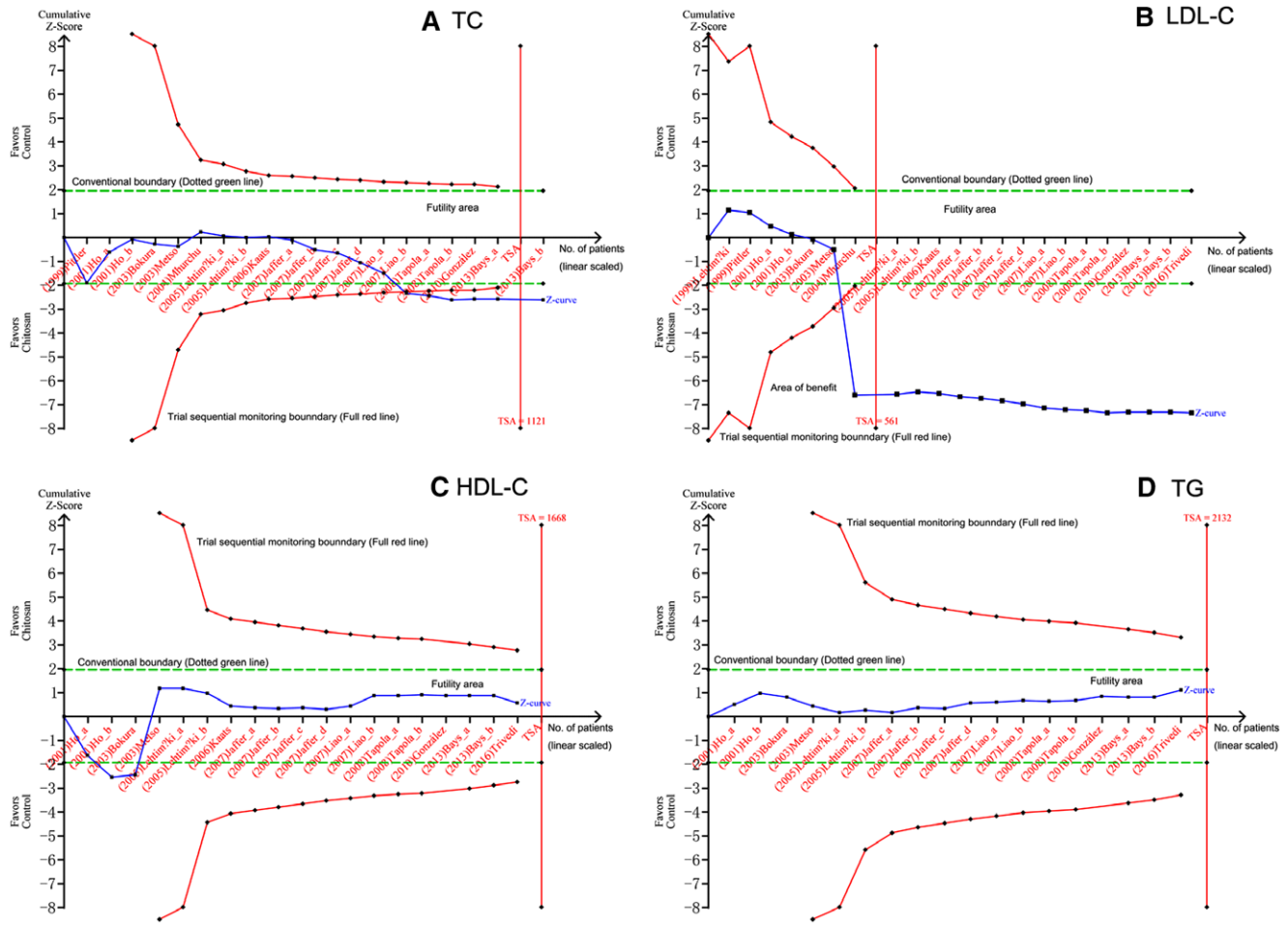

Figure 2. Trial sequential analysis on pooled result of effects of chitosan consumption on lipid profiles. A) TSA on pooled result of TC: the cumulative sample size over the RIS of 1121 and the cumulative *Z*-curve crossed both the conventional boundary and the trial sequential monitoring boundary for benefit. B) TSA on pooled result of LDL-C: the cumulative sample size over the RIS of 561 and the cumulative *Z*-curve crossed both the conventional boundary and the trial sequential monitoring boundary for benefit. C) TSA on pooled result of HDL-C: the cumulative sample size over the RIS of 1668 and the cumulative *Z*-curve did not cross both the conventional boundary and the trial sequential monitoring boundary. D) TSA on pooled result of TG: the cumulative sample size over the RIS of 2132 and the cumulative *Z*-curve did not cross both the conventional boundary and the trial sequential monitoring boundary. RIS, required information size.

-0.19 mmol L^{-1}; 95% CI: -0.27 to -0.12; $p = 0.0001$), but not in the shorter-term group. Subgroup analyses according to baseline TC showed that chitosan consumption significantly reduced TC and LDL-C levels in participants with a baseline TC >5.72 mmol L^{-1} compared with controls (TC: -0.24 mmol L^{-1}, 95% CI: -0.42, -0.07, $p = 0.006$; LDL-C: -0.14 mmol L^{-1}, 95% CI:-0.23, -0.05, $p = 0.003$). However, chitosan did not affect LDL-C in subjects with a baseline TC ≤ 5.72 mmol L^{-1} group. Finally, we also stratified studies according to the mean age (<50 years versus ≥50 years), and saw a significant change in TC (WMD: -0.48 mmol L^{-1}; 95% CI: -0.77 to -0.18; $p = 0.001$) and LDL-C (WMD: -0.28 mmol L^{-1}; 95% CI: -0.45 to -0.11; $p = 0.001$) in the subjects with mean age ≥ 50 years; meanwhile, a significant change in LDL-C was observed in subjects with the mean age <50 years (WMD: -0.18 mmol L^{-1}; 95% CI: -0.25 to -0.10; $p = 0.0001$).

3.6. Sensitivity Analyses

The pooled effect estimates on lipid profiles did not change substantially after leave-one-out sensitivity analyses (Supporting Information Figure S2). Sensitivity analysis that excluded lower-quality studies showed that the aggregated results similar to the overall results (TC: -0.17 mmol L^{-1}; 95% CI: -0.34 to -0.01; $p = 0.04$; LDL-C: -0.21 mmol L^{-1}; 95% CI: -0.27 to -0.15; $p = 0.0001$; HDL-C: -0.01 mmol L^{-1}; 95% CI: -0.04 to 0.03; $p = 0.79$; TG: -0.04 mmol L^{-1}; 95% CI: -0.16 to 0.08; $p = 0.47$).

3.7. Adverse Events

Chitosan was well tolerated, and the participants experienced no serious adverse events (AEs). Of the 14 included studies, 7 RCTs

Table 2. Subgroup and sensitivity analyses of TC, LDL-C, HDL-C, and TG stratified by previously defined study characteristics.

Variables	Arms	Total cholesterol			Arms	LDL cholesterol			Arms	HDL cholesterol			Arms	Triglycerides		
		WMD (95% CI)[a]	p	I^2 (%)		WMD (95% CI)[a]	p	I^2 (%)		WMD (95% CI)[a]	p	I^2 (%)		WMD (95% CI)[a]	p	I^2 (%)
Mean age																
<50 years	11	−0.02 (−0.13 to 0.09)	0.76	39	12	−0.18 (−0.25 to −0.10)[c]	0.0001	9	10	−0.00 (−0.04 to 0.03)	0.90	51	9	−0.04 (−0.16 to 0.07)	0.44	0
≥ 50 years	9	−0.48 (−0.77 to −0.18)[c]	0.001	57	7	−0.28 (−0.45 to −0.11)[c]	0.001	0	9	−0.04 (−0.13 to 0.04)	0.29	0	9	−0.12 (−0.37 to 0.13)	0.34	0
Intervention duration																
Shorter–term (< 12 weeks)	9	−0.46 (−0.80 to −0.12)[c]	0.008	71	9	−0.18 (−0.32 to −0.04)[c]	0.01	0	8	−0.02 (−0.08 to 0.05)	0.60	5	7	−0.04 (−0.26 to 0.18)	0.71	0
Longer–term (≥ 12 weeks)	11	−0.03 (−0.15 to 0.08)	0.59	40	12	−0.19 (−0.27 to −0.12)[c]	0.0001	7	11	−0.01 (−0.05 to 0.03)	0.74	44	11	−0.06 (−0.18 to 0.06)	0.30	0
Dose																
≤2.4 g d^{-1}	11	−0.34 (−0.58 to −0.10)[c]	0.006	70	11	−0.14 (−0.26 to −0.02)[c]	0.02	0	10	−0.07 (−0.11 to 0.00)	0.05	1	10	−0.02 (−0.15 to 0.10)	0.71	0
>2.4 g d^{-1}	9	−0.02 (−0.16 to 0.12)	0.80	39	10	−0.23 (−0.29 to −0.17)[c]	0.0001	0	9	0.06 (0.01 to 0.11)[c]	0.03	0	7	−0.13 (−0.32 to 0.05)	0.16	0
Study design																
Parallel	17	−0.25 (−0.45 to −0.05)[c]	0.01	76	18	−0.23 (−0.29 to −0.17)[c]	0.0001	0	16	0.03 (−0.01 to 0.07)	0.17	0	15	−0.14 (−0.29 to 0.02)	0.09	0
Crossover	3	−0.08 (−0.22 to 0.06)	0.25	0	3	−0.08 (−0.23 to 0.07)	0.30	0	3	−0.07 (−0.12 to 0.00)	0.05	40	3	0.01 (−0.13 to 0.14)	0.94	0
Mean baseline TC[b]																
≤5.72 mmol dL^{-1}	4	0.00 (−0.23 to 0.23)	0.99	51	4	−0.25 (−0.33 to −0.16)[c]	0.00001	6	2	0.03 (−0.05 to 0.10)	0.46	0	1	−0.51 (−1.33 to 0.33)	0.42	NA
>5.72 mmol dL^{-1}	16	−0.24 (−0.42 to −0.07)[c]	0.006	64	16	−0.14 (−0.23 to −0.05)[c]	0.003	0	16	−0.03 (−0.07 to 0.01)	0.17	32	16	−0.04 (−0.14 to 0.07)	0.51	0

a) WMD, weighted mean differences; CI, confidence interval.
b) In this subgroup analysis, the study conducted by Trivedi et al. was excluded, because the data of baseline TC was not available.
c) Indicates a significant result.

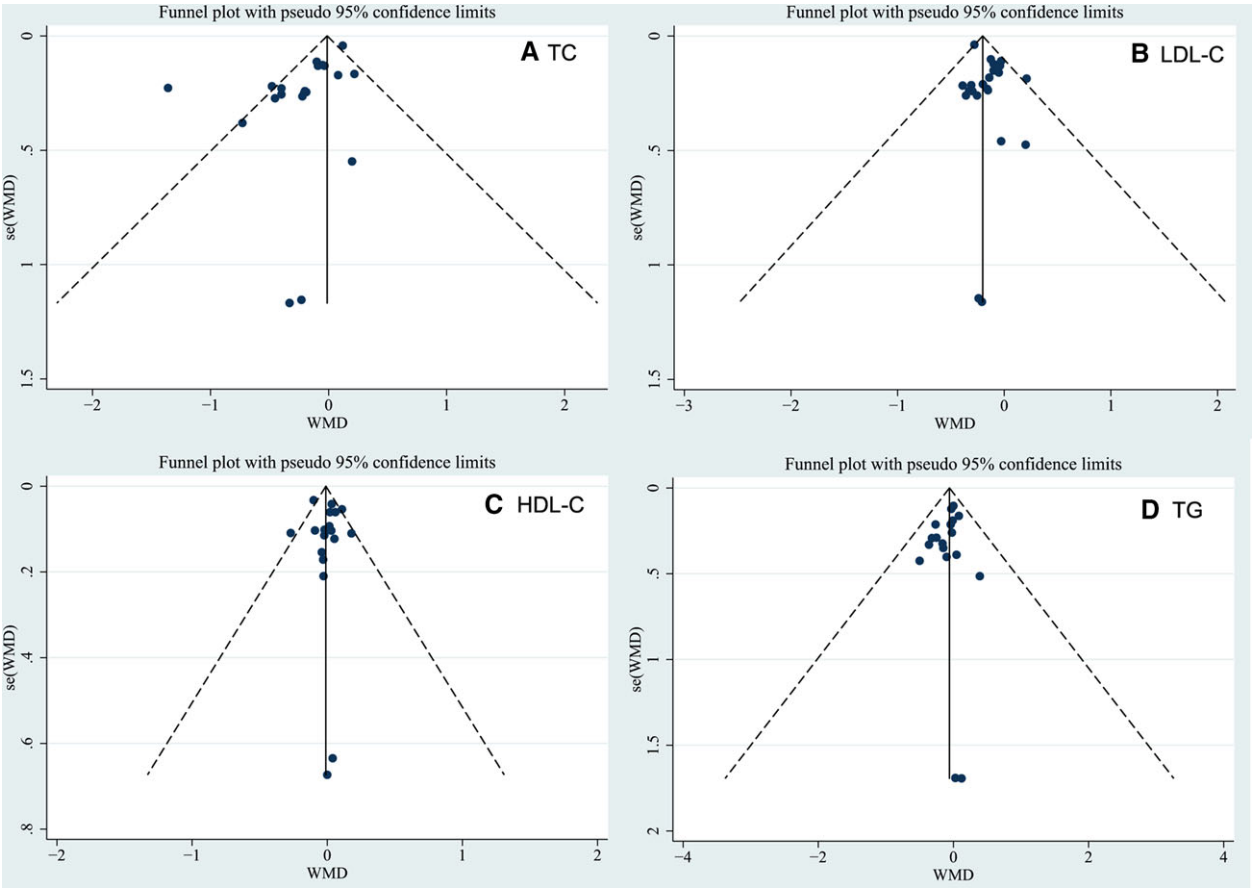

Figure 3. Funnel plots detailing publication bias in the studies selected for analysis.

reported AEs associated with chitosan supplementation including gastrointestinal complaints, constipation, body ache, rash, and diarrhea.

3.8. Publication Bias Diagnostics

The potential publication bias in the literature was examined by analyzing funnel plots and Egger's regression tests. In all trials, funnel plots were symmetrical (**Figure 3**). Egger's tests also suggests no evidence of publication bias in terms of TC ($p = 0.330$), LDL-C ($p = 0.121$), HDL-C ($p = 0.278$), and TG ($p = 0.344$).

4. Discussion

There is sufficient evidence to support the role of lipid-lowering therapies in the treatment and prevention of CVD and atherosclerosis-mediated cardiovascular events in wide-scale populations.[37] Lipid-lowering dietary or nutraceutical interventions are now considered potential protective therapies against CVD for many situations. These situations include those where patients have poor compliance of drugs, drug induced AEs, contraindications to drugs, or a personal preference for natural or alternative therapies. There are currently extensive RCT-based meta-analysis on the effects of chitosan on blood lipids levels, providing ample evidence for the beneficial effect of chitosan on TC and LDL-C levels. However, the consumption of chitosan did not appear to alter the levels of HDL-C and TG.

Our meta-analysis provides evidence for the use of chitosan as an adjunct to pharmacological therapy in patients with dyslipidemic. Chitosan was well tolerated, and the risk of AE did not substantively differ between chitosan and placebo treatments. This relationship is robust and consistent throughout sensitivity and subgroup analyses. Our analysis is not only a thorough synthesis of recent data the effects of chitosan on management of dyslipidemia but also identifies future research priorities.

A previous meta-analysis performed by Baker et al. and published in 2009, included six RCTs for analysis, and indicated that the use of chitosan significantly lowered TC but not LDL-C, HDL-C, or TG in hypercholesterolemia patients.[38] However, we argue that this conclusion may not be conclusive. As the authors clearly emphasized, the small number of included studies limits the strength of their conclusion. Our study has several advantages, over this previous meta-analysis, to make it more conclusive. Firstly, our analysis has more enlarged sample sizes than previously, giving us greater power to evaluate chitosan efficacy. Secondly, our study includes more clinical details, including a summary of side effects reported in eligible trials, as well as more

detailed dosages. Furthermore, we increased the robustness of our findings by applying TSA to evaluate the influence of random error and repetitive testing. Finally, our quality assessment suggested that there was no significant publication bias, and key subgroup and sensitivity analyses based on study design and patient characteristics were performed to identify the impact of chitosan on various parameters in the meta-analysis, unlike previously.

The important role of plasma LDL in atherosclerosis initiation and progression has been reported in both human and animal studies. According to the American Heart Association/American College of Cardiology (AHA/ACC) and the European Society of Cardiology (ESC) guidelines, the primary goal for CVD prevention and management is to reduce the risk of CVD by reducing patient LDL-C, blood pressure, BMI, and glucose to recommended levels. For each 1 mg dL^{-1} reduction in patient LDL-C concentration, the relative risk of a coronary heart disease event is decreased by 1%.[37] It appears that the greatest potential cardiovascular benefits from chitosan are due to its potential lipid-lowering effects. In our analysis, pooled results demonstrate that chitosan consumption results in a 7.73 mg dL^{-1} reduction in LDL-C; this reduction is statistically significant, and likely clinically significant due to the reduced risk of CVD. These findings supported in some subgroup analyses. In subgroups with residual cardiovascular risk (RCVR), individualized therapy, effective combination lipid-modifying drugs or a more aggressive glucose or blood pressure-lowering strategy should be employed.[39]

When we stratified studies according to the dose of chitosan intervention, the supplementation of chitosan significantly decreased the level of TC and LDL-C in low-dose chitosan group ($\leq$2.4 g d^{-1}). However, chitosan is associated with a statistically significant decrease in LDL-C and an increase in HDL-C levels in subjects who consumed >2.4 g of chitosan daily. No statistically significant effect was observed for TC. Although extensive searches and clear inclusion criteria were made in our present study, some differences in types and chemical compositions of chitosan, ethnicity, and diet habit still exist among the included trials. Among the included studies, due to the lack of original data of the reviewed studies, we were unable to determine the comparable degree of deacetylation of chitosan. Evidence shows that the physiological property of chitosan is probably determined by the degree of deacetylation of chitosan.[40] As the degree of deacetylation of chitosan increases, the fat digestibility seems to decrease. In addition, the subjects were requested to keep their alcohol and tobacco consumption in study with higher dose of chitosan consumption.[17] This may explain the unexpected result in our subgroup analysis. Future studies using a standardized chitosan-intervention protocol (i.e., including the consistency of chitosan source and dosage, duration of administration and designed rigorously with large sample sizes) are needed.

Although the present meta-analysis provides useful implications for clinical practice, several potential limitations in the current literature need to be acknowledged. Firstly, consistent with all meta-analyses, internal validity relies on the quality of individual studies. In this regard, most of the included studies had median sample sizes, potentially leading to an overestimation of treatment effects; smaller trials may be methodologically less robust and more prone to report larger effect sizes.[41] Secondly, influences of the other covariates, such as product quality and the bioavailability of chitosan in different therapies could not be fully determined due to a lack of detailed information. Additionally, trials selected in this study recruited subjects with different cardiovascular risk backgrounds (e.g., prediabetic, obese, and/or hypercolesterolemic) that could affect our results. Finally, the number of studies in this analysis examining the impact of chitosan on lipoprotein (a) [Lp (a)] was rather small. It would be valuable to have more data on this biomarker because epidemiological research has shown an independent association between the circulating concentration of Lp (a) and the risk of coronary heart disease and stroke.[42,43]

In conclusion, the results of our meta-analysis demonstrated a significant effect of chitosan supplementation on the reduction of TC and LDL-C levels, and this effect was even more evident in subjects with baseline TC >5.72 mmol dL^{-1}, a parallel study design, and subjects with mean age $\geq$50 years. There was no difference in side effect incidence between chitosan consumption and placebo, and no major AEs were reported. Therefore, chitosan consumption is a worthwhile dietary approach for preventing hypercholesterolemia, particularly in specific patient subgroups who cannot tolerate typical interventions. Further studies investigating the influence of chitosan administration on CVD-related morbidity and all-cause mortality are needed. Our findings are particularly significant in patients at high risk of CVD. The efficacy of chitosan in treatment of other chronic diseases, such as metabolic syndrome and diabetes, need to confirm the lipid-modifying effects of chitosan supplementation.

Abbreviations

AEs, adverse effects; CI, confidence interval; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RCTs, randomized control trials; RR, relative risks; TC, total cholesterol; TG, triglycerides; TSA, trial sequential analysis; WMD, weighted mean difference

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgments

H.H. and Y.Z. contributed equally to this work. Y.Z. acquired, analyzed, interpreted the data, and drafted the manuscript; H.C. completed the database, searched, selected, reviewed the articles, and extracted the data; D.L. collected and assembled the data, and performed the data analyses; H.H. conceived and designed the study, discussed the idea of the meta-analysis, critically reviewed the article for important intellectual content, and submitted the paper. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

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