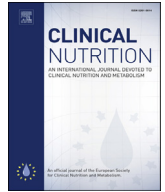




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Meta-analyses

A systematic review and meta-analysis of the impact of Spirulina supplementation on plasma lipid concentrations

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SUMMARY

Background & aims: The impact of Spirulina supplementation on plasma lipid concentrations has not been conclusively studied. Therefore the aim of the meta-analysis was to assess the effect of Spirulina supplementation on plasma lipid concentrations.

Methods: We searched PubMed and Scopus (up to July 03, 2015) to identify randomized controlled trials (RCTs) that investigate the effect Spirulina supplementation on plasma lipid concentrations. Meta-analysis and meta-regression were performed using random-effects models.

Results: Random-effect meta-analysis of data from 7 RCTs showed a significant effect of supplementation with spirulina in reducing plasma concentrations of total cholesterol (WMD: -46.76 mg/dL, 95% CI: -67.31 to -26.22 , $p < 0.001$), LDL-C (WMD: -41.32 mg/dL, 95% CI: -60.62 to -22.03 , $p < 0.001$) and triglycerides (WMD: -44.23 mg/dL, 95% CI: -50.22 to -38.24 , $p < 0.001$), and elevating those of HDL-C (WMD: 6.06 mg/dL, 95% CI: 2.37 – 9.76 , $p = 0.001$).

The impact of spirulina on plasma concentrations of total cholesterol (slope: -1.32 ; 95% CI: -8.58 to 5.93 ; $p = 0.720$), LDL-C (slope: -1.01 ; 95% CI: -8.03 to 6.02 ; $p = 0.778$), triglycerides (slope: -1.39 ; 95% CI: -4.26 to 1.48 ; $p = 0.342$) and HDL-C (slope: 1.79 , 95% CI: -0.48 to 4.05 ; $p = 0.122$) was independent of administered dose. Regarding duration of supplementation with Spirulina, significant associations were found with changes in plasma concentrations of total cholesterol (slope: -1.77 ; 95% CI: -3.48 to -0.07 ; $p = 0.042$), LDL-C (slope: -1.73 ; 95% CI: -3.40 to -0.06 ; $p = 0.042$) HDL-C (slope: 0.91 ; 95% CI: 0.68 – 1.14 ; $p < 0.001$) and triglycerides (slope: -1.39 ; 95% CI: -2.28 to -0.50 ; $p = 0.002$).

Conclusions: This meta-analysis showed a significant effect of supplementation with Spirulina in reducing plasma concentrations of total cholesterol, LDL-C, triglycerides and elevating those of HDL-C.

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1. Introduction

Spirulina is a filamentous, spiral-shaped, water blue-green microalga (Cyanobacterium). The most well-known species of spirulina researched in the scientific literature and safe for consumption are *Spirulina platensis*, *Spirulina maxima* and *Spirulina fusiformis* [1]. Spirulina is considered to be one of the most healing and prophylactic ingredients of nutrition in the 21st century [2,3] due to its nutrient profile, lack of toxicity and therapeutic effects [4]. Spirulina has been consumed as a food by North Africans and

Abbreviations: ACE, angiotensin I-converting enzyme; BMI, body mass index; CI, confidence interval; CHD, coronary heart disease; CMA, comprehensive meta-analysis; HMOX-1, heme oxygenase-1; GK, glucokinase; GSP, glycosylated serum protein; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, assessment-estimated insulin resistance; LDL-C, low-density lipoprotein cholesterol; NF-kB, nuclear factor KB; TC, total cholesterol; WMD, weighed mean difference; TG, triglycerides.

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Mexicans because it contains high amounts of antioxidants such as β -carotene, phycocyanin, microelements (K, Na, Ca, Mg, Fe, Zn), vitamins (tocopherols), eight necessary aminoacids, polyunsaturated fatty acids, especially γ -linolenic acid and phenolic compounds [5]. Like other nutraceuticals [6–8], Spirulina is recommended in arterial hypertension [9,10], insulin-resistance [2], diabetes mellitus [5,11], non-alcoholic fatty liver disease [12], malnutrition [13], anemia [14], allergic rhinitis [15], cancer [16] and in reduction of drug toxicity [17].

C-phycocyanin, a particular essential pigment of Spirulina, is used as a natural dye in food, cosmetics and pharmaceutical industry [18]. Phycocyanin contains an open-chain tetrapyrrole chromophore known as phycocyanobilin, which can activate atheroprotective heme oxygenase-1 (HMOX-1), a key enzyme in the heme catabolic pathway, in endothelial cells improving atherosclerosis in mice [19]. Moreover, phycocyanin has proven antioxidant, anti inflammatory and radical scavenging properties. Also, phycocyanin showed to decrease fasting blood glucose and glycosylated serum protein (GSP), being useful for diabetic patients [5]. According to experimental studies in alloxan-injured mice, phycocyanin decrease total cholesterol (TC) and triglycerides (TG) levels in serum, increase the hepatic glycogen level and maintain glucokinase (GK) expression in the liver [20]. Also, the angiotensin I-converting enzyme (ACE) inhibitor peptide Ile-Gln-Pro purified from Spirulina proved to be useful in the prevention and treatment of hypertension in rats [2,21]. Furthermore, clinical evidence showed that spirulina has a blood lipid lowering effect in healthy subjects, patients with heart disease, and in diabetic patients [22].

However, the evidence of the effects of Spirulina on plasma lipid parameters has not been conclusive. Therefore the aim of this study was to estimate the effect size of Spirulina supplementation on plasma lipid concentrations by pooling the reported results in randomized controlled trials (RCTs).

2. Methods

2.1. Search strategy

This study was designed according to the guidelines of the 2009 preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement [23]. A systematic literature search was performed in SCOPUS and Medline databases. The search terms (in titles and abstracts) were: (randomized controlled trials OR RCT OR randomized OR lipid OR total cholesterol OR LDL-cholesterol OR HDL-cholesterol OR triglycerides) and (Spirulina). To increase the accuracy of search, the wild-card term "*" was used. The search was limited to studies in human. The literature search in the above-mentioned databases was performed from inception to July 03, 2015. Hand-searching of the retrieved articles was performed to identify further relevant studies that were missed in the database search.

2.2. Study selection

The following criteria was used to identify eligible studies: (i) Randomized controlled trials with either case-control or case-cross-over design, (ii) investigation of the effects of spirulina or standardized spirulina-enriched extracts on plasma/serum lipid concentrations, (iii) providing sufficient information on baseline and end-trial plasma/serum lipid concentrations in both spirulina and control groups. Exclusion criteria were (i) experimental studies, (ii) uncontrolled studies, (iii) administration of non-standardized extracts or extracts containing negligible amounts of spirulina resulting in a daily intake of <5 mg, and (iv) lack of sufficient information on baseline or end-trial lipid concentrations.

In case of the latter item, authors of the article(s) were contacted and requested to provide numerical data.

2.3. Data extraction

The following items were extracted from the eligible studies: 1) first author's name; 2) publication year; 3) study location; 4) number of subjects receiving spirulina and control intervention; 5) age, gender and body mass index (BMI) of subjects in the spirulina and control groups; and 6) serum/plasma concentrations of lipid parameters including total cholesterol, LDL-C, HDL-C and triglycerides.

2.4. Quality assessment

Quality assessment of included studies was performed using Jadad scale. According to this scale the following parameters are appraised: randomization (0–2 points), blinding (0–2 points), and dropouts and withdrawals (0–1 point). The total quality score ranges between 0 and 5, representing the lowest and highest quality of study [24]. Jadad scores of ≤ 2 and ≥ 3 were reflected low- and high-quality studies, respectively [25].

2.5. Quantitative data synthesis

Comprehensive meta-analysis (CMA) V2 software (Biostat, NJ) was used for meta-analysis [26]. The units of all lipid factors including total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were collated in mg/dL. A multiplication by 38.6 and 88.5 was used to convert cholesterol (total cholesterol, HDL-C or LDL-C) and triglyceride units from mmol/L to mg/dL, respectively. Standard deviations (SDs) of the mean difference between pre-treatment and post-treatment values in each group were calculated using the following formula: $SD = \text{square root} [(SD_{\text{pre-treatment}})^2 + (SD_{\text{post-treatment}})^2 - (2R \times SD_{\text{pre-treatment}} \times SD_{\text{post-treatment}})]$, assuming a correlation coefficient (R) = 0.5. In case of reporting SEM, SD was estimated using the following formula: $SD = SEM \times \text{sqrt}(n)$, where n is the number of subjects.

The net between-group change in serum/plasma lipid concentrations in each study was calculated as follows: (value at end of follow-up in the treatment group – value at baseline in the treatment group) – (value at end of follow-up in the control group – value at baseline in the control group). Meta-analysis was performed using a random-effects model and the generic inverse variance weighting method. The choice of random-effects (instead of fixed-effects) model was the heterogeneity of studies in terms of type of spirulina supplement used, spirulina dose, duration of spirulina supplementation, and demographic characteristics of individual trials (underlying disease, age, gender, etc). Effect size was expressed as weighed mean difference (WMD) and 95% confidence interval (CI). In order to evaluate the influence of each study on the overall estimated effect size, a leave-one-out sensitivity analysis was conducted by iteratively removing each study and repeating the analysis.

2.6. Meta-regression

Random-effects meta-regression was performed under an unrestricted maximum likelihood model to explore the association between changes in plasma lipid (total cholesterol, HDL-C, LDL-C and triglycerides) concentrations with dose and duration of supplementation with spirulina.

2.7. Publication bias

The presence of potential publication bias was assessed using visual inspection of Begg's funnel plot asymmetry, and Begg's rank correlation and Egger's weighted regression tests. Duval & Tweedie "trim and fill" method was used to adjust the analyses for the effects of publication bias [27].

3. Results

3.1. Search results and trial flow

The initial screening for potential relevance removed the articles in whose titles and/or abstracts were obviously irrelevant (Fig. 1). After assessment, 7 RCTs achieved the inclusion criteria and were preferred for the final meta-analysis [22,28–33].

3.2. Characteristics of included studies

In total, 522 participants were randomized, of whom 312 were allocated to Spirulina supplementation group and 210 to control group in the selected studies. The number of participants in these trials ranged from 23 to 169. Included studies were published between 1996 and 2014, and were conducted in India (4 studies), Korea (2 studies) and Cameroun. A range of doses from 1 g/day to 10 g/day of Spirulina were administered in the included trials. Duration of supplementation with Spirulina ranged between 2 and 12 months. All trials were designed as parallel-group studies. One trial had four arms comparing Spirulina + diet modification, Spirulina + oral hypoglycemic drugs and diet modification, Spirulina + oral hypoglycemic drugs, insulin and diet modification

vs. usual medical care and treatment. Another trial had three arms comparing Spirulina 2 g/day supplementation, Spirulina 4 g/day supplementation vs. control. Demographic and baseline parameters of the included studies are shown in Table 1. Spirulina was safe and well-tolerated in all of the 7 RCTs included in this review, with no report of serious adverse events.

3.3. Quantitative data synthesis

Random-effect meta-analysis of data from 7 RCTs showed a significant effect of supplementation with Spirulina in reducing plasma concentrations of total cholesterol (WMD: -46.76 mg/dL, 95% CI: -67.31 to -26.22 , $p < 0.001$), LDL-C (WMD: -41.32 mg/dL, 95% CI: -60.62 to -22.03 , $p < 0.001$) and triglycerides (WMD: -44.23 mg/dL, 95% CI: -50.22 to -38.24 , $p < 0.001$), and elevating those of HDL-C (WMD: 6.06 mg/dL, 95% CI: 2.37 – 9.76 , $p = 0.001$) (Fig. 2). The estimated effect size of spirulina on total cholesterol, HDL-C, LDL-C and TG was robust, and not sensitive to any single study (Fig. 3).

3.4. Meta-regression analysis

Meta-regression analysis was conducted to evaluate the association between changes in plasma lipid concentrations and potential moderator variables including dose and duration of supplementation with Spirulina. The impact of Spirulina on plasma concentrations of total cholesterol (slope: -1.32 ; 95% CI: -8.58 to 5.93 ; $p = 0.720$), LDL-C (slope: -1.01 ; 95% CI: -8.03 to 6.02 ; $p = 0.778$), triglycerides (slope: -1.39 ; 95% CI: -4.26 to 1.48 ; $p = 0.342$) and HDL-C (slope: 1.79 , 95% CI: -0.48 to 4.05 ; $p = 0.122$) was independent of administered dose (Fig. 4).

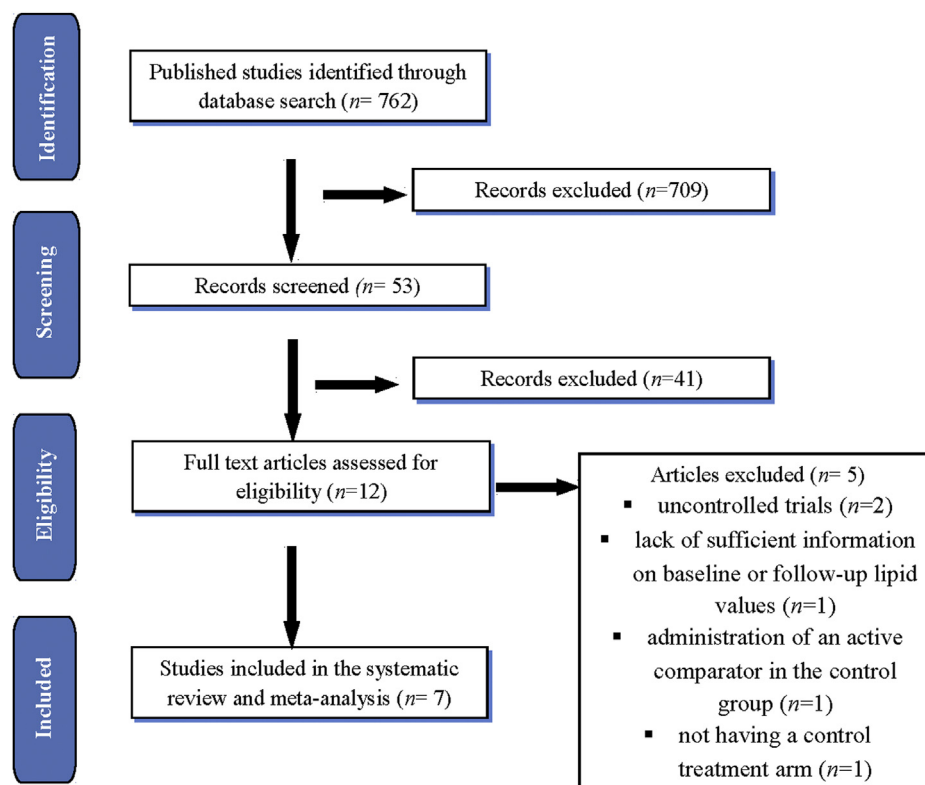


Fig. 1. Flow diagram of the study selection procedure showing the number of eligible randomized controlled trials for the meta-analysis of the impact of spirulina supplementation on plasma lipid concentrations.

Table 1
Demographic characteristics of the included studies.

	Anitha et al. [28]	Lee et al. [22]	Parikh et al. [29]	Park et al. [30]	Ramamoorthy et al. [31]	Samuels et al. [32]	Ngo-Matip et al. [33]	
Year	2010	2008	2001	2008	1996	2002	2010	
Jadad score	2	3	3	4	2	2	4	
Location	India	Korea	India	Korea	India	India	Cameroun	
Design	Parallel-group trial	Randomized placebo-controlled, parallel-group trial	Randomized placebo-controlled, parallel-group trial	Randomized double-blind placebo-controlled parallel trial	Parallel-group trial	Randomized parallel-group trial	Multicentre single-blind, randomized, parallel-group trial	
Duration of trial	12 weeks	12 weeks	2 months	16 weeks	3 months	2 months	12 months	
Inclusion criteria	Male volunteers with type-2 diabetes of 45–60 years of age	Patients with type 2 diabetes	Patients with type 2 diabetes	Volunteers aged > 60 years	Patients with ischemic heart disease, overweight blood cholesterol level of 250–400 mg/dl in the age group 40–60 y	Patients (age 2–13 years) with nephrotic syndrome	HIV antiretroviral-naïve patients	
Spirulina form	500 mg capsules	0.2 g pills of freeze-dried Spirulina	500 mg tablets	0.2 g pills of freeze-dried Spirulina	500 mg tablets	500 mg capsules	package of powder	
Spirulina intervention	1 g/day	8 g/day	2 g/day	8 g/day	2 g/day ^d ; 4 g/day ^e	1 g/day	10 g/day	
Participants	Case 40 ^a 40 ^b 40 ^c	19	15	41	10 ^d 10 ^e	15	82	
Age (years)	Control 40 Case 45–60*	18 52.1 ± 2.3**	10 53.8 ± 7.2	37 66.1 ± 1.2 [#]	NS 65.6 ± 1.4 ^{##}	8 7.0 ± 3.58	87 36.01 ± 9.44	
Male (%)	Control 100.0 ^a Case 100.0 ^b 100.0 ^c	47.4	60.0	58.5	NS	80.0	25.6	
BMI (kg/m ²)	Control 100.0 Case NS	61.1 23.8 ± 0.5**	60.0 25.2 ± 5.4	51.3 24.8 ± 0.7 [#]	NS 23.9 ± 0.5 ^{##}	62.5 15.72 ± 2.12	35.6 25.29 ± 4.54	
Total cholesterol (mg/dL)	Control 232 ± 10 ^a Case 234 ± 9.9 ^b 242 ± 10 ^c	203.1 ± 8.1**	201.3 ± 27.0	181.5 ± 7.1 [#]	200.5 ± 9.4 ^{##}	282.1 ± 16.59 ^d 309.8 ± 28.7 ^e	328.46 ± 132.46	228.7 ± 96.8
LDL-C (mg/dL)	Control 233 ± 10 Case 151 ± 11.2 ^a 152 ± 11 ^b 163 ± 11.6 ^c	186.9 ± 6.4** 129.4 ± 7.5**	201.8 ± 32.5 128.5 ± 23.1	179.1 ± 7.9 [#] 109.7 ± 8.6 [#]	213.7 ± 8.0 ^{##} 126.7 ± 8.3 ^{##}	280.84 ± 21.07 192.1 ± 15.9 ^d 220.5 ± 27.19 ^e	227.62 ± 76.94 225.20 ± 101.35	194.8 ± 80.1 127.0 ± 92.2
HDL-C (mg/dL)	Control 150 ± 11.5 Case 40 ± 7.4 ^a 40 ± 2.9 ^b 37 ± 4.7 ^c	106.9 ± 6.5** 48.8 ± 3.0**	127.8 ± 28.0 37.9 ± 7.7	112.9 ± 7.6 [#] 50.5 ± 4.1 [#]	126.7 ± 8.3 ^{##} 46.8 ± 1.8 ^{##}	191.8 ± 20.18 46.1 ± 4.95 ^d 44.6 ± 3.61 ^e	148.75 ± 55.15 57.52 ± 27.87	120.12 ± 75.8 48.0 ± 21.1
Triglycerides (mg/dL)	Control 42 ± 4.5 Case 202 ± 9.2 ^a 205 ± 9.2 ^b 211 ± 8.8 ^c	52.6 ± 3.2** 125.8 ± 14.1**	42.9 ± 6.2 163.9 ± 55.2	46.1 ± 3.8 [#] 106.5 ± 13.8 [#]	52.6 ± 4.0 ^{##} 135.5 ± 23.9 ^{##}	45.5 ± 3.91 220.1 ± 34.2 ^d 222.5 ± 33.57 ^e	53.0 ± 22.26 227.98 ± 121.0	53.6 ± 18.4 206.9 ± 122.7
	Control 203 ± 9.2	110.7 ± 14.1**	155.6 ± 46.6	100.6 ± 10.1 [#]	106.7 ± 14.7 ^{##}	218.1 ± 31.72	130.37 ± 51.21	117.6 ± 76.8

Values are expressed as mean ± SD. ** Values are expressed as mean ± SE.

Abbreviations: BMI: body mass index; NS: not stated; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; BMI: body mass index; *the value was provided for the total population; # male subjects; ## female subjects.

^a Denotes Spirulina + diet modification group.

^b Denotes Spirulina + oral hypoglycemic drugs and diet modification group.

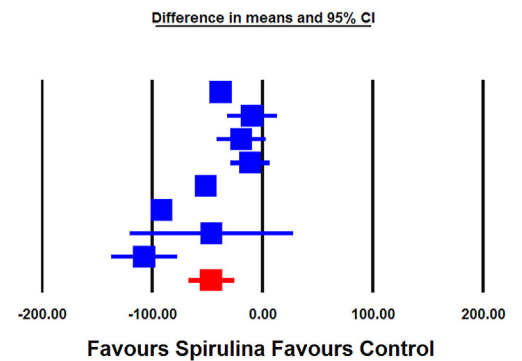
^c Denotes Spirulina + oral hypoglycemic drugs, insulin and diet modification group.

^d Denotes Spirulina 2 g/day group.

^e Denotes Spirulina 4 g/day group.

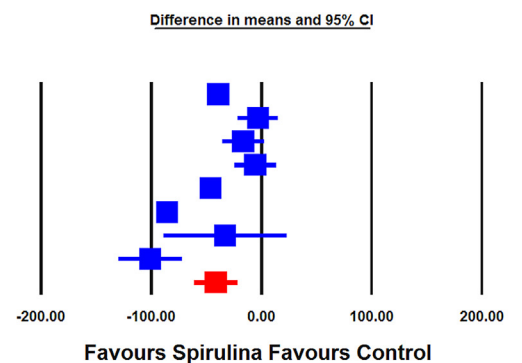
Total cholesterol

Study name	Difference in means	Standard error	Statistics for each study			Z-Value	p-Value
			Variance	Lower limit	Upper limit		
Anitha et al., 2010	-38.000	2.059	4.241	-42.036	-33.964	-18.452	0.000
Lee et al., 2008	-9.600	11.418	130.373	-31.979	12.779	-0.841	0.400
Parikh et al., 2001	-19.600	11.167	124.694	-41.486	2.286	-1.755	0.079
Park et al., 2008	-11.580	8.917	79.515	-29.057	5.897	-1.299	0.194
Ramamoorthy et al., 1996a	-51.500	2.907	8.450	-57.197	-45.803	-17.716	0.000
Ramamoorthy et al., 1996b	-91.900	3.481	12.117	-98.722	-85.078	-26.401	0.000
Samuels et al., 2002	-46.460	37.795	1428.498	-120.538	27.618	-1.229	0.219
Ngo-Matip et al., 2014	-107.400	15.112	228.362	-137.018	-77.782	-7.107	0.000
	-46.763	10.483	109.903	-67.310	-26.216	-4.461	0.000



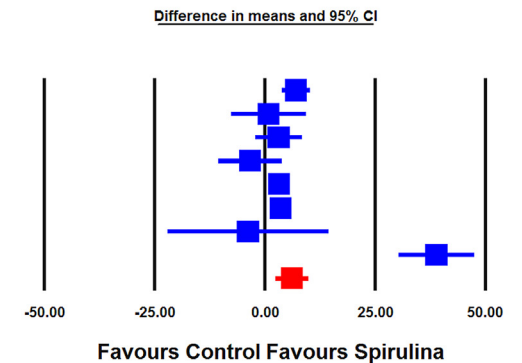
LDL-C

Study name	Difference in means	Standard error	Statistics for each study			Z-Value	p-Value
			Variance	Lower limit	Upper limit		
Anitha et al., 2010	-39.000	2.381	5.669	-43.666	-34.334	-16.380	0.000
Lee et al., 2008	-3.300	9.064	82.148	-21.064	14.464	-0.364	0.716
Parikh et al., 2001	-16.500	9.455	89.405	-35.032	2.032	-1.745	0.081
Park et al., 2008	-5.650	9.374	87.872	-24.023	12.723	-0.603	0.547
Ramamoorthy et al., 1996a	-46.100	2.930	8.585	-51.843	-40.357	-15.733	0.000
Ramamoorthy et al., 1996b	-85.700	3.523	12.408	-92.604	-78.796	-24.329	0.000
Samuels et al., 2002	-33.010	28.472	810.634	-88.813	22.793	-1.159	0.246
Ngo-Matip et al., 2014	-100.680	14.562	212.066	-129.222	-72.138	-6.914	0.000
	-41.324	9.846	96.944	-60.621	-22.026	-4.197	0.000



HDL-C

Study name	Difference in means	Standard error	Statistics for each study			Z-Value	p-Value
			Variance	Lower limit	Upper limit		
Anitha et al., 2010	7.000	1.602	2.567	3.859	10.141	4.369	0.000
Lee et al., 2008	0.800	4.276	18.281	-7.580	9.180	0.187	0.852
Parikh et al., 2001	3.100	2.664	7.095	-2.120	8.320	1.164	0.244
Park et al., 2008	-3.400	3.645	13.287	-10.544	3.744	-0.933	0.351
Ramamoorthy et al., 1996a	3.200	0.616	0.379	1.993	4.407	5.198	0.000
Ramamoorthy et al., 1996b	3.600	0.616	0.379	2.393	4.807	5.848	0.000
Samuels et al., 2002	-3.830	9.295	86.397	-22.048	14.388	-0.412	0.680
Ngo-Matip et al., 2014	38.900	4.313	18.605	30.446	47.354	9.019	0.000
	6.064	1.884	3.549	2.371	9.756	3.219	0.001



TG

Study name	Difference in means	Standard error	Statistics for each study			Z-Value	p-Value
			Variance	Lower limit	Upper limit		
Anitha et al., 2010	-43.000	2.195	4.818	-47.302	-38.698	-19.590	0.000
Lee et al., 2008	-44.800	20.265	410.667	-84.519	-5.081	-2.211	0.027
Parikh et al., 2001	-38.800	21.420	458.803	-80.782	3.182	-1.811	0.070
Park et al., 2008	-6.500	14.621	213.770	-35.156	22.156	-0.445	0.657
Ramamoorthy et al., 1996a	-43.300	1.939	3.762	-47.101	-39.499	-22.326	0.000
Ramamoorthy et al., 1996b	-45.800	2.370	5.617	-50.445	-41.155	-19.325	0.000
Samuels et al., 2002	-45.100	35.478	1258.670	-114.635	24.435	-1.271	0.204
Ngo-Matip et al., 2014	-99.900	16.476	271.474	-132.193	-67.607	-6.063	0.000
	-44.229	3.055	9.330	-50.216	-38.242	-14.480	0.000

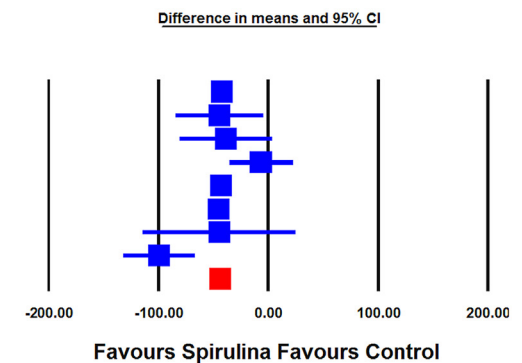
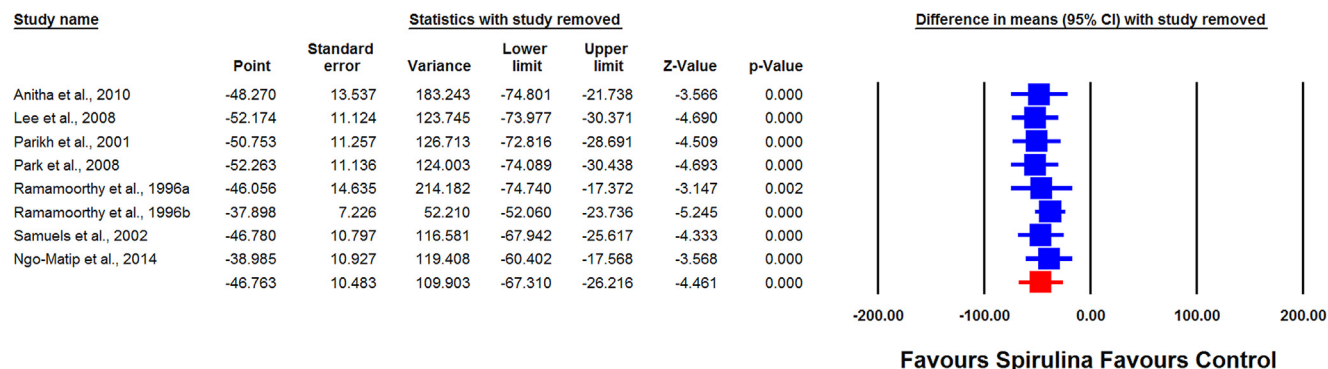
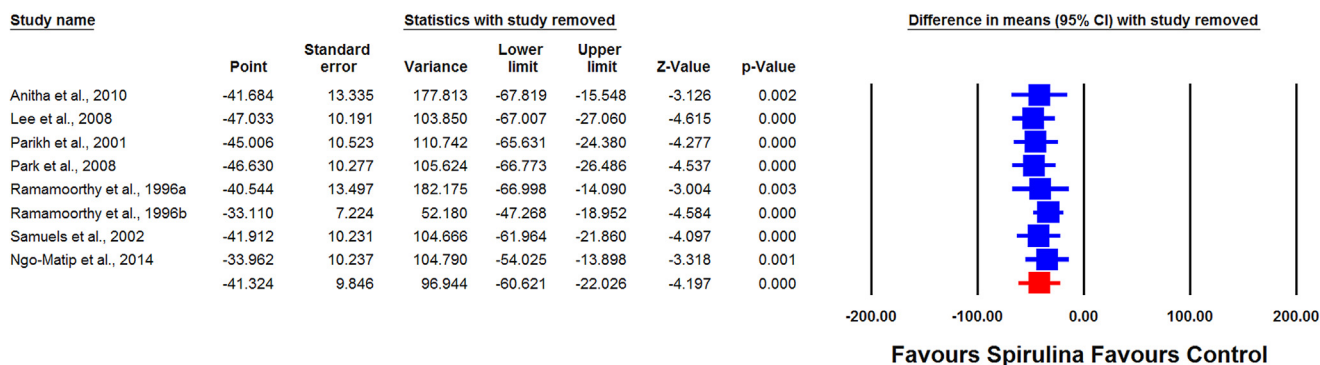


Fig. 2. Forest plot detailing weighted mean difference and 95% confidence intervals for the impact of spirulina supplementation on plasma lipid concentrations.

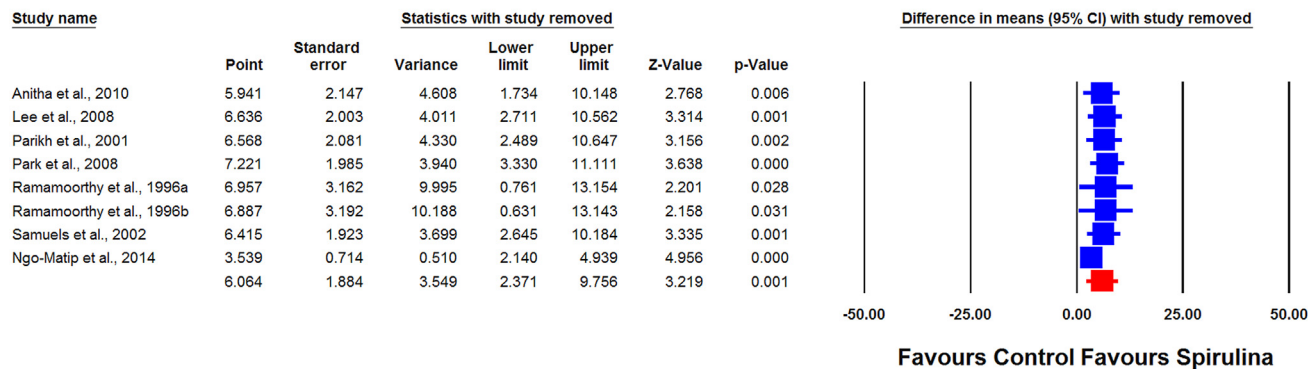
Total cholesterol



LDL-C



HDL-C



TG

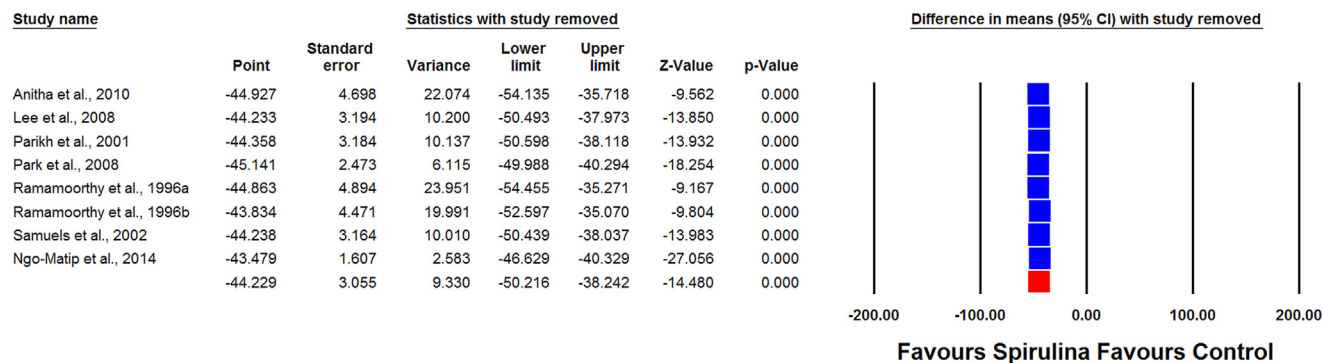


Fig. 3. Leave-one-out sensitivity analysis of the impact of spirulina supplementation on plasma lipid concentrations.

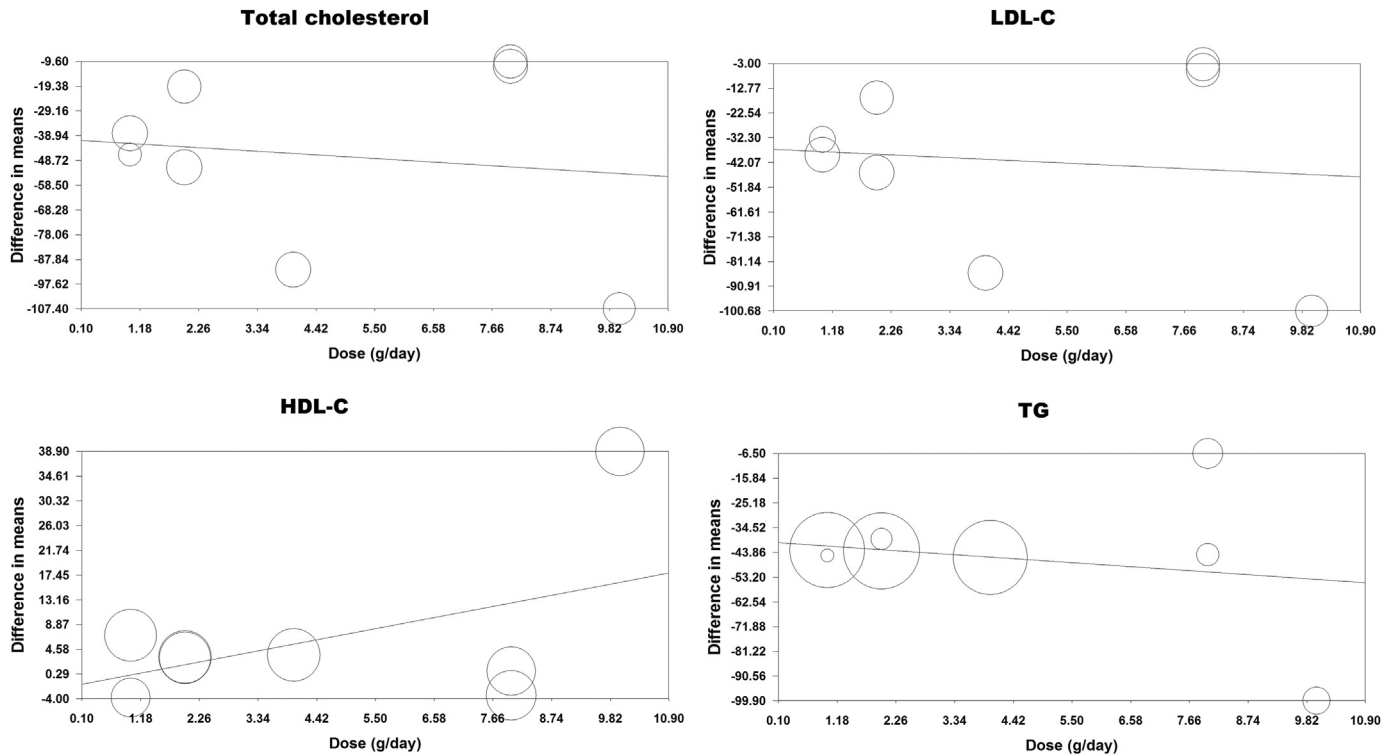


Fig. 4. Meta-regression plots of the association between mean changes in plasma lipid concentrations and administered spirulina dose. The size of each circle is inversely proportional to the variance of change.

With respect to duration of supplementation with spirulina, significant associations were found with changes in plasma concentrations of total cholesterol (slope: -1.77 ; 95% CI: -3.48 to -0.07 ;

$p = 0.042$), LDL-C (slope: -1.73 ; 95% CI: -3.40 to -0.06 ; $p = 0.042$) HDL-C (slope: 0.91 ; 95% CI: 0.68 to 1.14 ; $p < 0.001$) and triglycerides (slope: -1.39 ; 95% CI: -2.28 to -0.50 ; $p = 0.002$) (Fig. 5).

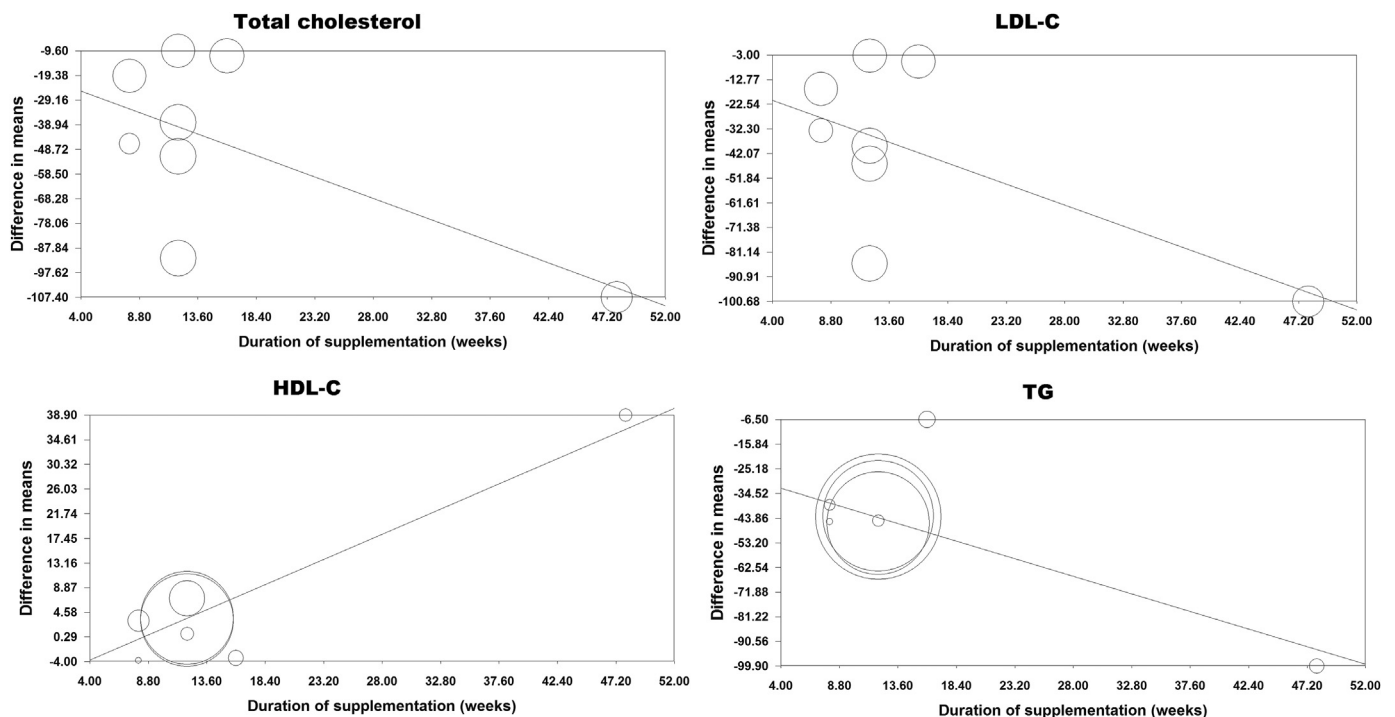


Fig. 5. Meta-regression plots of the association between mean changes in plasma lipid concentrations and duration of spirulina supplementation. The size of each circle is inversely proportional to the variance of change.

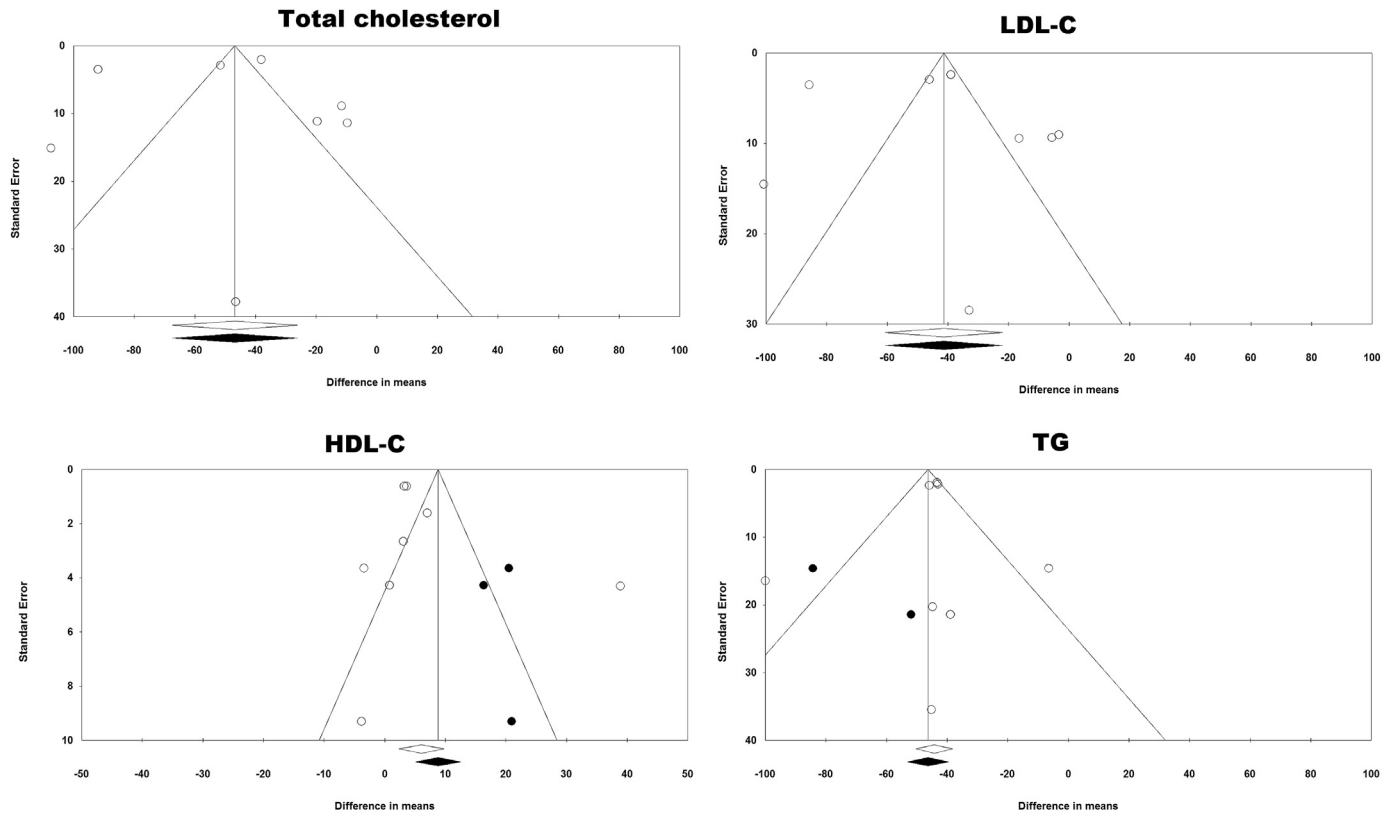


Fig. 6. Funnel plots detailing publication bias in the studies selected for analysis. Trim and fill method was used to impute for potentially missing studies. Open circles represent observed published studies; closed circles represent imputed unpublished studies.

3.5. Publication bias

Visual inspection of the funnel plot of the study precision (inverse SEM) by effect size (mean difference) suggested potential asymmetry that was imputed using trim-and-fill method. Potentially missing studies were those reporting reductions in plasma TG ($n = 2$), and elevations in HDL-C ($n = 3$) after spirulina supplementation. With respect to total cholesterol and LDL-C, no missing study was suggested. The effect size of spirulina on plasma levels of HDL-C (WMD: 8.80 mg/dL, 95% CI: 5.10 to 12.50) and TG (WMD: -46.30 mg/dL, 95% CI: -52.95 to -39.65) increased after imputation (Fig. 6).

In addition to visual inspection of funnel plots, presence of publication bias was explored using Begg's rank correlation test, Egger's linear regression test, and "fail safe N" test. None of these tests indicated evidence of publication bias for the impact of Spirulina on plasma concentrations of total cholesterol, LDL-C, HDL-C and TG (Table 2).

4. Discussion

To our knowledge, the current meta-analysis is the first to evaluate the effects of Spirulina supplementation on serum lipid parameters based on the results from RCTs. The results of this meta-analysis of available RCTs revealed a significant effect of supplementation with Spirulina in reducing plasma concentrations of total cholesterol, LDL-C and triglycerides, and increasing those of HDL-C. The impact of Spirulina on plasma concentrations of total cholesterol and triglycerides was independent of administered dose. Regarding duration of supplementation with Spirulina, significant associations were found in plasma concentrations of total cholesterol, LDL-C, HDL-C and triglycerides.

The active components responsible for hypolipidemic effects of Spirulina are not completely understood. The main ingredient of Spirulina, a protein named C-phycoyanin, reduce the lipids concentrations through scavenging free radicals, inhibiting lipid peroxidation, inhibiting NADPH oxidase expression and

Table 2
Assessment of publication bias in the impact of spirulina supplementation on plasma lipid concentrations.

	Begg's rank correlation test,			Egger's linear regression test					Fail safe N test
	Kendall's Tau ^a	z-value	p-value	Intercept	95% CI	t	df	p-value	n ^b
Total cholesterol	-0.18	0.62	0.536	0.06	-8.42 to 8.54	0.02	6	0.987	1449
LDL-C	-0.32	1.11	0.266	1.34	-7.01 to 9.69	0.39	6	0.709	1169
HDL-C	0	0	1.00	1.35	-3.00 to 5.70	0.76	6	0.476	148
Triglycerides	-0.11	0.37	0.711	-0.18	-2.38 to 2.01	0.21	6	0.844	1381

^a With continuity correction.

^b Number of theoretically missing studies.

increasing the activity of GSH peroxidase and superoxide dismutase [34,35]. The downregulation of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and NADH, which are cofactors in fat metabolism, might also explain the hypolipemic effects of Spirulina [34,35]. Another component isolated from Spirulina, glycolipid H-b₂, can inhibit the activity of pancreatic lipase in a dose-dependent manner [36]. Another significant component of Spirulina is gamma-linolenic acid (GLA), an omega-6 fatty acid known for regulation of prostaglandin and cholesterol synthesis [37]. It has been shown that Spirulina significantly increased the activity of lipoprotein lipase and hepatic triglyceride lipase in different experimental models [37]. Furthermore, Spirulina supplements have been reported to reduce calories and fats, and cholesterol-free source of protein which may lead to the lowering of intrahepatic triglyceride composition [38]. Dietary supplementation with this alga seems to decrease the intestinal assimilation of cholesterol and the reabsorption of bile acids in the ileum [39,40]. Spirulina might decrease inflammatory process through suppression of the activity of nuclear factor KB (NF-kB) and decrease of the production of pro-inflammatory cytokines [41,42]. All these biological processes might explain the underlying mechanisms involved in hypolipidemic effects of Spirulina.

No toxic effects on human physiology after acute or chronic doses of Spirulina were reported in toxicological studies [43]. Spirulina was considered harmless for consume, assuming that the purity of the supplement is preserved (1). In 2008 it was reported the first case of acute rhabdomyolysis after consumption of *Spirulina* as a dietary supplement [44]. After that, the Dietary Supplement Information Expert Committee (DSI-EC) of the United States Pharmacopeial Convention (USP) examined the data obtained from experimental and clinical trials and investigated those 31 side effects of Spirulina. DSI-EC allocated a Class A safeness score for *S. maxima* and *S. platensis*, therefore allowing the entrance of quality monographs for this supplement in USP–NF [45]. Another recent case report study has shown that Spirulina taken as a supplement capsule can induce anaphylaxis. However, before prescribing Spirulina, an allergenicity risk assessment should be realized [46]. In 2014, Food and Drug Administration (FDA) authorized the usage of the Spirulina extract as an organic blue color additive for drinks and foods [47].

The present meta-analysis has some limitations. Most significantly, there were limited number of eligible RCTs, most had modest numbers of patients and a poor design. Furthermore, the included studies were heterogeneous regarding factors like population characteristics, study design, Spirulina dose, and period of supplementation. Moreover, there is a considerable heterogeneity in the groups studied: patients with type 2 diabetes, patients with nephrotic syndrome, patients with ischemic heart disease females or volunteers over 60 years.

In conclusion, this meta-analysis showed a significant effect of supplementation with Spirulina in reducing plasma concentrations of total cholesterol, LDL-C, triglycerides and elevating those of HDL-C. Further well-designed trials are required to clarify the clinical value of Spirulina supplementation as add-on to conventional and novel lipid-lowering therapies in dyslipidemic patients.

Conflict of interest

This meta-analysis was written independently; No authors have any conflict of interest concerning the preparation of this analysis. No professional writer was involved in the preparation of this meta-analysis.

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