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Title: *Nigella Sativa* (Black Seed) Effects on Plasma Lipid Concentrations in Humans: a Systematic Review and Meta-Analysis of Randomized Placebo-Controlled Trials

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Abstract

The effects of *Nigella Sativa* (NS) on plasma lipid concentrations are controversial. A systematic review and meta-analysis of randomized controlled trials (RCTs) was conducted to obtain a conclusive result in humans.

PubMed-Medline, SCOPUS, Web of Science, and Google Scholar databases were searched (up to August 2015) to identify RCTs investigating the impact of NS on total cholesterol, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and triglycerides concentrations. A random-effects model and the generic inverse variance weighting method were used for quantitative data synthesis. Meta-regression, sensitivity analysis, and publication bias assessments were performed using standard methods.

A total of 17 RCTs examining the effects of NS on plasma lipid concentrations were included. Meta-analysis suggested a significant association between NS supplementation and a reduction in total cholesterol (weighed-mean-difference [WMD]: -15.65 mg/dL, 95% CI: -24.67, -6.63, p =0.001), LDL-C (WMD: -14.10 mg/dL, 95% CI: -19.32, -8.88, p <0.001), and triglyceride levels (WMD: -20.64 mg/dL, 95% CI: -30.29, -11.00, p <0.001). No significant effect on HDL-C concentrations (WMD: 0.28 mg/dL, 95% CI: -1.96, 2.53, p =0.804) was found. A greater effect of NS seed oil versus seed powder was observed on serum total cholesterol and LDL-C levels, and an increase in HDL-C levels was found only after NS seed powder supplementation.

NS has a significant impact on plasma lipid concentrations, leading to lower total cholesterol, LDL-C, and TG levels while increased HDL-C is associated with NS powder only. Further RCTs are needed to explore the NS benefits on cardiovascular outcomes.

1. Introduction

Nigella Sativa (NS), popularly known as black seed or black cumin, is an annual plant of the Ranunculaceae family which grows widely in many Middle Eastern countries and Southwest Asia. The seeds of NS are claimed to play antibacterial [1, 2], anti-inflammatory [3], immune-potentiating [4], antioxidant [5, 6], hypoglycemic [7, 8], antihypertensive [9], anti-obesity [10], bronchodilatory [11], neuro- and cardio-protective [12, 13], and antidiarrhoic effects [14], Increasing evidence also supports hypolipidemic properties of NS [13, 15-18]. The NS seeds contain many bioactive constituents, such as antioxidant compounds (mainly represented by thymoquinone and dithymoquinone), flavonoids, sterols, and polyunsaturated fatty acids [15], and the lipid-lowering effect is likely mediated by a synergistic action of its different components. Different mechanisms have been postulated, such as activation of the peroxisome proliferator-activated receptor gamma (PPAR-gamma) [19], increasing uptake of low-density lipoprotein cholesterol (LDL-C) by up-regulation of hepatic LDL receptors [20], *de novo* suppression of cholesterol synthesis [20], reduction in dietary cholesterol absorption, and prevention of lipid peroxidation [6],

There is growing interest in finding safe natural alternatives to common drugs used to treat dyslipidemia, one of the most important risk factor for cardiovascular diseases, particularly in patients resistant to or intolerant of statins [21]. Therefore, the lipid-lowering action of NS has been evaluated both in different experimental models, varying from normal [22] to dyslipidemic [23, 24], dysmetabolic [25], and diabetic animals [26], and in clinical studies, performed in healthy [27], dyslipidemic [28], dysmetabolic [29], and diabetic subjects [30]. Supplementation with NS has been associated with decreased concentrations of serum total cholesterol [23, 24, 26-29, 31-45], triglycerides [2, 23, 24, 26, 28, 31, 32, 34-41, 43, 44, 46], and/or increased concentrations of high-density lipoprotein cholesterol (HDL-C) [23, 25, 28, 32, 34-36, 39, 41-44].

With respect to clinical findings, although some randomized controlled trials (RCTs) have been performed, the reported results have been controversial [9, 47-62]. Because of the variable duration

of studies, preparation of NS employed, study designs, and recruited populations, it is difficult to draw definitive conclusions on the hypolipidemic activity of this natural supplement. The aim of this study was therefore to perform a systematic review of RCTs and conduct a meta-analysis to evaluate the effect of NS supplementation on plasma lipid concentrations.

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 [63]. PubMed-Medline, SCOPUS, Web of Science, and Google Scholar databases were searched using the following search terms in titles and abstracts: ("*Nigella sativa*" OR "black seed" OR "black cumin" OR thymoquinone) AND (placebo) AND (hyperlipidemia OR hyperlipidaemia OR hyperlipidemic OR hyperlipidaemic OR hypolipidemic OR hypolipidaemic OR dyslipidemia OR dyslipidaemia OR dyslipidemic OR dyslipidaemic OR hypercholesterolemia OR hypercholesterolaemia OR hypercholesterolemic OR hypercholesterolaemic OR hypocholesterolemic OR hypocholesterolaemic OR "low-density lipoprotein" OR "high-density lipoprotein" OR cholesterol OR triglycerides OR hypertriglyceridemia OR hypertriglyceridaemia OR hypotriglyceridemic OR hypotriglyceridaemic OR LDL OR LDL-C OR LDL-cholesterol OR HDL- OR HDL-C OR HDL-cholesterol). The wild-card term ''*'' was used to increase the sensitivity of the search strategy. No language restriction was applied. The literature was searched from inception to August 18th, 2015.

2.2 Study Selection

The following criteria were used to identify eligible studies: (i) randomized placebo-controlled trials with either case-control or case-cross-over design, (ii) investigation of the effects of NS or thymoquinone on plasma/serum lipid concentrations, (iii) providing sufficient information on

baseline and end-trial plasma/serum lipid concentrations in both NS and control groups. Exclusion criteria were (i) experimental studies, (ii) observational studies, (iii) uncontrolled studies, 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 necessary numerical data.

2.3 Data extraction

Eligible studies were reviewed, and the following data were abstracted: 1) first author's name; 2) year of publication; 3) country were the study was performed; 4) study design; 5) number of participants in the NS and control groups; 6) type and dose of NS supplement; 7) treatment duration; 9) age, gender, and body mass index (BMI) of study participants; and 9) data regarding baseline and follow-up plasma concentrations of total cholesterol, LDL-C, HDL-C, and triglycerides.

2.4 Quality assessment

A systematic assessment of bias in the included studies was performed using the Cochrane criteria [64]. The items used for the assessment of each study were as follows: adequacy of sequence generation, allocation concealment, blinding, addressing of dropouts (incomplete outcome data), selective outcome reporting, and other potential sources of bias. According to the recommendations of the Cochrane Handbook, a judgment of “yes” indicated low risk of bias, while “no” indicated high risk of bias. Labeling an item as “unclear” indicated an unclear or unknown risk of bias.

2.5 Quantitative Data Synthesis

Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ) [65]. Net changes in measurements (change scores) were calculated as follows: measure at end of follow-up − measure at baseline. For single-arm cross-over trials, net change in plasma concentrations of lipid indices were calculated by subtracting the value after control intervention

from that reported after treatment. All values were collated to mg/dL. Standard deviations (SDs) of the mean difference were calculated using the following formula: SD = square root [$(SD_{pre\text{-}treatment})^2$ + $(SD_{post\text{-}treatment})^2$ – (2R × $SD_{pre\text{-}treatment}$ × $SD_{post\text{-}treatment}$)], assuming a correlation coefficient (R) = 0.5. If the outcome measures were reported in median and range (or 95% confidence interval [CI]), mean and standard SD values were estimated using the method described by Wan et al [66]. When only the standard error of the mean (SEM) was reported, standard deviation (SD) was estimated using the following formula: SD = SEM × sqrt (*n*), where *n* is the number of subjects. In order to avoid unit-of-analysis error due to double-counting of subjects in the trials with more than 1 treatment arm, the control group was evenly (where possible) divided.

Net changes in measurements (change scores) were calculated for parallel and cross-over trials, as follows: (measure at the end of follow-up in the treatment group − measure at baseline in the treatment group) − (measure at the end of follow-up in the control group − measure at baseline in the control group). A random-effects model (using DerSimonian-Laird method) and the generic inverse variance method were used to compensate for the heterogeneity of studies in terms of study design, treatment duration, and the characteristics of populations being studied [67]. Effect sizes were expressed as weighted mean difference (WMD) and 95% confidence interval (CI). Inter-study heterogeneity was assessed using Cochran Q test and I^2 index. In order to evaluate the influence of each study on the overall effect size, sensitivity analysis was conducted using leave-one-out method, i.e. iteratively removing one study each time and repeating the analysis [68-70].

2.6 Meta-regression

A weighted random-effects meta-regression using unrestricted maximum likelihood model was performed to assess the association between the overall estimate of effect size and potential moderator variables including dose and duration of NS supplementation.

2.7 Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, Egger's weighted regression, and "fail safe N" tests. Duval & Tweedie "trim and fill" method was used to adjust the analysis for the effects of publication bias [71].

3. Results

3.1 Flow of studies

After multiple database searches, 52 published studies were identified, and the abstracts reviewed. Twenty-five did not meet the inclusion criteria and were excluded. Next, 22 full text articles were careful assessed and reviewed; of which 5 studies were excluded for not measuring plasma lipid concentrations (n=3), incomplete lipid data (n=1), and non-original article (n=1). Finally, 17 studies were eligible and included in the systematic review and meta-analysis. The study selection process is shown in **Figure 1**.

3.2 Characteristics of included studies

Data were pooled from 17 RCTs that included 1185 subjects, with 616 in the NS arm and 569 in the control arm. Included studies were published between 2008 and 2015. The clinical trials used different forms and doses of NS. Three studies investigated NS powder 1 g/day [49, 56, 57], two studies investigated NS powder 1.5 g/day [47, 50], one study investigated NS powder 1.6 g/day [58], three studies investigated NS powder 2 g/day [51, 61, 62], two studies investigated NS powder 2 spoons/day [52, 60], two studies investigated NS oil 5 ml/day [48, 55], one study investigated NS oil 100 mg/day [9], one study investigated NS oil 200 mg/day [9], one study investigated NS oil 1 g/day [53], and two studies investigated NS oil 3 g/day [54, 59]. The range of intervention periods was from 4 weeks [52, 62] up to 3 months [50, 54, 55, 58]. The most of included studies were parallel-group design [9, 47-57, 59-62], only one study was cross-over design [58]. Selected trials enrolled

subjects with metabolic syndrome [47, 56], overweight [51], obesity [50, 59], mild hypertension [9], hyperlipidemia [51, 52, 60-62], type 2 diabetes [53-55], menopausal women [56-58], and healthy subjects [48, 49] (**Table 1**).

3.3 Risk of bias assessment

Several of the included studies were characterized by lack of information about the allocation concealment and blinding of participants, personnel, and outcome assessors. Some trials did not provide sufficient information of sequence generation [9, 48, 50, 52, 53, 57, 60]. In addition, some trials had other biases related with the study design, such as open-label single arm [58] and single blind [52, 60]. However, most of evaluated studies showed low risk of bias according to incomplete outcome data and selective outcome reporting. Details of the quality of bias assessment are shown in **Table 2**.

3.4 Effect of NS supplementation on plasma lipid concentrations

Overall, the impact of NS supplementation on plasma concentrations of total cholesterol, LDL-C, HDL-C, and triglycerides was assessed in 15, 16, 16 and 18 treatment arms, respectively. NS supplementation was found to significantly reduce plasma concentrations of total cholesterol (WMD: -15.65 mg/dL, 95% CI: -24.67, -6.63, $p = 0.001$; **Figure 2**), LDL-C (WMD: -14.10 mg/dL, 95% CI: -19.32, -8.88, $p < 0.001$; **Figure 2**), and triglycerides (WMD: -20.64 mg/dL, 95% CI: -30.29, -11.00, $p < 0.001$; **Figure 2**), while no significant effect on HDL-C concentrations (WMD: 0.28 mg/dL, 95% CI: -1.96, 2.53, $p = 0.804$; **Figure 2**) was found. All these effects were robust in the sensitivity analysis (**Figure 3**), and the overall estimate of effect size was not significantly driven by a single study.

When the meta-analysis was stratified according to the type of NS supplement that was administered, a greater effect of NS seed oil versus seed powder was observed on serum total cholesterol and LDL-

cholesterol concentrations, while the effect of both supplement types on plasma triglycerides levels was comparable (**Figure 4**). With respect to plasma HDL-C concentrations, a significant elevation was found in the subgroups of studies with NS seed powder, but not NS seed oil (**Figure 4**).

3.5 Meta-regression

Meta-regression analysis was conducted to evaluate the association between changes in plasma lipid concentrations and potential confounders including dose and duration of supplementation with NS. No significant association was found between changes in lipid parameters and duration of supplementation with NS (**Figure 5**). With respect to dose, there were significant associations with changes in plasma total cholesterol (slope: 0.001; 95% CI: 0.0002, 0.015; $p = 0.044$) and HDL-C concentrations (slope: 0.004; 95% CI: 0.003, 0.005; $p < 0.001$), but not with LDL-C and triglyceride levels (**Figure 6**).

3.6 Publication bias

Visual inspection of funnel plots suggested an asymmetry in the meta-analyses of NS's effects on plasma lipid concentrations. Using "trim and fill" method, 2, 3 and 3 potentially missing studies were imputed for the meta-analyses of triglycerides, LDL-C, and HDL-C (**Figure 7**). Corrected effect sizes (following imputation of potentially missing studies) and the results of Egger's linear regression, Begg's rank correlation, and "fail safe N" tests are summarized in **Table 3.**

4. Discussion

The results of the present meta-analysis of RCTs showed that supplementation with NS significantly reduces plasma concentrations of total cholesterol, LDL-C, and triglycerides. This effect was greater with seed oil, while seed powder was found to be associated with a significant elevation in HDL-C levels.

The effects of NS seem to be predominant on LDL-C. The ethanol extract of NS is an agonist of the PPAR-gamma gene [19], whose activation appears to be associated with the enhanced expression of CD36 [72], a cellular scavenger receptor for atherogenic LDL, and ATP-binding cassette transporter A1, a reverse cholesterol transporter involved in the cholesterol efflux from macrophages [73]. Thymoquinone, an active ingredient of NS oil, can up-regulate hepatic LDL receptors [20], inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene [20], and down-regulate ApoB100 gene [74], thus leading to both increased clearance and reduced synthesis of LDL-C. NS could also stimulate bile acid excretion [75] and has a slight anorexic effect [76]. Moreover, many of phytochemicals present in the NS seeds may contribute to its hypocholesterolemic effects: β-sitosterol inhibits the intestinal absorption of dietary cholesterol [77, 78], anti-oxidants protect tissues from lipid peroxidation [6], and the rich content of unsaturated fatty acids may contribute to the reduction in and prevention of cholesterol oxidation [27].

Almost all previous experimental studies in animals [23, 24, 26, 31-44, 79-81], but one [25], showed a reduction in LDL-C after NS supplementation. Consistently, small clinical studies confirmed a significant LDL-C reduction after the consumption of NS in both healthy individuals [27] and dysmetabolic patients [28, 29, 45]. In our meta-analysis, seed oil showed a greater benefit on serum total cholesterol and LDL-C concentrations than seed powder. Owing to the different compositions and doses of NS preparations, along with the inter-study heterogeneities, it is difficult to justify this result with certainty. However, it has been demonstrated that, differently from the powder deriving from seed crushing, the preparation processes of the seed oil may lead to significant compositional changes in the active ingredients, with increased content of the thymoquinone, to which is attributed most of the biological activity of NS [82]. Controlled thermal processing of the seeds, responsible for thymoquinone accumulation at temperatures between 50 and 150°C, could explain the higher biological activity of the oil from heated seeds [82]. Furthermore, thymoquinone is soluble in fat, and the NS oil may be more effective than the aqueous extract form [59].

We found a highly significant effect of NS supplementation in reducing triglyceride concentrations, in line with many animal and clinical studies [2, 23, 24, 26, 28, 31, 32, 34-41, 43, 44, 46, 83-85], but not all [24, 25, 29, 33, 40, 45]. Possible mechanisms responsible for the triglyceride-lowering effect of NS may be due to its components. The high content of polyunsaturated fats may affect both the synthesis and catabolism of triglyceride-rich lipoproteins through increasing PPAR-gamma and lipoprotein lipase activities [59]. Triglyceride-rich lipoproteins exert pro-atherogenic effects, commonly attributed to their remnant lipoprotein particles [86, 87]. Remnants are involved in the mechanisms of endothelial dysfunction and atherosclerosis progression, and appear to be an independent risk factor for cardiovascular disease [86, 87]. Furthermore, the nigellamines A-(5) have been shown to reduce triglyceride levels *in vitro* similarly to clofibrate [88].

Finally, the impact of NS on HDL-C is more controversial: no overall significant effect was found in this meta-analysis, and data from literature are contrasting. Experimental and clinical studies have reported positive effects [23, 25, 28, 32, 34-36, 39, 41-44, 81, 83, 85], but others did not confirm these findings [2, 24, 26, 29, 31, 33, 34, 37, 38, 40, 45].

The divergent results may be due to differences in dosage and type of NS supplementation, dietary habits, physical activity level, duration of the intervention, ethnicity, laboratory and clinical characteristics of the patients studied. Indeed, an HDL-C increase was reported in studies where participants were assigned to an exercise program [51, 60], in experimental studies where NS determined a relevant decrement of triglycerides and insulin resistance, thus resulting in a lower clearance of HDL [25, 36, 54], or in studies administering seed powder [52]. A possible explanation for the superiority of seed powder on HDL-C may be the higher content of seeds of unsaturated fatty acids, mainly linoleic and oleic acids [16]. Regardless of the impact of NS supplementation on plasma HDL-C levels, it is noteworthy that the concept of HDL-C elevation for protection against atherosclerotic cardiovascular disease has been assailed by some recent large-scale trials [89-91]. In

fact, it is currently uncertain if HDL-C elevation could be considered as a treatment target to reduce the risk of cardiovascular outcomes [92].

It could be hypothesized that a longer period of NS supplementation might be necessary in order to impact on HDL-C values. Indeed, the RCTs included in this meta-analysis are short-lasting (maximum 12 weeks), while significant improvements were observed in clinical studies with 6 months supplementations [93].

A major point is that the value of a supplementation should be evaluated not only in term of surrogate endpoints, but also for its effects on morbidity and mortality. Many clinical studies have reported benefits of NS on fasting glucose, insulin resistance, and metabolic syndrome [30, 47, 50, 53-56], body weight and central obesity [10, 29, 50, 55, 59], arterial pressure [9, 94], and inflammation [50]. Lastly, additional cardio-protective effects have been demonstrated in experimental studies: NS inhibits the plaque formation [95] and arachidonic acid-induced platelet aggregation [96], improves endothelial function [97], reduces intima/media ratio [95], induces bradycardia [98], ameliorates cardiac hemodynamics [99], exerts cardio-protection against exogenous and endogenous toxic products [13]. Long-term RCTs are surely needed to evaluate the impact of NS on cardiovascular outcomes. These preliminary results, however, report a wide range of benefits other than the hypolipidemic effects, suggesting a potential use of NS as an adjunct to statin therapy, in consideration of both the significant residual cardiovascular risk in statin-treated individuals and the limitation of statins in achieving optimal LDL-C concentrations.

The safety of NS has been documented in clinical practice. Only a few adverse events have been reported in humans: two cases of contact dermatitis after topical use [100]. In addition to its pungent bitter taste, NS may cause mild, transient nausea and dyspepsia [47], exert a slight anorexic [76] and weight-loss effect [10, 29]. High doses of thymoquinone were found to induce hepatic oxidative stress in mice [101], but humans with solid tumors have tolerated a dose up to 2600 mg/day in a phase I clinical study, with no side effects [102]. NS seeds, particularly in the powdered form,

resulted in a potent hepato-protective effect in experimental models [6, 103], while a non-toxic increase in liver enzymes has been observed after NS oil administration in women [104]. In the included studies, there was no difference in the frequency of adverse effects between treated and placebo groups, and among all participants only one treated subject was reported to quit for nausea [47].

No significant association was found between changes in lipid parameters and duration of supplementation with NS in our meta-analysis. Ibrahim demonstrated that 1 month after cessation of supplementation, the lipid concentrations changed towards the pretreatment levels, thus suggesting the need of a life-long assumption [57]. On the other hand, in the available study with the longer follow-up (6 months), the effect of NS started after 4 days and continued for the entire follow-up [93].

A dose-dependent effect of NS was observed for changes in total cholesterol and HDL-C, but not for triglycerides and LDL-C. We showed in this meta-analysis that NS was more effective against triglyceride and LDL-C concentrations. It could be therefore hypothesized that any dosage was effective on these parameters, while higher doses of NS are needed to affect the other lipid variables, i.e. triglycerides and HDL-C.

RCTs with larger cohorts and longer follow-up are however needed to ascertain the most effective duration and dosage of NS supplementation, that at present are not defined.

4.1 Strengths and limitation

To the best of authors' knowledge, this is the first systematic review and meta-analysis of RTCs investigating the effect of NS on plasma lipid concentrations. However, a number of limitations should be mentioned. First of all, the heterogeneity of RCTs included in the meta-analysis have to be considered, as healthy individuals, patients with hypertension, type 2 diabetes, dyslipidemia, and metabolic syndrome were enrolled. Different preparations, doses, and durations of supplementation

were employed. Part of this inter-study heterogeneity was addressed by choosing a random-effects model for meta-analysis and performing subgroup and meta-regression analyses. Furthermore, some RCTs were not primarily designed to assess the effects of NS on lipid concentrations. Finally, the number of subjects studied in the present meta-analysis was relatively small, but the current pooled population size was sufficient to detect a significant lipid-lowering effect of NS. All observed effects in meta-analysis were robust in the sensitivity analysis, and the overall estimate of effect size was not significantly driven by a single study.

5. Conclusions

This present study provides for the first time a quantitative pooled estimate of the impact of NS supplementation on plasma lipid concentrations evaluated in RCTs, showing that NS significantly reduces plasma concentrations of total cholesterol, LDL-C, and triglycerides. These results are intriguing, considering the good safety profile and low cost of NS. Additional studies are required to define the optimal dosage and duration of this supplementation to obtain a favorable effect on lipid blood values. Finally, the value of adding NS supplements to conventional and novel LDL- [105-108] and triglyceride-lowering therapies [86, 109, 110] remains to be investigated in future studies.

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Table 1. Demographic characteristics of the included studies.

Author	Study design	Target Population	Treatment duration	n	Study groups	Age, years	Female (n, %)	BMI, (kg/m^2)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Total cholesterol (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)	Triglycerides (mg/dl)
Amin et al. (2015)	Randomized, double-blind, placebo-controlled	Men with metabolic syndrome	8 weeks	62	NS powder 1.5 g/day	45.1±11.7	0 (0.0)	27.4±3.1	131.8±20.2	82.5±12.1	184.3±33.6	110.2±28.0	34.3±7.8	169.5±44.3
				63	Placebo	41.5±12.8	0 (0.0)	27.5±4.1	125.5±16.7	76.8±11.5	180.8±23.3	119.5±27.3	33.7±7.4	163.6±42.7
Bin Sayeed et al. (2013)	Randomized, double-blind, placebo-controlled	Healthy male volunteers	9 weeks	20	NS powder 1 g/day	55.8±0.57	0 (0.0)	24.77±0.34	ND	ND	148.9±8.4	113.7±8.4	25.1±12.0	116.0±3.1
				20	Placebo	55.9±0.65	0 (0.0)	24.55±0.18	ND	ND	151.2±5.3	112.1±13.8	29.0±12.0	114.3±2.2
Datau et al. (2010)	Randomized, double-blind, placebo-controlled	Men with central obesity	3 months	19	NS powder 1.5 g/day	ND	0 (0.0)	ND	130.53±13.11	80.53±13.93	ND	ND	35.60±5.21	202.05±134.31
				20	Placebo	ND	0 (0.0)	ND	123.50±12.68	80.0±7.96	ND	ND	39.14±7.65	115.05±81.06
Dehkordi et al. (2008)	Randomized, double-blind, placebo-controlled	Men with mild hypertension	8 weeks	36	NS oil 100 mg/day	44.6±1.3	0 (0.0)	23.9±0.8	151.2±1.3	93.2±0.5	201.2±9.1	128.2±6.1	51.8±3.4	127.9±10.7
				39	NS oil 200 mg/day	43.7±1.3	0 (0.0)	24.1±0.8	149.5±1.3	95.1±0.8	200.7±8.2	127.7±6.8	52.7±3.1	118.0±9.7
				33	Placebo	43.1±1.4	0 (0.0)	24.5±0.7	148.2±1.2	94.5±0.8	189.7±8.7	123.5±5.4	47.2±2.9	121.2±10.3
Farzaneh et al. (2014)	Randomized, double-blind, placebo-controlled	Overweight females with hypercholes-terolemia	8 weeks	8	NS powder 2 g/day	34.14±10.54	8 (100.0)	25.39±0.75	ND	ND	221.5±31.34	119.1±11.96	45.62±10.02	167.88±18.61
				8	Placebo	33.0±4.34	8 (100.0)	25.85±1.45	ND	ND	232.75±12.85	120.5±10.39	48.37±8.61	173.8±50.43
Fatima et al. (2014)	Single-blind, placebo-controlled	Hyper-lipidemia	4 weeks	30	NS powder 2 spoons/day	ND	ND	ND	ND	ND	ND	ND	31.7±3.11	ND
				30	Placebo	ND	ND	ND	ND	ND	ND	ND	35.87±2.22	ND

Heshmati et al. (2015)	Randomized, double-blind, placebo-controlled	Type 2 diabetes	12 weeks	36	NS oil 3 g/day	45.3±6.5	54.3*	29.5±4.4	ND	ND	216.8±51.7	132.9±48.0	38.2±5.3	228.0±74.4
				36	Placebo	47.5±8.0	51.4*	28.6±4.3	ND	ND	228.3±49.0	140.6±44.8	38.5±5.5	244.4±59.8
Hosseini et al. (2013)	Randomized, double-blind, placebo-controlled	Type 2 diabetes	3 months	35	NS oil 5 ml/day	48.74±7.33	21 (60.0)	30.81±3.55	ND	ND	250.0±30.0	171.8±27.0	47.7±9.2	182.5±62.3
				35	Placebo	50.72±5.69	19 (54.2)	30.92±3.67	ND	ND	246.9±31.1	168.0±7.1	46.8±9.0	179.9±52.4
Ibrahim et al. (2014)[a]	Randomized, placebo-controlled	Menopausal women with metabolic syndrome	2 months	18	NS powder 1 g/day	ND	18 (100.0)	ND	ND	ND	233.1±40.4	179.7±35.8	60.9±10.0	133.7±31.6
				17	Placebo	ND	17 (100.0)	ND	ND	ND	234.2±40.7	186.7±23.1	52.5±10.9	132.6±42.8
Ibrahim et al. (2014)[b]	Randomized, placebo-controlled	Menopausal women	2 months	19	NS powder 1 g/day	53.22±2.16	19 (100.0)	27.18±4.34	129.33±15.44	77.13±9.16	235.5±40.2	179.8±34.4	50.7±8.5	155.9±31.0
				18	Placebo	53.71±3.57	18 (100.0)	27.75±4.38	138.40±18.90	83.93±15.73	234.3±39.4	177.5±18.6	51.0±9.7	152.3±46.9
Latiff et al. (2014)	Open label single arm, controlled	Peri-menopausal women	12 weeks	69	NS powder 1.6 g/day	50.1±7.6	69 (100.0)	26.31±4.89	122.32±15.25	79.07±7.76	225.1±47.2	165.9±204.216 6.3±198.0	60.3±14.7	125.8±74.4
				69	Placebo		69 (100.0)	26.03±4.66	121.42±14.89	78.42±8.48	225.8±46.0		58.8±17.4	124.9±70.0
Mahdavi et al. (2015)	Randomized, double-blind, placebo-controlled	Obese women	8 weeks	43	NS oil 3 g/day	41.5±11.7	43 (100.0)	32.4±1.5	120.5±10.3	7.7±0.7	203.1±42.2	129.1±32.3	48.8±11.6	130.2±65.9
				41	Placebo	39.3±9.9	41 (100.0)	32.6±1.5	120.4±10.4	7.9±0.6	191.7±41.1	119.2±33.0	49.3±13.4	115.5±64.7
Moeen-Ud-Din et al. (2014)	Single-blind, placebo-controlled	Hyper-lipidemia	6 weeks	27	NS powder 2 spoons/day	ND	ND	ND	ND	ND	ND	202.45±1.54	38.81±3.90	ND
				30	Placebo	ND	ND	ND	ND	ND	ND	189.15±3.90	36.11±2.11	ND
Qidwai et al. (2009)	Randomized, double-blind, placebo-controlled	Hypercholes-terolemia	6 weeks	39	NS powder 2 g/day	45.58±10.86	6 (10.0)	27.13±3.88	128.90±18.37	81.82±11.24	209.07±28.63	145.76±23.30	40.53±8.52	163.14±71.43
				34	Placebo	46.86±11.00	8 (14.0)	28.26±6.75	122.30±17.76	80.45±11.48	217.11±27.72	144.43±24.0	41.74±10.63	157.12±84.53

Sabzghabaee et al. (2012)	Randomized, placebo-controlled	Hypercholes -terolemia	4 weeks	37	NS powder 2 g/day	40.38**	17 (45.9)	25.01**	ND	ND	235.24±28.29	144.58±19.06	51.48±15.45	173.91±69.35
				37	Placebo	38.4**	16 (43.2)	23.19**	ND	ND	233.39±26.24	138.36±19.05	48.03±8.70	173.88±47.92
Hadi et al. (2015)	Randomized, placebo-controlled	Type 2 diabetes	8 weeks	23	NS oil 1 g/day	51.4±9.2	10 (43.5)	28.4±4.4	ND	ND	189.0±48.2	114.0±38.2	48.1±7.5	156.0±73.9
				20	Placebo	56.0±3.4	10 (50.0)	28.8±8.1	ND	ND	175.0±41.7	102.0±39.6	48.2±10.5	142±61.8
Amini et al. (2013)	Randomized, placebo-controlled	Healthy subjects with 200 mg/dL< total cholesterol< 300 mg/dL	8 weeks	35	NS oil 5 mL/day	42.3±13.8	17 (48.5)	ND	ND	ND	196.7±35.7	111.5±24.2	43.0±10.1	210.2±103.8
				35	Placebo	36.3±13.6	18 (51.5)	ND	ND	ND	184.2±28.2	112.4±18.1	42.4±6.4	191.6±6.4

Values are expressed as mean ± SD

*Percentage only

Abbreviations: NS, *Nigella sativa*; ND, no data; BMI, body mass index.

Table 2. Risk of bias assessment of the included studies according to the Cochrane guidelines.

Study	Sequence generation	Allocation concealment	Blinding of participants, personnel and outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Amin et al. (2015)	L	L	L	L	L	L
Bin Sayeed et al. (2013)	L	L	L	L	L	L
Datau et al. (2010)	U	U	U	L	L	U
Dehkordi et al. (2008)	U	U	U	L	L	U
Farzaneh et al. (2014)	L	U	U	L	L	U
Fatima et al. (2014)	U	U	U	H	U	U
Heshmati et al. (2015)	L	L	L	L	L	L
Hosseini et al. (2013)	L	U	U	L	U	U
Ibrahim et al. (2014)[a]	L	U	U	U	U	U
Ibrahim et al. (2014)[b]	U	U	U	L	L	U
Latiff et al. (2014)	H	H	H	L	L	H
Mahdavi et al. (2015)	L	L	L	L	L	L

Moeen-Ud-Din et al. (2014)	U	U	U	H	U	U
Qidwai et al. (2009)	L	L	L	L	L	L
Sabzghabaee et al. (2012)	L	U	U	L	L	U
Hadi et al., (2015)	U	U	L	L	H	L
Amini et al. (2013)	U	U	L	L	L	L

L, low risk of bias; H, high risk of bias; U, unclear risk of bias.

Table 3. Assessment of publication bias in the meta-analysis of NS effects on plasma concentrations of lipids.

	Corrected effect size[a]		**Begg's rank correlation test,**			**Egger's linear regression test**			**Fail safe N test**
	WMD	**95% CI**	Kendall's **Tau*[a]***	***z*-value**	***p*-value**	**Intercept**	**95% CI**	***p*-value**	***n*[b]**
Total cholesterol	-	-	0.11	0.59	0.553	-0.32	-3.53, 2.89	0.834	432
LDL-C	-17.21	-22.66, -11.76	-0.06	0.32	0.753	-0.50	-2.54, 1.55	0.610	982
HDL-C	-0.63	-2.76, 1.50	-0.14	0.83	0.405	0.99	-1.68, 3.65	0.445	-
Triglycerides	-17.80	-27.10, -8.50	0.14	0.77	0.44	-2.04	-3.44, -0.65	0.007	347

[a]With continuity correction; [b]Number of theoretically missing studies to bring the *p*-value to > 0.05.

FIGURE LEGENDS

Figure 1. Flow chart of the number of studies identified and included into the meta-analysis.

Figure 2. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of NS supplementation on plasma lipid concentrations.

Figure 3. Results of leave-one-out sensitivity analysis for the impact of NS supplementation on plasma lipid concentrations.

Figure 4. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of NS supplementation on plasma lipid concentrations in the subgroups of trials studying the effects of NS powder and black seed oil.

Figure 5. Random-effects meta-regression plots of the association between mean changes in plasma concentrations of lipids and NS dose.

Figure 6. Random-effects meta-regression plots of the association between mean changes in plasma concentrations of lipids and duration of NS supplementation.

Figure 7. Funnel plot displaying publication bias in the studies reporting the impact of NS supplementation on plasma lipid concentrations.