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The effect of L-arginine supplementation on lipid profile: a systematic review and meta-analysis of randomised controlled trials

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Abstract

A number of clinical trials have examined the effect of L-arginine on lipid profile in recent years; however, the results remain equivocal. Therefore, the present study aims to summarise and quantitatively examine the available evidence on the effectiveness L-arginine supplementation on lipid parameters using a systematic review and meta-analytic approach. Online databases including PubMed, Scopus, ISI Web of Science, Cochrane Library and Google Scholar were searched up to April 2019 for randomised controlled trials that examined the effect of L-arginine supplementation on lipid profile in adults. Treatment effects were expressed as weighted mean difference (WMD) and the corresponding standard error in concentrations of serum lipids. To estimate the overall effect of L-arginine supplementation, we used the random-effects model. In total, twelve studies were included in the systematic review. The meta-analysis revealed that L-arginine supplementation did not significantly change the concentrations of total cholesterol (WMD: −5·03 mg/dl; 95 % CI −10·78, 0·73; $P = 0·08$; inconsistency index (I^2) = 39·0 %), LDL (WMD: −0·47 mg/dl; 95 % CI −3·61, 2·66; $P = 0·76$; $I^2 = 0·0$ %), or HDL (WMD: 0·57 mg/dl; 95 % CI −1·28, 2·43; $P = 0·54$; $I^2 = 68·4$ %). A significant reduction was observed only in serum TAG levels (WMD: −7·04 mg/dl; 95 % CI −11·42, −2·67; $P < 0·001$; $I^2 = 0·0$ %). This meta-analysis concludes that L-arginine supplementation can significantly reduce blood TAG levels; however, there is insufficient evidence to support its hypocholesterolaemic effects. To draw straightforward conclusions regarding generalised recommendations for L-arginine supplementation for improving lipid profile, there is a need for more well-controlled trials targeting exclusively patients with dyslipidaemia.

Key words: **L-Arginine: Supplementation: Lipid profile: Systematic reviews: Meta-analyses**

CVD are the leading causes of death among non-communicable diseases, posing a significant health and economic burden worldwide[1,2]. The American Heart Association reported that 17·7 million people (representing 31 % of all global deaths) died from CVD in 2015, and this number is projected to rise to 23·6 million by 2030[3]. Dyslipidaemia has been identified as a major risk factor for CVD[4–6]. Thus, regulating and maintaining an optimal lipid profile is critical for the prevention of CVD. In this regard, statin therapy and diet modification are two of the most commonly prescribed approaches[7,8]. However, statins, among other commonly used lipid-lowering pharmacotherapies[9], have been established to pose some serious adverse effects, such as myopathies and hepatotoxicity[10,11]. Thus, there is a demand to identify viable, anti-lipid agents that are able to pose cardioprotective effects without inducing any side effects.

L-Arginine is a semi-essential amino acid which our body derives either from dietary sources or from endogenous metabolism[12,13]. L-Arginine is involved in several biochemical

Abbreviations: HDEL, hypoenergetic diet enriched in legumes; I^2, inconsistency index; RCT, randomised controlled trials; TC, total cholesterol; WMD, weighted mean difference.

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Table 1. PICOS criteria used to perform the systematic review and meta-analysis

Parameter	Criteria
Population	Adults
Intervention	Arginine
Comparator	Matched control group
Outcome	Circulating TC, TAG, LDL-cholesterol, and HDL-cholesterol
Setting or study design	Randomised controlled trials

PICOS, participants, interventions, comparisons, outcomes and study design; TC, total cholesterol.

processes, including polyamine synthesis, ammonia detoxification, immune modulation and secretion of hormones such as glucagon and growth hormone and insulin[14–16]. What is more, this amino acid produces nitric oxide (NO), a key molecule involved in the regulation of cell metabolism, insulin signalling and secretion, neurotransmission and immune system function[17,18]. It is suggested that L-arginine can be useful in improving lipid profile, due to its potential to increase NO production. Therefore, L-arginine has been investigated as a potentially cardio-protective compound, and seven meta-analyses concluded that it can be an effective tool in blood pressure management[19]. Several trials investigated the potential of L-arginine supplementation for the treatment of abnormal lipid profile; however, the results are inconsistent. For instance, some trials report that L-arginine supplementation induced a reduction in circulating concentrations of lipid parameters[20–23], while others report no significant effect[18,24–30]. Discrepancies in the findings may conceivably be attributed to the differences in study designs, characteristics of study participants, duration and the supplementation dosage applied in the trials. Therefore, we conducted a meta-analysis of those randomised controlled trials (RCT) to examine the efficacy of L-arginine supplementation as a lipid-lowering agent.

Methods

We conducted and reported the present systematic review and meta-analysis following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Supplementary Table S1)[31] for identification, screening, eligibility and inclusion of articles. The present study was not prospectively registered. Participants, interventions, comparisons, outcomes and study design are shown in Table 1.

Search strategy

Two independent investigators (A. H. and E. G.) performed a systematic search of all articles published until April 2019 in the following online databases: PubMed, Scopus, Cochrane Library, ISI Web of Science and Google Scholar. We used the following Medical Subject Headings and corresponding keywords: ('arginine' OR 'L-arginine') AND ('lipid' OR 'hyperlipidaemia' OR 'dyslipidemia' OR 'cholesterol' OR 'CHOL' OR 'hypercholesterolaemia' OR 'lipoprotein' OR 'hyperlipoproteinemia' OR 'high density lipoprotein' OR 'HDL' OR 'low density lipoprotein' OR 'LDL' OR 'triglyceride' OR 'TG') AND ('Intervention Studies' OR 'intervention' OR 'controlled trial' OR 'randomized' OR 'randomised' OR 'random' OR 'randomly' OR 'placebo' OR 'assignment'). The two reviewers also performed screening of the reference lists of relevant review articles and original papers that were selected for full-text review to identify potential eligible studies. Additionally, an email alert service was used to avoid missing any relevant articles. The language of the retrieved papers was restricted to English, while there were no restrictions regarding the year of publication.

Study selection

All studies retrieved from the electronic databases and reference lists were entered into endnote software (EndNote X6; Thomson Corporation) and duplicate studies were removed. In the next step, the titles and abstracts of the papers were examined by two independent reviewers (A. A. and E. G.) to exclude irrelevant articles. Afterwards, the full texts of the remaining publications were read and assessed according to the following four items: study design, participants, interventions and outcome measures. Finally, the studies were retained if they met the following inclusion criteria: (1) a randomised controlled design, (2) reporting the effect of L-arginine on at least one of the lipid profile parameters including total cholesterol (TC), TAG, LDL-cholesterol, and HDL-cholesterol and (3) intervention for more than 4 weeks. Studies were excluded if they (1) involved L-arginine supplementation in combination with some drugs or other types of supplements (minerals, vitamins or herbal supplements, unless a separate arm controlled the effect of the mixed substance), (2) reported duplicate data (in this case, the data with complete follow-up and outcome measures were included), (3) included adolescents as the population and (4) were not peer-reviewed articles (protocol or conference proceeding). Any disagreements regarding the process of study selection were resolved in consultation with the principal investigator (A. H.).

Data extraction

The major demographic and clinical data from each of the selected studies were screened and extracted independently by two investigators (A. H. and E. G.) using a predesigned Excel sheet. Any controversy was solved via discussion with a third, independent researcher to reach a consensus. The extracted information was as follows: the first author's last name, publication year, study design, country, sample size, participants mean age, sex, baseline BMI, follow-up duration, intervention duration (in weeks), type of intervention, dose of L-arginine (g/d), type of control, health status of the participants and main results. Corresponding authors were contacted in case of any missing data.

Quality assessment

The Cochrane Collaboration tool[32] was used for quality assessment, and it includes seven items, namely, randomisation sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective reporting and other biases. Each domain

was classified into three categories: low risk of bias, high risk of bias and unclear risk of bias. Finally, the overall quality of the studies was categorised into weak, fair or good, if <3, 3 or ≥ 4 domains were rated as low risk, respectively. Quality assessment was performed independently by two reviewers (A. A. and E. G.), while any disagreements were resolved by consulting the third reviewer (A. H.).

Statistical analysis

For carrying out the meta-analysis, we used STATA software (version 11; StataCorp). If outcome measures were reported in mmol/l, they were converted to mg/dl. The reviewers then extracted the mean difference between the baseline and endpoint data and the corresponding standard deviations in both intervention and control groups. If such data were not available, the mean difference was obtained by subtracting the mean value of the baseline point from that of the endpoint. If SD of the mean difference was not reported, it was calculated using the following formula: SD = square root ((SD pretreatment)^2 + (SD post-treatment)^2 − (2 × R × SD pretreatment × SD post-treatment)). To ensure meta-analysis was not sensitive to the selected correlation coefficient (R 0·5), all analyses were repeated using correlation coefficients of 0·2 and 0·8. Where only a standard error was reported, SD was estimated using the following formula: SD = SE × $\sqrt{n}$ (n being the number of subjects in each group). Using random-effects model developed by DerSimonian & Laird[33], the summary estimate was pooled as weighted mean difference (WMD) and 95 % CI. The inconsistency index (I^2) was used to quantify statistical heterogeneity in the meta-analyses, and values greater than 50 % were considered indicative of high heterogeneity. To identify the source of heterogeneity, subgroup analysis was conducted focusing on mean age, baseline BMI, dose of L-arginine supplementation, study duration and participant's health status. Sensitivity analysis was also performed to explore the extent to which inferences might depend on a particular study using the leave-one-out method (i.e. deleting one trail at a time and re-calculating the effect size). To assess publication bias, Begg[34] and Egger's[35] regression tests were performed. In all statistical analyses, the level of significance was set at $P < 0{\cdot}05$.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors. There was no human or animal involvement in the present study.

Results

Flow of study selection

A total of 2216 publications were identified after the search of the electronic databases, out of which 755 were removed as being duplicate (Fig. 1). By reviewing the title and abstracts of the remaining articles, 1438 publications not meeting the inclusion criteria were excluded. Subsequently, twenty-three full-text articles were carefully reviewed and eleven clinical trials were excluded because of the following reasons: five studies had a duration of supplementation period of less than 4 weeks; one study enrolled adolescents; two studies involved interventions that were a combination of other components together with L-arginine and the design did not enable evaluating L-arginine effect only; one study enrolled less than ten participants; and two articles reported the results from a same population. Finally, twelve trials[18,20–30] including fifteen treatment arms were considered eligible for the systematic review. However, one of the included articles[28] did not report the data required for the meta-analysis. We contacted the corresponding author of the study twice but did not receive any response, and therefore it was subsequently, excluded, leaving eleven studies[18,20–27,29,30] for inclusion in the meta-analysis.

Study and participant characteristics

Characteristics of the included trials are outlined in Table 2. In total, 631 participants were enrolled in the selected articles, out of which 359 individuals were allocated to L-arginine supplementation group and 272 subjects to the control group. These studies were published between 1996 and 2019 and were carried out in the Iran[20–23,29], Italy[27], Poland[18,24,26], Israel[28], UK[30] and Germany[25]. All studies except two[28,30] adopted a parallel study design. The mean age of the participants ranged from 20·86 to 64·5 years, and the mean baseline BMI varied from 23·67 to 38·35 kg/m^2. Only one[21] of included studies involved exclusively male population, two[28,29] involved women and the other trials involved populations of mixed sex. The follow-up period ranged from 4 to 77 weeks. In these studies, the daily supplementation dosage of L-arginine varied between 1 and 21 g/d. The health status of the included participants was mixed and included type 2 diabetes patients[22], postmenopausal women[28], patients with CVD[26], subjects with hypercholesterolaemia[30], obese individuals[18,20,24,29], subjects with hypertriacylglycerolaemia[25], individuals with the metabolic syndrome[23], healthy subjects[21] and those with impaired glucose tolerance and the metabolic syndrome[27]. No major adverse effects attributable to intervention or control were reported in RCT.

In a study by Pourghassem Gargari *et al.*[29], there were three intervention groups (arginine + hypoenergetic diet enriched in legumes (HDEL), arginine + HDEL + Se and HDEL + Se) and one control group (HDEL). We considered the result of the arginine + HDEL and HDEL groups as one arm and the result of the arginine + HDEL + Se and HDEL + Se groups as another arm. Furthermore, Rahimi & Naghizadeh[22] and Dashtabi *et al.*[20] included two different arginine doses in their trials (3 or 6 g/d); therefore, we considered these imputations as four different arms.

Quality assessment

Among twelve studies included in the present review, nine trials[18,20,21–29] were categorised as good quality and three trials[22,28,30] as fair quality. The details of the risk of bias in individual studies according to the domains used by the Cochrane Collaborations tool are provided in Table 3.

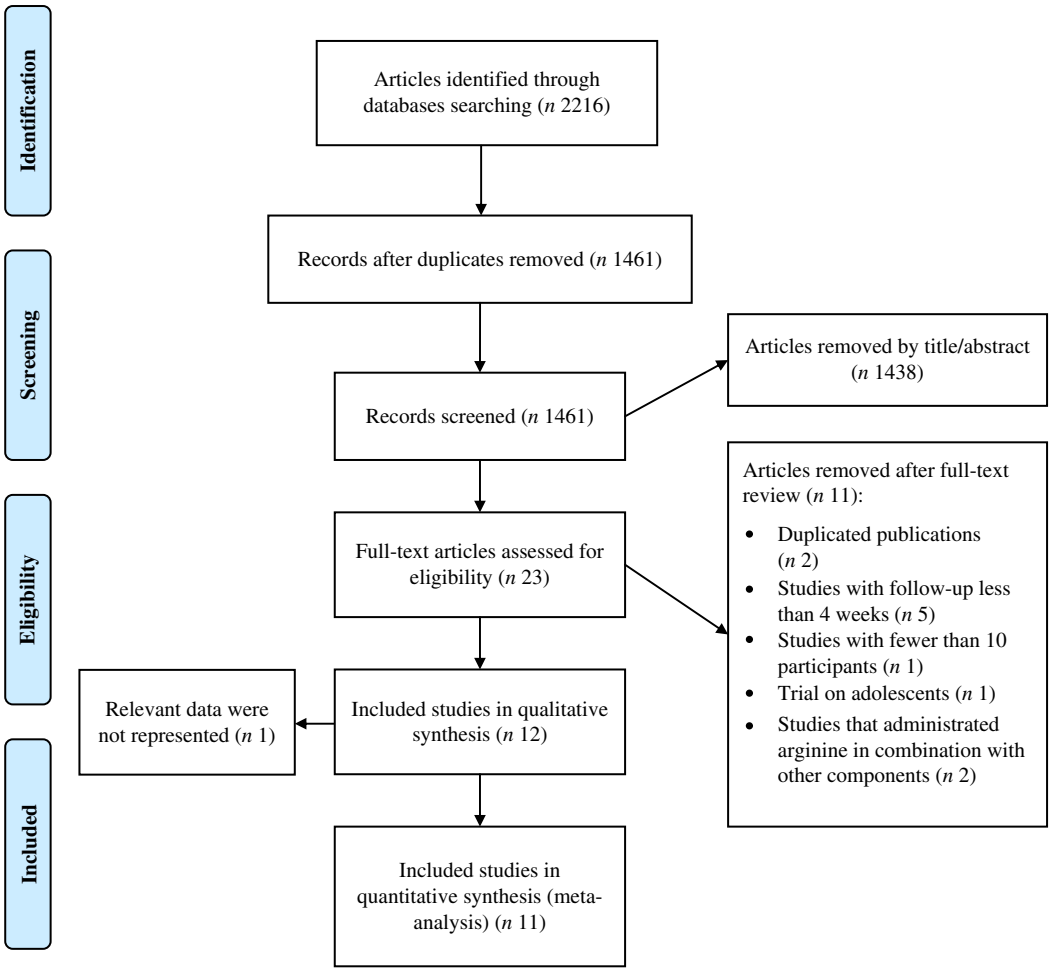

Fig. 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of study selection process.

Findings from the systematic review

The present systematic review revealed that three trials[20–22] reported that L-arginine supplementation managed to reduce TC levels, while eight studies[18,23,25–30] failed to find any significant effect on this parameter. In terms of changes in TAG levels, three trials observed a significant reduction after L-arginine supplementation in references [20,21,23], while eight studies[18,22,25–30] did not find such an effect. Evidence points out that arginine may pose favourable effects on LDL-cholesterol concentration as well – three trials[20–22] found that supplementation induced a decrease in plasma LDL-cholesterol, while other trials report no significant changes in this outcome[23–30]. Finally, L-arginine may induce favourable changes in HDL-cholesterol levels as confirmed in two trials;[20,21] however, the remaining studies[22–30] did not reach the same conclusion.

Findings from the meta-analysis

In total, we pooled the data from thirteen arms corresponding to ten studies[18,20–23,25–27,29,30] which included 561 participants, to estimate the effect of L-arginine supplementation on plasma TC levels. After using a meta-analysis random-effects model, we found that L-arginine supplementation did not significantly affect the serum TC levels (WMD: −5·03 mg/dl; 95 % CI −10·78, 0·73; $P = 0·08$) (Fig. 2). The between-study heterogeneity was non-significant ($P = 0·07$, $I^2 = 39·0$ %). Subgroup analysis based on participants' mean age, baseline BMI, study duration, participants health status and L-arginine dose confirmed that the effect is not statistically significant in any of the subgroups (Table 4). Findings from the sensitivity analysis revealed that the exclusion of any single study from the analysis did not alter the overall effect.

Thirteen arms from ten studies[18,20–23,25–27,29,30] including 561 participants reported the effect of L-arginine on serum TAG concentration. The pooled effect demonstrated a significant decrease in TAG levels following L-arginine supplementation (WMD: −7·04 mg/dl; 95 % CI −11·42, −2·67; $P < 0·001$) with a non-significant heterogeneity among included studies ($P = 0·59$, $I^2 = 0·0$ %) (Fig. 3). Subgroup analysis based on participants' mean age, baseline BMI, study duration, participants health status and L-arginine dose revealed that the effect was significant in studies that included participants with a mean age ≥50 years (WMD: −9·08 mg/dl; 95 % CI −16·70, −1·45; $P = 0·02$), a baseline BMI ≥30 kg/m^2 (WMD: −9·86 mg/dl; 95 % CI −16·22, −3·50; $P < 0·001$), L-arginine dose < 6 g/d (WMD: −7·89 mg/dl; 95 % CI −15·04, −0·73; $P = 0·03$), type 2 diabetes/

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Table 2. Characteristics of included studies

Authors	Country	Sample size	RCT design (blinding)	Sex	Mean age (years)	Mean BMI (kg/m^2)	Duration (weeks)	Target population	Intervention (name and daily dose)	Control	Results
Clarkson *et al.*[30]	UK	27	Crossover (yes)	Both	29	26	4	Subjects with hypercholesterolaemia	21 g/d arginine	Placebo	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↔
Blum *et al.*[28]	Israel	10	Crossover (yes)	Women	55	26·6	4	Postmenopausal women	9 g/d arginine	Placebo	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↔
Lucotti *et al.*[26]	Poland	30	Parallel (yes)	Both	64·5	34	26	Patients with CVD	6·4 g/d arginine	Placebo	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↔
Schulze *et al.* [25]	Germany	33	Parallel (yes)	Both	54·3	28·40	6	Subjects with hypertriacylglycerolaemia	3 g/d arginine	Placebo	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↔
Bogdanski *et al.*[24]	Poland	60	Parallel (yes)	Both	42·4	38·35	13	Obese	9 g/d arginine	Placebo	LDL-cholesterol ↔ HDL-cholesterol ↔
Rahimi & Naghizadeh[22]	Iran	33	Parallel (yes)	Both	50·89	29·07	13	T2DM	3 g/d arginine	Placebo	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↔
Rahimi & Naghizadeh[22]	Iran	35	Parallel (yes)	Both	51·98	28·55	13	T2DM	6 g/d arginine	Placebo	TC ↓ TAG ↔ LDL-cholesterol ↓ HDL-cholesterol ↔
Suliburska *et al.*[18]	Poland	88	Parallel (yes)	Both	42·3	36·45	26	Obese	9 g/d arginine	Placebo	TC ↔ TAG ↔
Pahlavani *et al.*[21]	Iran	52	Parallel (yes)	Male	20·86	23·67	7	Healthy	2 g/d arginine	Placebo	TC ↓ TAG ↓ LDL-cholesterol ↓ HDL-cholesterol ↑
Pourghassem Gargari *et al.*[29]	Iran	34	Parallel (yes)	Women	35·2	31·80	6	Obese	HDEL + 5 g/d arginine	HDEL	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↔
Pourghassem Gargari *et al.*[29]	Iran	34	Parallel (yes)	Women	35·3	32·45	6	Obese	HDEL + 5 g/d arginine + 200 μg/d Se	HDEL + 200 μg/d Se	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↔
Dashtabi *et al.*[20]	Iran	41	Parallel (yes)	Both	42·36	35·44	8	Obese	3 g/d arginine	Placebo	TC ↓ TAG ↔ LDL-cholesterol ↓ HDL-cholesterol ↔

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Table 2. (*Continued*)

Authors	Country	Sample size	RCT design (blinding)	Sex	Mean age (years)	Mean BMI (kg/m^2)	Duration (weeks)	Target population	Intervention (name and daily dose)	Control	Results
Dashtabi *et al.*[20]	Iran	42	Parallel (yes)	Both	43·23	34·09	8	Obese	6 g/d arginine	Placebo	TC ↓ TAG↓ LDL-cholesterol ↓ HDL-cholesterol ↑
Monti *et al.*[27]	Italy	56	Parallel (yes)	Both	56·4	35	77	Subjects with impaired glucose tolerance and metabolic syndrome	6·4 g/d arginine	Placebo	TC ↔ TAG ↔ LDL-cholesterol ↔ HDL-cholesterol ↕
Bahrami *et al.*[23]	Iran	56	Parallel (yes)	Both	50·7	30	13	Metabolic syndrome	5 g/d arginine	Placebo	TC ↔ TAG ↓ LDL-cholesterol ↕ HDL-cholesterol ↕

RCT, randomised controlled trial; TC, total cholesterol; T2DM, type 2 diabetes mellitus; HDEL, hypoenergetic diet enriched in legumes.

metabolic syndrome (WMD: −11·77 mg/dl; 95 % CI −19·39, −4·14; $P < 0·001$) and intervention duration ≥10 weeks (WMD: −9·21 mg/dl; 95 % CI −15·20, −3·21; $P < 0·001$) (Table 4). The sensitivity analysis demonstrated that by removing the study conducted by Bahrami *et al.*[23], the effect of L-arginine supplementation on TAG levels becomes non-significant (WMD: −4·65 mg/dl; 95 % CI −9·51, 0·19; $P = 0·06$).

The impact of L-arginine supplementation on LDL-cholesterol levels was assessed in ten trials[20–29,30], with thirteen treatment arms including 533 participants. The meta-analysis revealed that L-arginine supplementation did not significantly affect LDL-cholesterol levels (WMD: −0·47 mg/dl; 95 % CI −3·61, 2·66; $P = 0·76$), while the heterogeneity among the included studies was NS ($P = 0·53$, $I^2 = 0·0$ %) (Fig. 4). The subgroup analysis based on participants' mean age, baseline BMI, study duration, participants health status and L-arginine dose also showed that the effect is not statistically significant in any of the subgroups (Table 4). Furthermore, removing each individual study by sensitivity analysis did not change the pooled effect size.

Ten studies[20–27,29,30] including 533 participants from thirteen intervention arms measured changes in serum HDL-cholesterol concentrations following L-arginine supplementation. Pooled results from the random-effects model revealed that L-arginine supplementation had no significant effect on serum HDL-cholesterol levels (WMD: 0·57 mg/dl; 95 % CI −1·28, 2·43; $P = 0·54$) (Fig. 5). There was a significant heterogeneity among the studies ($P < 0·001$, $I^2 = 68·4$ %), and the subgroup analysis showed that baseline BMI (< 30 kg/m^2; $P = 0·96$; $I^2 = 0·0$ %), duration of follow-up (< 10 weeks: $P = 0·23$, $I^2 = 25·6$ %), participants health status (dyslipidaemia: $P = 0·69$, $I^2 = 0·0$ %) or (type 2 diabetes/metabolic syndrome: $P = 0·93$, $I^2 = 0·0$ %) and L-arginine dosage (< 6 g/d: $P = 0·96$; $I^2 = 0·0$ %) were significant contributors to the between-study heterogeneity. Besides, the subgroup analysis showed that L-arginine supplementation increases HDL levels in trials with a follow-up duration < 10 weeks (WMD: 2·04 mg/dl; 95 % CI 0·52, 3·56; $P = 0·01$) (Table 4). Findings from the sensitivity analysis revealed that the exclusion of any single study from the analysis did not alter the overall effect.

Publication bias

Although the visual inspection of funnel plots showed slight asymmetries, no significant publication bias was detected in the meta-analyses in the case of TC (Begg's test, $P = 0·54$; Egger's test, $P = 0·45$), TAG (Begg's test, $P = 0·54$; Egger's test, $P = 0·56$), LDL-cholesterol (Begg's test, $P = 0·39$; Egger's test, $P = 0·80$) or HDL-cholesterol (Begg's test, $P = 0·39$; Egger's test, $P = 0·86$).

Discussion

To the best of our knowledge, the present study is the first systematic review and meta-analysis that measured the effect of L-arginine supplementation on lipid profile by summarising the data from published RCT. Our results indicate that L-arginine supplementation was not able to induce changes in TC,

Table 3. Quality assessment of included studies based on Cochrane guidelines

Study	Random sequence generation	Allocation concealment	Blinding of participants, personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other sources of bias	Overall quality
Clarkson *et al.*[30]	Low	Unclear	Low	Unclear	Low	Unclear	Unclear	Fair
Blum *et al.*[28]	Low	Unclear	Low	Unclear	Unclear	Low	Unclear	Fair
Lucotti *et al.*[26]	Low	Low	Low	Low	Low	Low	Low	Good
Schulze *et al.*[25]	Low	Low	Low	Unclear	Low	Low	Unclear	Good
Bogdanski *et al.*[24]	Low	Low	Low	Unclear	Low	Low	Low	Good
Rahimi & Naghizadeh[22]	Low	Unclear	Low	Unclear	Low	Unclear	Unclear	Fair
Suliburska *et al.*[18]	Low	Low	Low	Unclear	Low	Low	Low	Good
Pahlavani *et al.*[21]	Low	Low	Low	Low	Low	Low	Low	Good
Pourghassem Gargari *et al.*[29]	Low	Low	Low	Low	Low	Low	Low	Good
Dashtabi *et al.*[20]	Low	Low	Low	Unclear	Low	Low	Unclear	Good
Monti *et al.*[27]	Low	Low	Low	Unclear	Low	Low	Low	Good
Bahrami *et al.*[23]	Low	Low	Low	Unclear	Low	Low	Low	Good

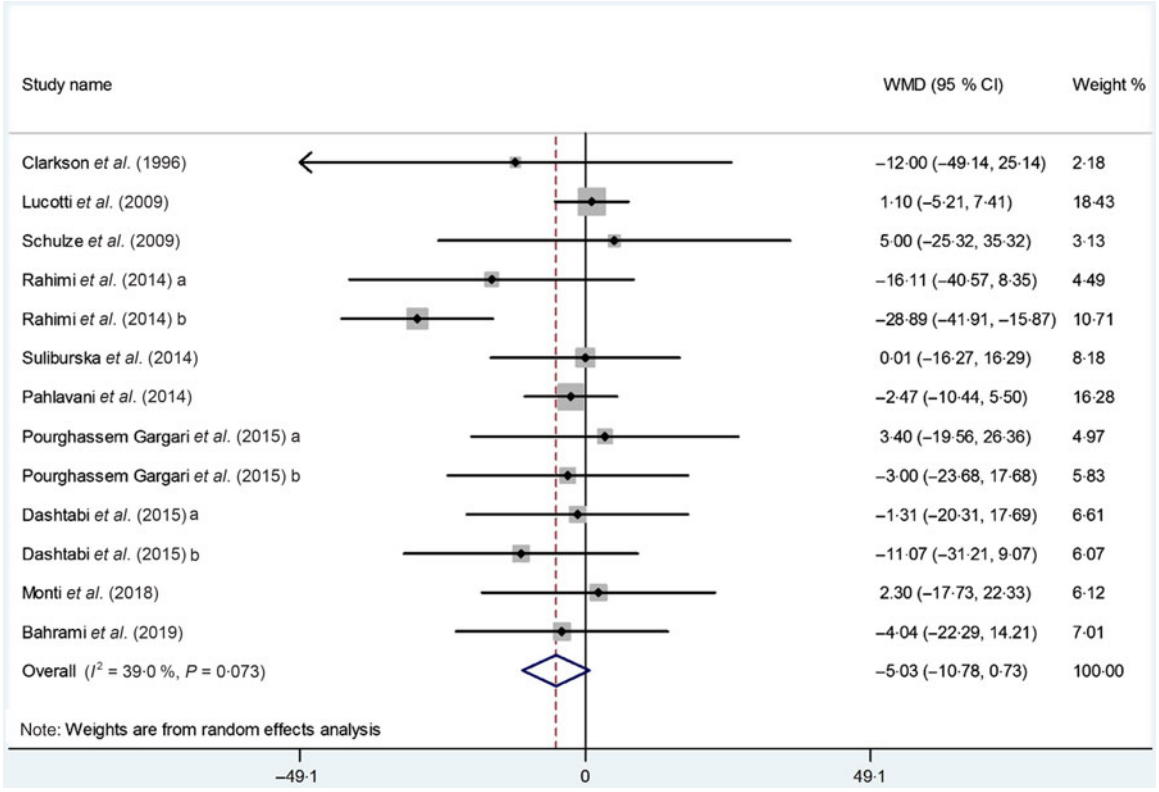

Fig. 2. Forest plot of the effect of L-arginine supplementation on total cholesterol. WMD, weighted mean difference.

LDL-cholesterol and HDL-cholesterol concentrations; however, it did induce a significant decrease in TAG levels. Subgroup analyses further confirmed that L-arginine supplementation imposed a significant TAG-lowering effect in studies that implemented long-term treatment (≥10 weeks) as well as in studies where participants' baseline BMI was ≥30 kg/m^2, mean age was ≥50 years, supplementation dosage was < 6 g/d and participants were type 2 diabetes or metabolic syndrome patients. Although the pooled effect size of L-arginine supplementation on HDL-cholesterol levels was NS, subgroup analysis revealed that this effect was significant only in studies that lasted longer than 10 weeks.

In this meta-analysis, we concluded that L-arginine was able to induce favourable changes only on TAG levels, while this was not observed in the case of other lipid parameters. The possible explanation for the observed lack of significant effect for all parameters except TAG might be the fact that the included population had only TAG levels above the recommended upper value, according to the definition of the metabolic syndrome. Thus, this observation may imply that individuals with higher TAG levels are better respondents to L-arginine therapy.

The biological plausibility of lipid-associated L-arginine implications comes from the existing relationship between this amino acid and glucose metabolism. Human clinical trials have

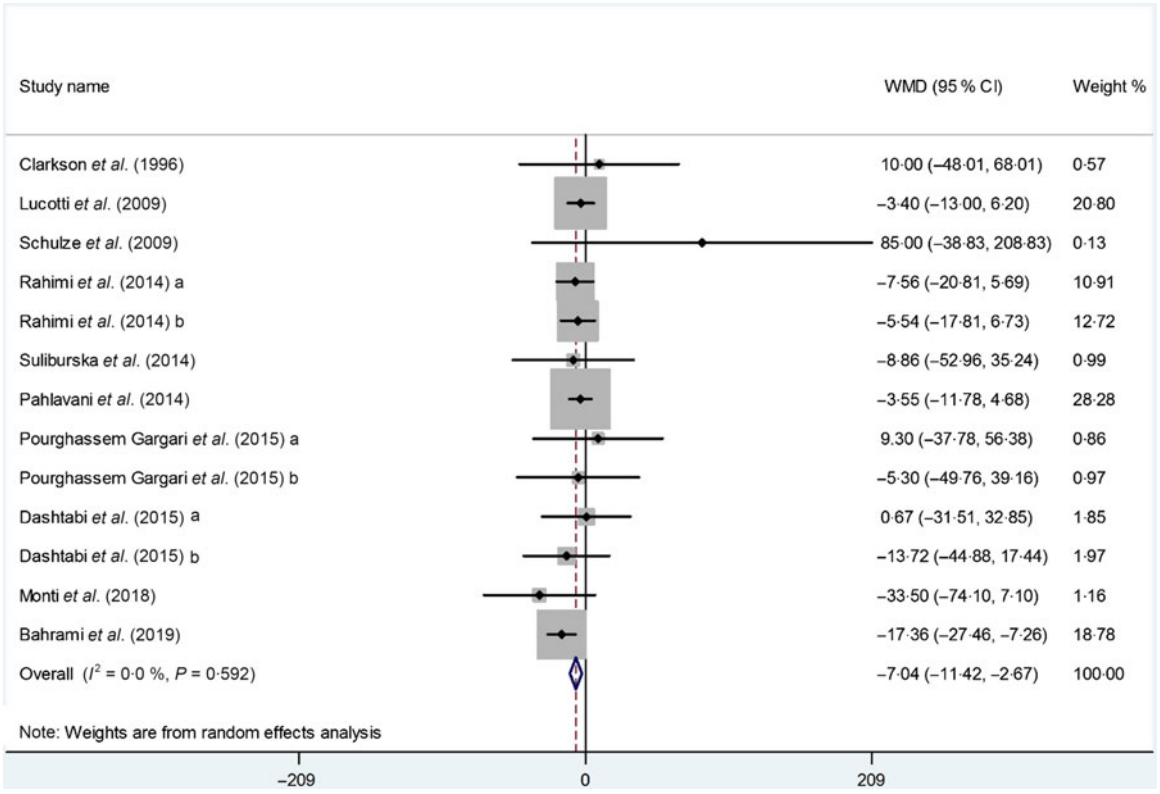

Fig. 3. Forest plot of the effect of L-arginine supplementation on TAG. WMD, weighted mean difference.

concluded that L-arginine can be an effective tool in reducing blood glucose levels in type 2 diabetes patients[36], increasing insulin sensitivity[37] and improving insulin resistance[38]. One of the proposed explanations for the observed results is that insulin resistance of the adipocytes can lead to an increased release of fatty acids into the circulation. Increased free fatty acid flux reaches the liver where it stimulates the assembly and secretion of VLDL, which results in hypertriacylglycerolaemia[38,39]. Another possible mechanism might be related to the lowering effect of L-arginine on blood glucose, the decrease in blood glucose levels leads to an increase in the concentration of cyclic AMP which in turn decreases the TAG levels[40,41]. Therefore, given the beneficial role of L-arginine in the glucose homeostasis, it is proposed that supplementation with this amino acid can lower serum TAG levels[41,42].

Other putative mechanisms may be related to the arginine NO synthase pathway. All NO synthase isoforms utilise L-arginine as a substrate, which undergoes a two-step metabolic conversion, yielding at the end L-citrulline and NO[43]. Elevated NO production consequently increases lipoprotein lipase activity[44]; and finally by performing hydrolysis of TAG, NO reduces TAG concentration in the plasma[25,45]. Animal study data also confirm L-arginine to be an effective lipid-lowering agent by decreasing the white fat expansion and improving serum TAG levels in rats[40,46].

A neutral effect of L-arginine supplementation on TC, LDL-cholesterol and HDL-cholesterol was observed in the present meta-analysis. Subgroup analysis of the effect of L-arginine supplementation on cholesterol or lipoprotein levels did not moderate the outcome. These findings are generally in line with the majority of individual studies selected for this review. Only three studies[20–22] showed a significant change in TC, LDL-cholesterol and HDL-cholesterol levels and others failed to find such a relationship. Furthermore, not all animal studies reported consistent results; as reducing[47,48] or even increasing effect of L-arginine supplementation on cholesterol level has been reported[49]. Madeira *et al.* reported that dietary L-arginine supplementation increases the concentration of total lipids, VLDL and TAG[50]. However, Hu *et al.*[51] reported that L-arginine supplementation decreases TAG and cholesterol levels in the plasma. In addition, He *et al.*[52] showed that L-arginine supplementation reduces VLDL, lipids and TAG concentrations in piglets. However, some animal studies reported that L-arginine might reduce cholesterol or lipoprotein levels by these mechanisms: (1) decrease the expression of hepatic 3-hydroxyl-3-methylglutaryl-CoA reductase mRNA, which shows interaction of L-arginine with cholesterol metabolism[53]; (2) increased the lipolysis as well as the oxidation of fatty acids and (3) increase plasma adiponectin levels which improved NEFA β-oxidation. On the other hand, others reported that possible TC increasing effect of L-arginine could be due to increased fat accretion in the carcass[54]. L-Arginine can also increase the level of HDL-cholesterol through its effect on inflammation[46,55,56].

L-Arginine has generally been well tolerated when administered in small doses (≤30 g/d)[57]. However, there were some reported benign side effects, which include abdominal pain, bloating, nausea and vomiting, airway inflammation, diarrhoea, hypotension, worsening of asthma and allergic reactions[57–59].

Table 4. Result of subgroup analysis of included studies in meta-analysis
(Effect sizes and 95 % confidence intervals; inconsistency indices (I^2); *P* values)

Subgrouped by	No. of trials	Effect size*	95 % CI	I^2 (%)	*P* for heterogeneity	*P* for effect size
TC						
Mean age (years)						
≥50	6	−7·47	−19·97, 5·04	72·5	<0·001	0·24
<50	7	−2·66	−8·46, 3·14	0·0	0·97	0·41
Baseline BMI (kg/m^2)						
≥30	8	−0·23	−5·01, 4·54	0·0	0·97	0·92
<30	5	−11·95	−26·64, 2·75	68·5	0·01	0·16
Intervention duration (weeks)						
≥10	6	−7·38	−18·78, 4·02	72·4	<0·001	0·20
<10	7	−2·72	−8·81, 3·36	0·0	0·96	0·43
Dose (g/d)						
≥6	6	−7·74	−19·74, 4·25	71·9	<0·001	0·26
<6	7	−2·69	−8·61, 3·23	0·0	0·94	0·37
Health status						
Dyslipidaemia	2	−1·80	−25·28, 21·69	0·0	0·48	0·88
T2D/MetS	4	−12·58	−28·19, 3·02	65·2	0·03	0·11
Other	7	−0·79	−5·09, 3·51	0·0	0·94	0·71
TAG						
Mean age (years)						
≥50	6	−9·08	−16·70, −1·45	36·7	0·16	0·02
<50	7	−3·56	−10·91, 3·79	0·0	0·98	0·31
Baseline BMI (kg/m^2)						
≥30	8	−9·86	−16·22, −3·50	0·0	0·50	<0·001
<30	5	−4·51	−10·54, 1·53	0·0	0·63	0·13
Intervention duration (weeks)						
≥10	6	−9·21	−15·20, −3·21	12·0	0·33	<0·001
<10	7	−3·09	−10·53, 4·35	0·0	0·81	0·38
Dose (g/d)						
≥6	6	−5·50	−12·58, 1·58	0·0	0·76	0·11
<6	7	−7·89	−15·04, −0·73	18·9	0·28	0·03
Health status						
Dyslipidaemia	2	26·73	−34·47, 87·93	13·5	0·28	0·39
T2D/MetS	4	−11·77	−19·39, −4·14	17·9	0·30	<0·001
Other	7	−3·46	−9·50, 2·23	0·0	0·99	0·22
LDL-cholesterol						
Mean age (years)						
≥50	6	−0·44	−7·23, 6·34	42·1	0·12	0·89
<50	7	−1·69	−6·31, 2·93	0·0	0·94	0·44
Baseline BMI (kg/m^2)						
≥30	8	2·39	−1·93, 6·71	0·0	0·75	0·21
<30	5	−3·66	−8·22, 0·90	0·0	0·53	0·10
Intervention duration (weeks)						
≥10	6	−0·17	−6·58, 6·24	42·5	0·12	0·95
<10	7	−2·00	−6·75, 2·76	0·0	0·96	0·38
Dose (g/d)						
≥6	6	−0·48	−7·82, 6·87	47·1	0·09	0·85
<6	7	−1·65	−6·21, 2·91	0·0	0·98	0·47
Health status						
Dyslipidaemia	2	6·1	−17·79, 29·79	0·0	0·99	0·62
T2D/MetS	4	−4·47	−11·68, 2·73	13·8	0·32	0·22
Other	7	0·82	−2·81, 4·44	0·0	0·58	0·66
HDL-cholesterol						
Mean age (years)						
≥50	6	−0·15	−2·77, 2·47	51·0	0·07	0·90
<50	7	1·29	−0·62, 3·21	58·0	0·05	0·21
Baseline BMI (kg/m^2)						
≥30	8	0·36	−2·03, 2·75	81·2	<0·001	0·76
<30	5	1·24	−1·51, 3·99	0·0	0·96	0·36
Intervention duration (weeks)						
≥10	6	−0·85	−3·28, 1·58	48·9	0·08	0·49
<10	7	2·04	0·52, 3·56	25·6	0·23	0·01
Dose (g/d)						
≥6	6	−0·25	−4·36, 3·87	86·0	<0·001	0·90
<6	7	1·18	−0·16, 2·52	0·0	0·96	0·08
Health status						
Dyslipidaemia	2	1·88	−5·26, 9·02	0·0	0·69	0·60
T2D/MetS	4	1·25	−1·09, 3·59	0·0	0·93	0·29
Other	7	0·21	−2·42, 2·85	83·8	<0·001	0·87

TC, total cholesterol; T2D, type 2 diabetes mellitus; MetS, metabolic syndrome.
* Calculated by random-effects model.

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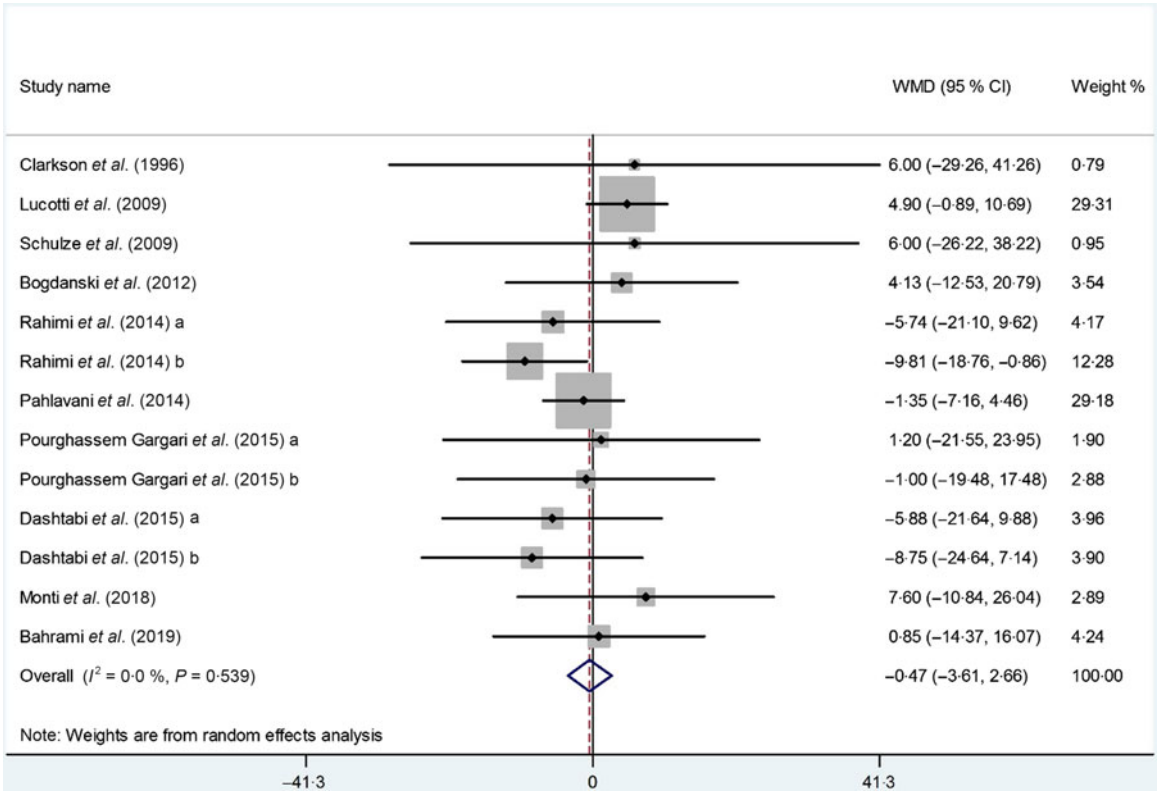

Fig. 4. Forest plot of the effect of L-arginine supplementation on LDL-cholesterol. WMD, weighted mean difference.

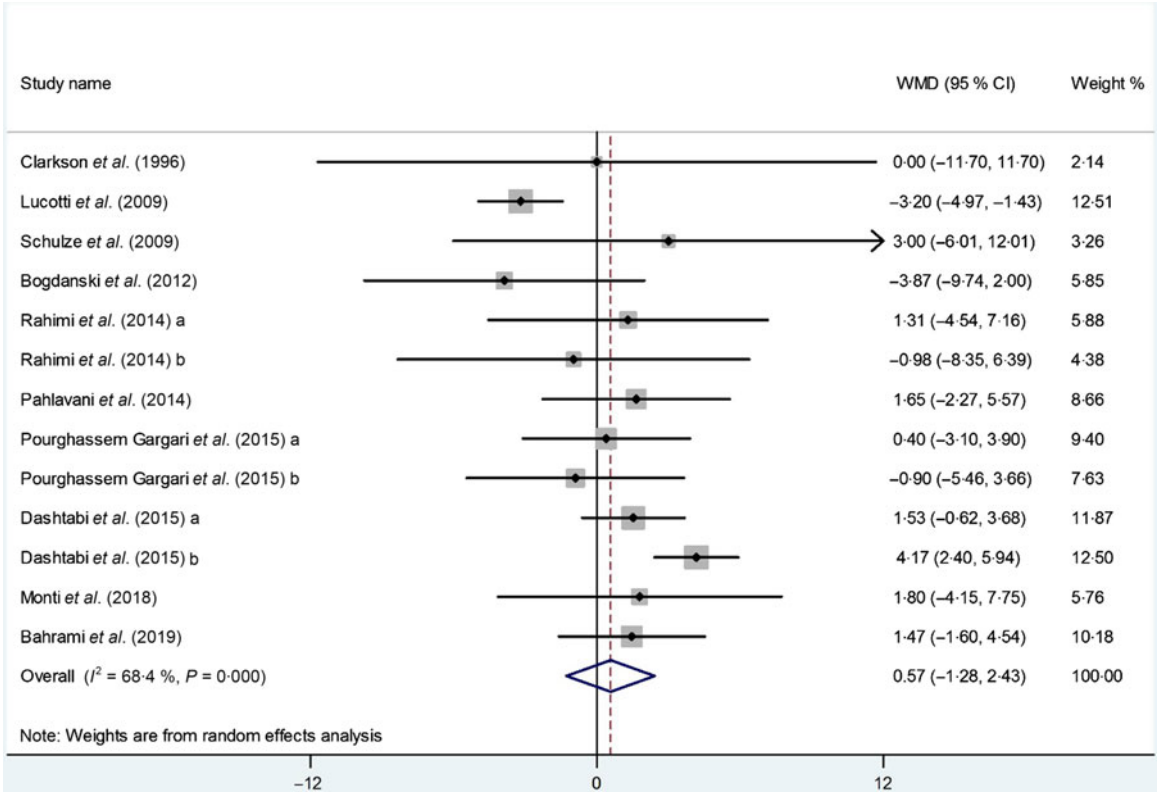

Fig. 5. Forest plot of the effect of L-arginine supplementation on HDL-cholesterol. WMD, weighted mean difference.

Furthermore, given that a major part of L-arginine is metabolised to ornithine and urea[57,60], patients with gout or renal function impairment should pay special caution when consuming it. Finally, due to its vasodilating properties, L-arginine has also been shown to interfere with certain medications (including Viagra and blood pressure medications) thus imposing negative reactions[57].

This meta-analysis has certain limitations that should be noted. First, the included studies involved individuals with different health status, resulting in a heterogeneous sample. Second, the sample sizes of individual trials were small, thus our results might more easily suffer from sample imbalances and an influence of baseline confounding factors. Third, the influence of sex remains unknown, since there was just one article that involved men. Women have different sex hormones compared with men that may affect lipid profile, thus implying that L-arginine may impose sex-dependent effects. Lastly, most RCT were not primarily designed to assess the effects of L-arginine on lipid concentrations. In order to draw straightforward conclusions regarding recommending L-arginine supplementation as a lipid-lowering agent, we need more RCT designed to specifically address this issue in a target population of patients with abnormal lipid profile.

The present study also has its strengths. It is only a systematic review and meta-analysis study to investigate the effect of arginine supplementation on lipid profile. Our systematic search makes it unlikely that large reports were missed, and error and bias were minimised by independent, duplicate decisions on the whole process of review by adhering to the PRISMA guidelines. Also, subgroup analysis and assessment of mean age, baseline BMI, dose of L-arginine, study duration and health status were done on the overall effect sizes.

Conclusion

The present systematic review and meta-analysis demonstrated that L-arginine supplementation leads to a significant reduction in TAG levels. However, no significant effect was observed in the case of other lipid parameters including TC, LDL-cholesterol and HDL-cholesterol. In order to confirm the results of our study, further clinical trials that exclusively examine the effects of L-arginine on participants with dyslipidaemia are required.

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A. H. and E. G. wrote the concept, design and carried out drafting of the present study. A. A. and E. G. performed searches of the electronic databases, screened the articles and extracted the data. A. H. performed the acquisition, analysis and interpretation of data. A. H. and E. G. critically revised the manuscript. A. P. performed a final revision and proofread of the article. All authors approved the final version of the manuscript. A. H. and E. G. are the guarantors of the present study.

The authors declare no conflict of interest.

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