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SYSTEMATIC REVIEWS AND META-ANALYSES

Effects of folic acid supplementation on C-reactive protein: A systematic review and meta-analysis of randomized controlled trials

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Abstract *Background and aim:* Given the contradictory results of previous randomized controlled trials (RCTs), we performed a systematic review and meta-analysis to quantify and summarize the effects of folic acid supplementation on C-reactive protein (CRP).
Methods and results: We performed a systematic search of all available RCTs conducted up to October 2018 in the following databases: PubMed, Scopus, and Cochrane. RCTs that investigated the effect of folate on CRP were included in the present study. Data were combined with the use of generic inverse-variance random-effects models. Statistical heterogeneity between studies was evaluated using Cochran's Q-test. Ten RCTs (1179 subjects) were included in the present meta-analysis. Pooled analysis results showed that folate supplementation significantly lowered the serum CRP level (weighted mean difference (WMD): −0.685 mg/l, 95% CI: −1.053, −0.318, $p < 0.001$). However, heterogeneity was significant ($I^2 = 96.7\%$, $p = 0.000$). Stratified analyses indicated that sex, intervention period, and type of study population were sources of heterogeneity. Following analysis, results revealed that the greatest impact was observed in women (WMD: −0.967 mg/l, 95% CI: −1.101, −0.833, $p = 0.000$), patients with type 2 diabetes mellitus (WMD: −1.764 mg/l, 95% CI: −2.002, −1.526, $p = 0.000$), and intervention period less than 12 weeks (WMD: −0.742 mg/l, 95% CI: −0.834, −0.650, $p = 0.000$).
Conclusion: This meta-analysis suggested that folic acid supplementation could significantly lower the serum CRP level. Folic acid leads to greater CRP lowering effect in women, patients with T2DM, and those with less than 12-week intervention.

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Introduction

Inflammation is an innate immunological response that is triggered by any kind of stimuli such as pathogens, irritants, and tissue injuries to localize damage to the tissue [1]. The mechanism of inflammation consists of an increase in the permeability of blood vessels and migration of leukocytes and other blood contents to the damaged site, which can cause redness, heat, and swelling [2]. Chronic inflammation can lead to inflammatory diseases such as atherosclerosis, rheumatoid arthritis, and asthma [3–5]. An increase in the concentration of acute phase reactants (APRs) accompanies inflammation and tissue injury [6]. C-reactive protein (CRP) is one of the most important APRs. CRP and many other APRs can influence multiple stages of inflammation [7–10]. During recent years, natural products have been reported to improve inflammation and thereby reduce the burden of diseases [11–13].

Although folic acid, folate, and vitamin B9 are often used interchangeably, folate is the water-soluble form of vitamin B (B9) that occurs naturally in foods including beef, liver, leafy vegetables, peas and beans, avocados, eggs, and milk, whereas folic acid is the synthetic form of folate [14,15]. Folic acid is one of the most important supplements given during pregnancy that can prevent many birth defects such as spina bifida. This is the reason why many health care providers recommend that all women of childbearing age receive a daily supplement of folic acid [16–20].

Folic acid is reported to improve insulin resistance [21] and oxidative stress [22,23]. In addition, folic acid supplementation improves hyperhomocysteinemia [24]. Folic acid may decrease the serum CRP level through the above-mentioned mechanisms. The effects of folic acid on CRP have been investigated in previous interventional studies, but the results are inconclusive. A lowering effect of folic acid on CRP has been reported in patients with T2DM [25], cervical intraepithelial neoplasm [26], and polycystic ovary syndrome (PCOS) [27]. However, other researchers reported that folic acid supplementation cannot change the serum CRP level [28,29]. To the best of our knowledge, no systematic review and meta-analysis have previously investigated the impact of folic acid on inflammation. Therefore, we conducted this study to evaluate the effects of folic acid supplementation on CRP across randomized controlled studies in adults.

Methods

Search strategy

The present meta-analysis was performed according to Systematic Reviews and Meta-Analyses statement guidelines for RCTs [30]. A systematic literature search was conducted using medical databases including PubMed, SCOPUS, Web of Science, Ovid, EMBASE, and Cochrane Library up to October 2018 under the following subject headings (MeSH) and non-MeSH keywords: “Folic Acid” OR “folate” “Vitamin M” OR “Vitamin B9” OR “Folacin” OR “Folvite” OR “Pteroylglutamic Acid” OR “folates” OR tetrahydrofolates” OR “Formyltetrahydrofolates” AND “C-Reactive Protein” OR “high-sensitivity CRP” OR “hs-CRP” OR “CRP” “high-sensitivity C-Reactive Protein” AND “Intervention Studies” OR “intervention” OR “controlled trial” OR “randomized” OR “randomised” OR “randomly” OR “placebo” OR “assignment” OR randomized controlled trial OR randomized clinical trial OR RCT OR blinded OR double blind OR double blinded OR “trial” OR “controlled clinical trial” OR Pragmatic Clinical Trial OR “crossover procedure” OR Cross-Over trial OR Double-Blind Method OR “equivalence trial” OR “double blind procedure”. The search was limited to RCTs assessing the influence of folic acid. The reference list of related articles was also hand-searched for additional relevant studies. Two independent investigators (H. K. V. and M. P.) screened titles and abstracts for relevant studies, and discrepancies were resolved using a third investigator.

Inclusion criteria

Published studies were included if they met the following criteria: (I) randomized controlled trials (RCTs) with either a parallel or a crossover design and only when placebo prescribed (II) conducted among adults (age $\geq$ 18 years); (III) prescribed folate supplementation, and (IV) reported sufficient information about CRP at baseline and the final of the intervention in both intervention and placebo groups. In case of any unavailable full-text article, we contacted the corresponding author, and if there was no response, the paper was excluded. We included trials that were performed with folic acid and folate; all other studies were excluded.

Quality assessment

The quality of eligible studies was evaluated independently by two investigators using the quantitative 5-point Jadad scale [31]. Studies with scores $\geq$3 and 2 were considered as high and low quality, respectively [32].

Data extraction

Eligible RCTs were reviewed independently by two authors, and the following data were extracted using a standardized electronic form: first author’s name, publication year, study location, sample size, dose of folic acid, study design, duration of intervention, patient’s status, and other information including age and sex. Mean and SD of outcome measures at study baseline, postintervention, and/or change between baseline and postintervention were recorded.

Statistical analysis

We evaluated the influence of folic acid supplementation on the change in CRP. Effect sizes for the meta-analysis were defined as the weighted mean difference (WMD;

value at the end of the trial minus the value at baseline) and 95% CI. In the event of no reported SD of the mean difference, it was calculated as follows: $SD^2 = [(SD1)^2 + (SD2)^2 - (2*R*SD1*SD2)]$. A correlation coefficient of 0.5 was used as this R-value [33]. Statistical heterogeneity between studies was examined using Cochran's Q-test and I^2 [34,35]. Predefined subgroup analyses were performed on gender (women and men), folic acid dose (<5 mg/l and ≥5 mg/l), supplementation duration (≤12 weeks and >12 weeks), and type of study population (healthy individuals, patients with type 2 diabetes mellitus, and subjects with specific female gynecologic diseases). Subgroup analyses for dose and duration were based on median cutoff of included studies. Subgroup analysis was performed with a fixed-effects model; the ideal form is when within-group heterogeneity is nonsignificant and between-group heterogeneity is significant before concluding for potential source of heterogeneity. Publication bias was assessed by visual inspection of funnel plot and Egger tests. Sensitivity analysis was also performed using the "metaninf" command in STATA to assess the effect of each individual study on the overall result after removing each study, in turn. All statistical analyses were conducted using STATA software (version 14).

Results

A total of 1436 reports were initially identified; after removing duplicates (n = 544), 892 articles remained. Out of 892 articles, 857 were excluded, as they were not RCTs, neither were they related to our present meta-analyses according to the inclusion criteria. A total of 35 potentially relevant articles were chosen for full-text evaluation and detailed examination. Among the full-text articles assessed, 25 studies were excluded for the following reasons: no CRP measurements performed (n = 7) and use of folate in combination with other components without an appropriate control group (n = 18), especially when combined with anti-inflammatory drugs [36]. Ultimately, 9 articles including 10 studies conducted in various types of populations met our inclusion criteria in this meta-analysis (Fig. 1).

Characteristics of the included studies

Study characteristics of the 9 eligible studies are summarized in Table 1. In one of the included trials, two different dosages of folic acid supplementation were administered (1 mg and 5 mg); hence, we considered it as two separate studies [27]. The range of folate supplementation dose was 0.8 mg/day [37] to 10 mg/day [28]. The trial duration was between 2 and 48 weeks [28,37]. Studies were conducted in different countries: four in Iran [25–27], three in the Netherlands [37–39], one in Canada [28], one in Italy [40], and one in the UK [29]. The enrolled subjects include healthy subjects [29,37,39,40], patients with type 2 diabetes mellitus (T2DM) [25,28,38,39], and those with female gynecologic diseases [26,27]. The range of quality scores for studies was between 3 and 5 points. Study conducted by Spoelstra-de Man et al [38] receive 5 score; because they used computer-generated random method.

Meta-analysis findings

Nine trials with 590 cases and 589 controls evaluated the effect of folic acid supplementation on the CRP level as an outcome measure [25–29,37–40]. The overall results from the random-effects model revealed a significant reduction in CRP levels following folic acid supplementation (WMD: −0.685 mg/l, 95% CI: −1.053, −0.318, $p < 0.001$) with significant heterogeneity ($I^2 = 96.7\%$, $p = 0.000$) (Fig. 2).

Subgroup analysis

Results of subgroup analyses are presented in Table 2. Stratified analysis showed that women experience higher reduction in the serum CRP level (−0.967 mg/l) after folic acid supplementation than men. The type of study population was another source of heterogeneity. The subgroups of healthy individuals and subjects with specific female diseases lacked heterogeneity. Furthermore, serum CRP levels were significantly lowered in patients with T2DM (WMD: −1.764 mg/l). Folic acid supplementation for equal to or less than 12 weeks caused a higher reduction in serum CRP levels (WMD: −0.742 mg/l) than for more than 12 weeks of supplementation (WMD: −0.144 mg/l). In addition, supplementation of a dose of ≥5 mg/day led to a higher reduction in CRP levels than that of <5 mg/day (WMD: −1.183 mg/l and −0.219 mg/l, respectively).

Publication bias

There was no significant publication bias as evidenced by either funnel plot asymmetry or Egger test (P = 0.182) (Supplemental Figure 1).

Sensitivity analysis

The present meta-analysis of folate showed a relatively high degree of heterogeneity across studies. The "metaninf" STATA command was used to assess the influence of each study on the pooled result (WMD) after omitting each study, in turn. Sensitivity analysis showed that overall estimate of effect size did not change significantly after elimination of individual studies; overall effect size without Asemi et al. [26] changed to −0.689 mg/l (95CI%: −1.06, −0.315). The same result found after Klerk et al. elimination; effect size minimally changed to −0.77 mg/l (95% CI: −1.22, −0.318) (Supplemental Figure 2).

Discussion

To the best of our knowledge, the present study is the first meta-analysis that investigates the most recent RCTs regarding the impact of folic acid supplementation on serum CRP concentration. A total of eight clinical trials

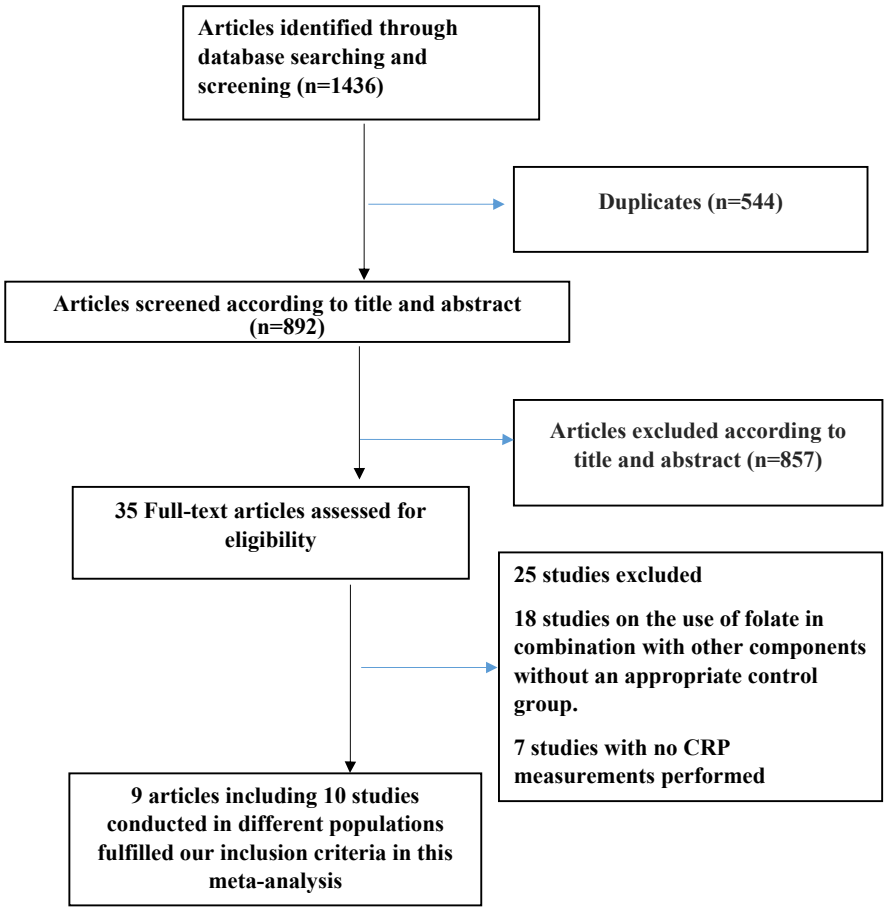

Figure 1 Flowchart of identification of included studies. The figure shows the progress of selection studies that fulfilled our inclusion criteria.

were included in this meta-analysis. Results of the included studies suggested that folic acid supplementation significantly lowered the serum CRP level. Subgroup analysis revealed that folic acid supplementation leads to higher significant reduction in women than in men, with an intervention period equal to or less than 12 weeks, and in patients with T2DM.

In concordance with our findings, taking a combination of 5 mg of folic acid with vitamin complex (5 mg of vitamin B2, 50 mg of vitamin B6, and 0.4 mg of vitamin B12) for 14 days has significantly reduced hs-CRP levels in patients with acute ischemic stroke [41]. Furthermore, in another study [42], low-dose folic acid supplementation (400 μg/day) for 7 weeks improved vascular function through effects on endothelial nitric oxide (NO) synthase and vascular oxidative stress in patients with coronary artery disease, although some researchers reported different results with no significant reduction in the serum CRP level after folic acid supplementation [43,44]. The discrepancies between the results of these studies may be related to different study designs along with controlling for cofounders. We showed that women experience higher reduction in CRP levels after folic acid supplementation than men. The female population is highly susceptible to folic acid deficiency [45], and the concentration of CRP in women is also higher than that in men [46–48]. These findings highlight the higher effect of folic acid supplementation in women than in men, on the one hand, because of possible deficiency and, on the other hand, a higher serum CRP level in this population.

Interestingly, in our meta-analysis, a 12-week intervention or less demonstrated a better response to folic acid intake regarding the serum CRP level. It could be related to bioavailability due to elongated treatment duration of folic acid supplementation. Our analysis showed that the reduction in CRP concentration level after treatment with folic acid was more in patients with T2DM than in healthy subjects. It was suggested that patients with T2DM have either higher or similar inflammation biomarker levels than healthy subjects [49–51]. Folic acid has been recently administered to patients with T2D during management, as it seems to modulate inflammatory biomarkers, endothelial function, and insulin resistance [52–55]. A previous meta-analysis showed that folate supplementation was effective in lowering the progression of carotid intima media thickness (cIMT), especially in patients with high CVD risk factors [56]. Folic acid supplementation also has been shown to improve endothelial function in subjects with hyperhomocysteinemia, hypercholesterolemia, and coronary artery disease [57–59]. A high homocysteine level has been reported in several clinical conditions associated with insulin resistance and high CVD risk

Table 1 Characteristics of eligible studies.

Author, year	Location	Study design	Participants	Gender (n)	Dose of Folic Acid	Duration (weeks)	Baseline CRP (mg/l)	After treatment CRP (mg/l)	Quality score
							Mean ± SD	Mean ± SD	
Talari, 2016	Iran	Randomized, Double-Blind, Placebo-Controlled Trial	Patients With T2DM, Overweight And CHD	Both (60)	5 mg	12	8.94 ± 5.47	7.75 ± 5.61	4
Asemi, 2016	Iran	Randomized, Double-Blind, Placebo-Controlled Trial	Women With Cervical Intraepithelial Neoplasia	Women (58)	5 mg	24	4.99 ± 4.24	4.79 ± 4.22	3
Bahmani, 2014	Iran	Randomized, Double-Blind, Placebo-Controlled Clinical Trial	Overweight And Obese Women With Polycystic Ovary Syndrome	Women (46)	1 mg	8	1.73 ± 0.58	1.47 ± 0.47	4
Bahmani, 2014	Iran	Randomized, Double-Blind, Placebo-Controlled Clinical Trial	Overweight And Obese Women With Polycystic Ovary Syndrome	Women (46)	5 mg	8	1.07 ± 0.34	0.86 ± 0.26	4
Title, 2006	Canada	Randomized, Placebo-Controlled, Cross-Over Study	Type 2 Diabetes	Both (38)	10 mg	2	3 ± 1.09	2 ± 0.51	4
Solini, 2006	Italy	Unmasked Randomized Placebo-Controlled Trial	Overweight Subjects	Both (60)	2.5 mg	12	0.84 ± 0.29	0.79 ± 0.29	3
Klerk, 2005	The Netherlands	Randomized Placebo Controlled Trial	Older Adults	Both (276)	0.8 mg	48	1.2 ± 0.4	1.2 ± 0.33	3
Mangoni, 2003	UK	Randomized, Double-Blind, Controlled Trial	Chronic Smokers	Both (24)	5 mg	4	2.2 ± 0.7	1.7 ± 0.7	3
Spoelstra-de Man, 2004	The Netherlands	Randomized, Double-Blind, Controlled Trial	Type 2 Diabetes Mellitus And Mild Hyperhomocysteinemia	Both (41)	5 mg	24	2.8 ± 1.04	3.47 ± 3.64	5
Durga, 2005	The Netherlands	Randomized, Double-Blind, Controlled Trial	Men And Postmenopausal Women With Homocysteine Concentrations Of 1.8 mg/L Or Higher	Both (530)	0.8 mg	52	1.5 ± 0.67	1.4 ± 0.55	4

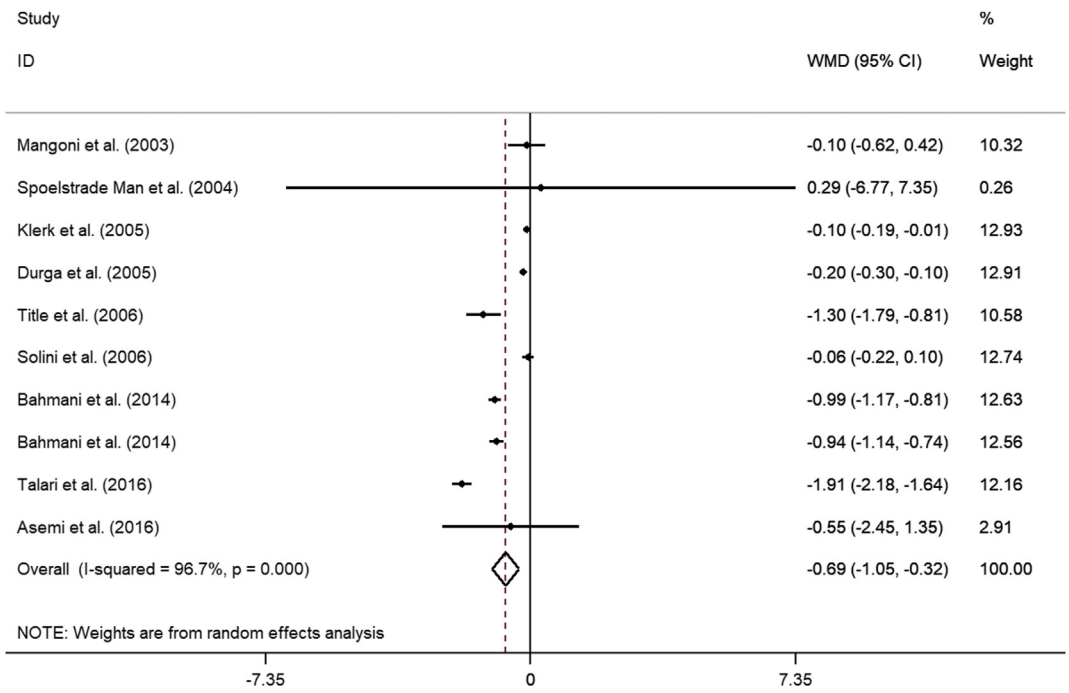

Figure 2 Forest plot of C-reactive protein change outcome. The figure shows the effects of folic acid supplementation on C-reactive protein.

factors [60]. The homocysteine-lowering effects of folic acid have also been well documented [61], which could be beneficial against atherosclerosis and its thrombogenic activity [29]. Apart from all the above-mentioned beneficial effects, we showed in the present study anti-inflammatory effects of folic acid supplementation in a comprehensive meta-analysis, which could be considered in patients who need folic acid. Although here we showed the lowering effect of folic acid on the serum CRP level, meaningful clinical effects must be determined in future studies.

Multiple mechanisms are involved in decreasing CRP concentration by folic acid supplementation. It is suggested that the decline in homocysteine level through folic acid supplementation may result in reduced oxidative stress [24]. Homocysteine is a marker for the presence of pathologic oxidative stress that enhances the expression of inflammatory factors by inducing nuclear factor kappa B (NF-kB) and poly(ADP-ribose) polymerase (PARP) activations [62,63]. In addition, previous studies revealed that folic acid may also act as an antioxidant through NADPH oxidase regulation, which mediates superoxide anion production [22,23]. Moreover, the decrease in insulin resistance following the supplementation of folic acid may lead to reduced synthesis of inflammatory factors [21].

Table 2 Subgroup analyses of folic acid supplementation on CRP levels.

	No.	WMD (95% CI)	Significance Test (S) of WMD	P-Heterogeneity Intergroup	I^2 (%)	P-Heterogeneity Between Sub-Groups[a]
Sex						<0.001
Women	3	−0.967 (−1.101, −0.833)	<0.001	0.853	0.0	
Both	7	−0.231 (−0.290, −0.172)	<0.001	<0.001	96.6	
Folic Acid Dose						<0.001
<5 mg/l	4	−0.219 (−0.277, −0.160)	<0.001	<0.001	96.3	
≥5 mg/l	6	−1.183 (−1.329, −1.037)	<0.001	<0.001	90.1	
Trial Duration						<0.001
≤12 Weeks	6	−0.742 (−0.834, −0.650)	<0.001	<0.001	97.0	
>12 Weeks	4	−0.144 (−0.221, −0.077)	<0.001	0.505	0.0	
Type Of Study Population						<0.001
Healthy Individuals	4	−0.130 (−0.191, −0.069)	<0.001	0.376	3.2	
Patients with Type 2 Diabetes Mellitus	3	−1.764 (−2.002, −1.526)	<0.001	0.087	59.0	
People With Specific Female Diseases	3	−0.967 (−1.101, −0.833)	<0.001	0.853	0.0	

[a] The ideal form is when within-group heterogeneity is nonsignificant and between-group heterogeneity is significant before concluding for potential source of heterogeneity.

However, in the present meta-analysis, there are some drawbacks to be acknowledged. Heterogeneity across included studies is the most important limitation of the present meta-analysis. The included trials were performed in subjects with different baseline medical conditions such as healthy, T2DM, cervical intraepithelial neoplasia, polycystic ovary syndrome, and mild hyperhomocysteinemia. In addition, the number of available studies was relatively small, and eligible trials were conducted in a small sample size. The number of studies in subgroup analysis was also small. Finally, the results of most studies were not adjusted for confounding factors, which can affect inflammatory markers in enrolled subjects, such as fruit and eatable intake. Dietary intake of folic acid could be another limitation across included studies. Accordingly, additional studies are required with an aim to evaluate the anti-inflammatory effect of folate.

In conclusion, this meta-analysis of randomized placebo-controlled trials suggested that folic acid supplementation significantly lowered the serum CRP level. Subgroup analysis also revealed that the greatest impact was among women, patients with T2DM, and for an intervention period of less than 12 weeks, but the results must be interpreted with caution, especially for subgroup analysis, because there was a lack of eligible studies. However, long-term well-designed dose-escalating trials are required because of insufficient evidence.

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Conflicts of interest

All the authors declared that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2018.11.006.

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