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Authors: Meysam Zarezadeh (Conceptualization) (Formal analysis) (Investigation) (Writing - original draft) (Supervision), Mohammad Reza Emami (Conceptualization) (Visualization), Hamed Kord-Varkane (Data curation) (Visualization) (Methodology), Seyed Mohammad Mousavi (Data curation) (Visualization), Hamed Alizadeh (Data curation) (Visualization), Omid Asbaghi (Data curation) (Visualization), Beheshteh Olang (Data curation) (Visualization) (Writing - review and editing), Masoud Khorshidi (Conceptualization) (Methodology) (Visualization) (Project administration)

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The effect of oral L-Arginine supplementation on asymmetric dimethylarginine levels: a systematic review and meta-analysis of randomized clinical trials

Authors

Meysam Zarezadeh¹, Mohammad Reza Emami², Hamed Kord-Varkane³, Seyed Mohammad Mousavi⁴, Hamed Alizadeh⁵, Omid Asbaghi⁶, Beheshteh Olang⁷, Masoud Khorshidi^{8*}

¹ Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran.

² Department of Nutrition, Faculty of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran.

³ Student Research Committee, Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁴ Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran.

⁵ Department of Occupational Health Engineering, Faculty of Health, Iran University of Medical Sciences, Tehran, Iran.

⁶ Nutritional Health and Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran.

⁷ Pediatric Gastroenterology, Hepatology and Nutrition Research Center, Research Institute for Children Health, Mofid Children's Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

⁸ Student Research Committee, Iran University of Medical Sciences, Tehran, Iran.

*; Corresponding author

***Corresponding Author: Masoud Khorshidi**

Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran.

Fax: +98-21-889 559 79

Telephone: +98-21-88955979

Email address: khorshidi.2065@gmail.com

Postal address: Iran University of Medical Sciences, Shahid Hemmat Highway, Tehran, Iran.

Abstract

Aims: Cardiovascular disease (CVD) is the main cause of mortality around the world. Asymmetric dimethylarginine (ADMA), as an inhibitor of nitric oxide synthase and cardiovascular risk factor, potentially can be increased by l-arginine intake. The aim of the present meta-analysis is to determine the effect of oral l-arginine supplementation on ADMA.

Methods: PubMed, Scopus, Cochrane Library and Web of Science databases were searched for the relevant randomized clinical trials up to Oct 2018. WMD and 95% CI was reported for the ADMA changes. Random-effect model was conducted for heterogeneous data. Sensitivity and subgroup analysis was performed in order to find the source of heterogeneity. Egger's and Begg's test and funnel plots was performed to identify existing publication bias. Jadad scale was used for rating included trials.

Results: A total of 519 articles after removing 111 duplicates were obtained from searching databases and 6 eligible trials were included to study. There was no significant effect of l-arginine supplementation on ADMA levels using random-effect analysis (WMD = -0.04 mg/dl; 95% CI: -0.39 – 0.31; P = 0.83, I² = 99.0%; P = <0.001) as well as using the subgroup analysis by mean age, intervention dosage and duration. The effect of sensitivity analysis was not significant.

Conclusion: This meta-analysis of randomized clinical trials showed no significant effect of L-arginine supplementation on ADMA levels.

Keywords: L-Arginine; Asymmetric dimethylarginine; Cardiovascular disease; Atherosclerosis; Hypertension; Meta-analysis.

Introduction

Cardiovascular disease (CVD) is the main cause of mortality around the world. The global burden of CVD was 26.9% of all deaths in 2010 [1]. Hypertension (HTN) is a potent risk factor for CVD. The global prevalence of HTN varies from 3.4% in rural Indian men to 72.5% in Polish women [2]. More than 90% of hypertensive cases are idiopathic [3], however, life style and dietary approaches have considerable impact on HTN risk [4, 5].

Asymmetric dimethylarginine (ADMA), as a risk factor for vascular dysfunction, inhibits eNOS (endothelial nitric oxide synthase) [6] and exacerbate the condition in patients are at risk of HTN. Methylated proteins, naturally produce ADMA after hydrolyzing which is degraded by dimethylarginine dimethylaminohydrolase (DDAH) and excrete to urine [7], however, in patients with CVD and diabetes due to elevated levels of oxidative stress, inflammation and oxidized low density lipoproteins (LDL), DDAH activity is reduced and as a result ADMA concentration increases in serum [8].

It has been shown that oral L-Arginine supplementation had significant impact on increasing NO (nitric oxide) production, vasodilation and blood flow [9, 10], however, potentially it can elevate

ADMA levels [11]. Published results by Jablecka and et al. study showed that supplementation with l-arginine increased ADMA levels in patients suffering mild hypertension [12]. Moreover, in Schneider and et al. study ADMA was increased in patients with peripheral arterial occlusive disease following l-arginine supplementation [13]. Nevertheless, some studies conclude that oral supplementation of l-arginine decrease ADMA levels [14-16] and some studies showed no effect of that (Bode-Boger, Schneider's CAD study) [13, 17].

According to the existing controversy among studies, we decided to conduct present meta-analysis to clarify the pure effect L-Arginine on ADMA levels. The aim of present study is to determine magnitude and direction of L-Arginine supplementation effect on ADMA levels.

Methods and materials

Searching

Following databases were searched for studies which reported L-Arginine supplementation effect on plasma or serum ADMA concentration: PubMed, Scopus, Cochrane Central Register of Controlled Trials and Web of Science. Relevant studies in English which was published up to Oct 2018 were included. Terms and keywords "L-Aarginine", "arginine", "L-Arg" and "Arg" were used in combination with "asymmetric dimethylarginine" and "ADMA" to search databases. Afterwards, the search was limited to randomized clinical trials by the following keywords: "randomly", "random", "randomized", "trial", "groups" and "placebo". In addition, the reference list of some related review articles was searched.

Study selection

Studies were eligible if they: 1) Were conducted at least as a single blind randomized clinical trial; 2) Used oral L-Arginine as intervention; 3) Reported ADMA levels in L-Arginine supplementation and placebo groups at least after intervention in form of mean and standard deviation (SD). Studies were excluded if they: 1) Administered L-Arginine by infusion; 2) Had not a placebo group; 3) Did not reported the SD; 4) Used l-arginine along with other interventions.

Data abstraction

Two reviewers (MZ and MKh) independently evaluated the articles and extracted data from the eligible studies and disagreements were discussed and resolved with third author (HKV). The following items abstracted from selected studies: first author of the study, publication year, study region, publisher journal, study population, gender of participants, number of enrolled participants, mean age, intervention dosage and duration, mean and SD of ADMA in supplement and placebo group after intervention.

Quality evaluation of the studies

Methodological quality of studies was assessed by Jadad scale [18]. Studies with score of ≥ 3 are defined as a high quality study. The scores were given according to following criteria reporting: withdrawals and dropouts, randomization and blinding procedure.

Statistical analysis

Fixed and random-effect model were used to calculate weight mean difference (WMD) and 95% confidence interval for including studies. The heterogeneity across studies was assessed by using Cochran's Q test and I^2 testes [19]. Subgroup analysis was performed to identify the source of

heterogeneity by age (two $<65y$ and $\geq 65y$ groups), intervention dosage ($>10g$ and $\leq 10g$) and duration ($>10w$ and $\leq 10w$). Data were recorded in form of mean \pm SD and standard errors and CIs was converted to SD wherever needed. Begg's test, Egger's test and funnel plots was used to evaluate the publication bias [20]. The sensitivity analysis was performed to assess influence of a single study in meta-analysis estimation and to identify the source of heterogeneity. Stata 14 (Stata Corporation, College Station, TX) was employed to perform all the statistical analysis. P-value < 0.05 was considered as significance level.

Results

Literature search

Electronic searching in databases resulted total of 519 articles after removing 111 duplicated citations. Finally, 6 trials were identified eligible and fully met the inclusion criteria [13-17]. The details of the study selection procedure are shown in **Figure 1**.

Study characteristics

Included studies were performed during 2001 to 2015. A total number 214 participants were enrolled which varied from 18 to 60 in studies. All included studies had parallel design. The mean age in studies was between 60 and 73. Moreover, the duration of intervention differed from 2 to 26 weeks and dosage of L-Arginine administration varied from 6.4 to 24 gr. Characteristics of the studies are shown in **Table 1**.

Effect of l-arginine on ADMA

The results of fixed-effect analysis showed that oral L-Arginine supplementation significantly increased ADMA levels (WMD = 0.27 mg/dl; 95% CI: 0.24 – 0.3; P = 0.000), however, after using random-effect model due to the high between-study heterogeneity ($I^2 = 99\%$, P = 0.000), the results was not statistically significant (WMD = -0.04 mg/dl; 95% CI: -0.39 – 0.31, P = 0.83; $I^2 = 99.0\%$, P = <0.001). Random-effect analysis results are shown in **Figure 2**.

In order to identify the source of heterogeneity, sensitivity analysis and subgroup analysis was conducted by mean age, intervention duration and dosage. The heterogeneity of the studies still remained unchanged in subgroups which indicated that none of the mentioned subgroups were the source of heterogeneity. Furthermore, there was no significant effect of l-arginine on ADMA in subgroup analysis. The results of subgroup analysis are shown in **Table 2**.

Sensitivity analysis was performed. Schneider study on patients with coronary artery disease (CAD) was excluded due to the its major impact on the results of the study. After removing CAD study, Random effect analysis was performed with 5 study, however, the results were not significant likewise (WMD = -0.05 mg/dl; 95% CI: -0.47 – 0.36; P = 0.802).

Publication bias

Egger's and begg's test was employed to identify any existing publication bias. Egger's test results showed that there was no publication bias with the included trials (t = -2.26, p = 0.087). There was

no publication bias, as well as, with the begg's test ($z = -0.94$, $p = 0.348$). Funnel plot of included studies, showed symmetric distribution around the mean difference except one study (**Figure 3**).

Discussion

To our knowledge, there is no meta-analysis was conducted to determine the effect of l-arginine supplementation on ADMA serum levels, so far. The results of present meta-analysis showed that oral l-arginine supplementation had no significant effect on ADMA levels.

ADMA, as an inhibitor of endothelial nitric oxide synthase (eNOS), is a major risk factor for cardiovascular complications [21]. Generally, in healthy individuals ADMA is produced by most body cells and is degraded by an enzyme named dimethylarginine dimethylaminohydrolase (DDAH) which means there is a balance between ADMA production and decomposition in healthy people [7], however, the activity of DDAH extremely decrease by hyperglycemia, hyperhomocysteinemia, oxidized LDL and pro-inflammatory cytokines [22]. Therefore, ADMA plays an important role in patients suffering from diabetes and cardiovascular disease due to the elevated levels of homocysteine, blood sugar, oxidized LDL and exceeding of oxidative stress and pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) [23]. Furthermore, it has been shown that ADMA induces production of reactive oxygen species (ROS) in endothelial cells [24]. Besides, ROS increases ADMA generation and expression of protein arginine N-methyltransferase (PRMTs), an enzyme that methylate proteins in arginine segments [25]. All mentioned processes leads to vascular dysfunction and atherosclerosis [26].

Altun ZS and et al. in an in vivo study showed that supplementation with L-Arginine significantly decreased ADMA levels in preeclamptic rats. it have been shown that oxidative stress

is elevated in preeclampsia [27]. It causes in excessive synthesis and decrease in degradation of ADMA. Also, in Kohli R and et al. study, supplementation with L-Arginine in streptozotocin-induced diabetic rats for two weeks, significantly increased plasma levels ADMA. Recent studies suggested that elevated levels of pro-inflammatory cytokines in diabetes decreased DDAH activity and expression and increased ADMA levels [28]. Nevertheless, in Lucotti and et al. study, supplementation with 6.4g L-Arginine for 72 weeks significantly decreased ADMA levels in cardiopathic patients [15]. Also, Sydow K and et al. showed that receiving 24g/d L-Arginine for 8 weeks decreased ADMA levels [16], however the amount was not significant. These results indicated that duration of supplementation with l-arginine might have substantial impact on ADMA levels in both human and animal studies.

Except of two included studies which reported ADMA as a primary outcome [14, 16], other studies reported it as a secondary outcome. Some studies despite the assessment of the ADMA, did not even reported the details [12, 29-32]. Although ADMA is neglected as a major risk factor for cardiovascular complications, we pooled the whole available data together to determine the pure effect of l-arginine on ADMA levels. Three of included studies with study population of stable angina, hyperhomocysteinemia and elderly individuals had 4-5-fold higher mean of ADMA than the other three which was due to the effect of homocysteine and age on ADMA levels. In Lucotti and et al. study [15], L-Arginine supplementation significantly decreased ADMA levels in comparison with placebo group.

Included trials were selected for the study by assessing precisely and setting principal inclusion and exclusion criteria. All trials were randomized blinded placebo controlled trials. Trials that used L-Arginine supplementation by infusion [33-35] and along with other interventions [36] were not included in the study. Nevertheless, there were several limitations. There was high between-study heterogeneity. One of the main contributors to the observed heterogeneity was inclusion of studies with various background diseases. Moreover, the reliability of present study is

dependent on included trials validity. Among the all included trials, only one trial reported withdrawals, randomization and blinding procedure as well [16]. Above all, several studies reported the results defectively. For example, one trial which contains 4 different groups of participants with different administered l-arginine dosages, reported the mean levels of ADMA in form of diagram without SD [12]. In case of availability of the mentioned data, a meta-regression analysis could have been conducted.

The results of present meta-analysis of blinded randomized clinical trials showed that l-arginine supplementation has no effect on ADMA levels, however, a new meta-analysis should be carried out preferably with large and long-term randomized clinical trials.

Conflict of interests

The authors declare that they have no conflict of interests.

Author agreement

MZ: Conceptualization, Formal Analysis, Investigation, Writing – Original Draft, Supervision

ME: Conceptualization, Visualization

HKV: Data Curation, Visualization, Methodology

SMM: Data Curation, Visualization

HA: Data Curation, Visualization

OA: Data Curation, Visualization

BO: Data Curation, Visualization, Writing – Review & Editing

MKh: Conceptualization, Methodology, Visualization, Project Administration

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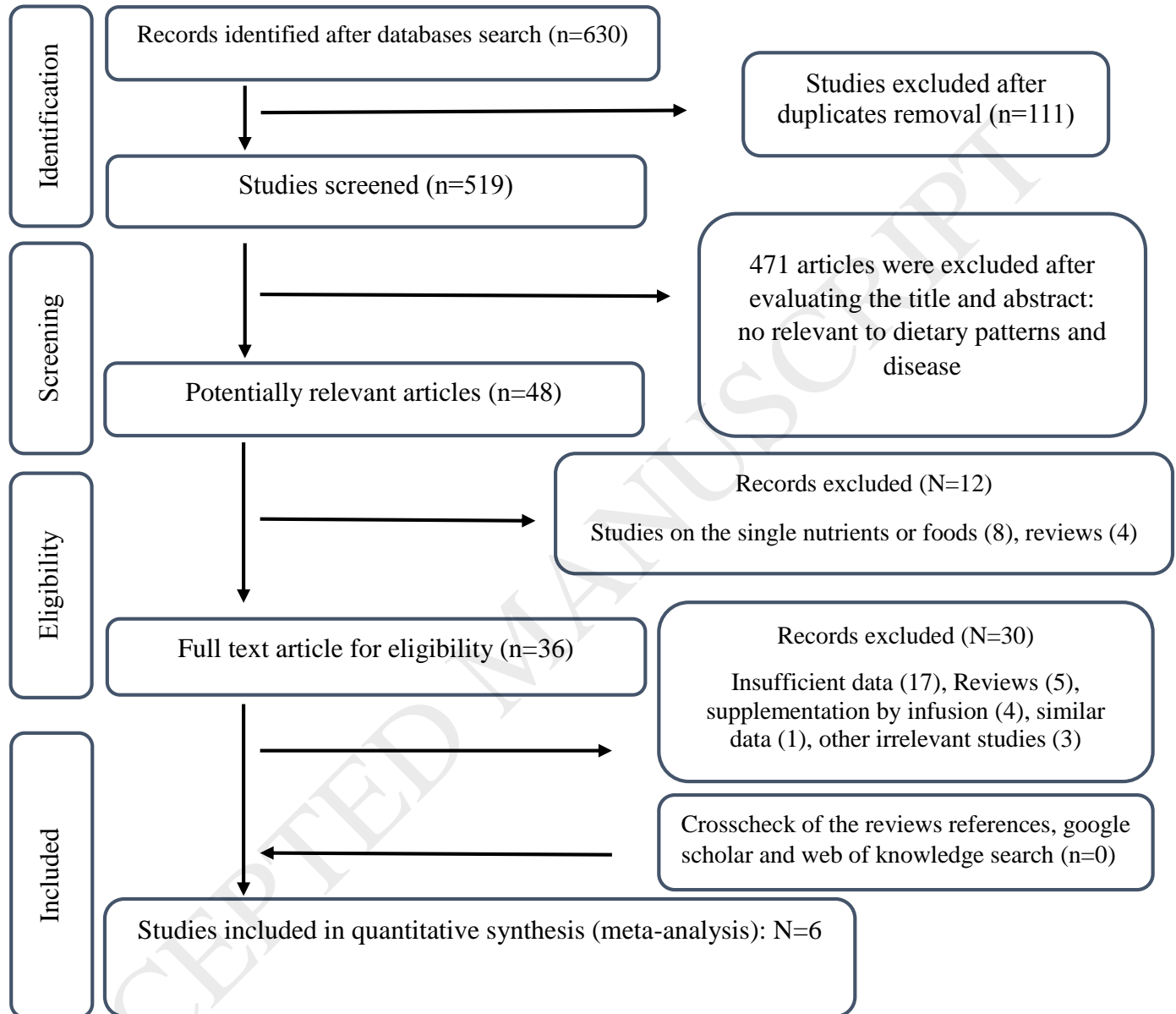
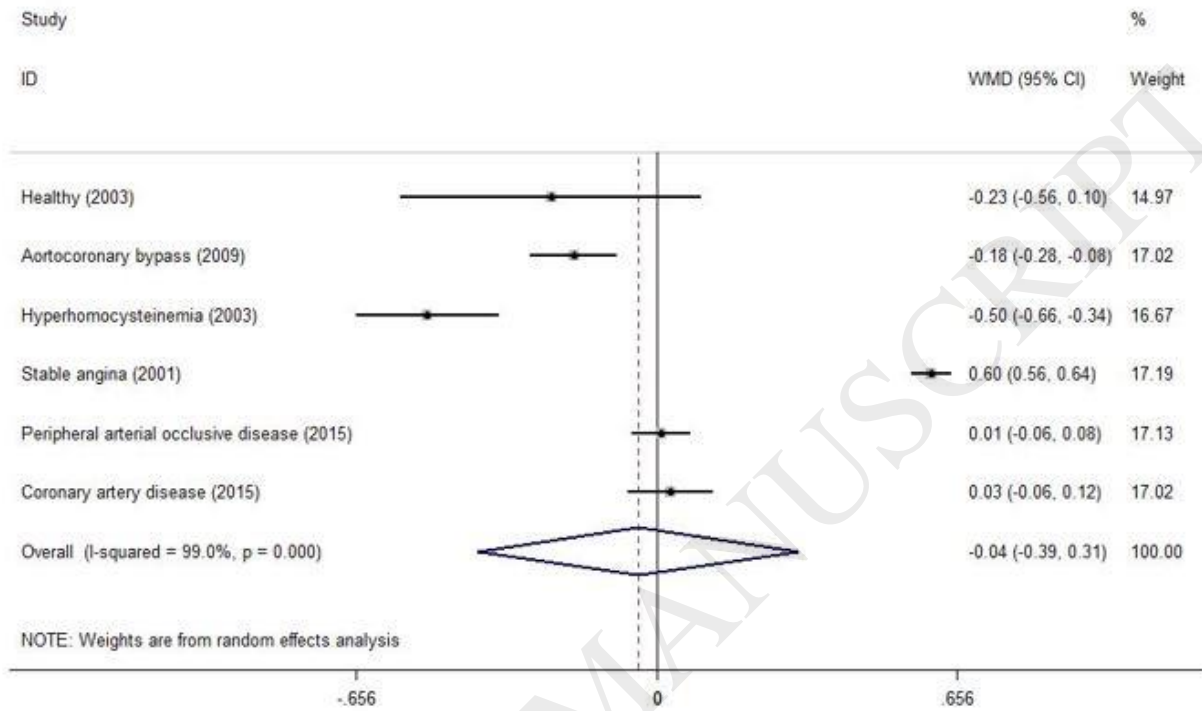
Figure 2. The procedure of study selection

Figure 2. The random - effect model analysis

Figure 2. The random-effect model analysis

Figure

3

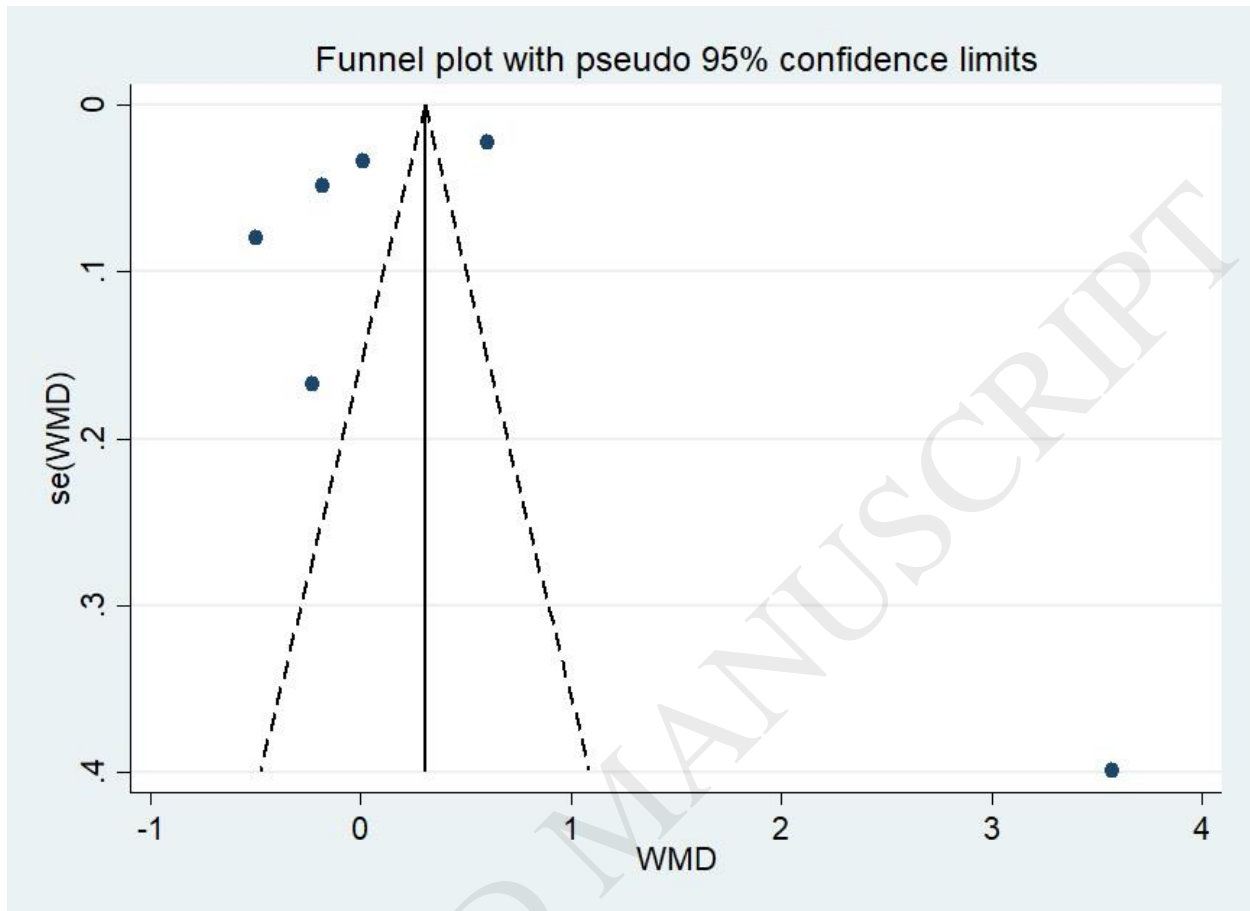


Table 1. General Characteristics of included studies

Study	Country	year	Study population	Duration (week)	Administration	L-arginine dosage (g/day)	Sample size	Mean age	Design	Jadad score
Bode-Boger	Germany	2003	Healthy	2	Oral	8	24	73	Parallel	3
Lucotti	Italy	2009	Aortocoronary bypass	26	Oral	6.4	32	65	Parallel	4
Schneider	Germany	2015	PAOD	12	Oral	9.96	40	67	Parallel	4
Schneider	Germany	2015	CAD	24	Oral	9.96	60	62	Parallel	4
Sydow	Germany	2003	HHCys	8	Oral	24	18	64	Parallel	5
Walker	UK	2001	Stable Angina	2	Oral	15	40	60	Parallel	3

Table 2. Pooled estimates of arginine effect on ADMA within different subgroups.

Group	No of comparisons	WMD (95% CI)	P value	P-heterogeneity	I^2 (%)
Total	6	-0.04(-0.39, 0.31)	0.498	<0.001	99.0
Arg dosage (g)					
≤10	4	-0.06 (-0.18, 0.05)	0.924	0.003	78.5
>10	2	0.05 (-1.03, 1.13)	0.296	<0.001	99.4
Intervention duration (week)					
≤10	3	-0.04 (-0.88, 0.80)	0.928	<0.001	99
>10	3	-0.04 (-0.17, 0.08)	0.474	0.002	84.1
Mean age					
<65	3	0.05 (-0.55, 0.64)	0.876	<0.001	99.2
≥65	3	-0.11 (-0.27, 0.06)	0.205	0.003	82.7