Increase in fasting vascular endothelial function after short-term oral L-arginine is effective when baseline flow-mediated dilation is low: a meta-analysis of randomized controlled trials

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ABSTRACT

Background: Previous trials suggest that oral L-arginine administration affects endothelial function. However, most of these studies were small, the conclusions were inconsistent, and the precise effects are therefore debatable.

Objective: The objective was to assess the effect of oral L-arginine supplementation on endothelial function, as measured with the use of fasting flow-mediated dilation (FMD).

Design: We conducted a meta-analysis of randomized, placebo-controlled L-arginine supplementation trials that evaluated endothelial function. Trials were identified in PubMed, Cochrane Library, Embase, reviews, and reference lists of relevant papers. The weighted mean difference (WMD) was calculated for net changes in FMD by using random-effect models. Previously defined subgroup analyses and meta-regression analyses were performed to explore the influence of study characteristics.

Results: Thirteen trials were included and evaluated. Because there was only one long-term study, we focused on short-term effects of L-arginine (12 studies, 492 participants). In an overall pooled estimate, L-arginine significantly increased FMD (WMD: 1.98%; 95% CI: 0.47, 3.48; P = 0.01). Meta-regression analysis indicated that the baseline FMD was inversely related to effect size (regression coefficient = −0.55; 95% CI: −1.00, −0.10; P = 0.016). A subgroup analysis suggested that L-arginine supplementation significantly increased FMD when the baseline FMD levels were <7% (WMD: 2.56%; 95% CI: 0.87, 4.25; P = 0.003), but had no effect on FMD when baseline FMD was >7% (WMD: −0.27%; 95% CI: −1.52, 0.97; P = 0.67).

Conclusion: Short-term oral L-arginine is effective at improving the fasting vascular endothelial function when the baseline FMD is low. Am J Clin Nutr 2009;89:1–8.

INTRODUCTION

Cardiovascular disease (CVD), the biggest burden and dominant chronic disease in many parts of the world, has been predicted to be the main cause of disability and death worldwide in the 21st century (1). Endothelial dysfunction is an early pathophysiological feature and an independent predictor of poor prognosis in most forms of CVD (2–4). Endothelial function has been shown to be influenced by several vascular risk factors. Treatment of these risk factors can restore endothelial function (5, 6). The L-arginine/nitric oxide (NO) pathway plays a critical role in maintaining normal endothelial function by causing blood vessel relaxation (vasodilatation). The semisessential amino acid L-arginine is the only substrate for NO synthesis in vascular endothelial cells. Therefore, this amino acid has the potential to improve endothelial function and is expected to play a role in the prevention or treatment of CVD and other vascular disorders (7). Preliminary reports suggest that arginine may be useful at improving medical conditions, including angina, atherosclerosis, coronary artery disease (CAD), erectile dysfunction, heart failure, and intermittent claudication/peripheral vascular disease (8–10).

Previously published trials have suggested that oral L-arginine supplementation affects endothelial function in hypercholesterolemic individuals, patients with CAD, healthy elderly individuals, and healthy young individuals who smoke (11–38). However, the sample size of these studies was small and the conclusions were inconsistent. As a result, the precise effects of arginine administration are still under debate.

In most of these studies, endothelial function was measured by fasting flow-mediated dilation (FMD), which represents the ability of the brachial artery to dilate in response to ischemia-induced hyperemia and reflects the local bioavailability of NO under a physiologic stimulation (38). The aim of the present review was to identify and combine all published randomized controlled trials that investigated the effects of L-arginine supplementation on FMD.

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SUBJECTS AND METHODS

Literature search
We systematically searched PubMed, Cochrane Library, Embase, reviews, and reference lists of relevant papers with the strategy of using the term ‘arginine’ paired with the following: ‘endothelial,’ ‘endothelium,’ ‘coronary heart disease,’ ‘CVD,’ ‘stroke,’ ‘cerebrovascular disease,’ ‘ischemia,’ and ‘trial.’

Study selection
We selected completed, published, and nonconfounded randomized placebo-controlled trials of oral L-arginine supplementation studies in which endothelial function was evaluated by estimating the fasting FMD in the brachial artery. Participants must have been treated with L-arginine for ≥3 d. Trials evaluating endothelial function with other methods were also included for systematic review but were not selected for meta-analysis, because these data could not be combined and compared quantitatively.

Quality assessment
Studies were assessed for quality of randomization, blinding, reporting of withdrawals, generation of random numbers, and concealment of allocation. Trials scored one point for each area addressed, with a possible score of between 0 and 5 (highest level of quality) (39).

Data extraction
All literature searches were independently reviewed by 2 authors (YB and LS) to identify relevant trials that met the inclusion criteria. Disparities were resolved by discussion. Data on trial size, population characteristics (age, sex, and baseline comorbidities), treatment regimen (dose of L-arginine, duration of treatment), and change in FMD levels were extracted.

Statistics and analysis
Our primary outcome was the net change in FMD due to L-arginine supplementation. Weighted mean differences (WMDs) and 95% CIs were calculated for net changes in FMD by using random-effect (DerSimonian and Laird) models (40). Statistical heterogeneity of treatment effects between studies was formally tested with Cochran’s test ($P < 0.1$). The $I^2$ statistic was also examined, and we considered $I^2 > 50\%$ to indicate significant heterogeneity between the trials (41). Previously defined subgroup analyses and meta-regression analyses were performed to explore heterogeneity in effects and the influence of study characteristics. Publication bias was assessed with funnel plots, Egger regression test (42), and fail-safe $N$ test (43). Statistical analyses were performed with Stata software (version 9.0; Stata Corporation, College Station, TX) and REVMAN software (version 5.0; Cochrane Collaboration, Oxford, United Kingdom).

RESULTS

Search results
A total of 1466 articles were identified in a combined search of the PubMed, Embase, and Cochrane Library databases and from a manual approach (search of previous studies cited in previous reviews and of reference lists from the identified articles); 1438 articles were excluded because they were not randomized controlled trials or their interventions were not relevant to the purpose of this meta-analysis (such as vitamin C or other agents intended to improve endothelial function). Full text assessment of the 28 potentially relevant articles resulted in 13 eligible randomized controlled studies (Figure 1). The most common reasons for exclusion were as follows: L-arginine was not supplied orally but intravenously in 5 trials (24–28) and intraarterially in 2 studies (23, 29) and that FMD levels were not reported. Four studies (20, 32–34) were not included because they were not placebo-controlled. One trial (22) was excluded because participants were only given one dose of L-arginine (15 g) together with a high-fat meal.

Study characteristics
We identified 13 trials (11–19, 35–38) with 589 subjects for inclusion in our study. Characteristics of the trials included in the analysis are shown in Table 1. Ten trials (11, 13–18, 35–37) had a crossover design. The trials varied in size from 10 to 36 subjects. As for the 13 studies that evaluated endothelial function, 4 trials (11, 13, 14, 17) investigated the effect of oral L-arginine on patients with stable CAD. The other 9 studies investigated the effect of L-arginine administration on children with chronic renal failure (15), patients with chronic heart failure (CHF) (12), healthy young men (18, 36), healthy individuals older than 70 y (16), clinically asymptomatic elderly subjects (38), patients with peripheral artery disease (19), or hypercholesterolemic patients (35, 37). The average age of the patients varied from 12 to 73.8 y. Doses of L-arginine in the included studies ranged from 3 to 24 g/d, and the treatment duration varied from 3 d to 6 mo. L-Arginine was administered 3 times/d in most of the studies (8 studies) and twice daily in 4 trials (14, 16, 37, 38), whereas 1 study (18) did not report the frequency of L-arginine administration.

Data quality
The quality scores of the trials varied from 2 to 5 (maximum score). All included trials were randomized, prospective, and placebo-controlled. Ten of the trials were crossover trials, and 10 were double-blinded. Four trials (12–14, 19) reported adequate details of withdrawals, whereas the others did not address this issue.

Effects of oral supplementation of L-arginine on FMD
Thirteen trials evaluated the effect of oral L-arginine supplementation on FMD level. The work of Bode-Böger et al (38) was separated into 2 trials (effect of L-arginine on subjects with high and low asymmetric dimethylarginine concentrations) for analysis. One study (12) did not directly report FMD in the
placebo-controlled group directly, but the data were able to be calculated from the information provided in the article. One study (18) reported the baseline and postsmoking FMD levels on the first, second, and third follow-up days. We extracted the postsmoking FMD data from the third day for meta-analysis to meet our inclusion criteria (1/C21 3d of L-arginine treatment).

The trial reported by Wilson et al (19) was the only study that evaluated the long-term effectiveness of L-arginine supplementation. The results of this trial were quite distinct from those of the short-term studies (heterogeneity test: $\chi^2 = 1538.28$, $I^2 = 99\%$, $P = 0.00001$).

First, the data were pooled from the 12 short-term studies. FMD levels were significantly higher in the L-arginine–supplemented subjects than in the placebo-treated subjects (13 comparisons, 493 participants; WMD: 1.98%; 95% CI: 0.47, 3.48; $P = 0.01$) (Figure 2). Significant heterogeneity for this outcome was found (heterogeneity $\chi^2 = 636.06$, $I^2 = 98\%$, $P < 0.00001$). The sources of heterogeneity were investigated by meta-regression methods. Meta-regression analysis of the data showed that the baseline FMD was negatively related to effect size (regression coefficient $= -0.55$; 95% CI: $-1.00$, $-0.1$; $P = 0.016$), which largely explained the heterogeneity of the effect. The dose of L-arginine (range: 3–24 g/d) and the average age of the participants were not effect modifiers.

Subsequently, we conducted a subgroup analysis according to baseline FMD. Because of the lack of reference ranges for FMD, we predefined the first to third quartiles of the baseline FMD ($<7\%$) for all included trials to be the low baseline FMD group, whereas the fourth quartile ($\geq 7\%$) was assigned as the high group. Characteristics of the subgroups are shown in Table 2. L-Arginine significantly increased FMD when the baseline FMD levels were $<7\%$ (10 trials, 365 participants; WMD: 2.56%; 95% CI: 0.87, 4.25; $P = 0.003$), but had no effect on FMD if the baseline endothelial function was normal (FMD $\geq 7\%$) (3 trials, 128 participants; WMD: $-0.27\%$; 95% CI: $-1.52$–$0.97$; $P = 0.67$) (Figure 2). L-Arginine was effective when baseline FMD was low, but the trend was not statistically significant when 4% (cutoff for first quartile) and 6% (cutoff for high median) were used as cutoffs or thresholds for the subgroup analysis (Table 3).

A sensitivity analysis according to other participant characteristics and study design features found that there were no effects due to mean age of the study population, dose of L-arginine, or study duration on the estimated change in FMD due to L-arginine supplementation (Table 3).

The intake of L-arginine seemed to have no significant effect on FMD (14 comparisons, 589 participants; WMD: 1.57%; 95% CI: $-0.32$, 3.46; $P = 0.1$) when the trial by Wilson et al (19) was included in the analysis.

Publication bias

Funnel plots of all short-term studies and 10 studies in the low baseline FMD group seemed asymmetric through visual examination, whereas a statistical analysis of funnel plots suggested no publication bias (Egger test, $P = 0.49$, Figure 3; Egger test, $P = 0.71$, Figure 4). A fail-safe N test indicated that it would take 2427 (for all studies) or 2383 (for the low baseline FMD...
subgroup) unpublished null result studies to bring the combined 
P to a nonsignificant level.

**DISCUSSION**

Our meta-analysis found that, as compared with placebo control, 
short-term supplementation with \( \text{L-arginine} \) was associated with 
a significant increase in brachial artery FMD, which indicated an 
improvement in endothelial function. The results of these trials 
were significantly heterogeneous. Therefore, we considered that 
the characteristics of subjects and study design may have influ-
enced the results of these studies. Meta-regression of heteroge-
neity for this outcome indicated that the heterogeneity was largely 
explained by the baseline FMD. Meta-regression analysis re-
vealed that baseline FMD was negatively related to effect size. In 
other words, as baseline FMD increased, the effect of \( \text{L-arginine} \) 
supplementation was gradually attenuated.

A subsequent subgroup analysis showed that the effect of 
\( \text{L-arginine} \) intake was positive when FMD was low at baseline 
but negative when FMD was high at baseline. These data sug-
gest that the intervention was able to restore the dysfunctional 
endothelium but could not further improve the endothelium func-
tion. The consistent results of the meta-regression and subgroup 
analyses reported herein add credence to the contrasting results 
in the different subgroups and suggest that the effect of arginine 
supplementation was greatly influenced by baseline FMD. This 
may influence the decisions of doctors, funding authorities, or 
medical institutions on the use of \( \text{L-arginine} \) in populations with 
different levels of endothelial function. Ultimately, this result 
appears to support the idea that we should provide \( \text{L-arginine} \) to 
those targeted patients who would benefit most rather than to all 
individuals.

To ensure comparability of the trials, we excluded trials in which 
the effect of \( \text{L-arginine} \) on endothelial function was not evaluated 
on the basis of FMD. Three studies (44–46) indicated that 
acetylcholine-induced increases in blood flow were enhanced 
with \( \text{L-arginine} \) therapy. Interestingly, one of these studies (45) 
documented that administration of \( \text{L-arginine} \) augmented the 
forearm blood flow response to acetylcholine in individuals 
with impaired endothelium-dependent vasodilatation, but not in 
healthy subjects. This finding is consistent with our results from 
the FMD study, which suggests that the effect of \( \text{L-arginine} \) sup-
plementation was influenced by the baseline endothelial profile.

The mechanism whereby \( \text{L-arginine} \) may improve endothelial 
function, especially when baseline FMD is low, remains un-
certain. Low FMD may result from decreased NO synthesis and

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>( g/d )</th>
<th>Frequency</th>
<th>Study duration</th>
<th>Participants</th>
<th>No. of subjects</th>
<th>Age (^1)</th>
<th>Study design</th>
<th>Baseline FMD (^2)</th>
<th>FMD (^2)</th>
<th>Side effects</th>
</tr>
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<tbody>
<tr>
<td>Clarkson et al (35)</td>
<td>1995</td>
<td>21</td>
<td>3</td>
<td>28</td>
<td>Hypercholesterolemic subjects</td>
<td>27</td>
<td>29 ± 5</td>
<td>R, PC, DB, CO</td>
<td>2.2 ± 1.4</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Adams et al (36)</td>
<td>1995</td>
<td>21</td>
<td>3</td>
<td>3</td>
<td>Healthy young men</td>
<td>12</td>
<td>31 ± 1</td>
<td>R, PC, DB, CO</td>
<td>6.5 ± 0.7</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Adams et al (11)</td>
<td>1997</td>
<td>21</td>
<td>3</td>
<td>3</td>
<td>CAD</td>
<td>10</td>
<td>40 ± 2</td>
<td>R, PC, DB, CO</td>
<td>1.8 ± 0.7</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Hambrecht et al (12)</td>
<td>2000</td>
<td>8</td>
<td>3</td>
<td>28</td>
<td>CHF</td>
<td>20</td>
<td>&lt;70</td>
<td>R, PC</td>
<td>6.5 ± 1.0</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Blum et al (13)</td>
<td>2000</td>
<td>9</td>
<td>3</td>
<td>30</td>
<td>Stable CAD</td>
<td>30</td>
<td>67 ± 8</td>
<td>R, PC, DB, CO</td>
<td>11.9 ± 6.3</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Maxwell et al (14)</td>
<td>2002</td>
<td>6</td>
<td>2</td>
<td>14</td>
<td>Stable CAD</td>
<td>36</td>
<td>65.9 ± 10</td>
<td>R, PC, DB, CO</td>
<td>5.5 ± 4.5</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Bennett-Richards et al (15)</td>
<td>2002</td>
<td>2.5–5/m²</td>
<td>3</td>
<td>28</td>
<td>Children with CRF</td>
<td>21</td>
<td>12 ± 3</td>
<td>R, PC, DB, CO</td>
<td>7.9 ± 2.3</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Bode-Böger et al (16)</td>
<td>2003</td>
<td>16</td>
<td>2</td>
<td>14</td>
<td>Healthy individuals</td>
<td>12</td>
<td>73.8 ± 2.7</td>
<td>R, PC, DB, CO</td>
<td>2.7 ± 1.2</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Abdelhamed et al (37)</td>
<td>2003</td>
<td>3.3</td>
<td>2</td>
<td>14</td>
<td>Hypercholesterolemic patients</td>
<td>47</td>
<td>53.5 ± 13.5</td>
<td>R, PC, DB</td>
<td>5.5 ± 3.3</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Yin et al (17)</td>
<td>2005</td>
<td>15</td>
<td>3</td>
<td>28</td>
<td>Stable CAD</td>
<td>31</td>
<td>58 ± 7</td>
<td>R, PC, open label, CO</td>
<td>4.2 ± 0.5</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Böger et al, GI-1 (38)(^6)</td>
<td>2007</td>
<td>3</td>
<td>2</td>
<td>21</td>
<td>Clinically asymptomatic elderly subjects</td>
<td>28</td>
<td>54.5 ± 7.9</td>
<td>R, PC, SB, CO</td>
<td>9.5 ± 3.2</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Böger et al, GI-2 (38)(^6)</td>
<td>2007</td>
<td>3</td>
<td>2</td>
<td>21</td>
<td>Clinically asymptomatic elderly subjects</td>
<td>28</td>
<td>59.6 ± 4.2</td>
<td>R, PC, SB, CO</td>
<td>6.1 ± 0.9</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Siasos et al (18)</td>
<td>2007</td>
<td>6</td>
<td>NA</td>
<td>3</td>
<td>Healthy individuals who smoked</td>
<td>10</td>
<td>24.3 ± 7.3</td>
<td>R, PC, DB, CO</td>
<td>3.1 ± 0.7</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Wilson et al (19)</td>
<td>2008</td>
<td>3</td>
<td>3</td>
<td>180</td>
<td>Peripheral arterial disease</td>
<td>96</td>
<td>73 ± 9</td>
<td>R, PC, DB</td>
<td>3.2 ± 0.8</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(^1\) NA, not available; CAD, coronary artery disease; CHF, chronic heart failure; CRF, chronic renal failure; R, randomized; C, controlled; PC, placebo controlled; DB, double blind; SB, single blind; CO, crossover; FMD, flow-mediated dilation.

\(^2\) All values are means ± SDs.

\(^3\) Study of group with high asymmetric dimethylarginine.

\(^4\) Study of group with low asymmetric dimethylarginine.

\(^5\) Study of group with high asymmetric dimethylarginine.

\(^6\) Study of group with low asymmetric dimethylarginine.
is therefore reversible with L-arginine supplementation, whereas subjects with a high FMD may already have sufficient NO activity, which explains the failure of L-arginine supplementation to increase FMD in such subjects (36). Furthermore, L-arginine may have additional favorable effects on endothelial function that are not mediated by an increase in the concentration of substrate. Studies have shown that oral L-arginine can enhance insulin and prolactin secretion (47–49), influence the immune system, and inhibit LDL oxidation (17, 50). It is uncertain whether any or all of these changes might contribute to the modulation of an individual’s response to oral L-arginine therapy.

The present study had several potential limitations. First, only 3 studies were combined for analysis in the high FMD group, which meant that the result of this group was less conclusive than that of the first group. Thus, before we ignore a beneficial effect of L-arginine supplementation on endothelial function in subjects with a high FMD, additional studies in healthy persons with normal vascular function are needed. Second, although a positive trend was documented, it was statistically nonsignificant when 4% and 6% were used as cutoffs for subgroup analysis. The lack of statistical significance might be due to the small number of studies in the low and high FMD strata. Therefore, we must be careful with the interpretation of the subgroup analysis until the rationale of the cutoffs is confirmed by additional large-scale, high-quality trials.

Third, because significant heterogeneity was observed in the treatment effects of all studies and in the subgroup with low FMD at baseline, both visual examination and statistical analysis of funnel plots did not provide sufficient evidence against a publication bias. However, a fail-safe N test was also performed to evaluate publication bias, which suggested that it would take 2427 (for all studies) or 2383 (for the subgroup with low FMD at baseline) unpublished null result studies to bring the combined P value to a nonsignificant level. The existence of that many

![FIGURE 2. Random-effects meta-analysis of weighted mean differences (95% CI) in flow-mediated dilation (FMD) with L-arginine treatment compared with placebo. Sizes of data markers indicate the weight of each study in the analysis. The subgroups were differentiated by FMD at baseline (<7% and ≥7%). Böger GI-1: study in subjects with high asymmetric dimethylarginine; Böger GI-2: study in subjects with low asymmetric dimethylarginine.](image)

**TABLE 2** Characteristics of subgroups according to baseline flow-mediated dilation (FMD)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Year</th>
<th>N</th>
<th>L-arginine Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>Weighted mean difference (random) (95% CI)</th>
<th>Weight (%)</th>
<th>Weighted mean difference (Random) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline FMD% &lt;7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarkson (35)</td>
<td>1995</td>
<td>27</td>
<td>5.60(3.00)</td>
<td>27</td>
<td>2.30(2.40)</td>
<td>7.7</td>
<td>3.30(1.85 to 4.75)</td>
</tr>
<tr>
<td>Adams (36)</td>
<td>1995</td>
<td>12</td>
<td>6.10(0.70)</td>
<td>12</td>
<td>6.50(0.70)</td>
<td>8.2</td>
<td>-0.40(0.96 to 0.16)</td>
</tr>
<tr>
<td>Adams (11)</td>
<td>1997</td>
<td>10</td>
<td>4.70(1.10)</td>
<td>10</td>
<td>1.80(0.70)</td>
<td>8.1</td>
<td>2.90(2.09 to 3.71)</td>
</tr>
<tr>
<td>Hambrecht (12)</td>
<td>2000</td>
<td>10</td>
<td>11.30(1.20)</td>
<td>8</td>
<td>6.50(1.00)</td>
<td>8.0</td>
<td>4.80(3.78 to 5.82)</td>
</tr>
<tr>
<td>Maxwell (14)</td>
<td>2002</td>
<td>36</td>
<td>8.00(4.50)</td>
<td>36</td>
<td>5.50(4.50)</td>
<td>7.1</td>
<td>2.50(3.33 to 4.67)</td>
</tr>
<tr>
<td>Bođer (16)</td>
<td>2003</td>
<td>12</td>
<td>5.73(1.19)</td>
<td>12</td>
<td>2.65(1.15)</td>
<td>8.0</td>
<td>3.08(2.14 to 4.02)</td>
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<td>Abdelhamid (37)</td>
<td>2003</td>
<td>20</td>
<td>3.90(0.70)</td>
<td>21</td>
<td>4.61(0.68)</td>
<td>8.2</td>
<td>-0.71(-1.13 to -0.29)</td>
</tr>
<tr>
<td>Yin (17)</td>
<td>2005</td>
<td>31</td>
<td>9.06(0.69)</td>
<td>31</td>
<td>4.19(0.46)</td>
<td>8.3</td>
<td>4.87(4.58 to 5.16)</td>
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<td>Böger GI-1 (38)</td>
<td>2007</td>
<td>15</td>
<td>9.89(1.51)</td>
<td>15</td>
<td>6.08(0.94)</td>
<td>8.1</td>
<td>3.81(2.91 to 4.71)</td>
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<td>Säss (18)</td>
<td>2008</td>
<td>10</td>
<td>4.70(0.70)</td>
<td>10</td>
<td>3.10(0.70)</td>
<td>8.2</td>
<td>1.60(0.99 to 2.21)</td>
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<td><strong>Subtotal (95% CI)</strong></td>
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<td></td>
<td></td>
<td>182</td>
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<td><strong>Baseline FMD% ≥7</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Blum (13)</td>
<td>2000</td>
<td>30</td>
<td>11.40(7.90)</td>
<td>30</td>
<td>11.90(6.30)</td>
<td>5.6</td>
<td>-0.50(-4.12 to 3.12)</td>
</tr>
<tr>
<td>Bennett-Richards (15)</td>
<td>2002</td>
<td>21</td>
<td>7.80(2.95)</td>
<td>21</td>
<td>7.94(2.26)</td>
<td>7.6</td>
<td>-0.14(-1.73 to 1.45)</td>
</tr>
<tr>
<td>Böger GI-2 (38)</td>
<td>2007</td>
<td>13</td>
<td>8.99(3.06)</td>
<td>13</td>
<td>9.47(3.16)</td>
<td>6.9</td>
<td>-0.48(-2.87 to 1.91)</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>64</td>
<td></td>
<td></td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>247</td>
<td></td>
<td></td>
<td>246</td>
<td></td>
<td>100</td>
<td>1.98(0.47 to 3.48)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intervention groups (n)</th>
<th>Low FMD (&lt;7%)1</th>
<th>High FMD (≥7%)2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>365</td>
<td>128</td>
</tr>
<tr>
<td>Age (y)</td>
<td>43.7 ± 6.13</td>
<td>62.5 ± 4.04</td>
</tr>
<tr>
<td>Baseline FMD (%)</td>
<td>4.3 ± 0.6</td>
<td>9.8 ± 1.2</td>
</tr>
<tr>
<td>L-Arginine dose (g/d)</td>
<td>12.0 ± 2.4</td>
<td>6.0 ± 1.7</td>
</tr>
<tr>
<td>Study duration (d)</td>
<td>17 ± 3.5</td>
<td>21.7 ± 4.6</td>
</tr>
</tbody>
</table>

1 First, second, and third quartiles for all trials.
2 Fourth quartile for all trials.
3 Mean ± SEM (all such values).
4 Significantly different from low-FMD group, P < 0.05 (Student’s t test).
unpublished studies is improbable; hence, this fail-safe N added greatly to the confidence of the results of our meta-analysis.

Fourth, only one study evaluated the association between long-term L-arginine treatment (>6 mo) and FMD levels. Therefore, the effectiveness and safety of long-term supplementation with L-arginine on endothelial function could not be quantitatively evaluated in our systematic review. When Wilson et al.’s (19) data were pooled with the short-term studies, a negative overall effect and a large degree of heterogeneity were found. Potential mechanisms of the unexpected findings in this trial should be examined carefully, and the long-term effects of L-arginine supplementation should be sought by more high-quality, double-blind, randomized clinical trials.

Fifth, the dose of L-arginine and mean age were statistically different between groups with high and low baseline FMD levels (Table 2) and varied largely in all studies. The potential influence of these factors was estimated by the sensitivity analysis and the meta-regression analysis, which indicated that the dose of L-arginine (range: 3–24 g/d) and the average age of the participants were not effect modifiers. It is an interesting point that low-dose L-arginine has been as effective as large doses, which suggests that daily nutritional doses may be enough to induce a favorable effect. Other explanations may be that either the dose range or the sample size was not large enough, which resulted in a relative lack of power to detect the effect. This phenomenon needs to be investigated in future studies.

Sixth, a major limitation of the existing evidence was that none of these trials showed an association between baseline L-arginine supplementation and endothelial function in unstable CAD patients. Furthermore, soft endpoints (net change of FMD) were used in these studies, whereas the effects of treatment on clinical outcomes were not examined.

Implications for clinical practice

Our meta-analysis suggests that individuals with apparently impaired endothelial function (low baseline FMD) are likely to benefit from short-term oral L-arginine intake. This beneficial effect seems not to be dose-dependent when the dose of L-arginine ranges from 3 to 24 g/d. However, its long-term effectiveness and safety are not clear. Future research efforts should concentrate on higher-quality and more rigorous randomized trials with longer follow-ups to resolve the uncertainty regarding the clinical effectiveness and safety of this type of intervention.

We thank Junhao Liu, J Isenburg, and BioMed Proofreading for their text editing.

The authors’ responsibilities were as follows—YB, LS, and RHui: conceived the idea for the study and developed the search strategy; and YB

### TABLE 3
Effect of L-arginine supplementation on flow-mediated dilation (FMD) stratified by previously defined study characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Intervention group</th>
<th>Effect (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline FMD&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7%</td>
<td>10</td>
<td>2.56 (0.87, 4.25)</td>
<td>0.01</td>
</tr>
<tr>
<td>≥7%, quartile 4</td>
<td>3</td>
<td>−0.27 (−1.52, 0.97)</td>
<td></td>
</tr>
<tr>
<td>Baseline FMD&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4%, quartile 1</td>
<td>4</td>
<td>2.21 (1.26, 3.15)</td>
<td>0.4</td>
</tr>
<tr>
<td>≥4%</td>
<td>9</td>
<td>1.39 (0.26, 2.53)</td>
<td></td>
</tr>
<tr>
<td>Baseline FMD&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6%, low median</td>
<td>7</td>
<td>2.33 (0.81, 3.85)</td>
<td>0.17</td>
</tr>
<tr>
<td>≥6%, high median</td>
<td>6</td>
<td>0.89 (−0.18, 1.94)</td>
<td></td>
</tr>
<tr>
<td>Mean age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55, low median</td>
<td>7</td>
<td>1.13 (−0.06, 2.50)</td>
<td>0.15</td>
</tr>
<tr>
<td>≥55, high median</td>
<td>6</td>
<td>2.65 (1.31, 3.99)</td>
<td></td>
</tr>
<tr>
<td>L-Arginine dose (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7, low median</td>
<td>7</td>
<td>1.10 (0.06, 2.15)</td>
<td>0.36</td>
</tr>
<tr>
<td>≥7, high median</td>
<td>6</td>
<td>1.95 (0.52, 3.38)</td>
<td></td>
</tr>
<tr>
<td>Study duration (wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4, low median</td>
<td>8</td>
<td>1.19 (0.08, 2.31)</td>
<td>0.17</td>
</tr>
<tr>
<td>≥4, high median</td>
<td>5</td>
<td>2.39 (1.06, 4.13)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> P for difference between strata (Student’s t test).<sup>2</sup> Different cutoffs of baseline FMD for the subgroup analysis were set based on the quartiles and medians for all trials.

FIGURE 3. Begg’s funnel plot (with pseudo 95% CIs) of all individual studies in the meta-analysis. Studies that evaluated the effect of L-arginine supplementation on flow-mediated dilation (FMD) were plotted with weighted mean differences (WMDs) on the vertical axis and the SEs of the WMDs along the horizontal axis. Graph symbols were sized by weights.

FIGURE 4. Begg’s funnel plot (with pseudo 95% CIs) of studies in the meta-analysis with low baseline flow-mediated dilation (FMD) levels (<7%). Studies that evaluated the effect of L-arginine supplementation on FMD were plotted with weighted mean differences (WMDs) along the vertical axis and the SEs of the WMDs along the horizontal axis. Graph symbols were sized by weights.
and LS; summarized the data and conducted the data analyses. All authors contributed to the data analysis, verification, and writing and revision of the manuscript. The authors had no conflict of interests to declare.

REFERENCES


