SYSTEMATIC REVIEW

REVISED Effectiveness of acute L-arginine supplementation on physical performance in strength training: a systematic review and meta-analysis

[version 2; peer review: 1 approved, 1 not approved]

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Abstract

Background: The oral administration of L-arginine has been related to improved physical performance due to a likely reduction in muscle fatigue, resulting from the vasodilator effect of nitric oxide on skeletal muscle. However, there is no precise and quantitative analysis of the information in the literature. The main objective of this study was to assess the effectiveness of L-arginine supplementation on physical performance in strength training with a systematic review and meta-analysis. We hypothesized that L-arginine supplementation would improve performance capacity and the effects involved in strength training.

Methods: The study period was from 2010 to 2020. The inclusion process established articles with well-designed human experiments that included only L-arginine supplementation (without any additional compounds) testing the effects of L-arginine supplementation on sports performance related to strength training; identical experimental conditions in placebo or control group; and publications in the last ten years (until December 31, 2020). Three studies were included that compared L-arginine supplementation with placebo in anaerobic performance tests. Test analysis supplementation with other supplements was removed and there was no gender, age, and ethnicity level.

Results: There was no significant heterogeneity ($p>0.05$) in the analysis of the three selected articles and the effects of L-arginine supplementation in muscular endurance; performance had a mean of 0.26 (95% CI = -0.129; 0.649; $p = 0.190$), peak torque with a mean of -0.002 (95% CI = -0.531; 0.527; $p = 0.99$) of the third series of exercises and, furthermore, when comparing the integrated effect (resistance

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rate with the peak torque) there was no difference with a mean of 0.168 (95% CI = -0.145; 0.481; p = 0.292).
Conclusions: Acute L-arginine supplementation provides no ergogenic effect on strength training performance.

Keywords
Aminoacid; Ergogenic; Resistance Exercises; Nitric Oxide; Anaerobic

This article is included in the Agriculture, Food and Nutrition gateway.

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REVISED **Amendments from Version 1**

Some additions were made to the writing to better elucidate the text. The contextualization of this work is based exclusively on the use of L-arginine and the use of other substances may mask the true effect, therefore, we cannot modify this context so that the N becomes greater and is based on research found in the literature. Given this, we recommend further studies so that we can understand the true effect of this substance.

Any further responses from the reviewers can be found at the end of the article

Introduction

Athletes and recreational resistance exercisers have commonly associated training with nutritional supplementation to improve sports performance, maximize muscle mass and strength gains.[1,2] Proteins and amino acids represent 35 to 40% of the ergogenic resources used by this population.[3] In this context, supplementation with L-arginine, classified as a non-essential amino acid, has been advocated for resistance training practitioners for different reasons.[4] First, for its potential to promote vasodilation and supply energy to active muscles, given its participation in the synthesis and availability of nitric oxide (NO).[5] Second, increasing the creatinine concentration in the skeletal striated muscle promotes improved physical performance and decreased muscle fatigue.[6] Third, by promoting an increase in growth hormone production, known as GH (growth hormone), that stimulates protein synthesis and, consequently, muscle mass.[4]

Despite being highly popular among exercise practitioners, few studies have examined the effect of oral administration of L-arginine on the ability to improve physical performance during resistance training, nor its role in increasing lean mass and maximizing gains force.[5] In addition, studies on the subjects used different protocols, dosages, populations, and associations with other compounds, presenting contradictory outcomes, making it challenging to make decisions about the use of L-arginine and drawing conclusions about the efficacy and safety of L-arginine supplementation.

Recently, Ojeda *et al.*[7] and Viribay *et al.*[8] performed studies of a systematic review with and without meta-analysis, respectively, on the effects of L-arginine on the physical performance of strength training practitioners. For the outcomes observed in the studies by Ojeda *et al.*[7] in respect to short-term supplementation and doses ranging from 0.075 to 8.0 $g \cdot d^{-1}$, it seemed to not influence variables such as nitrogen oxide (NOx), muscle oxygenation, oxygen consumption, and growth hormone secretion. The long-term outcomes, tested from three days to six weeks with maximum doses of 12 $g \cdot d^{-1}$, showed no effect and NO production during intermittent anaerobic exercise, nor in hemodynamic and vascular responses, maximal oxygen uptake (VO_2max), insulin, cortisol, growth hormone or insulin type 1 growth factor (IGF-1) in resistance exercise or strength. However, at the end of the research, there was an investigation that reported a significant effect of prolonged L-arg supplementation on exercise performance. Evidencing a faster recovery from muscle injuries caused by decreased levels of the enzyme lactate dehydrogenase (LDH) after training, and a positive impact on anaerobic performance, mainly due to the acceleration of muscle recovery processes. Both outcomes were performed with men and women in anaerobic and aerobic activities. For Viribay *et al.*[8] the effective dose of L-arginine supplementation in acute protocols should be adjusted to 0.15 g/kg (10–11 g) ingested 60–90 min before exercise to improve aerobic and anaerobic activities performed by men and women. Based on these findings, the authors indicated that most studies with L-arginine are not favorable to its use as a nutritional ergogenic resource and point to the need for further investigation on the subject. The recent recommendation of the International Society for Sports Nutrition draws attention to this[5] by indicating that L-arginine supplementation has a little scientific basis to ensure its safety and nutritional efficacy.

There is no clear and quantitative information analysis of the different effects involved in the anaerobic performance process, nor studies that use only the substance L-arginine, without adding any other compound, and this may be necessary to better understand the efficacy, dosage, and time of ingestion. Therefore, we cannot assume that some positive performance results are due only to L-arginine, as the association with other compounds simultaneously can mask the true and real effect since none of these protocols investigated the underlying mechanisms and/or not use of only the compost L-arginine[6,9,10] and that the samples are expanded when there is an association of the supplement with other compounds. This has a confounding factor in the efficacy of the substance L-arginine. We hypothesized that L-arginine supplementation would improve performance capacity and the effects involved in anaerobic activities.

Given the above, this study intends to advance with current knowledge by evaluating the effectiveness of L-arginine in the physical performance of resistance training practitioners from a systematic review and meta-analysis. Conducting a systematic review and meta-analysis on the effects of L-arginine supplementation in resistance exercise practitioners will contribute to current knowledge by i) analyzing the effects of supplementation on physical performance; ii) evaluate the

effectiveness of exclusive L-arginine supplementation in healthy and physically active populations; iii) investigate the dose-response relationship of oral L-arginine consumption.

Methods

This systematic review and meta-analysis were performed according to PRISMA® (*Preferred Reporting Items for Systematic Reviews and Meta-Analysis*) PICOS declaration guidelines and methodology, which defines the "Population," "Intervention," "Comparisons," "Results," and "Study Design." The review research was carried out considering the following phases:

I. Population: Adults who practice physical activity (male and female);

II. Intervention: Impact of L-arginine supplementation by strength training practitioners;

III. Comparisons: Changes or alterations in the results in the same sample of participants, when there is a comparison of variables related to the use of L-arginine compared to placebo in strength training;

IV. Outcomes: Any measures of physical performance and variables that relate to improvement in strength training (ex, peak strength, fatigue, number of repetitions, muscle performance);

V. Study design: Clinical trial.

The identification of the article selection process in the first phase was carried out in January 2021 in the Cochrane, PubMed, MEDLINE, LILACS, and SciELO databases, being carried out by the Mendeley software. For the selection of keywords, the descriptors in Health Sciences-*DeCS* were used, obtained through advanced searches in the union of search terms, including the "Mesh." The descriptors used were: "L-arginine"; "resistance exercises" and "strength training," a cognitive logical operator "AND," and "NOT," were used for the effective tracking of publications.

The inclusion process established that articles with the following characteristics would be used: (1) well-planned experiments that included L-arginine supplementation only (without any additional compounds); (2) studies with English and Spanish languages; (3) identical experimental conditions in the placebo or control group; (4) test the effects of L-arginine supplementation on sports performance related to strength training; (5) clinical trial; (6) publications in the last ten years (until December 31, 2020); (7) investigations carried out on humans; (8) results related to the use of L-arginine; (9) clear information about funding sources; and (10) absence of conflict of interest.

Texts with the following characteristics were excluded: (1) L-arginine supplementation with some other compound; (2) investigations in which the topic was not essentially the use of L-arginine; (3) different samples of the effects of using the L-arginine not mentioning its effects (articles that do not show L-arginine results, in particular); (4) data from studies without *Qualis* status and surveys that did not show results; (5) other types of outcomes exclusively from reviews, pneumatic, contagious, cancerous and transmissible diseases.

Two authors independently screened and agreed with the selected studies for eligibility (CP, ROG, and TRL). Likewise, after the inclusion/exclusion criteria are applied to each study, the source study data (including authors and year of publication), sample size, participant characteristics (level, race, and gender), supplement administration (dose and time), and final results of interventions were extracted independently (CP and ROG) using an Excel spreadsheet (Microsoft Inc, Seattle). Then, possible disagreements were resolved through discussion until a consensus was reached, or by third-party judgment (MJK). In this sense, the Kappa coefficient, which indicates the reliability between raters, among the authors was above 90 with a level of agreement "almost perfect".[11]

The Assessment of Multiple Systematic Reviews (AMSTAR2) tool was applied in the systematic review to assess the methodological quality of the studies.[12] The reviewers searched the studies for data that answered each question, and each reviewer's final ratings were placed on a spreadsheet to discuss agreements and disagreements, with a score assigned to each assessed study. To assess the quality of the evidence and the strength of the recommendations, the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system was used,[13] in which the research question was structured considering the domains of the acronym PICOS.

According to the Cochrane Collaboration Guidelines, to assess the risk of bias of randomized clinical trials, the RoB (Risk of Bias - Cochrane risk of bias tool revised for randomized trials) was used and evaluated by two authors independently, and disagreements were resolved by discussion and/or a third author. The list was separated into six

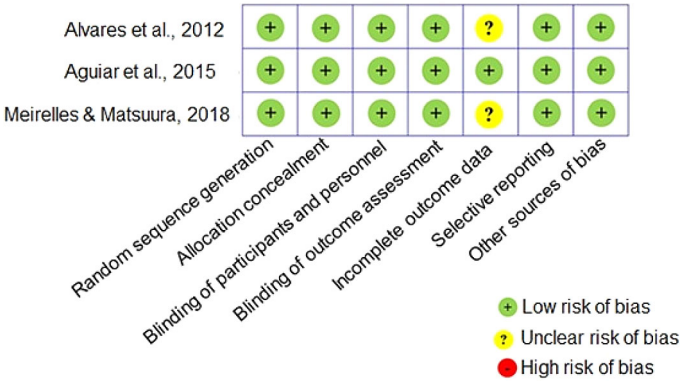

Figure 1. Risk of bias summary: Authors' judgments on each risk of bias item for all included studies.

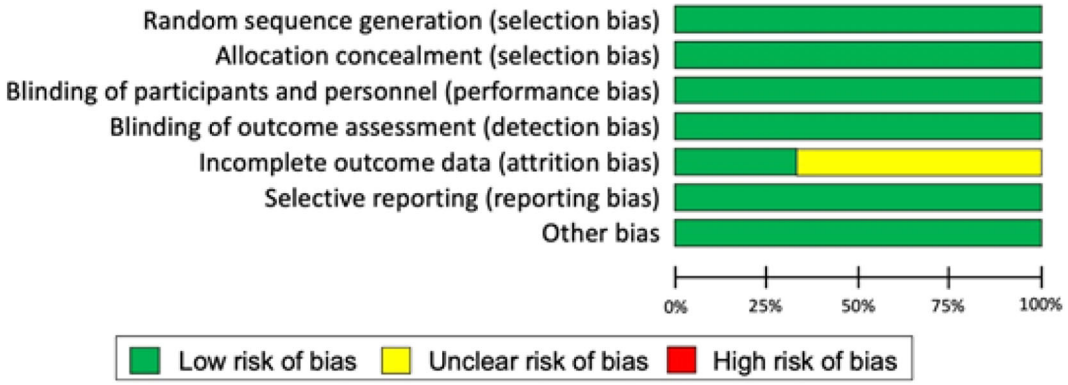

Figure 2. Risk of bias graph: authors' judgment on each risk of bias item presented as percentages across studies.

different domains: selection bias (random sequence generation, allocation concealment); performance bias (blinding of participants and researchers); detection bias (masking the outcome assessment); friction bias (incomplete result data); reporting bias (selective reporting); and other sources of bias.[14]

Domains were considered as 'low' if the criteria met a low risk of bias (probable bias that would not seriously alter the results) or 'high' if the criteria presented a high risk of bias (probable bias that seriously weakens confidence in the results), or it was considered "not clear" (a plausible bias that raises some doubts about the results), whether the risk of bias was unknown. Full details of each article and domain (Figures 1 and 2).

The Clinical Trials database was queried using the terms "descriptor" and "descriptors" to locate potential studies with L-arginine. Phase 2, 3, and 4 clinical studies enrolled in the aforementioned database were considered. For this review, approval by the ethics committee was not required due to the use of secondary data.

Statistical analyses

The meta-analysis was performed using the Comprehensive Meta-Analysis (CMA) software, version 3.3.070, including the three selected articles, one for each exercise series (first, second and third). The effect size was calculated based on the difference in performance between groups L-arginine and control group. We calculated the effect standardized by the standard deviation (SMD), since the variables were not presented in the same unit in all studies, for example, total work of the series and total work presented in Joule, peak torque in Nanometer, number of maximum repetitions and rate of resistance).

Some studies presented performance measures for more than one muscle group, each subgroup of these studies was included in the analysis as if it were a different study. To test the effect of L-arginine with no sample overlap, combined analyzes with all subgroups from each study were also presented and included in the analysis as a single effect per study.

Sensitivity analyzes were also performed for strength endurance measurements (total set work and total work, peak torque, maximum repetition number, and endurance rate) and peak torque (Nanometer). In the case of peak torque, as all studies presented data in the same measurement unit (Nanometer), we present the data in gross mean difference.

The inconsistency statistic (I^2) was calculated as an indicator of the percentage of the total variation observed within the studies due to real heterogeneity rather than chance, to avoid errors when using the Q statistic in the evaluation of heterogeneity.[14] I^2 values were included from 0 to 100%, representing a small amount of inconsistency between 25% and 50%, the average amount of heterogeneity between 50% and 75%, and a large amount of heterogeneity when the I^2 value was greater than 75%.[15] In this sense, low, moderate and high adjectives would be accepted referring to I^2 values of 25%, 50%, and 75%, respectively, although a restrictive categorization was not appropriate in all circumstances.[15,16]

Publication bias was analyzed using the Egger test and a p-value ≤ 0.05 was considered significant. Data were presented as standardized mean difference and 95% confidence interval, and we considered significant $p \leq 0.05$.

Results

Study identification

The studies that met the criteria to be included in this review are shown in the flowchart (Figure 3). The bibliographic search through electronic search identified a total of 1075 records according to the selected research equation, with

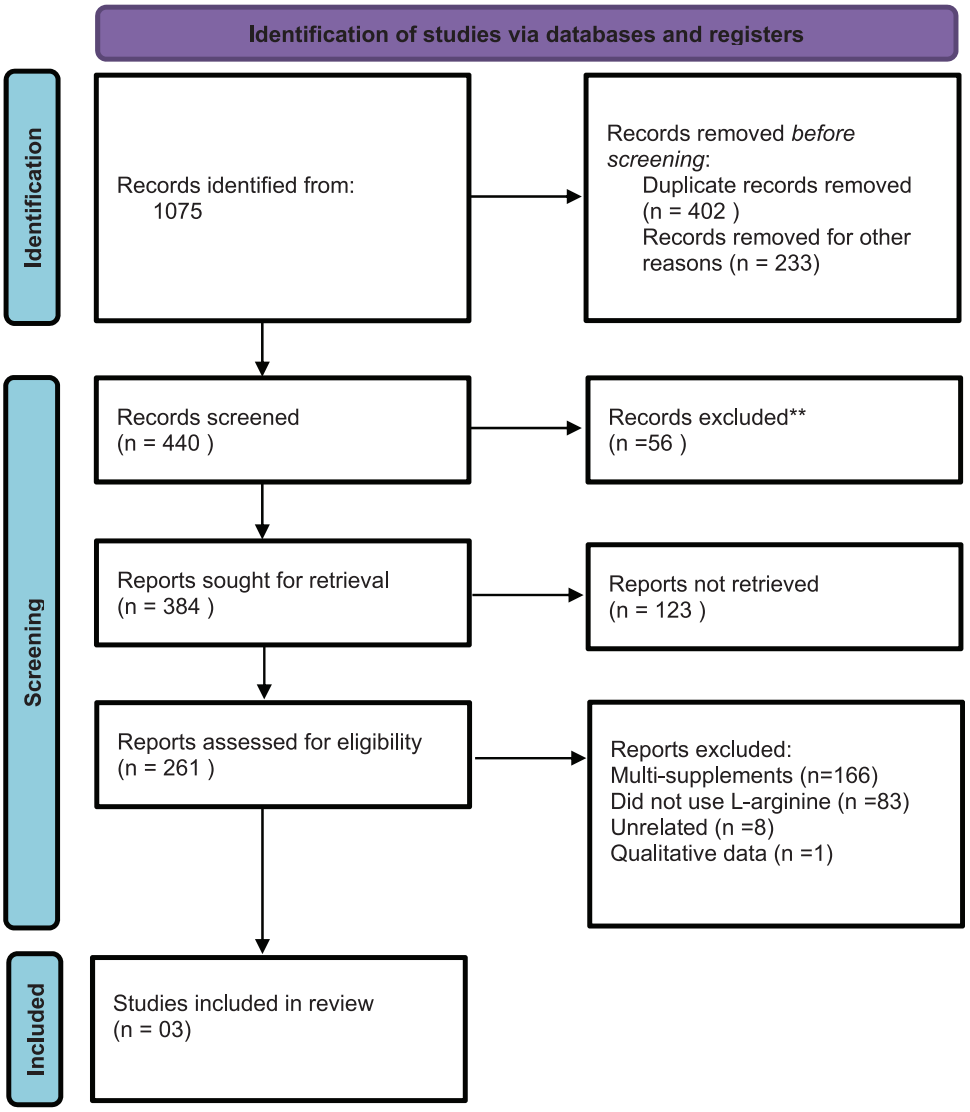

Figure 3. Flowchart of article selection for systematic review and meta-analysis (PRISMA, 2020).

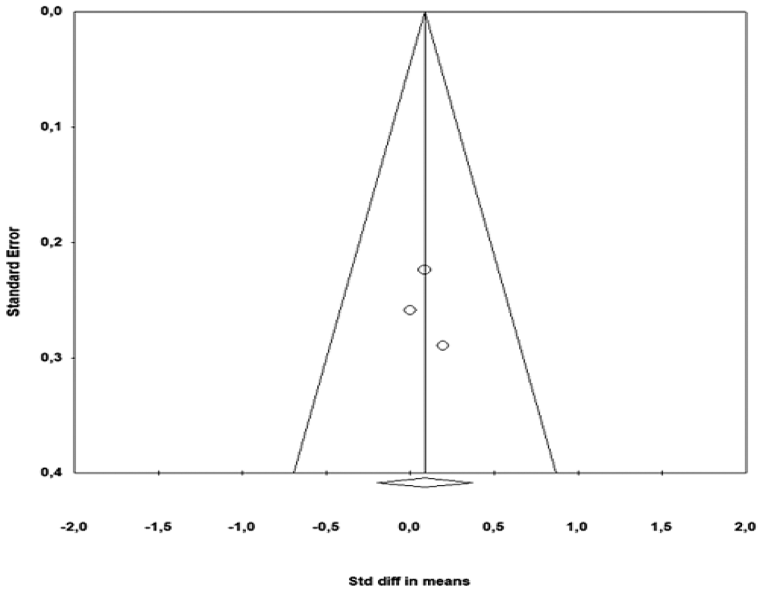

Figure 4. Funnel plot of publication bias of selected studies.

402 duplicate records being removed and 233 records removed for presenting animal tests, resulting in 440 records. Thus, after screening a total of 437 records were excluded from which: 52 were review studies, four inadequate results (use of the supplement without performing the physical activity), 123 performed on animals, 166 included more than one supplement, 83 did not use the substance L-arginine from the selected studies was removed from the quantitative synthesis and thus a total of three studies (n = 3) were included in the analysis.[17–19] In addition, the test of Egger ($p > 0.3$) and it was found that there was no significant effect for the risk of publication bias in the meta-analysis performed (Figure 4).

Characteristics of the studies

The publication period of the three studies ranged between 2012 and 2018 and the sample size ranged from 12 to 20 individuals. The average age ranged from 27 to 70 years, with two studies with a male population and one female, that is, in total the population represented was 57.44% male and 42.55% female. All studies were conducted in a randomized, double-blind, placebo-controlled, one-day (acute) period of intervention. The amount of L-arginine used was 6 g[17,19] and 8 g[18] per individual. Tests were performed on the upper limb,[17] bottom,[18] and upper and lower.[19] According to Table 1, the study by Meirelles and Matsuura[19] aimed to evaluate the acute effect of supplementation with L-arginine in the performance of strength in biceps flexion in three sets, evaluating the total work set, total, peak torque, and resistance rate. The study by Aguiar *et al.*[18] evaluated muscle performance in supplementation by measuring isokinetic strength data of the knee extensor and flexor muscles. And Alvares *et al.*,[17] analyzed the maximum number of repetitions of the lower and upper limbs in three sets.

Quality of evidence

The Quality of the evidence generated by the present study was assessed by the GRADE system, considering the assessment items suggested for clinical trials.[13] The risk of bias in the primary studies included was almost non-existent, as they used crossover designs, with a control group, double-blind, with secret and randomized allocations, with a one-week wash-out period.

There was no individual influence of any specific study on the summarized effects, with high homogeneity, low inconsistency (I^2 = 0.000%), in addition to the methodological similarity between the studies. The evidence was completely direct (well-defined cause and effect relationship) and no publication bias was observed, neither qualitatively through funnel graph analysis nor even in the hypothesis test (Egger). The only item that reduced the quality of evidence in this analysis was the low sample size (n = 30), however, there was still good precision, therefore, the level of evidence was identified as moderate (GRADE score = 3) for the effect gives L-arginine in strength performance.

Effect of L-arginine on performance: a meta-analysis

Figure 5 shows that L-arginine had no significant effect on physical performance. There was no significant heterogeneity ($p > 0.05$) in any analysis of the three selected articles and, therefore, we used fixed effects in all analyses. The effects of

Table 1. Summary of studies included in the systematic review that investigated the effectiveness of L-arginine in strength training from 2010 to 2020.

Author/ Year	Population	Intervention/ Usage time	Test	goal	Main conclusion
Alvares *et al*., 2012	15 trained men (59 ± 3.9 years – mean ± SD)	Randomized, double-blind, placebo-control. L-arginine group 6 g and placebo group 6 g Acute (1 day)	Upper limb strength exercise (5 reps)	Evaluate strength performance, muscle blood volume, and muscle oxygenation.	Supplementation did not increase strength performance and did not stimulate an increase in muscle oxygenation despite an increase in blood volume.
Aguiar *et al*., 2015	20 trained women (age: l-arginine group 70.6 ± 2.2 years and placebo 72.5 ± 1.6 years – mean ± SD)	Randomized, double-blind, placebo-control. L-arginine 8 g and placebo 8 g group Acute (1 day)	Lower limb strength exercise (3 sets of 8 repetitions)	Evaluate peripheral vasodilation and performance in muscle training	Supplementation does not provide an ergogenic effect on muscle and there is no interference with muscle strength.
Meirelles and Matsuura, 2018	12 men with experience and resistance training (age 27 ± 3 years, mean ± SD)	Randomized, double-blind, placebo-control. L-arginine 6 g and placebo 6 g group. Acute (1 day)	Lower and upper limb strength exercise (1 RM)	Evaluate strength performance and nitric oxide levels	Plasma nitrate levels did not change significantly after L-arginine or placebo supplementation in strength performance and nitric oxide levels.

gr: grams; RM: maximum repetition; SD: standard deviation.

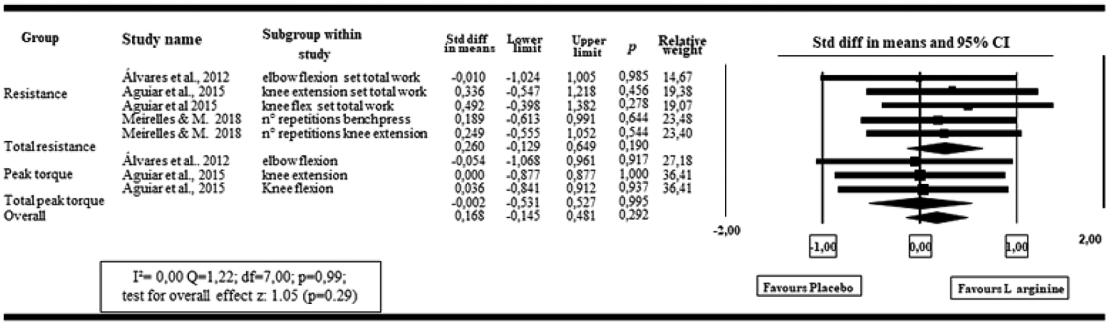

Figure 5. Effect of L-arginine comparing resistance and peak torque. Flex= flexion; ext = extension; N° = number; SD = standard difference; LL = lower limit; LS = upper limit; I^2 = I-squared; Q = Q-value; GL = degrees of freedom; Meirelles & M. 2018: Meirelles & Matsuura 2018.

L-arginine in the performance in muscular endurance it had an average of 0.26 (95% CI = −0.129; 0.649; $p = 0.190$), mean peak torque of −0.002 (95% CI = −0.531; 0.527; $p = 0.99$) of the third set of exercises and in addition, when comparing the integrated effect of resistance rate with peak torque, there was no significant difference with an average of 0.168 (95% CI = −0.145; 0.481; $p = 0.292$) (Figure 5).

The meta-analysis reported a small amount of inconsistency between the integrated effect studies reviewed ($I^2 = 0\%$; $p = 0.99$). The three analyzed studies and their subgroups had no positive effect on performance outcomes after L-arginine supplementation. In summary, there was no significant effect of L-arginine on performance under any condition tested.

Based on the analyses performed, it is inferred that L-arginine does not improve performance in any of the sets, in the measures of maximum strength or strength endurance, nor when the subgroups of the studies were combined in a single effect (integrated effect).

Discussion

This systematic review and meta-analysis aimed to assess the effectiveness of L-arginine on acute strength training performance. Our findings indicate that there was no significant effect of L-arginine on the production of maximum force and the endurance of force. Regarding dosage, differences of 6 and 8 g/day are observed, suggesting that both strategies may have similar physiological responses. Finally, L-arginine supplementation ingested 60–80 minutes before acute exercise (acute protocol) did not demonstrate performance improvement in strength training.

It is known that the L-arginine is related to NO production and increased blood flow, contributing to vasodilation and possibly an increase in performance in various activities.[20] However, of the three studies analyzed in our systematic review and meta-analysis, only the study by Álvares *et al*.,[17] observed increased blood flow with L-arginine supplementation. Furthermore, no study observed any difference in the concentrations of NO precursors with L-arginine supplementation. These findings justify why the three studies included in our review and meta-analysis did not demonstrate the ergogenic effects of L-arginine supplementation on strength training performance. As an example, Meirelles and Matsuura[21] investigated the effects of L-arginine supplementation on the number of maximum repetitions in the bench press (three sets of 70% of one-repetition maximum [1-RM]) and the leg extensor (three sets of 80% of 1-RM) in 12 physically active men. After the evaluations, the researchers observed no improvement in performance or changes in the concentrations of NO precursors with L-arginine supplementation.

The studies analyzed in this meta-analysis had no effect of supplementation of L-arginine on performance in strength training and was associated with lack of improvement in performance in the group L-arginine compared to the placebo group to the analyzed subgroups (extension exercises, flexion of lower and upper limbs together with the number of repetitions) of resistance rate and peak torque. Furthermore, not even the integrated effect was observed as a significant result when it contains an overlapping effect. However, our findings are not in line with other studies that observed increases in anaerobic performance[22,23] and aerobic[24] after supplementation with L-arginine; and in meta-analysis studies carried out by Viribay *et al*.,[8] published in 2020 and Ojeda *et al*.,[7] in the year 2019 on the effects of L-arginine supplementation in sports nutrition on physical performance have some contraversions in the results regarding the effectiveness of L-arginine.

As we can see, in the meta-analysis by Viribay *et al*.,[8] the study by Hurst *et al*.,[25] demonstrated that L-arginine supplementation (0.15 g/kg) increased time trial performance in a 16.1 km cycling event. This difference in results may be due to the characteristics of the analyzed task. The strength training protocols analyzed have a duration of ~2s (maximum strength assessment) to ~20s (example, 1 set of 10 repetitions maximum), and the cycling test analyzed by Hurst *et al*., had a duration of ~27 minutes. In long-term activities (i.e., > 1 min) increased blood flow and vasodilation is a very important factor in improving performance. In the study by Campbell *et al*.,[26] and Greer *et al*.,[27] L-arginine alpha-ketoglutarate were used in supplementation. Evidence indicates that L-arginine combined with alpha-ketoglutarate can improve performance in some activities, due to the increased production of adenosine triphosphate through the electron transport chain.[28] Specifically, alpha-ketoglutarate is a metabolite produced by the oxidative decarboxylation of isocitrate; a process that occurs in the Krebs cycle.[26,29] An exogenous supply of alpha-ketoglutarate through supplementation of L-arginine alpha-ketoglutarate (AAKG) can increase Krebs cycle flux, increasing the oxidation rate of acetyl-CoA.[28] In the study by Bailey *et al*.,[9] supplementation was carried out with L-arginine in association with maltodextrin (supplementation also used in the study by Liu *et al*.,[30] found in the meta-analysis by Ojeda *et al*.[7]). The possibility of reducing the rate of glycogen degradation or even increasing this content can help to reduce fatigue and thus support the capacity for exercise intensity without exhaustion since there is a link between the glycogen content muscle fiber and its ability to perform intense repeated contractions.[31,32] Muscles have complex carbohydrate stores made up of glucose molecules called glycogen, and the ingestion of maltodextrin, or any other carbohydrate, causes a decrease in the breakdown of liquid glycogen during long-term exercise.[33,34] This process maintains carbohydrate oxidation throughout the body and energy production, which can occur in both men and women.[35] The combination of this element together with proteins and/or amino acids can promote glycogen increase, recovery, and stimulate muscle protein synthesis after an intense exercise protocol.[28,31,36] As for strength exercises, for post-exercise muscle recovery of glycogen, carbohydrate combined with protein offer a greater contribution to sports performance, increasing the time for exhaustion, muscle strength, and muscle power.[37,38]

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together with proteins and/or amino acids can promote glycogen increase, recovery, and stimulate muscle protein synthesis after an intense exercise protocol.[28,31,36] As for strength exercises, for post-exercise muscle recovery of glycogen, carbohydrate combined with protein offer a greater contribution to sports performance, increasing the time for exhaustion, muscle strength, and muscle power.[37,38]

For this systematic review and meta-analysis, all included studies used only L-arginine without adding other supplements, which limits the possibility of obtaining synergistic or antagonistic effects in conjunction with other compounds. Studies confirm that L-arginine is related to NO synthesis and also its role as a cell-signaling molecule. However, due to the rapid oxidation by oxyhemoglobin to nitrate and nitrite, a fact that contributes to the half-life of the blood is greatly reduced and the quantification of NO production becomes more difficult.[39] According to current literature, there are some obstacles about protocols, doses, and supplementation time, which are controversial due to the results around L-arginine supplementation and its effects.[40]

This is the first meta-analysis investigating the acute effects of L-arginine supplementation on strength training performance. As for dosages and time of ingestion, due to the various current methods, it is suggested that more research should be carried out in the homogenization of L-arginine supplementation protocols regarding dosages and time of ingestion. In view of the few studies found that use only L-arginine, without any other substance that could mask the result, statistically, the number of studies (n = 03) analyzed in this systematic review and meta-analysis and the low sample number of each selected article related to L-arginine supplementation presupposes to represent a limitation with risk of bias, making them more sensitive to identify differences. However, for this review, the selection and analysis of studies, interpretation, and quantification of results represent strengths when evaluated by the AMSTAR guidelines, where GRADE scores and analysis were classified as "high quality"[12] and "moderate",[13] respectively.

In the same vein, this meta-analysis was carried out with homogeneous statistics, which constitutes an important point in favor. The choice to select studies that did not include systematic reviews implies raising evidence for further studies to be carried out in favor of the result, which aims to demonstrate the evident effect of positive performance with the use of L-arginine in physical tests related to muscle performance.

The dosage of L-arginine supplementation used in the studies may be a reason why changes were not observed in the concentrations of NO precursors and the performance improvement in strength training. The dosage of L-arginine used in the studies analyzed in our meta-analysis ranged between 6 and 8 g/day. However, Viribay *et al*. suggested L-arginine intake for 0.15 g/kg of body weight after performing a meta-analysis to assess the acute effects of L-arginine supplementation on the performance of aerobic and anaerobic predominance activities. . This dosage suggested by Viribay *et al*. agrees with the three studies analyzed in our meta-analysis, in which participants were supplemented with 6-8 g/day of L-arginine. Furthermore, the ergogenic effects with L-arginine supplementation depend on the time of ingestion/dosage and the onset of the analyzed activity, as blood levels of nitrate and nitrite increase 2.5–3 hours after supplement ingestion. rich in nitrate,[41] and the three studies analyzed in our meta-analysis ingested L-arg 60–80 minutes before starting the evaluations.

According to the results obtained, new dosages of L-arginine supplementation should be proposed, as well as studying the differences between elite and amateur athletes. Finally, a new research perspective should be considered studying the L-arginine absorption capacity involving mechanisms in physical activity practitioners, as this may represent a crucial understanding of the knowledge of supplementation.

Final considerations

Specific L-arginine supplementation does not provide an ergogenic effect on strength training performance. The effective dose of supplementation L-arginine, exercise protocols, and supplementation duration are not yet well understood and must be adjusted so that supposedly the anaerobic performance responses are positive. Because of this, some differences in supplementation and timing protocols were found that limited the drawing of robust conclusions, suggesting that published studies with meta-analysis lack credibility for this type of study. Thus, more clinical studies are needed to support systematic review and meta-analysis studies.

The publication bias obtained in studies of anaerobic performance means that practitioners should take these results with caution when applying the analyzed results to reality. Considering the importance of the theme and the sample size, the study has limitations due to the lack of bibliographic references expressed in the scientific world. Finally, we suggest further studies to compare the effects of exclusive L-arg supplementation without the association with other compounds on strength training performance, as the combination of compounds with L-arginine may mask the results of other studies analyzed.

However, this study should not be considered exhaustive and further research will be substantially important to strengthen future results. Such an effort is still needed and would pave the way for future studies with phytochemicals and pharmacology. For new scientific investments with this goal, we also recommend a more detailed investigation and one that fundamentally approaches the benefits of using L-arginine for human health. Finally, disseminating duly proven information about the use of L-arginine in practice will strengthen the values and richness of modern studies dealing with human health.

Registration and protocol
Review was not registered and protocol was not prepared.

Data availability
All data underlying the results are available as part of the article and no additional source data are required.

Reporting guidelines
Dryad: Effectiveness of acute L-arginine supplementation on physical performance in strength training: a systematic review and meta-analysis, https://doi.org/10.5061/dryad.2rbnzs7pd.

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Open Peer Review

Current Peer Review Status: ✗ ✓

Version 2

Reviewer Report 05 July 2022

https://doi.org/10.5256/f1000research.122261.r127157

✓ **Hector Rodriguez**
Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela

All of my suggestions have been fully addressed. For me it is okay for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pathophysiology of Chagas disease, cardiovascular pathology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 25 March 2022

https://doi.org/10.5256/f1000research.122261.r127156

✗ **Everson Nunes**
Protein Metabolism Laboratory, Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, ON, Canada

As I mentioned in my previous report, there are some methodological issues in this study. Also, the authors did not amend such issues in the updated version. Some examples:

- The meta-analysis model was not described in the methods.
- When meta-analyzing multiples outcomes per study a proper meta-analytic method should be applied (e.g., robust variance estimation or three-level model)[1].

- The choice of a fixed-effect model to conduct the analysis is wrong[1].
- There is no description of the method used to calculate SMD (i.e., Cohen's d or Hedges's g). This is important since the number of participants per study dictates the best method of choice[2].
- Figure 5 needs some work. The resolution of the figure I can access to review is very low, but it looks like commas have been used as decimal separators. It should be dots (ex: 0,001 should be 0.001).
- AMSTAR is still cited as a tool to access study quality. This is wrong as I mentioned before. AMSTAR is a tool to access the quality of systematic reviews[3].
- A real complete search strategy example was not included
- The discussion needs a lot of work to focus on the 3 studies returned from the systematic review.

Overall, the findings are still extremely underpowered. The systematic search returned 3 very heterogenic studies regarding the population age, sex, training status, and tests performed. Aside from that, the number of subjects in each study is small and when combined, the total number of subjects in the meta-analysis is too small to return a reliable effect size.

Based on that, we CANNOT conclude *"Specific L-arginine supplementation does not provide an ergogenic effect on strength training performance."* as stated by the authors. There is not enough data to conduct a proper meta-analysis using the selected inclusion and exclusion criteria.

Therefore, my professional opinion is that this paper lacks methodological and scientific quality and is not suitable for indexing.

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Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutritional supplements, sports nutrition, nutrition and resistance exercise, muscle physiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for

reasons outlined above.

Version 1

Reviewer Report 28 February 2022

https://doi.org/10.5256/f1000research.77594.r121618

Hector Rodriguez
Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela

In my opinion, It's too hard to get strong conclusions, with only three articles included. In fact, the same authors stated in the conclusion "***However, this study should not be considered exhaustive and further research will be substantially important to strengthen future results***". My suggestion is to consider a wide laps or more criteria to include more studies. For example, it would be possible to include the 166 records with L-arginine and another supplement.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Partly

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pathophysiology of Chagas disease, cardiovascular pathology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 07 December 2021

https://doi.org/10.5256/f1000research.77594.r100204

Everson Nunes
Protein Metabolism Laboratory, Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, ON, Canada

In the current paper, the authors proposed to assess the effectiveness of the acute ingestion of L-arginine on muscle strength and other related to muscle performance. I found some serious limitations in the current version of the paper.

Major comments:

How can we trust the conclusions of a meta-analysis based on 3 studies and 30 subjects? This should be clear in the discussion. I noticed that the authors included this as a limitation, but there is no discussion about how serious this limitation is.

Authors should be able to justify grouping distinct muscle groups (i.e., upper and lower body) and variables in only one measure to conduct this meta-analysis.

Please be aware that heterogeneity I2 statistics is based on Cochran's Q, which is hugely influenced by the number of studies. If the number of studies is too low (e.g. n=3), I2 will probably be low. Therefore, I am not sure if the result of this statistic can be trusted in the current paper.

Please provide a detailed search strategy for each database so readers can properly reproduce your search if needed.

Please describe the online tools or software used during the screening of the 1075 references.

Please include information about the model used for meta-analysis (e.g., fixed effect or random effects). In the methods, I found this only in the legend of figure 5. Also, I was not able to find information about what type of meta-analysis was conducted, is this a two-level or a three-level meta-analysis?

In the legend of figure 5, the authors stated that the fixed effect model was used. How can we use a fixed-effect model when study populations, doses, exercises, testing, and outcomes are not identical between studies? There are some relevant differences when comparing the 3 studies.

The authors stated using RoB2 tool to conduct RoB analysis (https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2?authuser=0). However, the domains and final reported RoB data do not match RoB2, but do match the original Cochrane RoB analysis (https://www.bmj.com/content/343/bmj.d5928)[1]. Please revise.

Please describe the software used to generate RoB figures.

It is not clear why the authors would use the "Assessment of Multiple Systematic Reviews (AMSTAR2)" in the current paper, since this is a tool to assess the quality of systematic reviews, not RCT (https://www.bmj.com/content/358/bmj.j4008)[2]. This tool is usually applied in umbrella reviews or reviews of systematic reviews. Please clarify why you are using this tool here. Also, there is no clear mention regarding the results of such analysis in the paper.

The authors state that "however, there was still good precision" in the quality of evidence analysis. What were the criteria for that? It is important to include in the methods what was considered a small, moderate, and big effect size or what would be considered as a relevant change.

A big part of the discussion does not fit the scope of the meta-analysis. The authors spent a lot of time citing and describing the use of L-arginine for other applications or other modalities of exercise.

General comments:

The version of figure 5 available to me does not look good for publication.

I found some minor punctuation and inconsistent use of "L-arg" in the text.

Overall, the authors should consider writing a paper including the systematic review and a narrative/scope review describing the 3 papers, but not a meta-analysis if we only have 3 studies on the topic.

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Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

Partly

Is the statistical analysis and its interpretation appropriate?

Partly

Are the conclusions drawn adequately supported by the results presented in the review?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutritional supplements, sports nutrition, nutrition and resistance exercise,

muscle physiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.