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The Effect of L-Citrulline Supplementation on Blood Pressure: An Updated Systematic Review and Meta-Analysis of Randomized Controlled Trials

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THE EFFECT OF L-CITRULLINE SUPPLEMENTATION ON BLOOD PRESSURE: AN UPDATED SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

by

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ABSTRACT

Cardiovascular disease remains one of the most prevalent diseases in the United States and has remained as the leading cause of death. Large Mendelian randomization studies have found significant correlations between high blood pressure and cardiovascular disease (CVD). In fact, high blood pressure is the single most important independent risk factor for CVD. The purpose of this study was to determine the effect of L-citrulline on blood pressure to determine whether it could be advised as an effective treatment for high blood pressure. L-citrulline is a naturally occurring amino acid that readily converts to L-arginine within the human body. L-arginine has shown promise in decreasing both systolic blood pressure (SBP) and diastolic blood pressure (DBP) significantly by potently increasing levels of nitric oxide (NO) in the body. L-arginine, however, displays poor oral bioavailability compared to L-citrulline. Thus, L-citrulline may be a more effective method in raising plasma arginine levels, increasing NO, and decreasing SBP and DBP. A thorough systematic review and meta-analysis was conducted to extrapolate this effect. Using online databases, hundreds of articles were screened, and ultimately 11 studies were chosen, encompassing 224 total participants. Results showed an overall significant effect of L-citrulline on both resting SBP (MD: -3.74; 95% CI [-6.74, -0.74]; p=0.01) and DBP (MD: -2.00; 95% CI [-3.93, -0.06]; p=0.04). Further analysis of funnel plots was used to determine publication biases and subgroup analysis was performed to determine specific trial moderators that could have affected the overall outcome. In most cases, L-citrulline displayed a significant effect on blood pressure, and more research is warranted to investigate its potential therapeutic effect on cardiovascular health.

ACKNOWLEDGEMENTS

I would like to thank Dr. Manish Gupta for serving as my Thesis Chair and research mentor and for his continued guidance and expertise throughout the entire process. I would also like to thank my committee members, Dr. Muthu Periasamy and Dr. Dinender Singla, for their supervision and insight. It is their passion for their research that has inspired my curiosity, and I am truly humbled to be supported by them. I want to also thank the UCF Office of Undergraduate Research and the Burnett Honors College for providing the structure and resources to complete my project.

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INTRODUCTION

High blood pressure, also known as hypertension, is the main risk factor for heart attacks and strokes, which are the leading causes of death in the United States [1]. In the world, it is estimated that about 1.13 billion people suffer from hypertension [3]. In addition, over nine million deaths have been associated directly with hypertension [4]. Blood pressure is the primary metric employed by clinicians to determine hypertension. Stage 1 hypertension is described as blood pressure at or above 130/80 mmHg while, Stage 2 hypertension is defined as blood pressure at or above 140/90 mmHg [5]. Despite there being more than a billion individuals with hypertension, about 95% of cases present with no direct cause of hypertension. This is termed essential hypertension [18]. Blood pressure is described as the product of total cardiac output and total peripheral resistance [18]. The incredible variability of blood pressure stems from the various mechanisms that can control it. Blood pressure is the culmination of many organ systems and serves to maintain homeostasis in response to internal and external stimuli. For the scope of this review and meta-analysis, an in-depth examination of the NO pathway with regard to L-citrulline is most appropriate.

BP Category	SBP		DBP
Normal	<120 mm Hg	and	<80 mm Hg
Elevated	120–129 mm Hg	and	<80 mm Hg
Hypertension			
Stage 1	130–139 mm Hg	or	80–89 mm Hg
Stage 2	≥140 mm Hg	or	≥90 mm Hg

Figure 1: Blood Pressure Categories for Adults according to the AHA [2]

LITERATURE REVIEW

Nitric Oxide (NO) is a gaseous signaling molecule that freely dissolves across membranes and is responsible for dilating blood vessels allowing for more blood flow and indirectly decreasing blood pressure by decreasing peripheral resistance to blood flow [6]. Endothelial function is dependent on NO availability for these functions [7]. Endothelial function is compromised as we age and become worse with conditions such as cardiovascular disease, diabetes, and hypertension. One proposed mechanism for endothelial dysfunction is the lack of the substrate L-arginine for use in NO production [8]. L-arginine and L-citrulline have been studied to correct this NO deficiency [9]. In a recent meta-analysis of 22 randomized-controlled trials, L-arginine supplementation showed potent antihypertensive effects [10]. Despite this, L-citrulline is being studied as possibly an even more potent amino acid for blood pressure and NO synthesis due to metabolism factors. L-arginine is metabolized by arginase in the stomach [11]. In addition, L-citrulline escapes renal and hepatic clearance of L-arginine [12]. L-citrulline supplementation has been shown in many studies to be able to avoid these pitfalls of L-arginine while also being able to increase L-arginine levels and NO production for a longer duration and to a much greater extent than L-arginine production [13, 14]. L-arginine is a conditionally essential amino acid, while L-citrulline is a nonessential amino acid naturally occurring in watermelon [17]. The mechanism by which this occurs is the conversion of L-arginine to L-citrulline via the enzyme endothelial Nitric Oxide Synthase (eNOS), releasing NO in the process [13]. As we age, the reduction in key enzymes such as eNOS and decreased L-arginine

bioavailability are strongly associated with the development of high blood pressure and cardiometabolic disease [15].

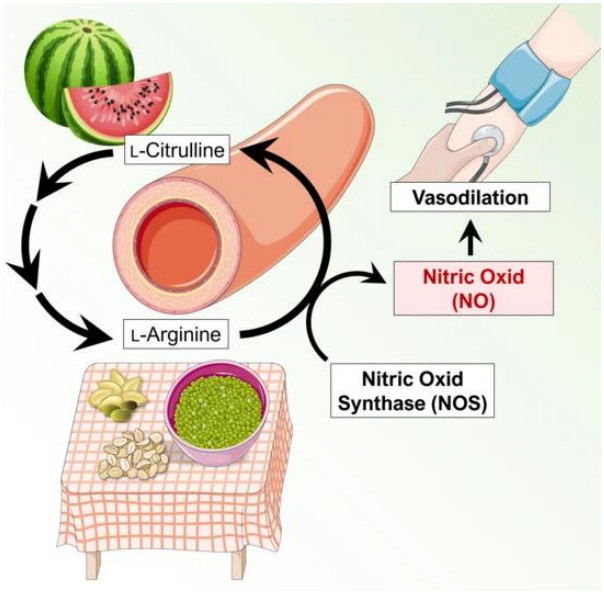

Figure 2: Graphical Abstract [16]

The exact mechanism of vasodilation starts with L-arginine and results in vascular smooth muscle relaxation. In the context of this review, oral supplementation of L-citrulline dramatically raises the plasma concentrations of L-arginine within vascular endothelial cells. [16]. L-arginine is then converted into L-citrulline and NO. NO diffuses into the vascular smooth muscle cell, where it triggers a signaling cascade that results in vasodilation. NO activates soluble guanylate cyclase (sGC), which converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) [16]. cGMP activates protein kinase G (PKG), which serves a synergistic function to decrease intracellular calcium concentrations. PKG activates sarcoplasmic calcium ATPases (SERCA) and plasma membrane calcium ATPases (PMCA) which pump calcium out of the intracellular space and into the sarcoplasmic reticulum and extracellular space, respectively [16]. In addition, PKG blocks inositol triphosphate receptors

(IP_3R) and voltage-dependent calcium channels (VDCC), which pump calcium into the intracellular space from the SR and extracellular space, respectively. This decrease in intracellular calcium activates the myosin light chain phosphatase and inhibits the myosin light chain kinase, which induces smooth muscle relaxation, vasodilation, and a decrease in BP by not allowing myosin to interact with actin within the endothelium [16].

As mentioned before, L-citrulline presents an effective way of increasing plasma arginine levels, even as opposed to direct supplementation of L-arginine. One study examined this very ability to determine which amino acid was favorable in raising plasma arginine levels. Schwedhelm et al. investigated the effect of various L-citrulline and L-arginine doses and formulations to determine which compounds were more desirable. The researchers found that two doses of 0.75 g of L-citrulline were equally as effective as raising plasma arginine levels as were three doses of 1g of L-arginine [13]. Bode-Böger et al. found a similar association with regard to dosing as well [19]. Despite there being more current research on L-arginine, these results showed that L-citrulline could achieve a similar effect of raising plasma arginine levels even with half the dose.

L-citrulline also displays antioxidant and anti-inflammatory properties that can protect the body from endothelial damage. These properties can help slow the progression of CVD by attenuating the damage caused by high blood pressure [15]. Three animal models of heart disease and endothelial damage were examined that showed positive effects of L-citrulline supplementation on metabolic parameters despite deleterious conditions. These studies showed that L-citrulline supplementation was able to reduce liver fat, decrease circulating triglycerides, preserve eNOS production, and help attenuate endothelial damage as a result of a high-fat and

high-carb diet [20, 21, 22]. In addition, L-citrulline indirectly protects endothelial cells from reactive oxygen species via the upregulation of eNOS [23]. This is because NO is able to directly neutralize hydroxyl radical formation caused by atherosclerosis [24]. L-citrulline has also been shown to help with inflammation via the modulation of inflammatory cytokines. In human studies, L-citrulline has been demonstrated to reduce circulating levels of inflammatory cytokines such as tumor necrosis factor alpha (TNF-a), interleukin 6 (IL-6), and c-reactive protein (CRP) [25]. With regards to adaptive immunity, l-citrulline supplementation has been shown to directly alter CD8+ and CD4+ metabolism and stabilize other immune cells [26].

From the literature, NO production serves as an important therapeutic target to mitigate the age-related loss of endothelial function and progression of high blood pressure and CVD. L-citrulline poses a unique promise to deal with this age-related decline directly and indirectly. L-citrulline drastically increases plasma arginine levels which is the substrate by which eNOS is able to produce NO within the endothelium and helps fight inflammation which can exacerbate and lead to increased endothelial damage. The subsequent meta-analysis will examine the effect of L-citrulline on blood pressure to better elucidate its effectiveness as an anti-hypertensive compound.

METHODS

Search Strategy, Eligibility, and Selection Criteria

The search strategy that will be employed will be to search relevant clinical trials on Google Scholar, PubMed, Scopus, and other compilations of academic papers. In addition, ongoing clinical trials posted on registers such as the NIH's database will be examined. Search criteria will include looking up "L-citrulline" or "citrulline" in addition to "blood pressure" or "systolic and diastolic blood pressure" and looking for trials that fit the criteria. Eligibility will look at the study as a whole and determine whether it is a good fit for this meta-analysis; randomized-controlled trials (RCTs) will be the gold standard for this. All studies examined met the following inclusion criteria: RCT study design; oral administration of L-citrulline only; at least one week of study duration; and sufficient baseline and final SBP and DBP data was reported.

Data Extraction

The data extraction went as follows: author's first name, publication year, study design, country of origin, the sample size of the intervention and control groups, baseline and observed SBP and DBP measurements, L-citrulline doses (g/day or mg/day), duration of supplementation, any previous notable clinical features, and quality characteristics.

Quality Assessment

The quality assessment that will be used is the 5-point Jadad Scale to determine the quality of the randomized-controlled trial [17]. The scale ranges from 0 to 5 and is used to designate the study as "high" or "low" quality. One point is given if the trial is randomized, and another if randomization is appropriate. Another point is given if the trial is blinded, and a fourth point if the blinding was appropriate. Lastly, a final point is given if dropouts are stated with an appropriate explanation. Scores of 3 or better are deemed as "high" quality studies, and lower scores indicate "low" quality RCTs.

Statistical Analysis

The statistical analysis will be performed using the Review Manager 5 software (RevMan 5) according to the Cochrane Guidelines [27]. For all outcomes, summary estimates of weighted mean differences (WMD) and 95 % CI were determined and visualized using forest plots. The inverse of the within-subject variance $(1/(\text{standard error})^2)$ was used as a weighting factor. Treatment effects were defined as weighted mean differences (WMDs), and 95% confidence intervals (CIs) were calculated to assess net changes in SBP or DBP values. A random-effects model was used to analyze the trials to account for heterogeneity. In addition, stratified analysis by age, dose, and study duration was conducted to examine any sources of heterogeneity. Finally, a funnel plot was used to assess publication bias. A $P < 0.05$ was considered to be statistically significant.

Calculations

For the purpose of calculations, resting SBP and DBP was measured. Differences were calculated by accounting for standard deviations (SD). If standard error (SE) was given, the SE was converted to SDs by the following formula: $SD = SE * \sqrt{n}$; where n is the number of participants. According to the Cochrane Guidelines, when subtracting two means with standard deviations, the final standard deviation will be given by the following formula:

$$SD = \sqrt{{SD_{Experimental}}^2 + {SD_{Placebo}}^2 - (2R SD_{Experimental}\, SD_{Placebo})}$$

Where R is the correlation coefficient. Many studies did not include a correlation coefficient, so we used existing studies to compute and average the correlation coefficient that was used in the calculations. The Cochrane Guidelines gave the formula as:

$$R = \frac{{SD_{Experimental}}^2 + {SD_{Placebo}}^2 - {SD_{Change}}^2}{2 SD_{Experimental}\, SD_{Placebo}}$$

For the calculations used in this meta-analysis, studies by Balderas-Munoz et al. 2012 and Gonzales et al. 2017 were used to calculate the correlation coefficient. The final correlation coefficient that was determined to be used for this meta-analysis was $R = 0.5$.

Abbreviations

BP: Blood pressure, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MD: Mean difference, WMD: Weighted mean difference, CI: Confidence Interval, I^2: Percent heterogeneity

RESULTS

Search Results and Study Characteristics

The search of multiple databases and registers of clinical trials resulted in 2813 articles and records. Many of the articles were duplicates (n = 1563), and after removal, 1250 records were screened for relevant information. Most records could be screened from the title alone, but some trials required the reading of the abstract. In totality, 1197 records were excluded as many articles contained irrelevant material to the study, presented studies on animal models, were not randomized trials, or were literature reviews. 53 records remained for retrieval, of which 5 records could not be accessed. 48 records were thoroughly assessed according to the inclusion criteria. Of these studies, 30 presented insufficient data, no control group, or acute supplementation, which made them ineligible for inclusion. In addition, 7 studies were excluded for concurrent supplementation with other compounds such as watermelon extract, l-arginine, or pharmaceutical drugs. In our analysis, 11 studies were chosen to be a part of the review [28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38]. A study selection flow diagram is depicted in Figure 3 according to the PRISMA guidelines [39]. According to the Cochrane Guidelines, the study from Gonzales et al. included 2 separate legs, and thus, it was considered two independent trials for our analysis [27, 31]. All trials were placebo-controlled and randomized, with 5 trials including only men [29, 30, 31, 32, 33] and 5 trials only including women [31, 34, 35, 36, 38]. 5 trials included healthy participants [29, 30, 32, 33, 38], 5 included healthy/hypertensive participants [31, 34, 35, 36, 37], and 1 study tested L-citrulline on participants with heart failure [28]. Study

duration varied from 1 to 16 weeks, and the dose of L-citrulline varied from 3-11 g/day. Study characteristics are described in further detail in Table 1. From the quality assessment, 9 studies were considered "high" quality [29, 31, 32, 33, 34, 35, 35, 36, 37, 38], and 2 studies were considered "low" quality [28, 30]. Study quality is summarized in Table 2.

Results from Previous Meta-analyses

This analysis differed from previous meta-analyses by including more recent studies [36, 37, 38] and by measuring only resting brachial SBP and DBP. The meta-analysis from Mahoobi et al. combined trials using L-citrulline and other watermelon extracts, which may have skewed results as watermelon extract may also contain nutrients such as lycopene, beta-carotene, and lutein [40]. Meta-analyses from Mirenayat et al., Yang et al., and Barkhidarian et al., also contained similar studies, but they did not include the three new studies listed above [41, 42, 43]. In addition, the meta-analysis by Yang et al. directly compared resting measurements with measurements taken during activity or cold exposure, which can dramatically influence the result [43]. Generally, these studies inflated the blood pressure lowering effect of L-citrulline if results from physical activity or cold exposure were used. Thus, this meta-analysis provides the most updated information regarding the effects of L-citrulline on blood pressure.

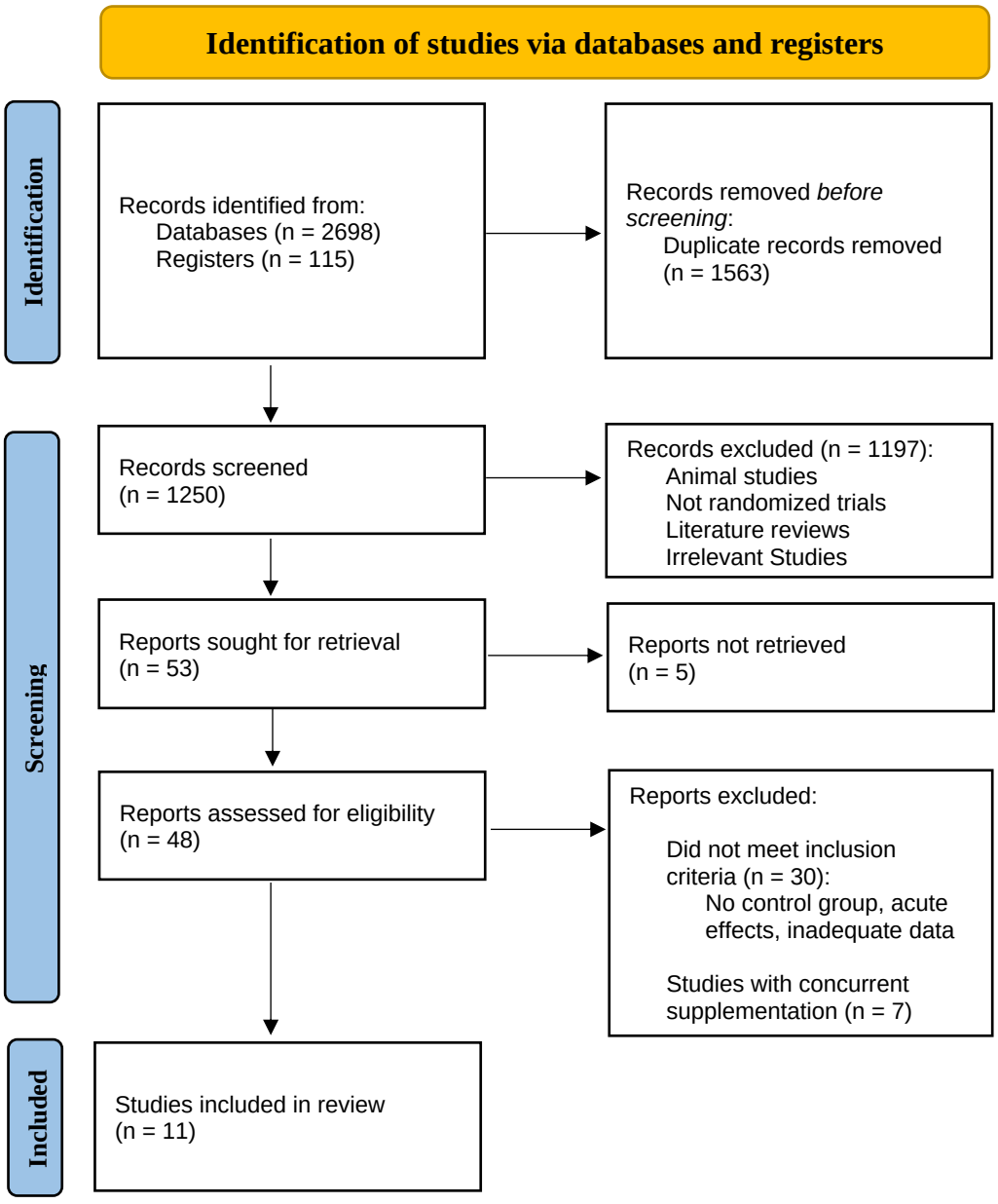

Figure 3: PRISMA Flow Diagram

First Author/ Year	Country	Study Design	Size (Males/Females) (Experimental)	Mean Age (years)	Study Duration (weeks)	Dose (g/day)	Notes about Participants	Results
Balderas-Munoz et al. (2012)	Mexico	Randomized, parallel controlled trial	24/11	67 ± 9	16	3	Systolic Heart Failure	SBP and DBP did not change significantly
Ochiai et al. (2012)	Japan	Double-blind, randomized, placebo-controlled parallel-group trial	15/0	58.3 ± 4.4	1	5.6	Healthy	SBP and DBP did not change significantly
Sanchez-Gonzalez et al. (2013)	USA	Randomized Crossover Trial	16/0	23 ± 12	2	7-11	Healthy	SBP and DBP decreased significantly
Gonzales et al. (2017) (2 trials)	USA	Double-blind, randomized, placebo-controlled crossover trial	12/0 0/13	71 ± 5 70 ± 5	2	6	Healthy, Hypertensive	DBP decreased significantly in male group only
Figueroa et al. (2010)	USA	Double-blind, randomized, two-period crossover trial	17/0	22 ± 4.1	4	6	Healthy	DBP decreased significantly in only the second period

Figueroa et al. (2016)	USA	Double-blind, randomized, placebo-controlled crossover trial	16/0	24 ± 6	2	6	Healthy	SBP and DBP did not change significantly
Wong et al. (2015)	USA	Randomized, controlled trial	0/27	58 ± 3	8	6	Healthy/ Hypertension	SBP and DBP decreased significantly
Wong et al. (2016)	USA	Randomized, controlled trial	0/23	58 ± 4.8	8	6	Healthy/ Hypertension	SBP and DBP decreased significantly
Maharaj et al. (2022)	USA	Double-blind, randomized, placebo-controlled parallel-group trial	0/14	61 ± 6	4	10	Hypertensive/ Postmenopausal	Aortic DBP decreased significantly
Jaime et al. (2022)	USA	Double-blind, randomized, placebo-controlled crossover trial	7/13	71.5 ± 7.3	2	6	Healthy/ Hypertension	SBP and DBP did not change significantly
Edwards et al. (2023)	USA	Double-blind, randomized, placebo-controlled crossover trial	0/16	66.9 ± 5.6	1	6	Healthy	DBP decreased significantly

Table 1: Characteristics of Included Trials

First Author/ Year	Randomization is mentioned	Randomization is appropriate	Blinding is mentioned	Blinding is appropriate	States reason and number of withdrawals	Total
Balderas-Munoz et al. (2012)	1	0	0	0	1	2
Ochiai et al. (2012)	1	0	1	0	1	3
Sanchez-Gonzalez et al. (2013)	1	0	1	0	0	2
Gonzales et al. (2017)	1	0	1	1	1	4
Figueroa et al. (2010)	1	0	1	0	1	3
Figueroa et al. (2016)	1	0	1	1	1	4
Wong et al. (2015)	1	1	0	0	1	3
Wong et al. (2016)	1	1	0	0	1	3
Maharaj et al. (2022)	1	0	1	1	1	4
Jaime et al. (2022)	1	0	1	1	0	3
Edwards et al. (2023)	1	0	1	1	0	3

Table 2: Quality Assessment According to the Jadad Scale

Meta-analysis

From the pooled data of 11 studies and 224 participants, the meta-analysis showed significant reductions in both SBP and DBP. SBP decreased by -3.74 mm Hg (95% CI [-6.74, -0.74]; p=0.01), and DBP decreased by -2.00 mm Hg (95% CI [-3.93, -0.06]; p=0.04). From the I^2 values, SBP and DBP both showed low heterogeneity. SBP had a slightly higher heterogeneity (I^2=29%, p=0.16) than DBP (I^2=14%, p=0.31), which may be attributed to the systolic pressure variation phenomenon. Forest plots for SBP and DBP are shown in Figures 4 and 5, respectively. In addition, calculations used for Forest plot analysis are listed in Table 3.

Publication Bias

To assess publication bias, a visual examination of funnel plots for SBP (Figure 6) and DBP (Figure 7) was used. Since both funnel plots were symmetric and 95% of studies remained within the plot area, this indicates that there was a low chance of publication bias within any one study.

Sub-group Analysis

To identify any sources of heterogeneity, a sub-group analysis was performed and summarized in Table 4. The analysis was stratified by mean age, dose, and study duration. The stratified analysis showed that L-citrulline supplementation was more effective on individuals

younger than 50 years of age for both SBP (MD: -4.02; 95% CI: [-12.6, 4.5]; P=0.008) and DBP (MD: -4.00; 95% CI: [-9.27, 1.26]; P=0.09). From the other parameters, 6 g/day seems to be the most effective dose, and supplementation durations should be around 4 weeks as perhaps the body may start tolerating L-citrulline and attenuating its effects.

First Author/Year	Baseline (Control) (mm Hg)	Final BP (Control) (mm Hg)	(n)	Difference (Control) (mm Hg)	Baseline BP (Experimental) (mm Hg)	Final BP (Experimental) (mm Hg)	(n)	Difference (Experimental) (mm Hg)
Balderas-Munoz et al. (2012)	S: 118 ± 15.8 D: 76.7 ± 11	S: Unclear D: Unclear	15	S: 7.08 ± 17.08 D: - 1.42 ± 14.12	S: 113 ± 17.2 D: 70.3 ± 11.5	S: Unclear D: Unclear	20	S: - 2.02 ± 9.36 D: - 3.22 ± 14.01
Ochiai et al. (2012) *	S: 130.9 ± 9.3 D: 84.6 ± 6.9	S: 127.9 ± 11.1 D: 81.8 ± 7.9	7	S: - 3 ± 10.3 D: - 2.8 ± 7.5	S: 135.5 ± 13.3 D: 82.4 ± 7.6	S: 131.6 ± 12.2 D: 81.9 ± 7.9	8	S: - 3.9 ± 12.8 D: - 0.5 ± 7.8
Sanchez-Gonzalez et al. (2013) *	S: 132 ± 8 D: 76 ± 12	S: 132 ± 8 D: 78 ± 12	16	S: 0 ± 8 D: 2 ± 12	S: 132 ± 8 D: 76 ± 12	S: 121 ± 8 D: 70 ± 12	16	S: - 11 ± 8 D: - 6 ± 12
Gonzales et al. (2017) (F)	S: 129 ± 15 D: 64 ± 7	S: 130 ± 10 D: 66 ± 6	12	S: 1.0 ± 8.0 D: - 2.0 ± 5.0	S: 127 ± 12 D: 65 ± 7	S: 128 ± 15 D: 64 ± 7	12	S: 0.1 ± 9.7 D: - 1.4 ± 4.2
Gonzales et al. (2017) (M)	S: 135 ± 11 D: 74 ± 9	S: 132 ± 11 D: 73 ± 6	13	S: - 2.7 ± 12.1 D: - 0.5 ± 7.6	S: 135 ± 14 D: 75 ± 9	S: 132 ± 13 D: 71 ± 6	13	S: - 2.5 ± 5.3 D: - 3.9 ± 5.3
Figueroa et al. (2010) *	S: 121 ± 12.4 D: 68 ± 8.2	S: 116 ± 8.2 D: 69 ± 8.2	17	S: - 5 ± 10.9 D: 1 ± 8.2	S: 120 ± 12.4 D: 67 ± 8.2	S: 119 ± 12.4 D: 69 ± 8.2	17	S: - 1 ± 12.4 D: 2 ± 8.2
Figueroa et al. (2016) *	S: 122 ± 8 D: 67 ± 4	S: 124 ± 8 D: 69 ± 4	16	S: 2 ± 8 D: 2 ± 4	S: 123 ± 12 D: 68 ± 8	S: 121 ± 12 D: 64 ± 8	16	S: - 2 ± 12 D: -4 ± 8
Wong et al. (2015)	S: 141 ± 2 D: 80 ± 8	S: 132 ± 16 D: 74 ± 12	14	S: - 9 ± 15.1 D: - 6 ± 10.6	S: 140 ± 9 D: 78 ± 7	S: 132 ± 9 D: 73 ± 6	13	S: - 8 ± 9 D: - 5 ± 6.6

Wong et al. (2016) *	S: 137 ± 13.3 D: 80 ± 9.9	S: 138 ± 13.3 D: 80 ± 9.9	11	S: 1 ± 13.3 D: 0 ± 9.9	S: 138 ± 13.9 D: 81 ± 13.9	S: 131 ± 17.3 D: 75 ± 13.9	12	S: - 7 ± 15.9 D: - 6 ± 13.9
Maharaj et al. (2022)	S: 136 ± 14 D: 79 ± 11	S: 139 ± 14 D: 79 ± 14	11	S: 3 ± 14 D: 0 ± 12.8	S: 139 ± 17 D: 83 ± 9	S: 135 ± 17 D: 81 ± 8	14	S: - 4 ± 17 D: - 2 ± 8.5
Jaime et al. (2022)	S: 138 ± 13 D: 77 ± 7	S: 138 ± 12 D: 78 ± 7	16	S: 0 ± 12.5 D: 1 ± 7	S: 137 ± 13 D: 77 ± 8	S: 139 ± 17 D: 77 ± 7	16	S: 2 ± 15.4 D: 0 ± 7.5
Edwards et al. (2023)	S: 131.6 ± 17.6 D: 73.6 ± 16.3	S: 133.4 ± 19.8 D: 77.9 ± 15.7	16	S: 1.8 ± 18.8 D: 4.3 ± 16	S: 131.1 ± 18.3 D: 78.8 ± 12.9	S: 129.9 ± 19.9 D: 73.6 ± 13.1	16	S: - 1.2 ± 19.2 D: - 5.2 ± 18.4

Table 3: Baseline and Final SBP and DBP Calculations

** - studies reporting standard error (SE), which were converted into standard deviation (SD) in the table.*

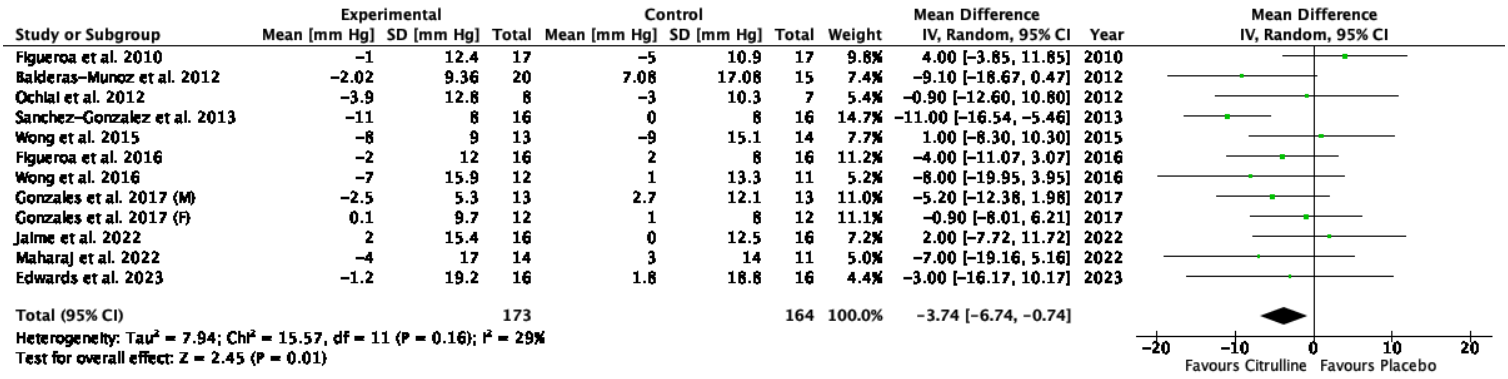

Figure 4: Forest Plot showing the effect of L-citrulline on SBP

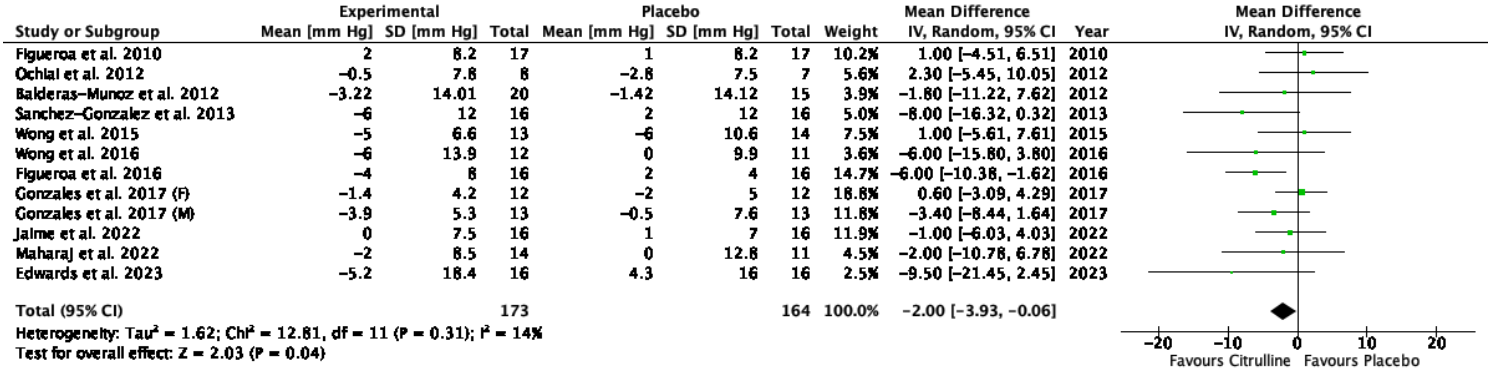

Figure 5: Forest Plot showing the effect of L-citrulline on DBP

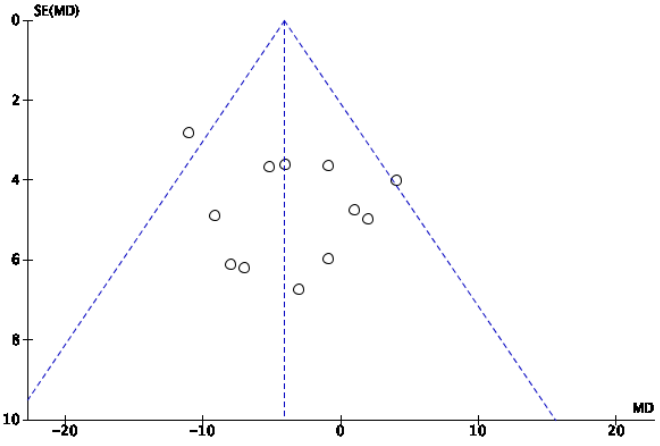

Figure 6: Funnel Plot of Studies for SBP

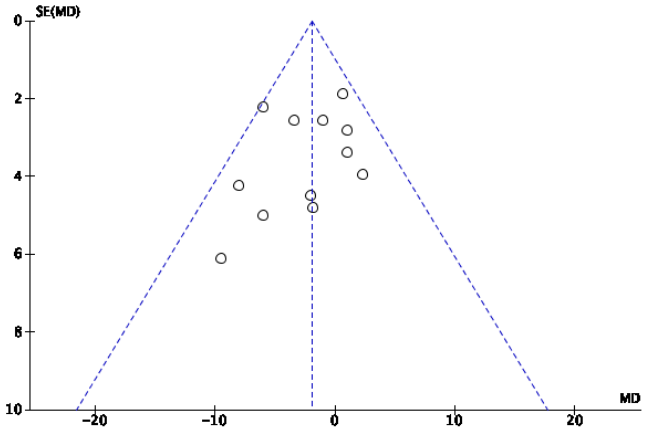

Figure 7: Funnel Plot of Studies for DBP

Subgroup	≥ 50 (Years old)	< 50 (Years old)	≥ 6 (g/day)	< 6 (g/day)	≥ 4 (Weeks)	< 4 (Weeks)
No. of Trials	8	3	9	2	5	6
SBP						
WMD (95% CI) (mm Hg)	-3.16 [-6.33, 0.02)	-4.02 [-12.6, 4.54]	-3.41 [-6.78, -0.04]	-5.72 [-13.63, 2.19]	-2.96 [-8.56, 2.63]	-4.33 [-7.94, -0.72]
P-Heterogeneity	0.74	0.008	0.12	0.29	0.17	0.21
I^2 *(%)*	0	79	37	12	37	29
P-Overall Effect	0.05	0.36	0.05	0.16	0.30	0.02
DBP						
WMD (95% CI) (mm Hg)	-1.04 [-3.11, 1.03]	-4.00 [-9.27, 1.26]	-2.33 [-4.47, -0.18]	0.64 [-5.34, 6.63]	-0.58 [-3.91, 2.74]	-2.73 [-5.54, 0.08]
P-Heterogeneity	0.69	0.09	0.24	0.51	0.76	0.12
I^2 *(%)*	0	59	22	0	0	41
P-Overall Effect	0.32	0.14	0.03	0.83	0.73	0.06

Table 4: Subgroup Analysis

DISCUSSION

Strengths, Limitations, and Future Directions

From the data, it is clear that L-citrulline does indeed induce a significant blood pressure lowering effect. The meta-analysis showed a reduction in SBP by around -3.74 mm Hg and DBP by around -2.00 mm Hg. Sub-group analysis showed that L-citrulline might benefit a younger population, with greater effects taking place during a shorter usage period.

While the results were significant, there are still some inherent limitations to our meta-analysis that need to be discussed. The most obvious limitation would be the lack of participant volume and standardization process. L-citrulline was given at varying doses that did not exceed 11 grams, and not enough participants were given enough varying doses to elucidate a specific dose response to L-citrulline. Furthermore, trials tended to be smaller, and trials of "low" quality were used in the analysis. However, the addition of new trials in this meta-analysis provided a more robust analysis compared to previous meta-analyses.

Despite L-citrulline's effect on blood pressure being significant, further research is warranted to determine the optimal dosage and even administration route for the amino acid. For example, Asklepion Pharmaceuticals is currently in the middle of a Phase 3 trial for intravenous L-citrulline administration that has shown promising preliminary results [44].

CONCLUSION

L-citrulline supplementation remains a promising new intervention to increase L-arginine levels within the body and indirectly increase NO levels within the endothelium. This is proven from the meta-analysis as L-citrulline significantly decreased resting SBP and DBP. This study demonstrates the potential therapeutical benefit of L-citrulline as a means to decrease high blood pressure via a different mechanism than current pharmaceutical drugs and offers clinicians a new tool to aid them in treating hypertension and CVD. These findings should be considered preliminary, and larger, better-designed trials should be untaken to determine L-citrulline's efficacy as well as its proper dosing, duration, and target populations.

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