

EFFECT OF L-ARGININE SUPPLEMENTATION WITH ENDOTHELIAL FUNCTION IN DIABETES MELLITUS AND CARDIOVASCULAR DISEASE: AN EVIDENCE BASED CASE REPORT

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Abstract

Background: Cardiovascular disease is the main complication that induces morbidity in diabetes mellitus patients. Endothelial function plays a role in these complications. Nitric oxide is a nitrogen-free radical produced from L-arginine, involved in several mechanisms for maintaining endothelial function to prevent cardiovascular disease and diabetes mellitus.

Methods: A search for relevant literature in PubMed, Cochrane, and ProQuest was conducted. After assessing the relevancy and eligibility of the literature, two pieces of literature were selected and critically appraised.

Results: There are two works of literature from systematic review and meta-analysis were found. The studies suggest pro and contra L-arginine supplementation with endothelial function in diabetes mellitus and cardiovascular disease.

Conclusions: L-arginine might be the alternative supplement for cardiovascular disease and diabetes mellitus, but further analysis in improved studies is needed to confirm.

Keywords: l-arginine, diabetes mellitus, cardiovascular disease, endothelial

Clinical Scenario

A 66-year-old woman came to a nutrition clinic for a consultation about her disease. She has a nutritional status of obesity grade 1 and has been suffering from diabetes mellitus for 20 years ago. She was hospitalized for a heart attack about one month ago. She has tried to change his diet, exercise, and take anti-diabetic medication regularly. She asked whether L-arginine supplementation could be potential therapy to support her treatment of diabetes mellitus and to prevent recurrent heart attacks.

Introduction

Diabetes mellitus (DM) is a chronic disease worldwide, particularly in developing countries.¹ Various epidemiological studies state that there is a significant increase in the incidence and prevalence of type 2 diabetes mellitus. The World Health Organization (WHO) predicts that there will be an increase in the number of people with type 2 diabetes in Indonesia from 8.4 million in 2000 to 21.3 million in 2035.²

Characteristics of diabetes mellitus are hyperglycemia that exists due to insulin

secretion disturbance, insulin action, or both.² Several studies have shown that diabetic patients have a higher risk of coronary artery disease (CAD), myocardial infarction, and other heart diseases.^{3,4} Endothelial dysfunction is a common condition in cardiovascular disease with diabetes mellitus.⁵ The mechanism of endothelial dysfunction caused by diabetes are the increased oxidative degradation of nitric oxide (NO) and the production of prostanoid vasoconstrictors.⁶ The availability of NO is one of the mechanisms that play a role in these complications.⁴

L-arginine is a conditional amino acid discovered in healthy adults however required in certain diseases, neonates, and infants.^{7,8} The sources of free arginine are from dietary intake (4-6 g of arginine per day), endogenous synthesis from citrulline (10-15% of total arginine production), and protein turnover (80% of the circulating arginine). Food sources of arginine come from watermelon juice, dairy products, eggs, nuts, seeds, algae, meats, rice protein concentrate, soy protein isolate, and relatively high include seafood.⁷ The synthesis of endogenous de novo involves the conversion of citrulline into arginine through a two-step enzymatic process relating enzyme arginosuccinate synthase (ASS) and arginosuccinate lyase (ASL) in the gut-

kidney axis. The synthesis of citrulline is derived from glutamine, glutamate, and ornithine in the mitochondria of enterocytes, released into circulation and taken up by kidneys for the synthesis of arginine.^{7, 8}

Arginine is a substrate for two enzymes, nitric oxide synthase (NOS) and arginase, which are essential for the formation of NO and urea, respectively. Arginine acts as a substrate for NO production by endothelial cells, thereby regulating vascular tone and cardiovascular homeostasis.⁹ Nitric oxide is produced from arginine by NOS enzymes that involve in the transfer of an electron from nicotinamide adenine dinucleotide phosphate (NADPH), then arginine substrate is oxidized to citrulline and NO.^{10, 11} Arginine is also related to the proliferation of T-cell and immune responses, as well as in creatine and collagen synthesis.¹² There are three isoforms of NOS, two of which endothelial (eNOS) and the third inducible NOS (iNOS) which are related to the inflammatory response.¹³

L-Arginine supplementation can restore arginine and NO levels by preventing the release of eNOS (thus reducing superoxide formation), providing adequate L-arginine for NO synthesis, boost the activity of guanosine triphosphate (GTP) siklohidrolase I, an enzyme that is stimulated by L-arginine and is the rate-limiting enzyme

for the synthesis of tetrahydrobiopterin (BH4), a cofactor for NOS activity. L-arginine and NO engage in protein synthesis by stimulating the mammalian target rapamycin (mTOR), peroxisome proliferator-activated receptor-gamma coactivator 1 α (PGC-1 α), and adenosine monophosphate (AMP)-activated protein kinase (AMPK) that affect mitochondrial biogenesis. The metabolism of L-arginine by arginase can lead to the synthesis of polyamines, essential for cell growth and angiogenesis. Increased vasodilation and vascularity induced by NO availability will improve nutrient and oxygen transport.^{14, 15}

Nitric Oxide is an active vasodilator that controls tissue blood flow and vascular resistance. Several studies reported that NO production is reduced in cardiovascular and metabolic diseases.¹⁵ Therefore, the aim of this evidence-based case report (EBCR) is to critically analyze the effect of supplementation of L-arginine for endothelial function in diabetes mellitus and cardiovascular disease.

Clinical Question

This case raises a question: does L-arginine supplementation has a better outcome manifestation for endothelial function in diabetes mellitus patient and cardiovascular disease?

Methods

Search Strategy

Literature search was conducted using advanced searching on PubMed, Cochrane, and ProQuest on June 20th, 2021. MeSH term was used in PubMed database search. The keywords used are ‘diabetes mellitus’ AND ‘cardiovascular disease’ AND ‘L-arginine’ AND ‘endothelial.’ (Table 1) After we got the result from this database, we screened by titles and abstracts, selected based on the inclusion criteria and filtered for duplicates and availability for full text. PICO-compatible literature was screened using full text eligibility criteria. Critical appraisal of the study was conducted using the guidance for randomized controlled trial (RCT) published by University of Oxford Centre for Evidence-Base Medicine (CEBM).

Eligibility Criteria

Article selection was based on the inclusion and exclusion criteria, which addressed the clinical question. The inclusion criteria were: 1) participants in the study were adults (aged \geq 18 years old) with a history of diabetes mellitus and or cardiovascular disease; 2) patients got L-arginine as intervention; 3) outcome measurement in the study is the markers of endothelial function; 4) the study design used was systematic review and/or meta-analysis and randomized

controlled trial; 5) published within the last 5 years. The exclusion criteria were: 1) research in the form of case control, reviews, comments, conference abstracts, letters, section in a book or non-clinical study; 2) study is not written in English language 3) research that was not done in humans.

Results

The search obtained 93 literatures from PubMed, 22 literatures from Cochrane and 607 literatures from ProQuest (**Table 1**). We filtered the literature by search engine filtering, screened the titles and abstract based on the inclusion criteria and filtered for duplicates. After reading full text, we used two literatures from systematic review and meta-analysis. The literatures are from Krause JR et al. and Gambardella et al. studies. The flowchart of searching and selection strategy can be seen in **Figure 1**. The eligible criteria description can be viewed in **Table 2**.

The study of Krause JR, et al (20018)¹⁶ has similar purpose to our clinical question which is to find out the effect of arginine supplementation for endothelial function in diabetes mellitus patients and cardiovascular disease. The literature show that there were no differences of endothelial function markers (the blood flow, NOx responses and ADMA responses) found between the supplementation of arginine and

placebo groups.¹⁶ The objective of Gambardella et al. (2020)¹⁷ study was obviously stated to evaluate the mechanism of arginine in the control of endothelial function and vascular in hypertension, aging, peripheral artery disease, ischemic heart disease, and diabetes mellitus. The literature supports the use of arginine supplementation for cardiovascular disease, particularly preventing worsening of atherosclerosis and hypertension.¹⁷

Critical Appraisal

The critical appraisal of this study for its validity, importance and applicability is describe in Table 3. We concluded that the level of evidence of this study based on Oxford CEBM is 1a.¹⁸

Discussion

There were two systematic review and meta-analysis included as useful article to answer the clinical question. The result of this study is still debate. Krause JR et al.⁵ compare supplementation of arginine with a placebo control group in subjects with any type of cardiovascular disease, and/or type 2 diabetes mellitus, and obesity. The intervention consisted of oral L-arginine supplementation (capsules, biscuits, bars, shakes, syrups) for a minimum duration of three days, but there was no limit to the maximum duration. The minimum dose of

arginine used was 1.2 g/day and the maximum was 15 g/day, while the most frequently used doses were 6.4 and 9.0 g/day. The comparison of control groups with the equal health conditions, receiving oral supplementation placebo for the same duration.

The outcome of this review was blood flow (assessed by flow-mediated dilation (FMD)) and biochemical findings (nitrite/nitrate (NO_x) rate and asymmetric dimethylarginine (ADMA)). Krause et al report that oral L-arginine supplementation has no improvements in blood flow and biochemical markers of endothelial dysfunction in patients with cardiovascular and or metabolic disorders. There were no differences of the blood flow (mean difference = 0.30 (95% CI: -0.85, 1.46), NO_x responses treatments (mean difference = 4.41 (95% CI: -0.50, 9.32) and ADMA response (mean difference = -0.04 (95% CI: -0.15, 0.08) found between the supplementation of arginine and placebo groups.

Gambardella et al.¹⁷ in their systematic review assessed the mechanism of arginine in the regulation of endothelial function and vascular in hypertension, aging, peripheral artery disease, ischemic heart disease, and diabetes mellitus. They assessed L-arginine supplementation in different clinical studies. The study of Gambardella et

al. reported that L-arginine therapeutic potential of endothelial function in CAD patients. They also report that arginine supplementation in the diabetic population has prophylactic treatment to prevent cardiovascular complications. Chronic administration of oral arginine has been shown to favor utilization of arginine for NO synthesis. Supplementation of oral L-arginine 3g/day improves endothelial function in hypertensive patients. A dose of 3g/day of L-arginine appears to be effective in favoring the utilization of arginine for NO synthesis, without toxic effects.

The limitations of recent evidence regarding L-arginine supplementation are that the analysis includes studies with a wide range of diseases and presents a high heterogeneity. The studies usually assess clinical outcomes without examining biochemistry and the number of different doses.

Conclusion

Cardiovascular disease is a major complication that induces morbidity in diabetic patients. Endothelial dysfunction plays a role in these complications. Nitric Oxide derived from arginine substrate, is an active vasodilator that controls tissue blood flow and vascular resistance for preventing cardiovascular disease and diabetes mellitus. Based on two systematic reviews and meta-

analyses, it can be concluded that L-arginine supplementation is still a matter of debate (pros and cons). L-arginine may be an alternative supplement for cardiovascular disease and diabetes mellitus, but further analysis in improved studies is needed to confirm.

Competing Interest

Authors declared no conflict of interest regarding this study.

List of Abbreviations

ADMA	: asymmetric dimethylarginine
AMP	: adenosine monophosphate
AMPK	: activated protein kinase
ASL	: arginosuccinate lyase
ASS	: arginosuccinate synthase
BH4	: tetrahydrobiopterin
CAD	: coronary artery disease
CEBM	: Centre for Evidence-Based Medicine
DM	: diabetes mellitus
EBCR	: evidence-based case report
eNOS	: endothelial nitric oxide synthase
FMD	: flow-mediated dilation
GTP	: guanosine triphosphate
iNOS	: inducible nitric oxide synthase
mTOR	: mammalian target rapamycin
NADPH	: nicotinamide adenine dinucleotide phosphate
NO	: nitric oxide
NOS	: nitric oxide synthase
PGC-1 α	: peroxisome proliferator- activated receptor-gamma coactivator 1 α
RCT	: randomized controlled trial
WHO	: World Health Organization

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Table 1. Resources and Search Strategy

Database	Search Strategy	Hits
PubMed	Search: ((diabetes mellitus [MeSH Terms]) AND ((cardiovascular disease [MeSH Terms]) AND ((L-arginine [MeSH Terms]) AND ((Endothelial [MeSH Terms])) Filters: Meta-Analysis, Observational Study, Randomized Controlled Trial, Systematic Review, Humans, English	93
Cochrane	#1 MeSH descriptor: [Diabetes mellitus] explode all trees 68561 #2 MeSH descriptor: [Cardiovascular disease] explode all 49343 #3 MeSH descriptor: [L-arginine] explode all trees 1538 #4 MeSH descriptor: [endothelial] explode all trees 17973 #5 #1 AND #2 AND #3 AND #4	22
ProQuest	(su (diabetes mellitus) AND (su (cardiovascular disease) AND su(L-arginine)) AND (su (endothelial function) Filters: Scholarly Journals, humans, English	607

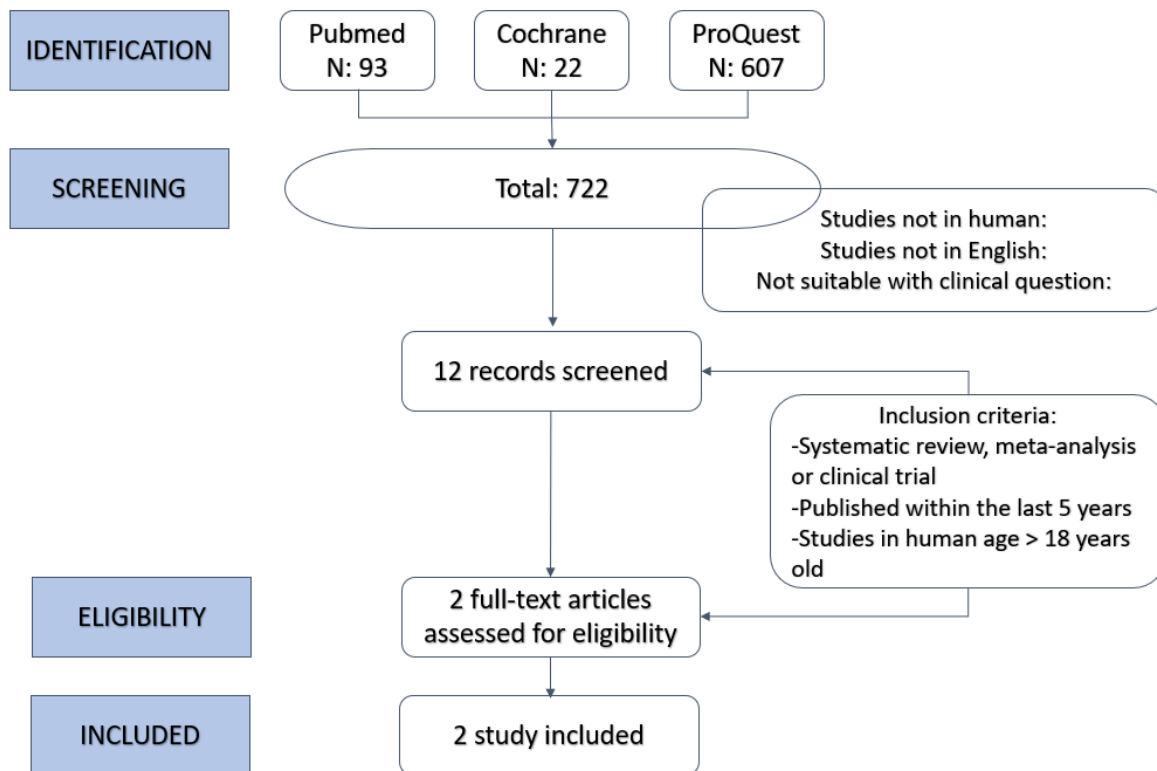


Figure 1. Flowchart of Literature Research

Table 2. Description of The Eligible Articles

	Krause JR, et al (2018) ¹⁶	Gambardella et al. (2020) ¹⁷
Research question of the study	To analyse the effects of L-arginine supplementation compared to placebo in individuals with cardiovascular disease, obesity, or diabetes	To analyse the functional role of arginine in the regulation of endothelial function and vascular in hypertension, ischemic heart disease, aging, peripheral artery disease, and diabetes mellitus.
Study design	Systematic review and meta-analysis of randomized controlled trials	Systematic review and meta-analysis of randomized controlled trials
Participants	Thirteen studies with 723 participants	Not clearly mentioned
Interventions	L-arginine or placebo treatments were orally administrated in all included studies via tablets or capsules, bars, or powder, and three studies did not report the form of administration	Intravenous infusion of arginine, oral supplementation and tap water
Outcome measurement	blood flow assessed by flow-mediated dilation (FMD), using the ultrasound technique. Secondary biochemical outcomes were nitrite/nitrate (NOx) rate and asymmetric dimethylarginine (ADMA), assessed by known enzymatic colorimetric assays	Blood flow assessed by flow-mediated dilation (FMD), nitrite/nitrate (NOx) rate and asymmetric dimethylarginine (ADMA)
Results	<ul style="list-style-type: none"> • There were no differences of the blood flow found between the supplementation of arginine and placebo groups • There were no differences was found in the mean difference of NOx responses between arginine or placebo treatments • There were no differences was found for the mean difference of ADMA responses comparing arginine and placebo treatments 	<ul style="list-style-type: none"> • L-arginine has therapeutic potential of endothelial function in CAD patients. • The use of arginine in the diabetic population, has a prophylactic treatment able to prevent cardiovascular complications of diabetes
Length of follow-up	three days - no limit set for maximum duration	three days - no limit set for maximum duration
Risk of bias	Minimal risk bias of individual studies (the lack of clarity regarding concealment of the allocation sequence, blinding of participants, and outcome assessment)	Not mentioned

Table 3. Critical Appraisal of Systematic Review and Meta-analysis

Parameters	Questions	Krause JR, et al (2018) ¹⁶	Gambardella et al. (2020) ¹⁷
Validity	Did the meta-analysis address a focused question?	Yes	Yes
	Were the criteria used to select articles for inclusion appropriate?	Yes	No
	Is it likely that important relevant studies were missed?	No	Yes
	Was the validity included studies appraised?	Yes	No
	Were the assessments of the reproducible?	Yes	No
	Were the results similar from study to study?	Yes	Yes
	Importance	What are the overall results of the meta-analysis?	<ul style="list-style-type: none"> • There were no differences of the blood flow found between the supplementation of arginine and placebo groups (mean difference = 0.30 (95% CI: -0.85, 1.46) • There were no differences was found in the mean difference of NOx responses between arginine or placebo treatments (mean difference = 4.41 (95% CI: -0.50, 9.32) • There were no differences was found for the mean difference of ADMA responses comparing arginine and placebo treatments (mean difference = -0.04 (95% CI: -0.15, 0.08)
How precise were the result?		Precise, 95% CI interval for TC and LDL are narrow	Not mentioned
Applicability		Can the results be applied to my patient care?	Yes
	Were all clinically important outcomes considered?	Yes	Yes
	Are the benefits worth the harm and costs?	Yes	Yes

CI: Confidence Interval