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The effect of L-arginine and citrulline on endothelial function in patients in heart failure with preserved ejection fraction

Arturo Orea-Tejeda[1, 2], Juan José Orozco-Gutiérrez[1], Lilia Castillo-Martínez[1, 2], Candace Keirns-Davies[4], Patricia Montaño-Hernández[1], Oscar Vázquez-Díaz[1], Adrián Valdespino-Trejo[1], Oscar Infante[3], Raúl Martínez-Memije[3]

[1]Heart Failure Clinic, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Mexico City, Mexico
[2]Cardiology Department, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Mexico City, Mexico
[3]Instrumentation Department, Instituto Nacional de Cardiología "ICh", Mexico City, México
[4]Massachussets General Hospital, Boston, USA

Abstract

Background: *To evaluate the effect of the amino acids L-arginine and citrulline on endothelial function in patients in stable diastolic and right heart failure using photoplethysmography.*

Methods: *Thirty patients from the Heart Failure Clinic of the Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" underwent photoplethysmography using the hyperemia technique. Index finger flow was assessed at baseline and after ischemia every 30 s by maximum amplitude time (MAT), total time of the curve (TT) and the index of the two (MAT/TT < 30 = normal) before and after the administration of L-arginine (8 g/day in two doses, n = 15) or citrulline (3 g/day in one dose, n = 15) for 60 days in addition to optimal pharmacological treatment.*

Results: *There were no statistically significant differences between the two groups at baseline. After the intervention, the MAT/TT index of all patients normalized in each evaluation period with statistically significant differences. Basal L-arginine group = 38.75 ± 11.52, final 23.32 ± 6.08, p = 0.007 and basal citrulline group = 41.4 ± 13.47, final 23.65 ± 6.74, p = 0.007 at 60–90 s. Post-ischemia: basal L-arginine 36.60 ± 11.51, final 18.81 ± 15.13, p = 0.004 and basal citrulline = 49.51 ± 15.17, final 27.13 ± 7.87, p = 0.003.*

Conclusions: *The administration of L-arginine and citrulline has a beneficial effect on endothelial function as shown by the normalized MAT/TT index. It probably improves systemic and pulmonary hemodynamics, which could help in the treatment of diastolic heart failure.* (Cardiol J 2010; 17, 5: 464–470)

Key words: L-arginine, citrulline, heart failure, endothelial function

Address for correspondence: Dr. Lilia Castillo-Martínez, Investigation Coordinator of Cardiology Department at Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Providencia 1218-A 402 Col. del Valle, Benito Juárez, CP 03100 Mexico City, Mexico, tel./fax: (5255) 55-13-93-84, e-mail: caml1225@yahoo.com.mx

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Introduction

Congestive heart failure (CHF) is a multisystem clinical syndrome with considerable morbidity and mortality. The patho-physiology involves interactions among multiple types of damage, including ventricular remodeling, over-activation of the neuro-hormonal system and cytokines, as well as endothelial dysfunction [1].

Endothelial dysfunction plays a fundamental role in various conditions such as systemic hypertension and heart failure. For now, no specific treatment is available [1].

Oral administration of L-arginine has been shown to increase endothelium-dependent vasodilatation by the liberation of nitric oxide (NO) in patients with hypercholesterolemia. It has also been associated with clinical improvement in patients with atheromatosis of the left anterior descending coronary artery [2].

L-arginine is a semi-essential basic amino acid formed from citrulline and ornithine that participates as a fundamental intermediary in the urea cycle. It is a precursor in the formation of nitric oxide by the enzyme nitric oxide synthase. It directly increases the formation of NO and indirectly stimulates the liberation of growth hormone, which acts through insulin-like growth factor 1 (IGF-1) [3]. L-arginine generates an intermediate called agmatine that acts on alpha 2 clonidine type receptors. All of these effects are achieved with oral doses ranging from 5.6 to 12.6 g/day (maximum 30 g) [3].

Citrulline is an alpha amino acid that is metabolized to L-arginine in the vascular endothelium, kidney and other cells. Oral citrulline has been found to be more effective than L-arginine in producing an increase in the blood levels of L-arginine; a dose of 3.8 g/m^2 body surface area increases the peak concentration of L-arginine by 227% four hours after administration, compared to a 90% increase with the same dose of L-arginine. The explanation is that citrulline does not undergo intestinal or hepatic metabolism because it is not a substrate for arginase, and therefore does not induce its expression and activation. Consequently, L-citrulline holds promise in the treatment of endothelial dysfunction, and perhaps in cardiovascular disease, in which L-arginine deficiency and bioavailability of NO is involved [4, 5].

Photoplethysmography is a simple, low cost, optically based technique that provides a way to evaluate vascular function by detecting changes in blood flow and pulse in tissular microvascular space [6]. It can evaluate endothelial function indirectly by sensing vasodilatation in the index finger. The change in pulse amplitude in the digital wave is the result of flow mediated vasodilatation resulting from nitric oxide liberation [7] and is considered to be a complex response to ischemia. It is expressed as a change in the microvasculature, in this case in the fingers, and reflects endothelial function [8]. Some studies also associate alterations in pulse amplitude as a response to flow mediated vasodilatation and endothelial dysfunction in the coronary arteries [9–11].

This study was designed to evaluate the effect of L-arginine or citrulline malate administration on endothelial function (flow mediated vasodilatation) using photoplethysmography in heart failure patients with preserved ejection fraction (HFpEF).

Methods

Study population

From January to December 2008 outpatients who came to the Heart Failure Clinic of the Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" (INCMNSZ) were recruited. Patients were aged 18 or over, men and non-pregnant women with a confirmed diagnosis of heart failure with preserved ejection fraction. HFpEF was characterized by signs and symptoms of heart failure and by echocardiogram with left ventricular ejection fraction ≥ 45%, shortening fraction ≥ 28%, left atrial diameter > 45 mm, ventricular septal thickness > 12 mm, posterior wall thickness > 12 mm, slow, inverted, pseudonormal or restrictive transmitral Doppler flow pattern [12] and right ventricular dysfunction defined as an ejection fraction ≤ 35% measured by radioisotopic ventriculography [13, 14]. The patients were stable and in New York Heart Association (NYHA) functional classes I–III. Patients were excluded if they presented ischemia that was susceptible to revascularization, myocardial infarction, unstable angina or a myocardial revascularization procedure, including percutaneous transluminal coronary angioplasty and aorto-coronary by-passes. Other exclusion criteria included a cerebrovascular event within the last three months, dysfunctional prosthetic valve, obstructive or non-obstructive hypertrophic cardiomyopathy, uncorrected congenital heart disease, active myocarditis, history of resuscitation after sudden death, and severe arrhythmias.

All patients received standard CHF therapy (diuretics, angiotensin converting enzyme inhibitors, angiotensin II antagonists, aldosterone receptor blockers, digitalis and beta-adreno-receptor blockers).

Patients were consecutively randomized to a group with oral L-arginine supplementation

(3 g powder daily, n = 15) or an oral citrulline malate group (3 g powder daily, n = 15). The duration of amino acid administration and follow-up was two months.

The present study was approved by the Institutional Ethics Committee of Biomedical Research in Humans of the INCMNSZ. All patients were informed regarding the purpose of the study and signed informed consent forms.

Measurement

Photoplethysmography. A baseline digital photoplethysmographic wave was recorded for 30 s. The forearm was then compressed with a sphygmomanometer cuff for 5 min using a pressure of 30 mm Hg above the systolic arterial pressure recorded (ischemic phase). The compression was then released and the digital photoplethysmographic wave was recorded for 120 s. The wave was analyzed at 30 s intervals for comparison with the baseline values. The most representative waves were selected from the recording of each interval, and the maximum amplitude time (MAT) and total time (TT) were measured in order to calculate the MAT/TT index. A MAT/TT index of less than 30 was considered normal, as proposed in other studies [9, 15].

All evaluations were performed at the beginning, and two months after initiating supplementation, by a cardiologist who was blinded to the patient's study group.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and categorical variables as absolute and relative frequency. For comparisons between the two groups at baseline, χ^2 or Fisher's exact test for categorical and the Mann-Whitney U test for continuous variables were used. To compare the changes from baseline to two months (end of study), the Wilcoxon signed rank test were used for continuous variables. A p value of < 0.05 was considered statistically significant. All analyses were performed using a commercially available package (SPSS for Windows, version 10.0 1999 Chicago: SPSS Inc.).

Results

Of the 30 patients included in the study, two were eliminated from the arginine group. One developed gastrointestinal symptoms, and the other was hospitalized for a non-cardiovascular cause. One patient in the citrulline group underwent non-cardiovascular surgery. Thus, 13 subjects in the L-arginine group were analyzed and 14 in the citrulline group. With the exception of the patient with gastric intolerance, the supplements were well tolerated.

Table 1 lists the baseline clinical characteristics of the study groups. It is evident that the principal co-morbidities were systemic hypertension, obesity and diabetes mellitus. There were no significant differences between the two groups at baseline. Echocardiograms for the two groups were also similar (Table 2).

The principal echocardiographic changes that were evident after two months of therapy with the amino acids were a statistically significant fall in pulmonary arterial pressure and decreased right ventricular diastolic diameter and left ventricular systolic diameter. These changes were more noteworthy in the arginine group and approached statistical significance (Table 3).

The changes in endothelial function revealed by photoplethysmography are shown in Table 4. Statistically significant decreases in maximum amplitude time, total time and the MAT/TT can be seen in all of the intervals in both groups. MAT/TT indices achieved normal values at the end of the intervention in both groups (Table 4).

In Figures 1 and 2, the changes observed in the MAT/TT indices in both groups after intervention are shown. After two months they had normalized in all intervals, a statistically significant finding.

Discussion

The well-documented process of progressive deterioration of cardiac performance in CHF has been ascribed to multiple causes [16]. At a functional level, reduced coronary endothelial vasodilator function in CHF has been well established [17]. A consequence of endothelial dysfunction is the inability of a vessel to dilate in response to physiological stimuli such as increases in blood flow, reflecting impaired flow-dependent, endothelium-mediated vasodilation (FDD) [18].

Studies of endothelial function, both in peripheral and coronary circulation, in clinical and experimental CHF have consistently shown evidence of diminished endothelium-dependent vasodilator function [19]. Inasmuch as endothelial vasodilator function is involved in controlling tissue perfusion, impaired exercise-induced release of NO may contribute to reduced exercise capacity in chronic heart failure with more severe symptoms [20].

This impaired FDD in heart failure is a generalized abnormality that occurs in both peripheral

Table 1. Baseline clinical characteristics of the study population.

Variables	Arginine (n = 15)	Citrulline (n = 15)	P
Age (years)	63.07 ± 14.5	66.80 ± 10.7	0.30
Women	7 (46.7%)	6 (40%)	0.78
Men	8 (53.3%)	9 (60%)	
NYHA I	8 (53.3%)	4 (26.7%)	0.14
NYHA II	6 (40%)	11 (73.7%)	0.14
NYHA III	1 (6.7%)	0 (0%)	0.14
Right heart failure	14 (93.3%)	10 (66.7%)	0.08
History of AMI	1 (6.7%)	6 (40%)	0.04
Ischemic heart disease	6 (40%)	7 (46%)	0.71
Diabetes mellitus	8 (53.3%)	8 (53.3%)	0.64
Systemic hypertension	14 (93.3%)	13 (86.7%)	0.54
COPD	1 (6.7%)	1 (6.7%)	0.75
Atrial fibrillation	3 (20%)	3 (20%)	0.67
Pulmonary arterial hypertension	10 (71.4%)	13 (86.7%)	0.29
Obstructive sleep apnea	4 (30.8%)	1 (8.3%)	0.18
Hypothyroidism	4 (26.7%)	6 (40%)	0.35
Dyslipidemia	7 (70%)	5 (50%)	0.32
ACEI	2 (13.3%)	6 (40%)	0.49
ARA	12 (80%)	9 (60%)	0.06
Aspirin	6 (40%)	5 (33.3%)	0.50
Thiazide diuretic	6 (40%)	3 (20%)	0.21
Loop diuretic	4 (26.7%)	5 (33.3%)	0.07
Nitrates	2 (13.3%)	4 (26.7%)	0.07
Statins	3 (20%)	5 (33.3%)	0.002
Fibrates	2 (13.3%)	3 (20%)	0.62
Spironolactone	13 (86.7%)	10 (66.7%)	0.27
Beta-blocker	12 (80%)	12 (80%)	0.07
Amiodarone	1 (6.7%)	3 (20%)	0.45
Digitalis	5 (33.3%)	3 (20%)	0.34

Data is presented as mean ± standard deviation or n (%); AMI — acute myocardial infarction; NYHA — New York Heart Association; COPD — chronic obstructive pulmonary disease; ACEI — angiotensin converting enzyme inhibitor; ARA — angiotensin receptor antagonist

Table 2. Baseline echocardiographic findings of the study groups.

Echocardiographic findings	Arginine (n = 15)	Citrulline (n = 15)	P
LVEF (%)	60.2 ± 8.09	60.0 ± 9.49	0.18
Filling pattern:			0.35
Slow	7 (50%)	8 (57.1%)	X
Pseudonormal	3 (21.4%)	2 (14.3%)	X
Inverted	2 (14.3%)	0 (0%)	X
LVSD [mm]	31.2 ± 5.57	32.1 ± 6.37	0.88
LVDD [mm]	47.8 ± 6.34	41.8 ± 10.32	0.92
Left atrial diameter [mm]	41 ± 9.33	37.9 ± 7.62	0.34
Ventricular septal thickness [mm]	11.6 ± 2.16	12.6 ± 2.38	0.11
Posterior wall thickness [mm]	10.6 ± 1.84	11.6 ± 2.02	0.71
PASP [mm Hg]	57.2 ± 19.89	53.7 ± 8.40	0.86
Systemic blood pressure:			
Systolic pressure [mm Hg]	137.5 ± 20.05	138.1 ± 16.0	0.92
Diastolic pressure [mm Hg]	86.0 ± 14.3	81.82 ± 10.78	0.41

Data is presented as mean ± standard deviation or n (%); LVEF — left ventricular ejection fraction; LVSD — left ventricular systolic diameter; LVDD — left ventricular diastolic diameter; PASP — pulmonary arterial systolic pressure

Table 3. Comparison of baseline and final echocardiographic findings for treatment groups.

Echocardiogram	Arginine (n = 13)	P	Citrulline (n = 14)	P
LVEF (%):				
Baseline	60.6 ± 8.62	0.74	60.0 ± 10.3	0.61
2 months	61.32 ± 7.67		58.0 ± 9.76	
LVSD [mm]:				
Baseline	30.8 ± 5.45	0.07	30.3 ± 5.41	0.20
2 months	29.0 ± 5.90		28.7 ± 5.45	
Ventricular septal thickness [mm]:				
Baseline	11.86 ± 2.23	0.32	12.4 ± 2.27	0.26
2 months	12.28 ± 1.98		13.02 ± 1.82	
Posterior wall thickness [mm]:				
Baseline	11.27 ± 1.85	0.31	12.4 ± 2.27	0.12
2 months	10.86 ± 1.55		11.0 ± 1.56	
RVDD [mm]				
Baseline	41.0 ± 7.29	0.07	42.17 ± 6.43	0.18
2 months	37.8 ± 5.83		40.33 ± 6.59	
PASP [mm Hg]:				
Baseline	56.33 ± 9.98	0.02	56.67 ± 7.96	0.02
2 months	44.07 ± 16.49		47.67 ± 8.59	
Systemic systolic pressure [mm Hg]:				
Baseline	137.5 ± 20.05	0.002	138.1 ± 16.0	0.006
2 months	117.8 ± 10.87		122.9 ± 19.0	
Systemic diastolic pressure [mm Hg]:				
Baseline	86 ± 14.3	0.005	81.82 ± 10.78	0.05
2 months	70.5 ± 10.37		74.36 ± 7.03	

Data is expressed as mean ± standard deviation; LVEF — left ventricular ejection fraction; LVSD — left ventricular systolic diameter; RVDD — right ventricular diastolic diameter; PASP — pulmonary arterial systolic pressure

Table 4. Comparison of baseline and final endothelial function for treatment groups.

Photoplethysmographic indices	Arginine (n = 13)	P	Citrulline (n = 14)	P
MAT/TT, pre-ischemia:				
Baseline	38.75 ± 11.52	0.007	41.4 ± 13.47	0.007
2 months	23.32 ± 6.08		23.65 ± 6.74	
MAT/TT, 0–30 s post-ischemia:				
Baseline	38.91 ± 9.31	0.005	40.90 ± 9.27	0.001
2 months	21.32 ± 16.43		32.10 ± 6.45	
MAT/TT, 30–60 s post-ischemia:				
Baseline	39.86 ± 12.47	0.004	42.96 ± 8.82	0.003
2 months	21.32 ± 16.43		32.10 ± 6.45	
MAT/TT, 60–90 s post-ischemia:				
Baseline	36.60 ± 11.51	0.004	49.51 ± 15.17	0.003
2 months	18.81 ± 15.13		27.13 ± 7.87	
MAT/TT, 90–120 s post-ischemia:				
Baseline	33.47 ± 7.67	0.018	49.82 ± 18.39	0.047
2 months	14.74 ± 17.67		25.60 ± 4.65	

Data is presented as mean ± standard deviation; MAT/TT — maximum amplitude time/total wave time

and coronary circulation and appears to be due to the reduced availability of NO [21]. Endothelial dysfunction is also associated with accelerated progression of heart failure [20]. Patients with CHF and more severe endothelial dysfunction would have a higher incidence of hospitalization for decompen-

sated CHF, cardiac transplantation, or cardiac death than those with better preserved endothelium-dependent relaxation [20].

Recchia et al. [22] showed that the cardiac production of NO declined during the development of heart failure in the rapid pacing canine model of CHF.

Functional studies of cardiac NO synthase inhibition or endocardial removal include impaired diastolic relaxation [23], which is commonly observed in CHF. Numerous investigators have demonstrated that under conditions of L-arginine depletion, NO synthase is capable of generating superoxide radicals [24], and there is evidence that inhibition of NO synthase results in impaired myocardial perfusion during adenosine-induced hyperemia, suggesting that endothelium-derived NO plays a significant role in the regulation of myocardial perfusion [25] and also contributes to increased exercise capacity after physical training in patients with CHF [26].

Depleted L-arginine was apparent in our study by the MAT/TT index obtained from photoplethysmography, inasmuch as the index before intervention was above normal values, as described in other studies [6, 15]. As other investigators have reported [23], endothelial dysfunction was confirmed in HFpEF [27], since the baseline values of both systemic and pulmonary pressure were increased, even with antihypertensive treatment.

These alterations of the vasculature impact the physiology of the heart and lead to the deterioration of function, with diminished right ventricular ejection fraction and increased diastolic diameter. It is probable that the left ventricle maintains its ejection fraction within normal limits, at least temporarily, because it manages low filling volumes.

There are reports of decreased systemic and pulmonary pressure with the consumption of arginine and citrulline [28, 29]. In this series, statistically significant improvement in these values was also found, supporting the concept of improved endothelial function because of a greater availability of nitric oxide. This probably led to a reduction in right ventricular afterload, resulting in the improvement in ejection fraction demonstrated by radioisotopic ventriculography after exercise and decreased right ventricular diastolic diameter in the echocardiogram that tended toward statistical significance.

Since improvements in systemic and pulmonary arterial pressure and right ventricular function were similar in the two groups, these results indicate that citrulline is not less effective than arginine. Moreover, the fact that citrulline was similar to arginine but required a lower dose, and was associated with better gastric tolerance, would suggest that it may be the best option in some patients.

In patients with HFpEF and right heart failure it is plausible that normalization of the MAT/TT index is evidence of more efficient FDD, and use of these amino acids as adjuvants to conventional heart failure treatment could optimize cardiac effort with the best impact after exercise.

Limitations of the study

This study lacked a control group, and the number of patients was small. However, it is noteworthy that in spite of the small sample the results were statistically significant when subjects were compared before and after intervention.

We also recognize that the intervention time was short, and probably with a longer follow-up some variables could have similar findings to that observed with right heart failure and with statistical significance.

Conclusions

The administration of arginine and/or citrulline in patients in HFpEF improves endothelial function because of the effects on FDD (post-ischemia MAT/TT index).

Systolic and diastolic arterial pressures as well as pulmonary arterial systolic pressure decrease significantly after arginine or citrulline administration, which improves right ventricular diastolic diameter.

More studies are necessary to validate these effects in a greater number of patients and their impact on patients with diminished systolic function.

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