ORIGINAL ARTICLE

Arginine Therapy and Cardiopulmonary Hemodynamics in Hospitalized Children with Sickle Cell Anemia

A Prospective, Double-blinded, Randomized Placebo-controlled Clinical Trial

Richard Onalo[1], Antoinette Cilliers[2], Peter Cooper[3], and Claudia R. Morris[4,5,6]

[1]Cardiology Unit, Department of Paediatrics, Faculty of Clinical Sciences, University of Abuja, Abuja, Nigeria; [2]Division of Paediatric Cardiology, Department of Paediatrics, Chris Hani Baragwanath Academic Hospital, and [3]Department of Paediatrics and Child Health, University of the Witwatersrand, Johannesburg, South Africa; [4]Division of Pediatric Emergency Medicine, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia; [5]Children's Healthcare of Atlanta, Atlanta, Georgia; and [6]Center for Clinical and Translational Research of Children's Healthcare of Atlanta and Emory University, Atlanta, Georgia

ORCID ID: 0000-0001-6019-2858 (C.R.M.).

Abstract

Rationale: Acute changes in cardiopulmonary hemodynamics that include tricuspid regurgitant jet velocity (TRV) elevation measured by Doppler echocardiography are often encountered during sickle cell vasoocclusive pain and acute chest syndrome (ACS). Arginine and nitric oxide depletion develop in patients with these complications. Arginine administration may therefore improve nitric oxide bioavailability and potentiate pulmonary vasodilatation.

Objectives: To evaluate effects of L-arginine supplementation on Doppler indices of cardiopulmonary hemodynamics in children with sickle cell anemia experiencing pain.

Methods: This was a prospective, double-blinded, randomized placebo-controlled trial of oral arginine in children with sickle cell anemia age 5–17 years hospitalized with severe pain and/or ACS.

Measurements and Main Results: Blood biomarkers and Doppler echocardiographic indices of cardiopulmonary hemodynamics were measured before and after supplementation. The mean change in TRV, pulmonary artery systolic pressure, mean pulmonary artery pressure, and other indices of cardiopulmonary hemodynamics were tested with paired Student's t test and correlated with markers of arginine bioavailability using Pearson correlation. Sixty-six children were randomized into arginine versus placebo groups. An elevated TRV $\geq$ 2.5 m/s was seen in 40 (61%) patients. A Day 5 Doppler echocardiogram was performed in 47 patients who remained hospitalized. A greater reduction in median TRV occurred in the arginine group than placebo (22.2%, $n = 22$ vs. 3.8%, $n = 25$; $p < 0.01$). A larger percentage increase in global arginine bioavailability was associated with a lower TRV after 5 days of supplementation ($r = -0.533$; $P = 0.001$). Significant differences in multiple indices of cardiopulmonary hemodynamics and mean N-terminal pro B-type brain natriuretic peptide were also noted after arginine therapy.

Conclusions: Oral arginine supplementation improves cardiopulmonary hemodynamics during sickle cell disease vasoocclusive pain and ACS.

Clinical trial registered with Pan African Clinical Trial Registry https://pactr.samrc.ac.za/Search.aspx (PACTR201611001864290).

Keywords: arginine; tricuspid regurgitation jet velocity; PH; sickle cell vasoocclusive pain crisis; acute chest syndrome

(Received in original form August 19, 2021; accepted in final form April 15, 2022)

Supported by the Tertiary Education Trust Fund (R.O.) and in part by NIH National Center for Complementary and Integrative Health grant K24AT009893 (C.R.M.).

Author Contributions: All authors made substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work. R.O. drafted the manuscript, and A.C., P.C., and C.R.M. revised it critically for important intellectual content. All authors provided final approval of the version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data sharing statement: For original data, please contact richardonalo@yahoo.com.

Correspondence and requests for reprints should be addressed to Claudia R. Morris, M.D., Professor of Pediatrics and Emergency Medicine, the Wilbur Fisk Glenn Jr. Distinguished Faculty Chair for Clinical and Translational Research, Division of Pediatric Emergency Medicine, Department of Pediatrics, Emory University School of Medicine, 1760 Haygood Drive NE W458, Atlanta, GA 30322. E-mail: claudia.r.morris@emory.edu.

This article has a related editorial.

Am J Respir Crit Care Med Vol 206, Iss 1, pp 70–80, Jul 1, 2022

Originally Published in Press as DOI: 10.1164/rccm.202108-1930OC on April 15, 2022
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Acute changes in cardiopulmonary hemodynamics that include tricuspid regurgitant jet velocity elevation measured by Doppler echocardiography are often encountered during sickle cell vasoocclusive pain and acute chest syndrome. Arginine and nitric oxide depletion develop in patients with these complications, associated with pain severity.

What This Study Adds to the Field: Oral arginine supplementation improves cardiopulmonary hemodynamics during sickle cell disease vaso-occlusive pain and acute chest syndrome. Given the safety, tolerability, and low cost of this nutritional supplement, the potential value of arginine therapy in low-resource environments common to sub-Saharan Africa and throughout the world is high.

Pulmonary hypertension (PH) is common in sickle cell disease (SCD) and is associated with increased hemolytic rate and early mortality (1–7). Tricuspid regurgitant jet velocity (TRV) ⩾ 2.5 m/s measured by Doppler echocardiogram (echo) can identify patients at risk for PH, although a right heart cardiac catheterization is needed to make the definitive diagnosis. However, TRV is a noninvasive and useful screening tool for PH while also identifying patients with SCD with a high hemolytic rate and those at risk for early death (1, 3, 4, 8–15).

Abnormal Doppler-derived indices of cardiopulmonary hemodynamics in SCD are well established in the literature (1–6, 8–14), although the clinical implications for children remain more elusive compared with adults (15, 16). Pediatric studies evaluating echocardiographic markers suggestive of PH have documented an elevated TRV > 2.5 m/s in 10–25% of children with SCD (8, 11, 12, 17–19). In the longitudinal PUSH (Pulmonary Hypertension and the Hypoxic Response in SCD) study by Gordeuk and colleagues, a 14% prevalence of a TRV > 2.5 m/s was found among children with the SS genotype at baseline. Those with higher TRV had higher biomarkers of hemolysis and worse 6-minute-walk capacity. Of children with a normal TRV, 15% developed TRV elevation during a 22-month follow-up period. Increased mortality risk occurred among children with a TRV > 2.6 m/s (11, 12). Because even mild elevations in pulmonary artery pressures are poorly tolerated in patients with SCD (20), a better understanding of mechanisms that contribute to elevated pulmonary artery pressures are needed.

Painful vasoocclusive episodes (VOE) and acute chest syndrome (ACS) in SCD are often associated with worsening anemia, enhanced red cell adhesion, hypoxemia, and vasoconstriction, which could promote or exacerbate PH. Elevations in TRV are common during VOE (21, 22) and ACS (20), and up to 13% of affected individuals may develop cor pulmonale (20). During VOE, echo measurements of TRV have been shown by Machado and colleagues to significantly increase from a mean of 2.4 m/s in steady state to 2.9 m/s during VOE in an adult study performed in the United States (22). In a separate study, Mekontso Dessap and colleagues reported that the prevalence of TRV ⩾ 2.5 m/s rose from 32% in steady state to 60% during ACS (20).

Arginine is a nutritional amino acid and the obligate substrate for production of nitric oxide (NO), a potent vasodilator. Mechanistically, abnormal arginine and NO bioavailability contribute in part to the vasculopathy that occurs in SCD (7, 23–25). Specifically, arginine depletion is associated with PH risk (23, 26), early mortality (23, 27), ACS, and degree of pain severity in adults and children with SCD (28–30). Low plasma arginine concentrations also predict the need for pediatric hospitalization for VOE (28). Heightened hemolytic rate during sickle cell crises contributes to the release of cell-free hemoglobin and erythrocyte-derived arginase into circulation, which rapidly depletes both NO and arginine (7, 24). Arginine replacement therapy may therefore represent a strategy to replete NO (2, 31, 32).

Oral arginine improved Doppler-estimated pulmonary artery systolic pressure (PASP) by >15% in patients with SCD and PH risk identified by TRV elevation (26). Clinical trials in humans have also demonstrated the therapeutic benefits of arginine therapy in individuals without SCD with idiopathic and secondary PH (31). In the report by Mehta and colleagues (33), a 28% reduction in pulmonary vascular resistance (PVR) and a 16% reduction in the mean pulmonary artery pressure (mPAP) was documented in 10 adult patients with PH (secondary to chronic ischemic cardiomyopathy) undergoing right heart cardiac catheterization after infusion of 500 mg/kg parenteral arginine. Their findings were further corroborated by another report of improved oxygenation and decreased PVR in infants with persistent PH of the newborn (34). Additional evidence of the benefits of arginine therapy was illustrated by Nagaya and colleagues (35) in a placebo-controlled study involving 10 adults with PH, in which a significant reduction in mPAP and PVR occurred in response to arginine therapy.

Currently, standard drugs used in the management of PH, such as bosentan and sildenafil, are expensive and are not readily available in many areas of Nigeria. In addition, there are numerous reports of disappointing results with the use of these drugs in clinical studies targeting patients with SCD (3). Arginine represents a safe and inexpensive nutritional supplement that may have value, particularly in low-resource environments. Promising phase 2 randomized controlled trials (RCTs) of both oral and parenteral arginine (30, 36, 37) to treat children with SCD hospitalized with pain have demonstrated an impact on total opioid use, pain scores, time to crisis resolution, and/or length of hospital stay. Improved blood pressure (38), decreased oxidative stress, and improved mitochondrial function have also been documented (39). This study aimed at evaluating the impact of L-arginine supplementation on cardiopulmonary hemodynamics in children hospitalized with VOE and ACS.

Some of the results of this study have been previously reported in the form of abstracts and publications from the parent trial (36, 38).

Materials

Study Design and Participants

This study was part of a parent trial designed as a prospective, phase 2, double-blind, placebo-controlled RCT of oral arginine supplementation given for pain control involving children (5–17 years old) with SCA hospitalized for VOE with or without ACS that took place in Nigeria over a 2-year

period (February 2017–February 2019). Inclusion criteria included sickle-related severe pain defined by a Numerical Pain Rating Score of ⩾7 on a 0-to-10-point severity scale (36). This study was approved by the appropriate institutional review boards of the University of Abuja Teaching Hospital and National Hospital, Abuja, Nigeria, where the study was performed. Written informed consent was obtained from all participants' parents or guardians as applicable. The study was registered at the Pan African Clinical Trial Registry (PACTR201611001864290) (36). All patients were homozygous for hemoglobin-SS. Patients with a known or suspected congenital heart disease were excluded from the study.

Sample Size Calculation

Sample size calculation was based on the data published by Morris and colleagues on arginine therapy targeting patients with SCD at risk for PH (26). Using an assumed PASP of 64 mm Hg, power of 80%, and a significance level of 5%, a sample size of 33 patients each in the arginine and placebo groups (total of 66 patients) was adequate to detect a mean change of 10 mm Hg in Doppler-estimated PASP, with the assumption that the response of PASP would be similar to that observed by Morris and colleagues (26).

Statistical Analysis

Patients discharged before completion of the 5-day study protocol were treated as missing at random; to handle missing data, the complete-case analysis approach was used such that only patients with paired data (before and after supplement) were included in the final statistical analysis of cardiopulmonary hemodynamics.

Descriptive statistics were reported as mean ± SD or mean or median with interquartile range (IQR) for continuous data and counts with percentages for categorical data. Mean values of heart rates, blood pressures, and oxygen saturation in the two study groups were compared using Student's *t* test. A paired *t* test was used for pre- versus postsupplement comparisons for variables with normal distribution. Normality was tested using the Shapiro-Wilk test, and the Wilcoxon signed-rank test was used for comparing the indices of cardiopulmonary hemodynamics and plasma concentrations of arginine and other biomarkers of cardiac function among the patients. Pearson correlation coefficient was used when appropriate.

Data were analyzed using STATA 14 (StatCorp, LP). A *P* value of less than 0.05 was set as the level of statistical significance.

Intraobserver reproducibility of echocardiographic measurements was tested using Bland-Altman agreement analysis as was previously reported (21).

Study Procedures

Eligible children admitted into the Emergency Paediatric Unit with the diagnosis of sickle cell crisis consistent with acute VOE with and without ACS (defined by chest pain and breathing difficulty, with new infiltrates on chest radiography) were recruited and had transthoracic echo performed through previously described methods (21, 26) within 6 hours of admission. Blood samples for plasma arginine, citrulline, and ornithine were obtained at the time of intravenous access for medications and on Day 5 of the study; the global arginine bioavailability ratio (GABR, defined as plasma concentration of arginine/[citrulline + ornithine]) was measured using high-performance liquid chromatography with tandem mass spectrometry as previously described (23, 40). Blood samples were analyzed for plasma N-terminal pro B-type brain natriuretic peptide (NT-proBNP) and cardiac troponin-I by means of a fully automated electrochemiluminescence immunoassay method using the Maglumi 800 analyzer (Snibe Diagnostics, Shenzen Mindray Biomedical).

Enrolled patients were randomized into an oral L-arginine or placebo (sucrose) arm using a block randomized design (block size of four), in which patients were randomized by the study center in a 1:1 ratio of placebo to L-arginine as soon as they presented to the emergency department or within 6 hours of admission in a double-blinded fashion. The study drug or placebo was dissolved in red grape juice and ingested immediately at the dose of 300 mg/kg/d in three divided doses (100 mg/kg/dose) for 5 days (15 doses) or until discharge, whichever came first, as previously described (36). Patients received standard unit protocol management for VOE and/or ACS. Blood samples and echocardiography were repeated on Day 5 of admission after completion of the supplementation protocol. Transthoracic echocardiography was done using a General Electric Vivid-e ultrasound machine with a 6S transducer probe, capacity for image storage and offline analysis, and an integrated calculation package. Standard two-dimensional, M-mode, and Doppler echocardiograms were performed by the researcher in the supine and left lateral decubitus positions, and measurements were done according to the American Society of Echocardiography guidelines. Pulse wave Doppler Tei index of the left and right ventricles was determined from the ratio of the summation of isovolemic relaxation and contraction times to the ejection time. The pulmonary artery acceleration time (PAAT) was obtained from the pulse wave Doppler signal just proximal to the pulmonary valve, and the ratio of PAAT and right ventricular (RV) ejection time computed. Mitral and tricuspid annular plane systolic excursions were measured using the standard M-mode technique with the cursor placed at the lateral site of the annulus from the apical four-chamber view and interpreted using published normative values. Linear measurements of internal left ventricular diameter and septal and inferior-lateral wall thickness at end-diastole and end-systole, respectively, were obtained using M-mode measurements from the parasternal short-axis view just below the tips of the mitral valve leaflets. Simultaneously recorded electrocardiograph was used as a reference to accurately determine end-systole and end-diastole.

Continuous-wave Doppler sampling of the peak TRV was used to estimate the RV systolic pressure and hence pulmonary artery systolic pressure in the absence of RV outflow tract obstruction with the use of the modified Bernoulli equation. Estimated pulmonary artery end-diastolic pressures were obtained by applying the modified Bernoulli equation to the pulmonary regurgitation end-diastolic velocity Doppler signal. The mean right atrial pressure was estimated according to the degree of collapse of the inferior vena cava with inspiration: 5 mm Hg for a collapse of >50%, 10 mm Hg for a collapse of 25–50%, and 15 mm Hg for a collapse of <25%. All measurements were made online by a single investigator using an average of three cardiac cycles. The images were saved and reanalyzed offline by the same researcher to obtain a second reading. The values obtained were tested for intraobserver variability using Bland-Altman analysis.

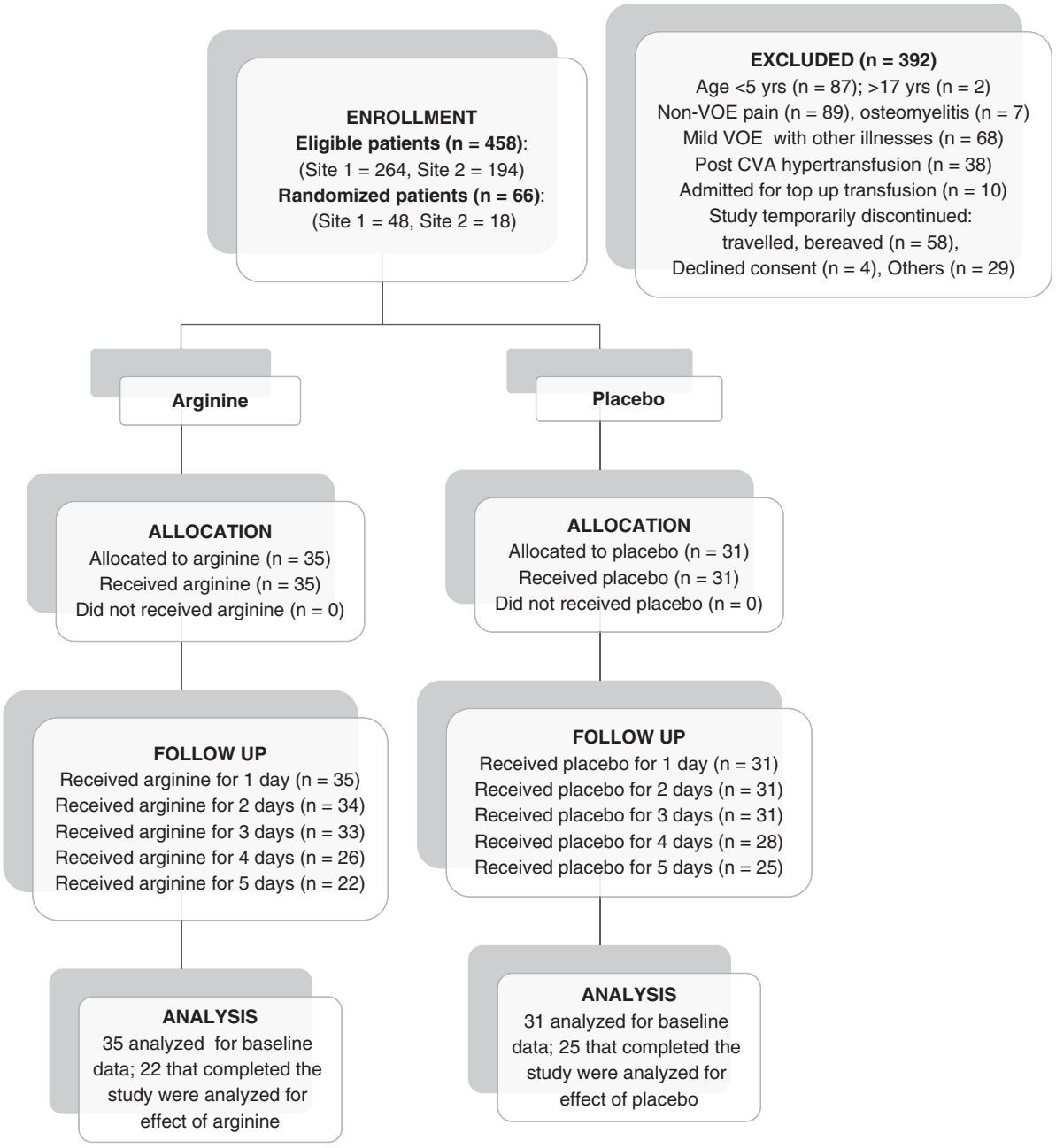

Figure 1. Consolidated Standards of Reporting Trials flow diagram. The enrollment and randomization process of participants in impact of arginine on cardiovascular changes in crisis trial. Other reasons for exclusion: guardian not available to give consent ($n = 3$), needed immediate blood transfusion ($n = 6$), readmission ($n = 7$), hepatitis ($n = 5$), treatment commenced before diagnosis was made ($n = 5$), inadvertently missed ($n = 2$), and acute kidney injury ($n = 1$). CVA = cerebrovascular accident; VOE = vasoocclusive episodes.

Results

Baseline Characteristics of the Study Patients

A total of 66 children with SCA were included. The details of patient enrollment and randomization are shown in the consort flow diagram (Figure 1). Baseline demographics and characteristics of patients in the two study arms are in Table 1.

The mean patient age was 10.6 ± 3.3 years, and 58% were male. Echo indices of cardiopulmonary hemodynamics were measured before and after supplementation. Baseline values of TRV, mPAP, and other echocardiographic indices are summarized in Table 1 for all participants and those randomized to receive either arginine ($n = 35$) or placebo ($n = 31$). The mean TRV at presentation was 2.4 ± 0.7 m/s, and the median was 2.6 m/s (IQR, 2.2–2.8 m/s).

Overall, an elevated TRV ⩾ 2.5 m/s was seen in 40 (61%) patients, and an estimated

Table 1. Baseline Characteristics of Patients Randomized into the Two Study Groups

Baseline Parameters	All (*N* = 66)	Arginine Group (*n* = 35)	Placebo Group (*n* = 31)	*P* Value
Sex, male	38 (58)	18 (51)	20 (65)	0.578
Age, yr	10.6 ± 3.3	10.8 ± 3.2	10.5 ± 3.6	0.721
Weight, kg	31.0 ± 11.6	30.3 ± 8.3	31.7 ± 14.5	0.627
Height, cm	140.7 ± 16.4	139.9 ± 15.6	141.7 ± 17.5	0.660
Oxygen saturation	96 ± 3	95 ± 4	96 ± 4	0.315
Hemoglobin at presentation, g/dl	7.2 ± 1.4	7.1 ± 1.2	7.3 ± 1.6	0.565
Arginine, μM	101 ± 49	91 ± 47	111 ± 50	0.099
Ornithine, μM	237 ± 176	250 ± 190	223 ± 162	0.539
Citrulline, μM	13 ± 6	13 ± 7	13 ± 6	>0.999
Arginine/ornithine	0.53 ± 0.34	0.49 ± 0.35	0.54 ± 0.33	0.554
GABR	0.49 ± 0.3	0.45 ± 0.32	0.51 ± 0.29	0.430
Pulse rate, bpm	104 ± 16	102 ± 17	106 ± 15	0.317
Systolic BP, mm Hg	121 ± 13	123 ± 13	119 ± 12	0.201
Diastolic BP, mm Hg	75 ± 14	76 ± 14	73 ± 12	0.357
Echocardiograph indices				
TRV ≥ 2.5 m/s	2.4 ± 0.7	2.4 ± 0.5	2.3 ± 0.9	0.571
	40 (61)	19 (54)	21 (68)	0.581
PRV > 2.2 m/s	1.3 ± 0.7	1.2 ± 0.7	1.4 ± 0.7	0.251
	7 (10.6)	4 (11.4)	3 (9.7)	>0.999
RAP > 0 mm Hg	11.1 ± 3.1	10.5 ± 3.4	11.4 ± 2.7	0.318
	22 (33.3)	13 (59.1)	9 (36.0)	0.440
PASP ≥ 30 mm Hg	35.1 ± 11.0	35.5 ± 10.4	34.6 ± 11.7	0.742
	50 (75.8)	25 (71.4)	25 (80.6)	0.851
PADP ≥ 25 mm Hg	20.1 ± 8.5	20.4 ± 9.0	19.9 ± 8.1	0.814
	11 (16.7)	9 (25.7)	2 (6.5)	0.103
mPAP ≥ 25 mm Hg	25.4 ± 7.7	25.4 ± 8.1	25.4 ± 7.3	>0.999
	34 (52)	15 (43)	19 (61)	0.398

Definition of abbreviations: BP = blood pressure; GABR = global arginine bioavailability ratio; mPAP = mean pulmonary artery pressure; PADP = pulmonary artery diastolic pressure; PASP = pulmonary artery systolic pressure; PRV = pulmonary regurgitation; RAP = right atrial pressure; TRV = tricuspid regurgitation velocity.
Parameters are described as mean ± SD or *n* (%) as appropriate.

mPAP ⩾ 25 mm Hg was calculated in 34 (52%) patients. All baseline characteristics were similar between the arginine and placebo groups.

A total of 19 patients were discharged before receiving 15 doses of study drug (13 who received arginine vs. 6 who received the placebo). Using the complete-case analysis approach, 47 patients (22 in the arginine group and 25 in the placebo group) were further analyzed.

Eleven patients required supplemental oxygen: six in the arginine group and five in the placebo group. The mean oxygen saturation of patients who received supplemental oxygen was 89% ± 4% (range, 82–94%) in the arginine arm and 90% ± 3% (range, 86–94%) in the placebo arm ($P = 0.554$).

Effect of Arginine Supplementation on Echo Indices of Cardiopulmonary Hemodynamics

A Day 5 echo after completion of study drug was performed as per protocol in 47 patients. The median (IQR) TRV declined 22% in the arginine arm compared with 3.8% in the placebo arm (Table 2 and Figure 2). Looking specifically at children with an elevated TRV ⩾ 2.5 m/s before study drug, there was a greater reduction in the proportion of children with a high TRV in the arginine arm than in the placebo arm (82% vs. 25%; $P < 0.001$); the mean change in TRV was 0.90 ± 0.17 m/s versus 0.23 ± 0.10 m/s ($P < 0.001$), arginine versus placebo, respectively. Improvements in other indices of pulmonary hemodynamics tended to be higher in the arginine group than in the placebo group (Table 2). Mean tricuspid annular plane systolic excursion *z* score deteriorated in the placebo group from 0.9 ± 2.5 at baseline to −0.2 ± 2.2 ($P = 0.04$). Elevated NT-proBNP concentrations decreased after 5 days, with the decline being much more pronounced in the arginine group than in the placebo group ($P < 0.001$). When the indices of cardiopulmonary hemodynamics were stratified according to severity, the prevalence of abnormal values (TRV ⩾ 2.5 m/s; PASP > 40 mm Hg; mPAP > 30 mmHg), although similar at baseline, were lower in the arginine group at completion of supplementation (Table 3). Trends were noted in PASP:systolic blood pressure ratio > 0.4, PAAT < 90 ms, PAAT:ET ratio < 0.31, and other variables reported in Table 2 that were not statistically significant (data not shown). The reductions in prevalence of abnormal indices of pulmonary hemodynamics were higher in patients treated with arginine than in their counterparts who received placebo (Table 4).

Correlation between Doppler-derived Cardiopulmonary Hemodynamics and Improved Arginine Bioavailability

A larger percentage increase in plasma arginine was associated with a lower TRV after 5 days of supplementation (Figure 3). Pearson correlation analysis showed a positive correlation between the percentage increase in plasma arginine and TRV after 5 days of study drug, with a $\rho = -0.341$ ($P = 0.045$). The GABR, an index of arginine availability at the cellular level (23), also showed a statistically significant inverse relationship with TRV ($\rho = -0.533$; $P = 0.001$).

Table 2. Effect of Arginine Supplementation on Echocardiography-derived Cardiopulmonary Hemodynamics and Cardiac Biomarkers Compared with Placebo

	Arginine ($n=22$)			Placebo ($n=25$)		
	Before Arginine	**After Arginine**	***P* Value**	**Before Placebo**	**After Placebo**	***P* Value**
Echocardiography-derived indices						
TRV, m/s	2.7 (2.5 to 2.9)	2.1 (1.8 to 2.3)	**<0.001**	2.6 (2.2 to 2.8)	2.5 (1.8 to 2.6)	**0.037**
PRV, m/s	1.4 (0.9 to 1.9)	1.1 (0.9 to 1.6)	0.282	1.6 (0.9 to 1.9)	1.6 (1.3 to 1.9)	0.689
IVC index, %	25.2 (17.6 to 36.4)	50.0 (23.0 to 55.1)	**0.019**	27.5 (18.2 to 34.5)	25.8 (20.0 to 35.9)	0.556
RAP, mm Hg	10 (10.0 to 15.0)	5 (5.0 to 10.0)	**0.008**	10 (10.0 to 15.0)	10 (10.0 to 15.0)	>0.999
PASP, mm Hg	40.6 (34.4 to 43.6)	21.2 (9.7 to 29.7)	**0.002**	36.8 (30.1 to 42.9)	32.3 (22.8 to 38.7)	**0.018**
PADP, mm Hg	16.9 (13.3 to 26.3)	9.7 (7.2 to 18.5)	**0.005**	21.0 (15.9 to 24.5)	20.0 (15.1 to 24.6)	0.587
mPAP, mm Hg	25.0 (19.8 to 32.1)	14.7 (12.8 to 20.1)	**<0.001**	26.1 (20.6 to 30.2)	21.4 (15.6 to 25.6)	0.128
RV fractional area change, %	46.4 (38.1 to 51.6)	45.5 (40.6 to 50.0)	0.550	45.6 (39.1 to 49.0)	43.2 (38.4 to 45.8)	0.372
TAPSE *z* score	0.7 (−1.7 to 2.2)	1.2 (−1.8 to 2.1)	0.820	1.9 (−0.2 to 2.7)	−0.1 (−1.7 to 0.8)	0.07
MAPSE *z* score	−0.3 (−1.6 to 0.8)	−0.5 (−1.4 to 1.4)	0.676	0.5 (−0.4 to 2.2)	0.2 (−1.6 to 0.1)	0.447
PAAT, ms	104.8 (79.9 to 119.9)	113.1 (99.8 to 119.8)	0.360	89.9 (73.2 to 116.5)	93.2 (83.2 to 116.5)	0.475
PAAT:ET ratio	0.4 (0.3 to 0.4)	0.4 (0.3 to 0.4)	0.719	0.4 (0.3 to 0.5)	0.4 (0.3 to 0.4)	0.551
PASP:SBP ratio	0.3 (0.3 to 0.4)	0.2 (0.2 to 0.3)	**0.001**	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.871
RV Tei index	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.770	0.2 (0.2 to 0.3)	0.3 (0.2 to 0.3)	0.500
LV Tei index	0.3 (0.2 to 0.4)	0.4 (0.3 to 0.4)	0.140	0.3 (0.3 to 0.4)	0.4 (0.3 to 0.4)	**0.014**
SBP	125 (115 to 140)	107 (100 to 110)	**<0.001**	120 (110 to 130)	105 (100 to 115)	**0.006**
DBP	80 (70 to 90)	68 (60 to 70)	**0.018**	75 (64 to 80)	65 (60 to 70)	**0.06**
Cardiac biomarkers						
ProBNP, pg/ml	50.6 (2.0 to 310.4)	33.1 (2.0 to 61.1)	**0.020**	32.1 (2.0 to 195.0)	10.5 (2.0 to 69.3)	**0.010**
	159.2 ± 189.6	38.7 ± 51.2	**0.012**	478.3 ± 1,047.3	26.7 ± 37.3	0.071
Trop I, ng/ml	0.05 (0.03 to 0.20)	0.06 (0.01 to 0.09)	**0.020**	0.03 (0.01 to 0.98)	0.04 (0.01 to 0.2)	0.463
	1.4 ± 4.0	0.2 ± 0.4	0.184	0.7 ± 1.8	0.3 ± 0.7	0.422
Amino acids						
Arginine, μM	81 (66 to 107)	168 (122 to 252)	**<0.001**	93 (62 to 132)	125 (86 to 160)	0.06
Ornithine, μM	198 (126 to 280)	283 (220 to 388)	**<0.001**	208 (148 to 348)	240 (144 to 468)	0.570
Citrulline, μM	11 (8 to 15)	13.4 (8.4 to 19.5)	0.440	11 (9 to 14)	13 (10 to 17)	0.685
Arginine/ornithine	0.5 (0.3 to 0.6)	0.6 (0.4 to 0.7)	0.06	0.4 (0.3 to 0.6)	0.5 (0.2 to 0.9)	0.795
GABR	0.4 (0.3 to 0.6)	0.6 (0.4 to 0.7)	**0.05**	0.4 (0.3 to 0.6)	0.4 (0.2 to 0.8)	0.871

Definition of abbreviations: DBP = diastolic blood pressure; ET = ejection time; GABR = global arginine bioavailability ratio; IVC = inferior vena cava; LV = left ventricle; MAPSE = mitral annular plane systolic excursion; mPAP = mean pulmonary artery pressure; PAAT = pulmonary acceleration artery time; PADP = pulmonary artery diastolic pressure; PASP = pulmonary artery systolic pressure; proBNP = N-terminal pro B-type brain natriuretic peptide; PRV = pulmonary regurgitation velocity; RV = right ventricle; SBP = systolic blood pressure; TAPSE = tricuspid annular plane systolic excursion; Trop I = troponin I; TRV = tricuspid regurgitation velocity.
Data are presented as median (interquartile range) or mean ± SD. Only paired data on patients with hospital stay ≥5 days are included.
Bold is for analyses that are statistically significant ($P \leq 0.05$).

Discussion

Elevated echocardiographic-derived TRV commonly develops in patients with SCD experiencing acute pain (21, 22). Although often a self-limited condition, it has been poorly tolerated in some adults with SCD (20), as PH may compromise oxygenation and worsen a sickling crisis (41). No therapies currently target the transient elevation of TRV during VOE and ACS. In the present study, >60% of patients enrolled had an elevated TRV at the presentation of their pain crisis, which is much higher than the baseline reported prevalence of 10–25% in children with SCD (11, 12, 17). However, our observations are similar to the report of Machado and colleagues (22), who demonstrated an increase in both the prevalence and severity of PH risk in patients with SCD during periods of acute stress, such

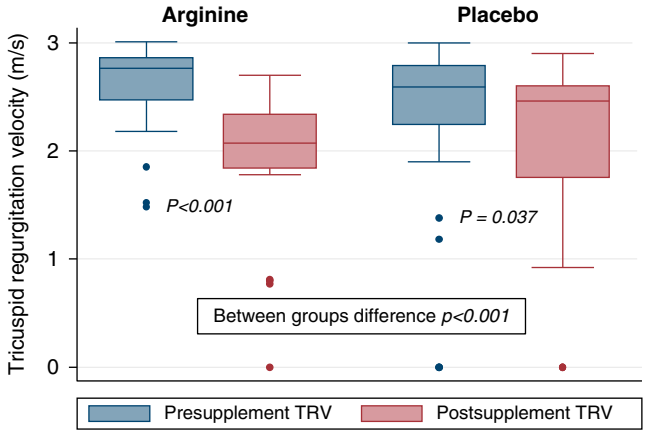

Figure 2. Change in median tricuspid regurgitation jet velocity (TRV) in patients treated with arginine compared with placebo after 5 days of supplementation. Boxplot of TRV by treatment group showing the marked change in the 25th and 75th percentile as well as the median value of the mean pulmonary artery pressure in the arginine group ($n=22$) when compared with the changes in the placebo group ($n=25$). $P<0.001$.

Table 3. Stratification of Indices of Cardiopulmonary Hemodynamics According to Severity before and after Arginine Supplementation

Cardiopulmonary Indices	Number and Proportion of Children			Number and Proportion of Children		
	Before Arginine	After Arginine	*P* Value	Before Placebo	After Placebo	*P* Value
TRV, m/s						
<2.5	5 (22.7)	19 (86.4)	**0.035**	9 (36.0)	13 (52.0)	0.56
2.5–2.9	15 (68.2)	3 (13.6)		15 (60.0)	12 (48.0)	
≥3	2 (9.1)	0 (0.0)		1 (4.0)	0 (0.0)	
PASP, mm Hg						
<30	5 (22.7)	17 (77.3)	**0.030**	5 (20.0)	10 (40)	0.293
30–40	6 (27.3)	4 (18.2)		8 (32.0)	9 (36.0)	
41–50	11 (50.0)	1 (4.5)		12 (48.0)	6 (24.0)	
mPAP, mm Hg						
<25	11 (50.0)	21 (95.5)	**0.004**	11 (44.0)	16 (64.0)	0.516
25–29	3 (13.6)	1 (4.5)		7 (28.0)	6 (24.0)	
≥30	8 (36.4)	0 (0.0)		7 (28.0)	3 (12.0)	
≥2.0	2 (9.1)	1 (4.5)		3 (12.0)	1 (4.0)	

Definition of abbreviations: mPAP = mean pulmonary artery pressure; PASP = pulmonary artery systolic pressure; TRV = tricuspid regurgitation velocity.
Data are presented as *n* (%).
Bold is for analyses that are statistically significant ($P \leq 0.05$).

as pain crisis and exercise. A better understanding of the mechanisms contributing to acute cardiopulmonary hemodynamic abnormalities may lead to future therapeutic agents that target acute elevation in TRV that commonly occurs during VOE and ACS. Although the cause of TRV elevation remains elusive, an increase in cardiac output, left ventricular volume overload, and NO-related vasculopathy are among the reported culprits (2, 3, 5, 23, 42). TRV elevations often improve after resolution of the sickle-related acute event (20, 22); however, a greater decrease in TRV was noted in this study after arginine therapy.

In the present study, a larger percentage increase in plasma arginine at the end of the study was associated with a lower TRV after 5 days of supplementation, supporting the previously reported association of arginine bioavailability and TRV (23). Although not synonymous with PH, TRV elevation identifies PH risk and increased hemolytic rate. The relationship between TRV and arginine bioavailability may be related to the pulmonary vasoconstrictive tendency created by a depleted NO state of SCA often exacerbated during VOE and ACS (28, 29). An increase in RV afterload increases RV end-systolic pressure, which is transmitted through to the right atrium, resulting in an increased TRV.

In the present study, oral arginine supplementation was associated with an 18.4% greater decrease in median TRV than placebo. A similar observation was recently reported by El-Masrya and colleagues (43), who compared the efficacy of arginine with that of sildenafil in lowering pulmonary pressures among 60 children with β-thalassemia in an RCT and found a 12.8% reduction in mean TRV in the arginine group and a comparative noninferiority efficacy of arginine to sildenafil. Other studies in adults without SCD report similar reductions in PAP of >15% after intravenous infusions or oral arginine (26, 33). This current study adds to a growing body of evidence suggesting benefits of arginine therapy for adults and children with SCD experiencing an elevated TRV, a condition known to be associated with hemolysis and mortality risk (3, 7). We speculate that this may represent a previously unrecognized additional mechanism of action for arginine therapy related to improved pain (30, 36), as decreased PAP could improve oxygenation and potentially decrease painful erythrocyte sickling.

Plasma arginine concentrations and global arginine bioavailability are low in patients with SCD experiencing VOE and ACS (28, 29). Prior studies demonstrate an association of arginine deficiency with pain severity (25, 28) as well as TRV elevation (23, 26). In addition, several phase 2 RCTs have demonstrated the efficacy of supplemental arginine to improve pain in hospitalized children with SCD (30, 36, 37). This study adds to the literature demonstrating an impact of arginine therapy on TRV and other echo-derived indices of cardiopulmonary hemodynamics as well.

The ratio of plasma arginine concentration to that of ornithine + citrulline identified as a biomarker of increased mortality risk in SCD has also been proposed as a measure of arginine bioavailability in cardiovascular disease (40, 44). Tang and colleagues found GABR to be a more robust predictor of adverse cardiovascular events, including stroke, repeat myocardial infarction, and mortality, than cholesterol. (40). Low arginine bioavailability also identifies increased risk of death in patients with malaria (45) and has recently been implicated in severe coronavirus disease (COVID-19) infection and multisystem inflammatory syndrome in children (46). Furthermore, Shao and colleagues (44) reported low GABR in patients without SCD with PH associated with systolic heart failure. Arginine bioavailability appears to incorporate cardiovascular risks beyond SCD.

In addition to the impact of arginine on TRV, other important indices of echo-derived cardiopulmonary hemodynamics, including PASP, PASP:systolic blood pressure ratio, and mPAP, were also reduced after arginine therapy to a greater extent than placebo. A significant improvement in mean NT-proBNP was also observed in the arginine arm compared with a nonsignificant

Table 4. Reduction in Number and Proportion of Children with Abnormal Cardiopulmonary Hemodynamics before and after Arginine Supplementation

Echocardiography-derived Indices	Number and Proportion of Children with Abnormal Indices of Cardiopulmonary Hemodynamics						*P* Value for % Reduction Comparing Arginine vs. Placebo
	Arginine Group			Placebo Group			
	Before (*n* [%])	After (*n* [%])	% Reduction	Before (*n* [%])	After (*n* [%])	% Reduction	
TRV ⩾ 2.5 m/s	17 (77.3)	3 (13.6)	82.4	16 (64.0)	12 (48.0)	25.0	**<0.001**
PASP ⩾ 30 mm Hg	17 (77.3)	5 (22.7)	70.6	20 (80.0)	15 (60.0)	25.0	**0.002**
mPAP ⩾ 30 mm Hg	11 (50.0)	1 (4.5)	91.0	14 (56.0)	9 (36.0)	35.7	**<0.001**
PAAT < 90 ms	9 (40.9)	3 (13.6)	54.6	12 (48.0)	13 (52.0)	0.0*	**<0.001**
PAAT:ET < 0.31	5 (22.7)	3 (13.6)	40.1	9 (36.0)	9 (36.0)	0.0	**0.004**
PASP:SBP > 0.4	4 (18.2)	1 (4.5)	75.3	4 (16.0)	4 (16.0)	0.0	**<0.001**
TAPSE $z < -2$SD	5 (22.7)	3 (13.6)	40.1	5 (20.0)	4 (16.0)	20.0	0.131
MAPSE $z < -2$SD	6 (27.3)	1 (4.5)	83.5	2 (8.0)	4 (16.0)	0.0†	**<0.001**
RV Tei > 0.4	5 (22.7)	1 (4.5)	80.2	2 (8.0)	1 (4.0)	50.0	**0.031**
LV Tei > 0.45	6 (27.3)	3 (13.6)	50.2	2 (8.0)	6 (24.0)	0.0‡	**<0.001**
RVFAC < 33%	6 (27.3)	5 (22.7)	16.8	3 (12.0)	6 (24.0)	0.0†	**<0.001**
Pro-BNP > 160 pg/ml	9 (40.9)	3 (13.6)	66.7	8 (32.0)	3 (12.0)	62.5	0.764
Trop I > 2.0 ng/ml	2 (9.1)	1 (4.5)	50.0	3 (12)	1 (4.0)	66.7	0.246

Definition of abbreviations: ET = ejection time; LV = left ventricle; MAPSE = mitral annulus planar systolic excursion; mPAP = mean pulmonary artery pressure; PAAT = pulmonary artery acceleration time; PASP = pulmonary artery systolic pressure; proBNP = N-terminal pro B-type brain natriuretic peptide; RV = right ventricle; RVFAC = right ventricle fractional area change; SBP = systolic blood pressure; TAPSE = tricuspid annulus planar systolic excursion; Trop I = troponin I; TRV = tricuspid regurgitation velocity.
Bold is for analyses that are statistically significant ($P \leq 0.05$).
*Did not reduce but increased by 8.2%.
†Did not reduce but increased by 100%.
‡Did not reduce but increased by 200%.

trend in the placebo arm. Improvements in mean troponin-1 were likely limited by a small sample size; however, what is noteworthy is the high frequency of abnormal NT-proBNP and troponin-1 identified in these children with acute sickle pain. Biomarkers of cardiac dysfunction are rarely obtained in patients with SCD; however, Akkus and colleagues recently reported elevated troponins in 20% of adults with SCD VOE/chest pain tested, associated with lower platelet count, higher biomarkers of hemolysis (lactate dehydrogenase, aspartate aminotransferase, and bilirubin; $P < 0.01$), and TRV ⩾ 3 m/s ($P = 0.03$) (47). The impact of acute VOE on cardiopulmonary function in SCD warrants further attention.

The mechanisms of action of arginine are multifactorial (24, 25, 31, 39), including NO-related vasculopathy (32). A linear relationship between plasma arginine concentrations and NO production after administration of arginine has been demonstrated previously, irrespective of baseline plasma arginine concentrations (48), a phenomenon referred to as the "arginine paradox", which has been confirmed using *in vitro* experimentation (49). Theoretically, an increase in substrate availability should not alter the rate of NO production because of the low saturation constant for endothelial NO synthase (eNOS) (approximately 5 μM), which is why it is called a paradox. (49). In addition, release of the erythrocyte-derived arginase 1 enzyme after hemolysis of sickled red blood cells (23, 26) further limits substrate availability for the eNOS, because the affinity constant (K_m) of arginine for arginase is much lower than that of eNOS (50). Therefore, saturating the enzyme system with arginine quickly ensures that the arginase maximum velocity (V_{max}) is achieved, thus allowing eNOS to function at maximum capacity to replete NO and subsequently cause vasodilation. In relation to patients with SCD, even the sickest patients with severe arginine deficiency should have sufficient L-arginine available to fully saturate the binding site of the eNOS enzyme. However, one also needs to take into consideration the K_m for the arginine CAT (cationic amino acid transporter), which is responsible for facilitating intracellular transport of arginine. The K_m of L-arginine for CAT is 100–150 μM, which is around normal physiologic concentrations of arginine in circulation (31). Even minor fluctuations in plasma arginine

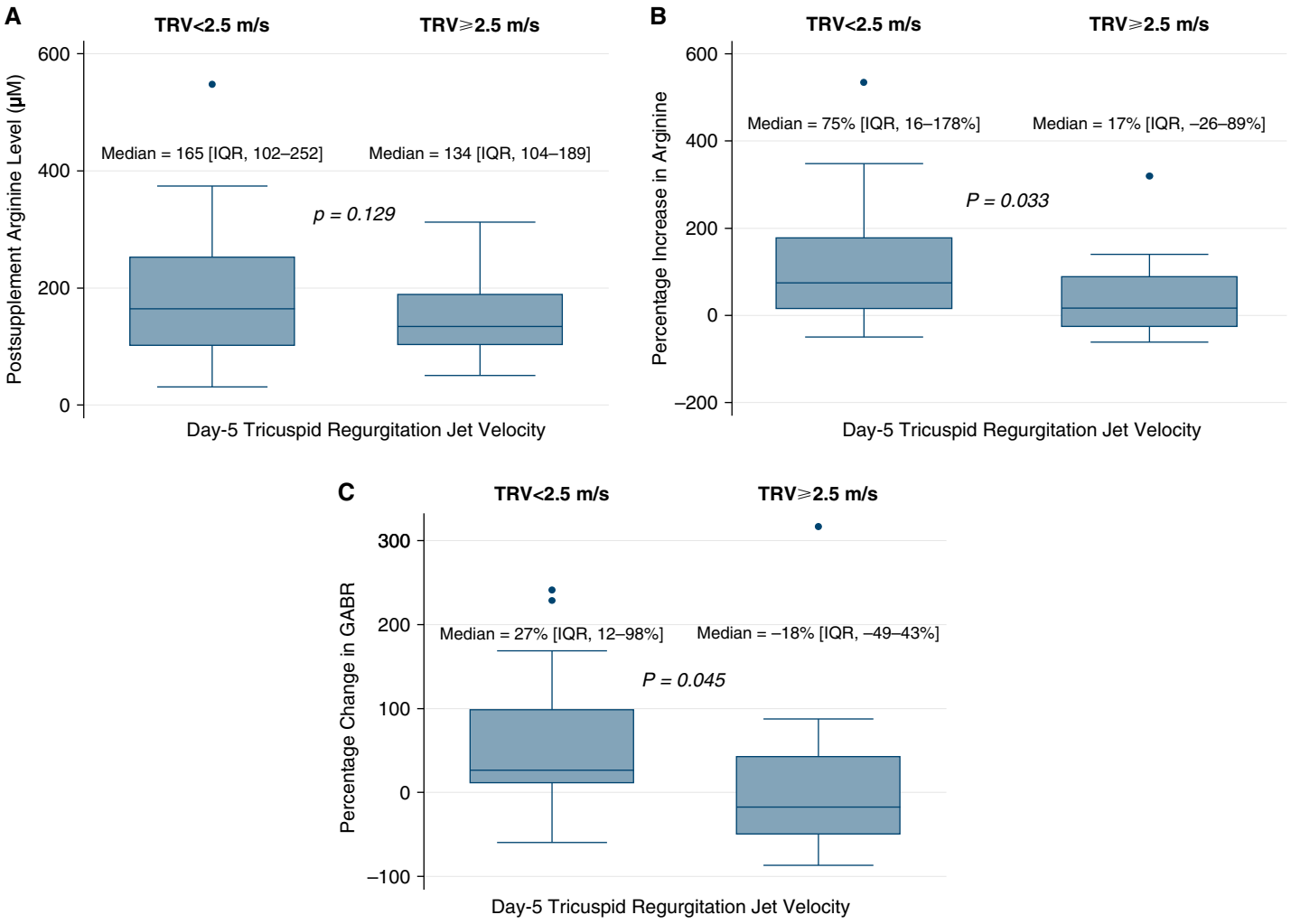

Figure 3. Relationship between (*A*) postsupplement plasma arginine concentrations, (*B*) percentage increase in plasma arginine, (*C*) global arginine bioavailability ratio, and the 5-day end-of-study tricuspid regurgitation jet velocity (TRV) measured by Doppler echocardiography. Patients experiencing the greatest improvement in TRV also experienced a greater increase in arginine bioavailability reflected by change in plasma arginine concentration ($P = 0.03$) and global arginine bioavailability ratio (GABR; $P = 0.045$) independent of study arm. IQR = interquartile range.

concentration may impact intracellular transport, intracellular arginine bioavailability, and ultimately NO generation. These additional facts help explain the arginine paradox in relation to supplementation studies in patients with SCD known to develop an acquired arginine deficiency (24, 26, 30, 36, 38, 39).

Lack of right heart cardiac catheterization data, regarded as the gold standard to confirm the presence of PH, is a major limitation of this study. However, not only is cardiac catheterization not available in participating hospitals, but the ethics of performing this invasive procedure in hospitalized children with SCA may be questionable. In addition, the authors acknowledge the shortcomings of indirect assessment of mPAP and other cardiac indices using echo-derived parameters. Other factors that may have influenced the accuracy of the echocardiographically derived measurement are the effects of respiration, which could not be adequately controlled, as some of the younger patients were unable to consistently obey the command to hold their breath at end-expiration. Furthermore, the acquisition of TRV in the clinical setting is notoriously difficult. Another limitation is the fact that this study was conducted at only two sites within Nigeria and involved a relatively small sample size, which may impact the capacity to generalize the findings to other institutions and countries. Oxygen required in a small group of patients may have impacted pulmonary hemodynamics; however, any effect should have been balanced through the randomization process. Finally, approximately one-third of the patients randomized were discharged home before Day 5 completion of the study protocol because their pain improved sufficiently to be managed at home, the majority of whom were randomized to arginine. Consequently, a postsupplement echo was not available in this group of patients. However, a comparison at Day 5 of the patients with more severe symptoms requiring a longer hospital stay was possible, and >70% of this subgroup had an elevated

TRV at the time of hospital admission. Notably, patients with an elevated TRV were more likely to require at least 5 days of hospitalization for treatment of their acute event, an observation not previously documented.

In conclusion, L-arginine may play an important role in improving cardiopulmonary hemodynamics in patients with SCD during VOE and ACS. Large-scale trials are needed to understand the implications of this effect in patients with SCD. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Uche Nnebe-Agumadu and O. O. Oniyangi for their clinical support. They also thank the clinical staff, nurses, and physicians working at the Emergency Paediatric Units of the University of Abuja Teaching Hospital and National Hospital, Abuja; O. M. Damilare and J. Figueroa for statistical support; and B. C. Vorster, D. V. Onalo, Y. Zubairu, and A. U. Ayodele-Kehinde for their contributions to the success of this clinical trial. The authors also thank the patients with sickle cell anemia and their families, without whom this study would not have been possible.

References

1. Gladwin MT, Sachdev V, Jison ML, Shizukuda Y, Plehn JF, Minter K, *et al.* Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N Engl J Med* 2004;350:886–895.
2. Shilo NR, Morris CR. Pathways to pulmonary hypertension in sickle cell disease: the search for prevention and early intervention. *Expert Rev Hematol* 2017;10:875–890.
3. Klings ES, Machado RF, Barst RJ, Morris CR, Mubarak KK, Gordeuk VR, *et al.*; American Thoracic Society Ad Hoc Committee on Pulmonary Hypertension of Sickle Cell Disease. An official American Thoracic Society clinical practice guideline: diagnosis, risk stratification, and management of pulmonary hypertension of sickle cell disease. *Am J Respir Crit Care Med* 2014;189:727–740.
4. Gladwin MT. Prevalence, risk factors and mortality of pulmonary hypertension defined by right heart catheterization in patients with sickle cell disease. *Expert Rev Hematol* 2011;4:593–596.
5. Sachdev V, Kato GJ, Gibbs JS, Barst RJ, Machado RF, Nouraie M, *et al.*; Walk-PHASST Investigators. Echocardiographic markers of elevated pulmonary pressure and left ventricular diastolic dysfunction are associated with exercise intolerance in adults and adolescents with homozygous sickle cell anemia in the United States and United Kingdom. *Circulation* 2011;124:1452–1460.
6. Maitra P, Caughey M, Robinson L, Desai PC, Jones S, Nouraie M, *et al.* Risk factors for mortality in adult patients with sickle cell disease: a meta-analysis of studies in North America and Europe. *Haematologica* 2017;102:626–636.
7. Kato GJ, Steinberg MH, Gladwin MT. Intravascular hemolysis and the pathophysiology of sickle cell disease. *J Clin Invest* 2017;127: 750–760.
8. Hagar RW, Michlitsch JG, Gardner J, Vichinsky EP, Morris CR. Clinical differences between children and adults with pulmonary hypertension and sickle cell disease. *Br J Haematol* 2008;140:104–112.
9. Parent F, Bachir D, Inamo J, Lionnet F, Driss F, Loko G, *et al.* A hemodynamic study of pulmonary hypertension in sickle cell disease. *N Engl J Med* 2011;365:44–53.
10. Mehari A, Alam S, Tian X, Cuttica MJ, Barnett CF, Miles G, *et al.* Hemodynamic predictors of mortality in adults with sickle cell disease. *Am J Respir Crit Care Med* 2013;187:840–847.
11. Nouraie M, Darbari DS, Rana S, Minniti CP, Castro OL, Luchtman-Jones L, *et al.* Tricuspid regurgitation velocity and other biomarkers of mortality in children, adolescents and young adults with sickle cell disease in the United States: the PUSH study. *Am J Hematol* 2020;95: 766–774.
12. Gordeuk VR, Minniti CP, Nouraie M, Campbell AD, Rana SR, Luchtman-Jones L, *et al.* Elevated tricuspid regurgitation velocity and decline in exercise capacity over 22 months of follow up in children and adolescents with sickle cell anemia. *Haematologica* 2011;96: 33–40.
13. Gladwin MT, Barst RJ, Gibbs JSR, Hildesheim M, Sachdev V, Nouraie M, *et al.*; walk-PHaSST Investigators and Patients. Risk factors for death in 632 patients with sickle cell disease in the United States and United Kingdom. *PLoS One* 2014;9:e99489.
14. Anthi A, Machado RF, Jison ML, Taveira-Dasilva AM, Rubin LJ, Hunter L, *et al.* Hemodynamic and functional assessment of patients with sickle cell disease and pulmonary hypertension. *Am J Respir Crit Care Med* 2007;175:1272–1279.
15. Morris CR. Vascular risk assessment in patients with sickle cell disease. *Haematologica* 2011;96:1–5.
16. Paul R, Minniti CP, Nouraie M, Luchtman-Jones L, Campbell A, Rana S, *et al.* Clinical correlates of acute pulmonary events in children and adolescents with sickle cell disease. *Eur J Haematol* 2013;91:62–68.
17. Minniti CP, Sable C, Campbell A, Rana S, Ensing G, Dham N, *et al.* Elevated tricuspid regurgitant jet velocity in children and adolescents with sickle cell disease: association with hemolysis and hemoglobin oxygen desaturation. *Haematologica* 2009;94:340–347.
18. Onyekwere OC, Campbell A, Teshome M, Onyeagoro S, Sylvan C, Akintilo A, *et al.* Pulmonary hypertension in children and adolescents with sickle cell disease. *Pediatr Cardiol* 2008;29:309–312.
19. Chaudry RA, Cikes M, Karu T, Hutchinson C, Ball S, Sutherland G, *et al.* Paediatric sickle cell disease: pulmonary hypertension but normal vascular resistance. *Arch Dis Child* 2011;96:131–136.
20. Mekontso Dessap A, Leon R, Habibi A, Nzouakou R, Roudot-Thoraval F, Adnot S, *et al.* Pulmonary hypertension and cor pulmonale during severe acute chest syndrome in sickle cell disease. *Am J Respir Crit Care Med* 2008;177:646–653.
21. Onalo R, Cooper P, Cilliers A, Nnebe-Agumadu U. Cardiovascular changes in children with sickle cell crisis. *Cardiol Young* 2020;30: 162–170.
22. Machado RF, Mack AK, Martyr S, Barnett C, Macarthur P, Sachdev V, *et al.* Severity of pulmonary hypertension during vaso-occlusive pain crisis and exercise in patients with sickle cell disease. *Br J Haematol* 2007;136:319–325.
23. Morris CR, Kato GJ, Poljakovic M, Wang X, Blackwelder WC, Sachdev V, *et al.* Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA* 2005;294:81–90.
24. Morris CR, Hamilton-Reeves J, Martindale RG, Sarav M, Ochoa Gautier JB. Acquired amino acid deficiencies: a focus on arginine and glutamine. *Nutr Clin Pract* 2017;32:30S–47S.
25. Bakshi N, Morris CR. The role of the arginine metabolome in pain: implications for sickle cell disease. *J Pain Res* 2016;9:167–175.
26. Morris CR, Morris SM Jr, Hagar W, Van Warmerdam J, Claster S, Kepka-Lenhart D, *et al.* Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? *Am J Respir Crit Care Med* 2003; 168:63–69.
27. Cox SE, Makani J, Komba AN, Soka D, Newton CR, Kirkham FJ, *et al.* Global arginine bioavailability in Tanzanian sickle cell anaemia patients at steady-state: a nested case control study of deaths versus survivors. *Br J Haematol* 2011;155:522–524.
28. Morris CR, Kuypers FA, Larkin S, Vichinsky EP, Styles LA. Patterns of arginine and nitric oxide in patients with sickle cell disease with vaso-occlusive crisis and acute chest syndrome. *J Pediatr Hematol Oncol* 2000;22:515–520.
29. Lopez BL, Kreshak AA, Morris CR, Davis-Moon L, Ballas SK, Ma XL. L-arginine levels are diminished in adult acute vaso-occlusive sickle cell crisis in the emergency department. *Br J Haematol* 2003;120:532–534.
30. Morris CR, Kuypers FA, Lavrisha L, Ansari M, Sweeters N, Stewart M, *et al.* A randomized, placebo-controlled trial of arginine therapy for the treatment of children with sickle cell disease hospitalized with vaso-occlusive pain episodes. *Haematologica* 2013;98:1375–1382.
31. Morris CR. Alterations of the arginine metabolome in sickle cell disease: a growing rationale for arginine therapy. *Hematol Oncol Clin North Am* 2014;28:301–321.

32. Morris CR, Kuypers FA, Larkin S, Sweeters N, Simon J, Vichinsky EP, *et al.* Arginine therapy: a novel strategy to induce nitric oxide production in sickle cell disease. *Br J Haematol* 2000;111: 498–500.
33. Mehta S, Stewart DJ, Langleben D, Levy RD. Short-term pulmonary vasodilation with L-arginine in pulmonary hypertension. *Circulation* 1995;92:1539–1545.
34. McCaffrey MJ, Bose CL, Reiter PD, Stiles AD. Effect of L-arginine infusion on infants with persistent pulmonary hypertension of the newborn. *Biol Neonate* 1995;67:240–243.
35. Nagaya N, Uematsu M, Oya H, Sato N, Sakamaki F, Kyotani S, *et al.* Short-term oral administration of L-arginine improves hemodynamics and exercise capacity in patients with precapillary pulmonary hypertension. *Am J Respir Crit Care Med* 2001;163:887–891.
36. Onalo R, Cooper P, Cilliers A, Vorster BC, Uche NA, Oluseyi OO, *et al.* Randomized control trial of oral arginine therapy for children with sickle cell anemia hospitalized for pain in Nigeria. *Am J Hematol* 2021;96:89–97.
37. Sridharan K, Sivaramakrishnan G. Adjuvant interventions with opioids for vaso-occlusive crisis in sickle cell disease: a mixed treatment network meta-analysis of randomized controlled clinical trials. *J Opioid Manag* 2020;16:267–275.
38. Onalo R, Cilliers A, Cooper P. Impact of oral $_{L}$-arginine supplementation on blood pressure dynamics in children with severe sickle cell vaso-occlusive crisis. *Am J Cardiovasc Dis* 2021;11:136–147.
39. Morris CR, Brown LAS, Reynolds M, Dampier CD, Lane PA, Watt A, *et al.* Impact of arginine therapy on mitochondrial function in children with sickle cell disease during vaso-occlusive pain. *Blood* 2020;136: 1402–1406.
40. Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *J Am Coll Cardiol* 2009;53: 2061–2067.
41. Gladwin MT, Vichinsky E. Pulmonary complications of sickle cell disease. *N Engl J Med* 2008;359:2254–2265.
42. Mushemi-Blake S, Melikian N, Drasar E, Bhan A, Lunt A, Desai SR, *et al.* Pulmonary haemodynamics in sickle cell disease are driven predominantly by a high-output state rather than elevated pulmonary vascular resistance: a prospective 3-dimensional echocardiography/ Doppler study. *PLoS One* 2015;10:e0135472.
43. El-Masrya H, El-Shanshory M, Mahmoud AM, Tolba O, Elsharkawya H. Comparative therapeutic effect of the L-arginine and sildenafil on beta-thalassemia children with increased tricuspid regurgitant jet velocity. *Pediatr Hematol Oncol J* 2020;5:25–29.
44. Shao Z, Wang Z, Shrestha K, Thakur A, Borowski AG, Sweet W, *et al.* Pulmonary hypertension associated with advanced systolic heart failure: dysregulated arginine metabolism and importance of compensatory dimethylarginine dimethylaminohydrolase-1. *J Am Coll Cardiol* 2012;59:1150–1158.
45. Gramaglia I, Sobolewski P, Meays D, Contreras R, Nolan JP, Frangos JA, *et al.* Low nitric oxide bioavailability contributes to the genesis of experimental cerebral malaria. *Nat Med* 2006;12:1417–1422.
46. Rees CA, Rostad CA, Mantus G, Anderson EJ, Chahroudi A, Jaggi P, *et al.* Altered amino acid profile in patients with SARS-CoV-2 infection. *Proc Natl Acad Sci USA* 2021;118:e2101708118.
47. Akkus NI, Rajpal S, Hilbun J, Dwary A, Smith TR, Mina G, *et al.* Troponin elevation in sickle cell disease. *Med Princ Pract* 2021;30:437–442.
48. Mariotti F, Petzke KJ, Bonnet D, Szezepanski I, Bos C, Huneau JF, *et al.* Kinetics of the utilization of dietary arginine for nitric oxide and urea synthesis: insight into the arginine-nitric oxide metabolic system in humans. *Am J Clin Nutr* 2013;97:972–979.
49. Bode-Böger SM, Scalera F, Ignarro LJ. The L-arginine paradox: importance of the L-arginine/asymmetrical dimethylarginine ratio. *Pharmacol Ther* 2007;114:295–306.
50. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. *Biochem J* 1998;336:1–17.