

EFFICACY OF CITRULLINE SUPPLEMENTATION TO DECREASE THE RISK OF PULMONARY HYPERTENSION AFTER CONGENITAL HEART DISEASE SURGERY. A LOCAL EXPERIENCE

EFICACIA DEL SUPLEMENTO DE CITRULINA PARA DISMINUIR EL RIESGO DE HIPERTENSIÓN

PULMONAR POST-CIRUGÍA DE CARDIOPATÍA CONGÉNITA. UNA EXPERIENCIA LOCAL

EFICÁCIA DA SUPLEMENTAÇÃO DE CITRULINA PARA DIMINUIR O RISCO DE HIPERTENSÃO PULMONAR APÓS CIRURGIA DE CARDIOPATIA CONGÊNITA. UMA EXPERIÊNCIA LOCAL

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La hipertensión pulmonar es una complicación grave, frecuente después de una cirugía cardíaca. Concentraciones elevadas de citrulina en plasma disminuyen el riesgo de hipertensión pulmonar del paciente en el postoperatorio. Sin embargo, en nuestro país no está aún indicada la suplementación de este compuesto como terapia preventiva. En este trabajo se evaluó la administración de citrulina oral a un grupo de pacientes pediátricos intervenidos de cirugía cardíaca, comparándolo con un grupo de pacientes control a los cuales no se les administró citrulina. Este es el primer estudio en nuestra población que analiza distintos biomarcadores asociados a hipertensión pulmonar para conocer posibles asociaciones entre los valores de citrulina plasmática y riesgo del paciente de presentar crisis hipertensivas en el postoperatorio de la cirugía cardíaca congénita. Un hallazgo importante fue que los niveles de citrulina en el plasma de los pacientes antes de la cirugía fueron mucho más bajos que los valores normales esperados para su grupo de edad, esto podría deberse a un estado nutricional deficiente de los pacientes estudiados. Sólo los sujetos que recibieron citrulina superaron el nivel mínimo recomendado y ninguno de ellos presentó hipertensión pulmonar; mientras que en el grupo control, el 67% de las mediciones de presión superó los niveles recomendados. En conclusión, los resultados de este estudio indicarían que la suplementación de citrulina oral es eficaz para reducir la hipertensión pulmonar postoperatoria, recomendando su uso en los protocolos de cirugía cardíaca congénita.

Key Concepts

A) What is known about the subject:

Pulmonary hypertension is a serious potential complication after cardiac surgery. Previous studies associated elevations in plasma citrulline concentrations above age-specific values with a lower risk of postoperative pulmonary hypertension.

B) What does this work contribute?

This is the first study in our population that analyzes intermediate metabolites for biomarkers of pulmonary hypertension that could demonstrate the patient's condition as an application of translational medicine. We reaffirm that citrulline supplementation is effective in reducing postoperative pulmonary hypertension.

Resumen:

La hipertensión pulmonar (HP) es una causa importante de morbimortalidad entre pacientes con cardiopatía congénita (CHD) y también una complicación potencialmente grave después de la reparación quirúrgica. La citrulina oral, precursora de la síntesis de óxido nítrico, es segura y eficaz para disminuir el riesgo postoperatorio de HP.

Objetivo: El presente estudio tuvo como objetivo investigar en pacientes pediátricos los cambios plasmáticos de los metabolitos citrulina, arginina, homocisteína y óxido nítrico y la presión arterial pulmonar (PAP) antes y después de la cirugía cardíaca para describir el estado de nuestra población con respecto al riesgo de HP y buscar posibles biomarcadores para su detección y tratamiento temprano.

Resultados principales / Discusión: 16 pacientes pediátricos argentinos con CHD sometidos a bypass cardiopulmonar fueron aleatorizados en dos grupos: (A) con y (B) sin suplementación perioperatoria de citrulina. Encontramos que los niveles medios de citrulina en plasma antes de la cirugía fueron más bajos en ambos grupos con respecto a los valores referenciales, probablemente debido a un estado nutricional deficiente de nuestros pacientes; solo el grupo A superó el nivel mínimo recomendado para evitar la HP. Además, ninguno de los pacientes en el grupo A mostró una PAP media superior a 20mmHg, mientras que en el grupo B, el 67% de las mediciones fue mayor que el nivel de referencia.

Conclusiones: Reafirmamos que la suplementación con citrulina es efectiva para reducir la HP postoperatoria y que los biomarcadores podrían evidenciar el estado del paciente como una aplicación de medicina traslacional.

Palabras clave: citrulina; hipertensión pulmonar; óxido nítrico; cardiología.

Abstract:

Pulmonary hypertension (PH) is a major cause of morbi-mortality among patients with congenital heart disease (CHD) and also a potentially severe complication after surgical repair. Oral citrulline, a precursor to NO synthesis, is safe and efficacious for decreasing the risk of postoperative PH.

Objective: The aim of the present study was to investigate in pediatric patients the changes of plasma citrulline, arginine, homocysteine and nitric oxide (NO) metabolites and pulmonary artery pressures (PAP) pre-post cardiac surgery in order to describe our population status with regard to the risk of pulmonary hypertension and look for potential biomarkers for early detection and treatment.

Main results/Discussion: 16 Argentine pediatric patients with CHD undergoing cardiopulmonary bypass were randomized in two groups: (A) with and (B) without perioperative citrulline supplementation. We found that plasma citrulline median levels before surgery were lower in both groups respect to referential values, probably due to the poor nutritional status of our patients; only group A surpassed post-surgery the minimum recommended level to avoid PH. Furthermore, none of the patients in group A showed mean PAP higher than 20 mmHg, whereas in group B, 67% of the measurements were \geq than the reference level.

Conclusions: We reaffirm that citrulline supplementation it is effective in reducing postoperative pulmonary hypertension and biomarkers could evidence patient status as a translational medicine application.

Key words: citrulline; pulmonary hypertension; nitric oxide; cardiology.

Resumo

A hipertensão pulmonar (HP) é uma das principais causas de morbimortalidade entre pacientes com cardiopatía congénita (DCC) e também uma complicação potencialmente grave após o reparo cirúrgico. A citrulina oral, precursora da síntese do NO, é segura e eficaz para diminuir o risco de HP pós-operatória.

Objetivo: O objetivo do presente estudo foi investigar em pacientes pediátricos as alterações dos metabolitos plasmáticos da citrulina, arginina, homocisteína e óxido nítrico (NO) e pressões da artéria pulmonar (PAP) pré-pós-cirurgia cardíaca, a fim de descrever nosso status populacional com relação ao risco de hipertensão pulmonar e procure potenciais biomarcadores para detecção e tratamento precoces.

Principais resultados / Discussão: 16 pacientes pediátricos argentinos com DCC submetidos à circulação extracorpórea foram randomizados em dois grupos: (A) com e (B) sem suplementação peri-operatória de citrulina. Descobrimos que os níveis médios de citrulina plasmática antes da cirurgia eram menores nos dois grupos em relação aos valores referenciais, provavelmente devido ao mau estado nutricional de nossos pacientes; somente o grupo A superou no pós-operatório o nível mínimo recomendado para evitar HP. Além disso, nenhum dos pacientes do grupo A apresentou PAP média superior a 20 mmHg, enquanto no grupo B, 67% das medidas foram \geq acima do nível de referência.

Conclusões: Reafirmamos que a suplementação com citrulina é eficaz na redução da hipertensão pulmonar no pós-operatório e os biomarcadores podem evidenciar o status do paciente como aplicação de medicamento translacional.

Palavras-chave: citrulina; hipertensão pulmonar; óxido nítrico; cardiologia

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INTRODUCTION

Pulmonary hypertension is a serious potential complication after cardiac surgery, being caused by multiple etiological factors: environmental, genetic and nutritional¹. It is very important to elucidate the contribution of each of these three components to the development of this complication, in order to minimize the risks or at least predict them more clearly. So far, recommended prevention measures include induced alkalosis, sedation, paralysis, and administration of oxygen²⁻⁴. In addition, the treatment options are inotropic and parenteral vasodilators or inhalation of nitric oxide (NO) at a high cost. NO causes vasodilatation of the pulmonary vasculature mediated by cyclic guanosine monophosphate. The endogenous NO is produced from the metabolism of citrulline and arginine, amino acids generated by the urea cycle (Figure 1).

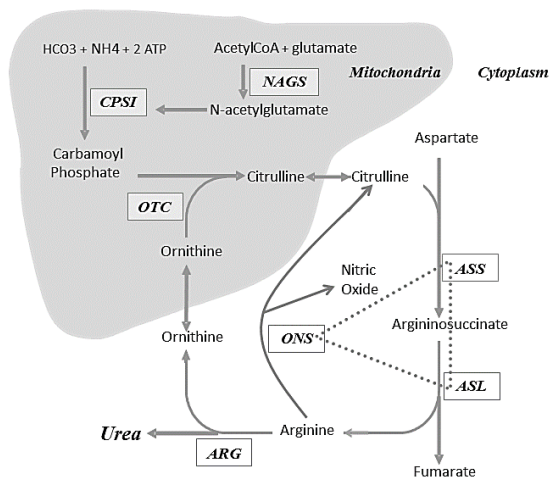


FIGURE 1. Urea cycle. Enzymes involved and synthesis route of nitric oxide. NAGS: N-acetylglutamate synthase; CPSI: carbamoylphosphate synthase; OTC: ornithine transcarbamylase; ASL: arginine succinate lyase; ARG1: arginase 1; NOS: nitric oxide synthase.

Extracorporeal circulation leads to significant reductions in postoperative concentrations of citrulline and arginine, and pulmonary endothelial dysfunction. The administration of oral citrulline is effective in maintaining plasma arginine concentrations and has no recognized toxicity^{5,6}. Previous studies associated elevations in plasma citrulline concentrations above age-specific values with a decreased risk of postoperative pulmonary hypertension. Therefore, they conclude that an increase in the doses tested of oral citrulline supplementation, resulting in consistent citrulline concentrations in excess of 37 $\mu\text{mol/L}$, could prevent postoperative pulmonary hypertension⁷.

The aim of the present study is to determine whether oral citrulline supplementation in the perioperative period: 1) is effective in increasing plasma citrulline concentrations above the recommended 37 $\mu\text{mol/L}$; 2) if it is associated with decreased risk of postoperative pulmonary hypertension; and 3) report the local experience in the application of this preventive tool in PH.

POPULATION

The study included patients from the Hospital de Niños de la Santísima Trinidad (Córdoba, Argentina), undergoing reconstructive Congenital Heart surgery and were at risk of PH (2011-2015 period). Cardiopulmonary bypass surgery included: repair of ventricular septal defect (VSD, 40%), atrioventricular canal (CAV, 33%), and univentricular heart with bidirectional Glenn anastomosis and/or modified Fontan-Kreutzer anastomosis (CUV, 27%).

The exclusion criteria were: significant narrowing of the pulmonary artery, previous placement of the stent in the anterior pulmonary artery, previous angioplasty of the pulmonary artery, atrial-ventricular valve insufficiency, mitral atresia, total obstructive pulmonary anomalous venous return.

Prior to surgery, each patient was randomly assigned to one of the following groups:

- i. Group with oral citrulline administration (OCG) in five doses of 3 g/m² (total dose of 15 g/m²) with the first dose administered during anesthesia induction, the second upon arrival in the intensive care unit, and subsequently every 12 hours.
- ii. Control group with placebo administration (PG). This group was matched by age, weight and CHD to the treatment group.

The project was evaluated and approved by the CIEIS of the Child and the Adult, Hospital Ethics Committee, Ministry of Health of Córdoba Province. The informed consent of the parents was obtained in the preoperative evaluation.

Sampling: 5 ml of blood in two tubes with EDTA and heparin, obtained from each patient at 4 time points: immediately before the incision and at 0, 24 and 48 hours in the intensive care unit.

METHODS

Concentration of plasma citrulline, arginine and homocysteine, was determined by HPLC (Hewlett Packard Serie 1100)⁸⁻¹⁰. Plasmatic NO metabolites quantification by chemiluminescence with a Sievers 280 nitric oxide analyzer (GE Analytical Instruments, Boulder, Colorado). Pulmonary (PAP) and systemic blood pressure (PAS) measurements were made using a pulmonary catheter placed in the operating room; in case of obstruction of the pulmonary catheter with a biventricular repair, the pulmonary non-invasive pressure was measured with Doppler echocardiography (sPAP according to tricuspid regurgitation gradient + right middle atrial pressure depending inferior vena cava inspiratory collapse and mPAP according to pulmonary insufficiency gradient at the beginning of diastole + mean pressure of the right atrium)^{11,12}. Postoperative pulmonary and systemic pressure was recorded every 6 hours for 48 hours in the Cardiovascular Intensive Therapy Unit. In biventricular circulation, pulmonary hypertension is defined as mean pressure > 20 mmHg¹³. In univentricular circulation, 20 mmHg already means pulmonary hypertension so it is recommended consider pulmonary hypertension when mean pressure gradient between the pulmonary artery and the left atrium greater than 6 mmHg¹⁴. Statistical analysis: The average concentration of each metabolite over time was compared between groups by analysis of variance. Under the Akaike (AIC) and Bayesian (BIC) criteria, the best fit model was selected for each response variable. The level of significance was set at 0.05. In case of detecting significant differences, for the comparisons between the means, the DGC test was applied. The InfoStat v2015/16 software was used¹⁵.

RESULTS AND DISCUSSION

From the total number of patients who met the inclusion criteria ($n = 18$), the planned follow-up was completed in 15 patients (10 OCG and 5 PG). Sex distribution was homogeneous (53% M, 47% F) and the average age was 24.5 months (range 3-48 months). In this clinical research study, the sample size was limited; however, this is the first assessment of a previously probed protocol⁷ in our population, with a modification on the test dose. Although the sample size was small, the results showed strong statistical power with significant differences in the majority of measurements between the two groups, and it was possible to find a clear trend as observed previously, further studies may give statistical significance in those values.

Citrulline

In both groups of children, the average level of basal citrulline (prior to incision) was $17.93 \mu\text{mol/L}$ IQR 10-27, with a standard error of 3.75 in the OCG and 5.29 in the PG. This value was in the lower limit of the normal values of reference for age (VN = 16-32 $\mu\text{mol/L}$) and, really far from the values found in another cohorts (32 $\mu\text{mol/L}$ IQR 25-44)⁷. This would indicate a deficient nutritional status of the individuals and a possible predisposition to greater complications if they are not supplemented during the postoperative period.

Over time, the average concentration of citrulline depended on the treatment (significant interaction, $p = 0.02$). While in the OCG the concentration was always increasing, in the PG the concentration at 12 hours postoperatively (critical rescue point to prevent pulmonary hypertension) was lower than that determined in the immediate postoperative period and below the recommended values. The majority of patients in OCG far exceeded the recommended concentration of 37 $\mu\text{mol/L}$ to prevent pulmonary hypertension⁷, but not in the PG (42 $\mu\text{mol/L}$ and 16 $\mu\text{mol/L}$, respectively; $p = 0.039$; Figure 2a).

Arginine

The basal levels of plasma arginine were within the normal range (VN = 44-120 $\mu\text{mol/L}$) in both groups, with great inter-individuals variability. The average concentration of arginine over time was dependent on treatment ($p = 0.0015$). In the PG, the values decreased abruptly 12 hours after surgery (Figure 2b). This behavior differs from that observed by Smith et al.⁷, who reported the abrupt fall of this amino acid immediately in the postoperative period. In contrast, in the OCG, the average post-surgery arginine concentration remained without significant changes over time.

Homocysteine

In both groups, baseline homocysteine levels were within the normal range (VN $\leq 12 \mu\text{mol/L}$). Homocysteine levels increased immediately in the postoperative period, followed by a marked decrease at 12 hours post-surgery in both groups (Figure 2c). Homocysteine had a very marked tendency to decrease in the PG, while in the OCG maintained more homogeneous values, restoring its initial values (pre-surgery) at 24 hours.

Nitric Oxide (NO)

The NO measurements showed in both groups a normal baseline value and a decrease, below the normal values, at 12 hours after the postoperative period (25.90 $\mu\text{mol/L}$ PG and 34.49 $\mu\text{mol/L}$ OCG, NV for children $<6y = 47.2 + 11.2 \mu\text{mol/L}$) (Figure 2d). The mean post-surgery NO concentration remained more stable in the OCG than in the PG; with a drastic decrease in PG at 12 hours, a critical risk of hypertension moment.

Pulmonary Artery Pressure (PAP)

In both groups, a curve with a maximum peak of PAP is observed in the postoperative period reaching its minimum at 24 hours (Figure 2e). There were no significant differences between the average values of both groups; even so it was possible to observe a more stable behavior and with lower values in the treatment group than in the placebo before surgery (14 mmHg vs 19 mmHg, $p = 0.15$), immediately in the postoperative period (17 mmHg vs 22 mmHg, $p = 0.41$) and at 12 hours (16 mmHg vs 19 mmHg, $p = 0.67$). On the other hand, none of the individuals in the OCG had elevated PAP, the values were much lower both in the immediate postoperative period and at 12 hours than previously reported⁷. By contrast, in the PG, 67% of the measurements were equal to or greater than the normal pulmonary arterial pressure in the postoperative period. Three patients of the PG exceeded 20 mmHg and presented plasma citrulline concentrations at 12 hours lower than the normal value (10, 20 and 26 $\mu\text{mol/L}$; VN for children $<6y = 30 \mu\text{mol/L}$ IQR 23-37)¹⁶.

Systemic Arterial Pressure (SAP)

The values of SAP increased in the immediate postoperative period of cardiac surgery in both groups, with a statistically significant difference between them at this point (67 mmHg IQR 63-73 vs 81 mmHg IQR 75-82, $p = 0.025$, treated group vs. placebo, respectively). At 24 hours after surgery, the systemic blood pressure in the OCG remained constant while in the PG a marked decrease was observed (Figure 2f).

Therefore, complications regarding SAP are not expected after the administration of oral citrulline, but, on the contrary, it seems to have greater stability in the course of recovery, achieving faster return to basal values.

Correlation between the variables studied

In the OCG we only observe correlation of PAP with arginine concentration ($p = 0.02$). In the PG a statistical correlation of PAP with NO levels was found ($p = 0.01$), and it was possible to associate the concentrations of this compound with plasma levels of homocysteine ($p = 0.02$). This finding supports the role of homocysteine as a biomarker of PAP, allowing to provide a better prognosis and treatment in patients undergoing cardiovascular surgery. In addition, PAP was found in direct association with the levels of arginine and/or NO in all patients, which highlights the importance of the intermediate metabolites of this cycle to determine homeostasis.

All the studied metabolites showed a differential behavior of greater stability over time in the OCG and even in the PAP and PAS observed; this seems to be related to a lower tension in the urea cycle due to greater availability of the primary metabolite, citrulline, which would allow the generation of intermediate metabolites.

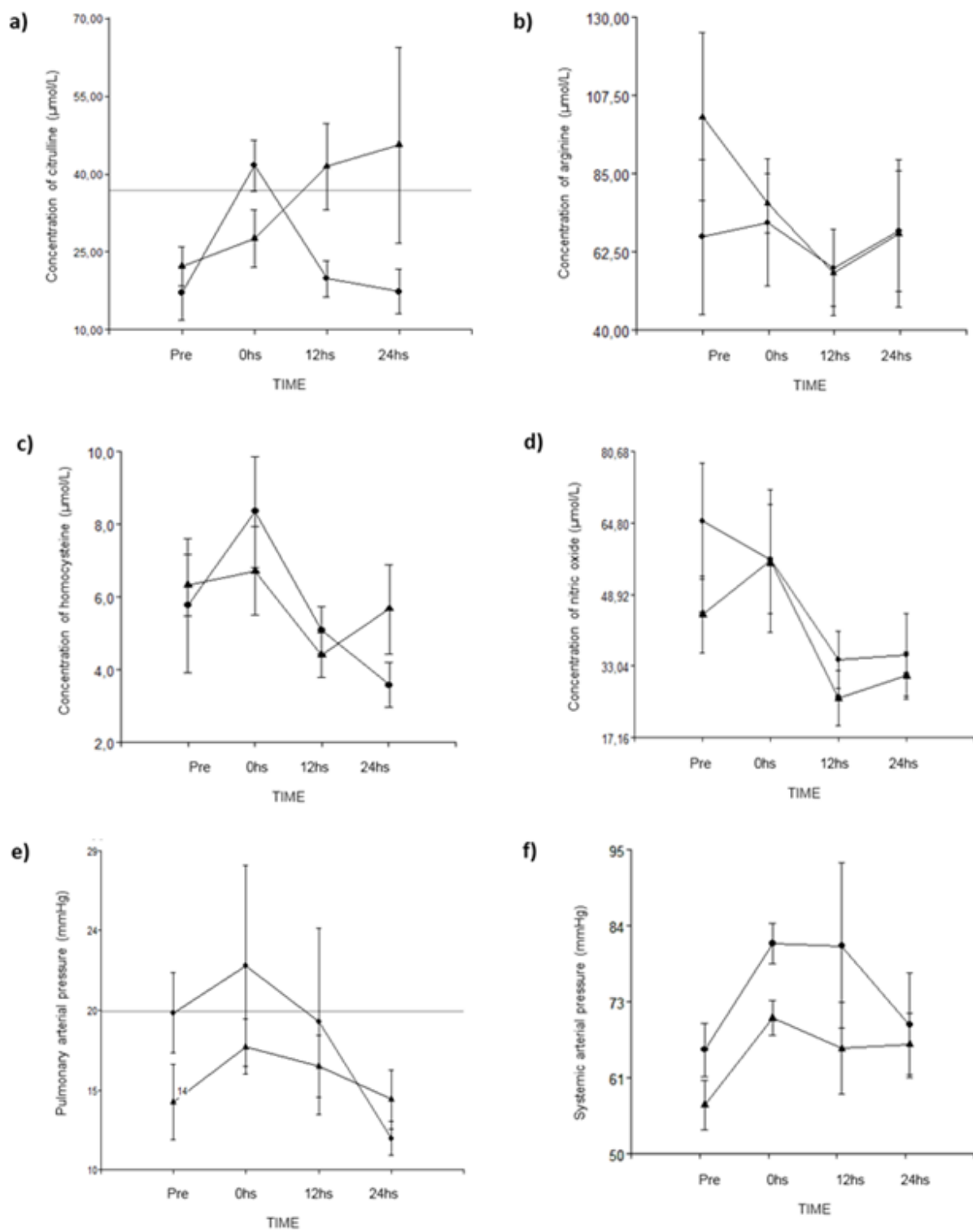


FIGURE 2. Average concentrations of a) Citrulline, b) Arginine, c) Homocysteine, d) Nitric Oxide, and e) Median pulmonary arterial pressure, and f) Median systemic arterial pressure; according to the time of determination in each patient of the group treated with oral citrulline (triangle, ▲) and the placebo group (circle, ●). The line demarcates the normal values for each measurement

CONCLUSIONS

Oral citrulline administration of citrulline in Congenital Heart Disease repair demonstrated the ability to prevent PAP. A higher dose acted more effectively than those previously used and reported. Also, it is highlighted the finding of citrulline deficiency of our cohort, this is an added component in our population, emphasizing the importance of citrulline for the care and evolution of the patient in the intensive care unit.

Limitations of liability: The responsibility of the present work is only of the authors.

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Originalidad del trabajo

Este artículo es original y no ha sido enviado para su publicación a otro medio de difusión científica en forma completa ni parcialmente.

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