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The effects of short term L-citrulline supplementation in the immediate preparation period on the blood morphotic indicators in the population of amateur runners

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A Study Design

B Data Collection

C Statistical Analysis

D Manuscript Preparation

E Funds Collection

Key words: cytruline, blood count, morphological blood parameters, semi-professional runners, post-exercise response

Abstract

Background and Study Aim: The main aim of the study was to asses the effects of short term L-citrulline supplementation in the immediate preparation period on the blood morphotic indicators in the population of amateur runners.

Material and Methods: Population of 24 physically active semi-professional runners preperign for the long distance runn were assigned to two groups to determine the effect of 14-days of 6000 mg per day cytruline supplementation in the immediate preparation period on the blood morphotic indicators in the population of amateur runners. The study was completed by 12 subjects receiving 6 000 mg per cytruline daily (in six 1g gelatin capsules), and 10 subjects receiving placebo gelatin capluses fulfilled with potato flour. The morphological blood parameters were messured from the venous blood collected into 5-ml tubes containing EDTAK2 as the anticoagulant before and after two weeks of supplementation.

Results: There were no significant differences found between the groups with respect to changes in the levels of the studied parameters, except for creatine kinase.

Conclusions: Results point to suggest that a two-week L-citrulline supplementation period not affect resting changes in blood morphology parameters.

Introduction

It has been suggested that increasing the synthesis of nitric oxide (NO) modulated through dietary supplementation could enhance muscular performance, improve resistance to fatigue during physical activity, and affect body homeostasis (Cutrufello, et al., 2015).

Consequently, a variety of supplements containing NO precursors have been introduced to the market, that may affect the level of exercise capacity and modulate the performance enhancement (Gonzalez et al., 2023). Nitric oxide serves as a dynamic signaling molecule that is crucial for numerous cellular and cardiovascular functions. Specifically, nitric oxide has the potential to decrease the oxygen demands of exercise, reduce the adenosine triphosphate (ATP) expenditure associated with muscle contraction, enhance mitochondrial efficiency, and optimize calcium handling (Thompson et al., 2019; Gonzalez et al., 2023). One of the most important direct NO precursors is the L-arginine. In the contrast of oral L-arginin supplementation on of the best methods to increase L-

arginin content is the L-cytrulline supplementation. Citrulline can be converted back into L-arginine with minimal pre-systemic breakdown, positioning it as a potential candidate for supplementation aimed at enhancing nitric oxide production (Besco et al., 2012). Citrulline is an on of the many importain amino acid that is involved in various metabolic processes and that may affect a large number of tissues. It can be synthesized in the body or obtained from external sources, ex. consumtion of citrulline-rich foods such as watermelon, cantaloupe, or pumpkin (Bendahan et al. 2002; Villareal et al., 2018). High-intensity physical activity leads to a buildup of ammonia in the bloodstream, which subsequently activates the enzyme phosphofructokinase. This activation enhances the rate of glycolysis (Fitts, 1994). The elevation of glycolysis during high-intensity physical activity leads to anaerobic glycolysis, which causes a buildup of blood lactate and heightened fatigue. Morover such situation affect blood morphological homeostasis, and may lead to many unfavorable morphological changes

Therefore, the aim of this study was to evaluate how two-weeks L-cytruline supplementation affect basic morphological homeostasis.. In addition, the effect of L-cytruline supplementation on blood inflamation and muscle damage parametrers was evaluated .

MATERIALS AND METHODS

ETHICS

This study was conducted following approval of the Iindependent bioethics committee for scientific research at the Medical University of Gdansk (consent No. NKBBN/450/2020), [2022-07-29]). All researchers involved in the study have respected the principles of the Helsinki Declaration. Prior to the study, the participants gave written informed consent to participate in the study, with an option to withdraw at any time for any reason.

STUDY DESIGN

The study was designed as a double-blind randomized controlled trial with parallel groups. The supplementation protocol involved a 14-day (7 times per week) supplementation program with *6000 mg* of L-cytruline of semi-professional long distance runners (Kuyavian-Pomeranian Voivodeship, Poland). During the initial study visit, the subjects' age, body composition, and height were recorded. All participants were examined by a professional physician and were healthy. Before and after 14 days of supplementation, venous blood samples were taken at erly morning houers for morphological analyzes. Before the study, the L-cytruline supplements were analysed and confirmed high content of active substance and compliance with quantitative and qualitative requirements of the supplement at the Masdiag Laboratory (Warsaw, Poland).

PARTICIPANTS

Participants (n = 24) were semi-professional long distance runners preperign for the long distance runn on distance 42 195 were assigned to two groups to determine the effect of 14 days of *6000 mg* per day cytruline supplementation. Before study enrollment, they were screened by laboratory assistants with respect to the following exclusion criteria: history of morphological or cardiac problems, orthopedic problems, intake of ergogenic nutritional supplements nd drugs within 6 months prior to the study. The subjects were randomly assigned to the supplemented (n = 12) or control groups (n = 12) using an online randomization software. The study was completed by 12 subjects receiving *6000 mg* (3 time per *2000 mg*) cytruline daily (in six 1000 mg gelatin capsules), and 10 subjects receiving same number equel looking placebo gelatin capluses fulfilled with potato flour

During the two week experiment two participants were excluded due the injurie during preparation period and termination of the start preparation.

All subject during the experiment were asked to adopt a similar eating pattern based on a diet individually specified for their age group and the intensity of physical activity. The characteristics of each group are provided in Table 1.

Table 1. **The characteristics of participants before and after supplementation with a L-cytruline of (mean ± SD).**

Variable	Supplemented (*n* = 12)		Control (placebo) (*n* = 10)	
	Before	**After**	**Before**	**After**
Age (years)	37.02± 0.61	–	42.05 ± 0.52	–
Body height (cm)	180.00 ± 3.24	–	182.12 ± 1.21	–
Body mass (kg)	76.23 ± 5.22	75.54 ± 6.17	79.86 ± 5.46	78.11 ± 5.11
Body mass index	23.27 ± 1.75	23.04 ± 1.66	24.33 ± 1.38	24.15 ± 1.24
Fat mass (%)	14.80 ± 1.39	14.42 ± 1.31	15.25 ± 1.43	14.92 ± 1.53

* Significant difference vs. control group at P < 0.05 before supplementation

SUPPLEMENTATION

Both groups completed a 14-day (7 times per week) supplementation program with 6 000 mg of L-cytruline. The dose was determined based on maximal dietary recommendations for the consumption within a margin of dietary safety, i.e., no gastrointestinal disturbances associated with the dose were observed in pilot studies in a population of 30 young physically active students (men, [21.23 ± 1.23]) that were performed before the start of the current study. The control group received the same number of identical-looking rice-floured gelatin capsules as the supplemented group.

The L-cytruline and control supplements were analysed and confirmed purity, quantitative and qualitative requirements of the supplement at the Masdiag Laboratory (Warsaw, Poland), and placed in identical black plastic boxes marked with coded information on the type of preparation and recommended dose.

BLOOD COLLECTION AND ANALYSIS

To determine the morphological blood parameters (red blood cells, RBC; haemoglobin, HGB; haematocrit, HCT; mean corpuscular volume, MCV; mean corpuscular haemoglobin, MCH; and mean corpuscular haemoglobin concentration, MCHC), venous blood samples were collected two time points (two weeks before the star and a day before the start) in early morning houers before anly meal into 5-ml BD Vacutainer® blood collection tubes containing EDTAK2 (anticoagulant). Morphological examinations were performed using flow cytometry on Sysmex XS-1000i apparatus (Kobe, Japan).

STATISTICAL ANALYSIS

Data were analyzed using a set of basic statistical tests **(a**verage, minimum, maximum, standard deviation were calculated**)**. In order to check the significance of differences between groups, a test of significance of differences for independent groups was used. The significance threshold was set at $P < 0.05$. Statistical analysis was performed using Statistica 1**2** software (TIBCO Software Inc. Palo Alto, CA, USA).

RESULTS

Baseline values of hematological and muscle damage parameters before and after 14-L-cytruline intervention presented in Table 2. Two-way analysis of variance showed that while in the Supplemented group there was a slight decrease in baseline CK levels after the 14-day L-cytruline supplementation intervention (11.26%, $p<0.01$), in the Control group there was a significant increase (17.75%, $p<0.01$),.

Table 2 Baseline values of hematological and muscle damage parameters before and after two-weeks of L-cytruline supplementation (mean ± SD).

Variable	Unit	Supplemented (n = 12)		Control (placebo) (n = 10)	
		Before	After	Before	After
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WBC	**× 10^3 μL**	5.92 ± 1.31	5.54 ± 1.22	5.61 ± 1.53	6.11 ± 1.98
LY	**× 10^3 μL**	2.10± 0.68	2.07 ± 0.86	2.01 ± 0.24	2.09 ± 0.27
MO	**× 10^3 μL**	0.57 ± 0.13	0.59 ± 0.20	0.53 ± 0.75	0.55 ± 0.32
NEU	**× 10^3 μL**	2.76 ± 0.59	2.87 ± 1.48	2.86 ± 1.40	2.95 ± 0.25
RBC	**× 10^6 μL**	4.92 ± 0.32	4.91 ± 0.35	4.91 ± 0.20	4.95 ± 0.31
Hb	**g·dL^{-1}**	15.11 ± 0.52	15.20 ± 0.75	15.31 ± 0.62	15.11 ± 0.78
HCT	**%**	43.63 ± *3.63	43.22 ± 1.95	42.21 ± 0.07	42.45 ± 0.01
MCV	**fL**	87.35 ± 0.93	87.21 ± 3.74	87.12 ± 2.50	86.12 ± 3.22
MCH	**pg**	34.63 ± 1.11	35.01 ± 1.47	30.63 ± 1.01	30.76 ± 1.04
MCHC	**g·dL^{-1}**	35.17 ± 0.77	35.12 ± 0.63	35.38 ± 0.71	36.14 ± 0.92
Plt	**× 10^3 μL**	243.54 ± 53.22	242.22 ± 41.22	260.09 ± 55.74	257.14 ± 52.14
MPV	**fL**	10.62 ± 0.91	10.60 ± 0.90	10.61 ± 1.05	10.60 ± 1.05
RDW	**%**	12.78 ± 0.34	12.69 ± 0.87	12.97 ± 1.25	12.42 ± 1.00
SII Index	**×10^9 cells/L**	311.54 ± 87.12	324.01 ± 125.11	315.16 ± 142.24	347.22 ± 142.51
CK	**U/L**	284.59 ± 159.18	252.30 ± 188.12*	276.18 ± 68.43	325.63 ± 58.39*
CRP	**mg/L**	0.91± 0.30	0.90 ± 0.40	1.02 ± 0.49	1.10 ± 0.70

Note: Suplemented, L-cytruline group; Control, placebo group; WBC, white blood cells; LY, lymphocytes; MO, monocytes; NEU, neutrocytes; RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean concentration of corpuscular hemoglobin concentration; Plt, platelets; MPV, mean platelet volume; RDW, red blood cell distribution width; SII, Systemic immune-inflammation index; CK, creatine kinase; CRP, c-reactive protein.

*Significant difference vs. before 14-days of supplementation intervention at $p < 0.01$.

DISCUSSION

The effects of citrulline supplementation on strength and power outcomes have been studied extensively in recent years (Perez-Guisado et al., 2015; Trexler et al., 2019; Gonzalez et al., 2020; Vårvik et al., 2021; Viribay et al., 2022).

Unfortunately, it is still very difficult to find and information regarding the impact of L-cytruline supplementation on basic morphological parameters. Results of the current analysis indicate that citrulline supplementation may not affect blood morphological parameters in comparison to placebo supplemented grop.

It may me due the short period of supplementation. But we have to have in mind that analysed values were the basic ones so we could not showe infulance of L-cytruline on post-exercises morphological cahges. Generay we have observed some changes in inflammation parameters – cratine

kinase. In case of supplemented population, we observed slight decrease in baseline CK (11.26%, $p<0.01$), and in the control group there was a significant increase (17.75%, $p<0.01$). Simillar changes were be presented on the animal mice model in reserch conducted by Ghozali et al. 2024. Presented results were consistent with the role of CK as a marker for muscle damage, and they indicated that L-citrulline might have a protective effect against muscle damage, even in the pres-start training period. In conclusion, L-citrulline supplementation may demonstrates promis in attenuating muscle damage and souch findings highlight the potential therapeutic role of L-citrulline in enhancing muscle recovery and performance.

Overall, we have showed that short-term supplementation of L-citrulline has shown to be safe and well-tolerated. In whole analysed L-cytruline population we did not report any stomach discomfort as a side effect after ingestion. It may be due the fact that like it was shown by, Moinard et al. (2008) L-citrulline supplementation is not inducting any gastrointestinal distress in daily doses up to 15 g.

A major limitation of the current study is the short-term nature of presented intervention. Given the positive effects observed from some of the results, further reserch should be performed using different doses of L-citrulline, especially on exercise performance in a variety of populations who may benefit from it.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding authors A.S., K.K and B.S.Data are in the form of excel and pdf files.

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