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Watermelon Juice: Potential Functional Drink for Sore Muscle Relief in Athletes

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ABSTRACT: L-Citrulline is an excellent candidate to reduce muscle soreness, and watermelon is a fruit rich in this amino acid. This study investigated the potential of watermelon juice as a functional drink for athletes. An in vitro study of intestinal absorption of L-citrulline in Caco-2 cells was performed using unpasteurized (NW), pasteurized (80 °C for 40 s) watermelon juice (PW) and, as control, a standard of L-citrulline. L-citrulline bioavailability was greater when it was contained in a matrix of watermelon and when no heat treatment was applied. In the in vivo experiment (maximum effort test in a cycloergometer), seven athletes were supplied with 500 mL of natural watermelon juice (1.17 g of L-citrulline), enriched watermelon juice (4.83 g of L-citrulline plus 1.17 g from watermelon), and placebo. Both watermelon juices helped to reduce the recovery heart rate and muscle soreness after 24 h.

KEYWORDS: *L-citrulline, bioavailability, cellular transport, anaerobic performance, Caco-2 cell, pasteurization, cellular absorption*

INTRODUCTION

Until recently, L-citrulline attracted little interest among the nutrition community, almost certainly because it is a non-protein amino acid and was viewed solely as a metabolic intermediary in the urea cycle. Also, L-citrulline is almost absent from natural foods, watermelon being a notable exception.[1] The elevated content of L-citrulline, a major hydroxyl radical scavenger, allows wild watermelon to resist drought-mediated oxidative stress in harsh environments.[2] These antioxidant properties, together with the ability to generate nitric oxide (NO), make citrulline an excellent candidate for the treatment of pathological situations characterized by oxidative stress and decreased arginine availability,[3] for example, hypertension, heart failure, atherosclerosis, sickle cell disease,[4−6] and sexual stamina and erectile functions.[7] Other benefits associated with L-citrulline intake include improving athletic performance due to NO synthesis and increasing glucose transport in skeletal muscle.[8] Citrulline malate was shown to increase levels of arginine and ornithine (which are important for muscle growth) and influence levels of growth hormone.[9] For example, in aged rats, 1 week refeeding with an L-citrulline-enriched diet (at 5 g/kg day) induced an increase in absolute muscle protein synthesis rate together with an increase in protein.[10] A dietary supplement rich in L-citrulline also helps in smooth muscle relaxation.[11] Pérez-Guisado and Jakeman[12] found that citrulline malate (8 g/day) enhances athletic anaerobic performance and relieves muscle soreness. L-Citrulline also accelerates lactic acid removal, allowing better physical performance, that is, more intense training and faster recovery after each workout.[13]

Today, the food industry is researching natural source foods, and their enrichment with bioactive compounds, as substitutes of pharmacological products. Watermelon juice, naturally rich in L-citrulline, is an excellent option for athletes who want to improve their sports performance. However, a prerequisite for this juice should be the adequate bioavailability of L-citrulline in the juice composition and after thermal pasteurization. Methods for assessing the bioavailability include in vitro models such as the Caco-2 Cell prediction of intestinal absorption, which has been adapted to test a number of foods.[14]

The aim of the present study was to determine the in vitro L-citrulline bioavailability from a synthetic standard or natural watermelon juice (pasteurized or not) and, according to these results, determine the effect of a potential functional watermelon juice in an in vivo experiment in athletic performance using a test of maximum effort cycle ergometer.

MATERIALS AND METHODS

In Vitro Study in Pasteurized or Nonpasteurized Watermelon Juice Enriched with L-Citrulline. *Reagents.* Dulbecco's modified Eagle's medium (DMEM), nonessential amino acids (NEAA), fetal bovine serum, and antibiotic solution (penicillin and streptomycin) were obtained from GIBCO (Eggenstein, Germany). Sodium pyruvate trypsin solution−EDTA, Hank's salts with Ca^{2+} but not Mg^{2+} (HBSS), and Hank's salts without phenol red were

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purchased from Sigma (Madrid, Spain). L-Citrulline was purchased from Acofarma (Barcelona, Spain). Caco-2 cells (HTB-37) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The Caco-2 cell line derives from a human colon adenocarcinoma, which has morphological and functional similarities to human intestinal epithelial cells. These cells were stored in a bank of cell cultures frozen in liquid nitrogen.

Watermelon Juice Preparation. Ten seedless watermelons (*Citrullus lanatus* cv. 'Motril') were purchased at commercial maturity from a local supermarket (Cartagena, Spain). The fruits were transported (300 m) to the laboratory and stored at 10 °C after washing with 100 mg L^{-1} sodium hypochlorite and drying using absorbent paper. Pulp was liquefied (Moulinex FruttiPro-BKA1, Spain) and transferred to a sterile container. This juice was unpasteurized natural watermelon (NW treatment) or was pasteurized at 80 °C for 40 s and immediately cooled on ice to 8 °C (pasteurized watermelon (PW) treatment) using a thermoresistometer (Mastia, Cartagena, Spain). The pasteurization temperature and time were chosen on the basis of preliminary data for obtaining optimal quality in red color, lycopene, antioxidant, and phenolic content of the watermelon juice.[15] Juices were transferred to sterile glass containers and stored at 4 °C until analysis (3 days later). A standard of L-citrulline dissolved in Milli-Q water was used as a control.

Cell Culture. Caco-2 cells were extracted from the nitrogen tank and introduced into a bath at 37 °C to accelerate thawing. Cells were transferred to a 25 cm^2 flask with DMEM and incubated in a controlled atmosphere at 37 °C, 5% CO_2, and 95% relative humidity (RH) for 21 days, until the cells reached an optimum level of differentiation.[16] Cells adhered to the flask and grew until they covered the surface, forming a cell monolayer, and the medium was changed every 2−3 days with DMEM. When the cells reached confluence, they proceeded to trypsinization and dilution of cell suspension. Trypsinization was done to dislodge the cells and transfer to larger surfaces, using proteolytic agents such as trypsin. For the dilution of the cell suspension, the cells were diluted in DMEM to a concentration of 5×10^5 cells cm^{-2}. For absorption studies, cells were seeded on polycarbonate inserts (Costar Transwell plate inserts, 12 mm polycarbonate, pore diameter = 0.3 mm; Costar, Tewksbury, MA, USA).

Measurement of Transepithelial Electrical Resistance (TEER). Evaluations of epithelial integrity and maturity of the monolayers were performed by measuring TEER. This was measured using a voltmeter with an EndOhm-12 chamber (EVOM, World Precision Instruments, Berlin, Germany). Values are expressed in standard units of ohms per square centimeter and presented as the mean of triplicate measurements. For calculation, the equation TEER = $R_{total} \times A$ was used (R_{total} is the measured resistance in ohms and A is the surface area in cm^2).

Absorption of L-Citrulline in Caco-2 Cells, Absorption Speed, and Apparent Permeability. Transport assay was performed by incubating an aliquot of each sample (0.2 mL) in the apical part of the polycarbonate insert. All samples were prepared to obtain a concentration of 0.685 mM of L-citrulline. This concentration was similar to kinetic parameters of citrulline uptake (K_m the Michaelis−Menten constant)[17] allowing work in nonsaturable intervals and knowledge of the difference among treatments. At 4, 8, and 15 min cellular transport was performed in triplicate; these times were used according to Bahri et al.,[17] who reported that absorption at 37 °C is linear up to 12 min. After incubation (37 °C, 5% CO_2, 95% RH), the amount of L-citrulline remaining in the apical portion was determined by HPLC-MS.[1] Absorption was calculated by comparing initial and final citrulline contents (after cell transport), and absorption speed was determined as the amount of citrulline (ng) through the polycarbonate insert per second.

The permeability coefficient (P_{app}) across Caco-2 cell monolayers was calculated using the following equation: P_{app} (cm/s) = $(dQ/dt)(1/AC_0)$, where P_{app} is the apparent permeability coefficient (cm/s), dQ/dt is the rate of transport of L-citrulline ($\mu g\ s^{-1}$), A is the monolayer growth surface area (cm^2), and C_0 is the initial concentration the L-citrulline in the donor side ($\mu g\ mL^{-1}$).[18]

In Vivo Study (Athletic Performance). *Subjects.* Students of Sport Sciences at the University of Murcia (7 men, mean ± SD age, 22.7 ± 0.8 years; body mass, 68.9 ± 3.8 kg; height = 170.8 ± 3.6 cm; body mass index, 24.0 ± 0.6 kg m^{-2}) volunteered to participate in this investigation. The subjects were not competitive athletes, but they participated regularly in different sports. The study was approved by the Ethical Committee of the University of Murcia (Spain). All details of the study were explained to the participants, who also gave their written consent. Subjects completed a questionnaire about their personal history of cardiovascular diseases. Cardiopulmonary auscultation and resting blood pressure (Stethoscope, Quirumed S.L, Madrid, Spain) were assessed. Also, a baseline electrocardiogram (BTL-08 SD6 model, Columbia, USA) and spirometry at rest (Spiro analyzer ST-250R, Fukuda, Madrid, Spain) were performed. Furthermore, an anthropometric evaluation was performed according to the International Society for the Advancement of Kinanthropometry (ISAK).[19] These determinations were performed on volunteers to determine inclusion or exclusion criteria, each subject undergoing a medical examination. All of these parameters were normal in all seven subjects, who were not taking any medication or suffering from injuries that could interfere with athletic performance.

Study Design. On the day prior to the completion of the test, participants did not practice any vigorous physical exercise or consume alcoholic beverages or stimulants. In addition, subjects were required to ingest a diet rich in carbohydrates at least 3 h before the start of testing. As Mandel et al.[20] recommended, subjects ingested 500 mL of beverage 1 h before the test. Unpasteurized drinks were composed of (1) natural watermelon juice (NW) containing 1.17 g of citrulline from the proper watermelon, (2) enriched watermelon juice (EW) (6 g/500 mL = 1.17 g citrulline from watermelon + 4.83 g of citrulline added), and (3) sample placebo (infusion of fruit-flavored plants composed of rosehip, hibiscus, rooibos, apple pulp, natural flavors, and strawberry pieces. Placebo samples had a color similar to watermelon juice and 5.8 g/100 mL of sugar, equivalent to total sugars, sucrose, glucose, and fructose, obtained by HPLC for watermelon juice. There is no recommendation for citrulline intake, but a dose of 6 or 8 g of citrulline malate has been used by other authors.[9,12] The composition of each treatment is reported in Table 1. To minimize any expectancy effects, participants were not informed of the potential role of the different treatments on outcomes.

Table 1. Physicochemical Characteristics and L-Citrulline Content[a]

	NW	EW	placebo
sugar content (%)	5.80 ± 0.03	5.80 ± 0.00	5.80 ± 0.06
density (g/mL)	1.03 ± 0.00	1.03 ± 0.01	1.02 ± 0.01
L	28.01 ± 0.10	24.20 ± 0.09	21.85 ± 0.02
°hue	37.42 ± 0.03	37.21 ± 0.04	34.47 ± 0.06
chroma	15.94 ± 0.01	16.75 ± 0.09	11.04 ± 0.00
citrulline (g/L)	2.33 ± 0.13	12.33 ± 0.18	

[a]Values are means (n = 3) ± SE. NW (natural unpasteurized watermelon juice), EW (enriched unpasteurized watermelon juice). Sugars contents = sum of glucose, fructose, and sucrose. Luminosity (CIE L^*), hue angle ($°h = \tan^{-1}(b^*/a^*)$), chroma = $[(a^*)^2 + (b^*)^2]^{1/2}$.

The effect of each drink was tested on each test subject, with a separation of 5 days between each test. Allocation of drink order was randomized for each subject. For each test, participants were fitted with a chest strap and heart rate monitor (Monark, CR 2032, Taiwan), and each repetition was monitored. The exercise protocol commenced with a standardized 5 min warmup on a cycle ergometer at 75 W (Ergoline GmbH, Bitz, Germany). Participants completed 11 min for each test on a cycle ergometer (Monark, 874 E, Vansbro, Sweden) at a resistance of 4.5 and 5 kg depending on the subject's weight. That involved completing eight repetitions of 30 s exercise intervals, separated by 1 min of rest and a final 3 min recovery. This protocol of

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alternating intense physical activity followed by a short break was obtained from Pérez-Guisado and Jakeman,[12] as citrulline malate supplementation would be less effective in enhancing the performance of short aerobic exercises sessions or anaerobic sessions with sufficient rest.

During resistance exercise intervals, blood lactate samples were taken at 0, 2, 4, 6, 8, and 3 min of recovery. Blood samples were drawn by puncture (on the ring finger of the left-hand) using a sterile lancet (Dr. Lange, Warsaw, Poland) and dispensed into collection capillaries of 0.01 mL (Dr. Lange, Warsaw, Poland). Immediately, blood samples were transferred into cuvettes (LKM 140, Dr. Lange Cuvette Test, Berlin, Germany), containing anticoagulant, for the immediate determination of lactate concentrations using an automated analyzer (Miniphotometer 8/8 plus, LP 20, Dr. Lange, Berlin, Germany).

After each cycle (8 per test), ratings of perceived exertion were assessed using the Borg scale (6−20).[21] After the end of each session (NW juice, EW juice, and placebo), the subjects self-reported their soreness 24 and 48 h after the physical exertion on a scale from 1 to 5 as follows: 1, no soreness; 2, minimal soreness with no impact on immediate training; 3, medium soreness with minimal impact on immediate training; 4, high soreness with negative impact on immediate training; 5, maximum soreness with physical disability for immediate training.[12]

Statistical Analyses. The distribution of data in the in vivo experiment was initially assessed by the normality test of Shapiro−Wilk. Statistical analysis was performed using the nonparametric test. In both experiments, to compare the values obtained with each of the substances supplied, respective Friedman ANOVA was performed. Statistical analyses were performed with SPSS for Windows version 15.0. Data are presented as the mean ± standard error. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Intestinal Absorption in Caco-2 Cells. *Influence of the Monolayer in the Transepithelial Electrical Resistance.* Minor differences were observed in the TEER values (data not shown), most probably due to the small variability of the monolayers noted by Brown et al.[22] The established culture conditions allowed development of viable cell monolayers, uniform, differentiated, and functional, that is, with selective permeability. The measured values of TEER (data not shown), greater than 450 cm^2, were within the ranges used by Lentz et al.[23] and Yamashita et al.[24] The TEER across Caco-2 cells increases with culture time, reaching a maximum after about 10−15 days, but full formation of transport systems requires 21−24 days.[25] In this study Caco-2 cells were incubated for 21 days until the cells reached an optimum level of differentiation.

Absorption Percentage of L-Citrulline in Caco-2 Cells. Figure 1 shows the Caco-2 absorption of L-citrulline from NW and PW juices and control (L-citruille + water). Absorption percentage was statistically significant for the studied treatments, with NW juice attaining the highest absorption percentage (18.87%) at 8 min, being at that time significantly different from the other two treatments. For the PW juice and the control, the highest absorptions of L-citrulline were obtained in the first 4 min (13.19 and 11.85%, respectively). These results show two interesting facts. First, the absorption of L-citrulline in the NW juice was greater than in the control. This indicates that the watermelon juice is a more suitable vehicle for the transport and bioavailability of L-citrulline than a pure standard, that is, a pharmacological formulation. Second, comparison of the absorption obtained for NW and PW juice showed that the application of heat treatment as pasteurization (80 °C/40 s) decreased the absorption percentage of L-citrulline content in the same food matrix.

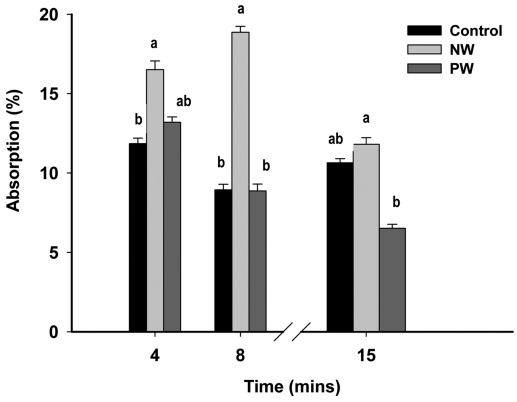

Figure 1. Absorption of L-citrulline from watermelon juice by Caco-2 cells. Values are means ($n = 3 \pm$ SE). Control (L-citrulline); NW, unpasteurized natural watermelon juice; PW, pasteurized watermelon juice. The same letter at each time point indicates no statistical difference according to the LSD test $p \leq 0.05$.

The relationship between processing treatment and bioavailability depends on each bioactive compound and food matrix.[26] For example, heat treatment (90 °C/30 s) produced a significant decrease in tocopherol and carotenoid bioaccessibility in all three different beverages and increased the bioavailability of ascorbic acid.[27] However, in amino acids such as L-citrulline, heat treatment could react with other compounds present in the watermelon juice (such as sugars) to form nutritionally unavailable derivatives, the most common examples of which are Maillard products, reducing the absorption percentage. A similar response has been reported in lysine: when a processed food or feedstuff is subjected to heating, the lysine present can be altered to nutritionally unavailable derivatives.[28,29] Van Barneveld et al.[30] found a 73% decrease in the available lysine content of field peas heated from 110 to 165 °C when determined using an animal growth assay. In our case, 32% of the previously available L-citrulline was not absorbed in the pasteurized juice.

Absorption percentages found in this work for citrulline in the watermelon juice (13.19−18.87%) were lower than those found in the bioavailability of *trans*-β-carotene in cassava (23.85%) and sweet potato (25.82%) but similar to that in beans (32.33%).[31] Actually, the bioaccessibility of carotenoids from main dietary sources was highly variable, ranging from <0.1% (β-carotene from raw tomato) to almost 100% (α-tocopherol from white bread).[32] The bioaccessibility of carotenoids has been also studied in watermelon using an in vitro model,[33] obtaining 2.7% for lycopene, 30.2% for β-carotene, and 64.3% for phytoene. These previous results show the importance of considering influential factors such as the type of bioactive compound, food matrix, food processing, and isomerization in the bioavailability results.[32] However, it is important to remember that although in vitro models approximate the bioavailability of functional compounds, the actual in vivo cell reality is different.

Absorption Speed. An important parameter to know in bioavailability studies is the absorption speed of the compound. Faster absorption during the first minute avoids competition with other compounds and enhances any beneficial effects of the functional compound concerned. Figure 2 shows the absorption speed of L-citrulline in Caco-2 from watermelon juice. Juice preparations were statistically different at each time point. In all cases, NW juice was absorbed more quickly than

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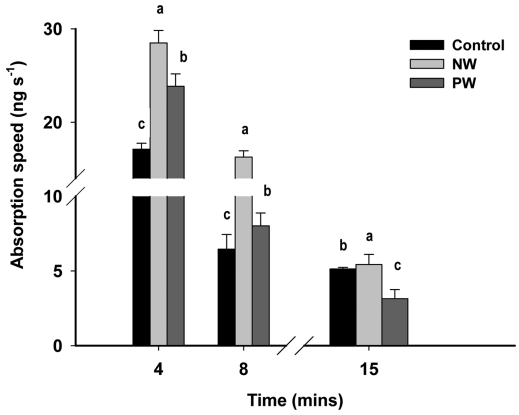

Figure 2. Absorption speed of L-citrulline from watermelon juice in Caco-2 cells. Values are means (n = 3 ± SE). Control, L-citrulline; NW, unpasteurized natural watermelon juice; PW, pasteurized watermelon juice. The same letter at each time point indicates no statistical difference according to the LSD test at $p \leq 0.05$.

the standard or PW juice. In all samples, the absorption speed decreased with time at 15 min of cellular transport, and there was a greater reduction. This shows that L-citrulline bioavailability may be limited by its intestinal absorption. This reduction in the absorption speed could be explained by the nonlinearity absorption at that time.[17]

Apparent Permeability. Permeability values of the analyzed samples are shown in Figure 3. Samples tested showed

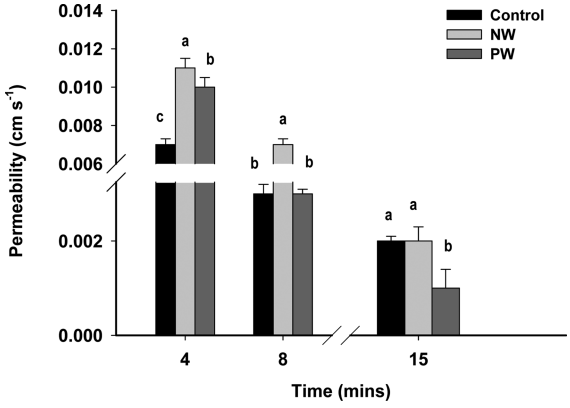

Figure 3. Permeability (P_{app}, cm/s) of L-citrulline from functional watermelon juice in Caco-2 cells. Values are means (n = 3 ± SE). Control, L-citrulline; NW, unpasteurized natural watermelon juice; PW, pasteurized watermelon juice. The same letter at each time point indicates no statistical difference according to the LSD test at $p \leq 0.05$.

significant differences, with citrulline present in the NW juice showing higher permeation of cells compared to the PW juice and standard. All treatments showed reduced permeability over time. Reductions of 40.90% in the 8th minute and 80% in the 15th minute were obtained for the NW juice.

Some authors prefer to use the permeability coefficient (P_{app}) instead of the absorption speed to quantify the absorption process in situ in animal models, because absorption speed does not encompass the surface of useful absorption, which varies in the different areas of the gastrointestinal tract that are being tested.[34] However, in this study, absorption speed and permeability presented similar behaviors. Absorption (%), absorption speed, and permeability of Caco-2 cells were higher when the L-citrulline was supplied as a NW juice. The pasteurization treatment affected the percentage, speed, and permeability of absorption.

In Vivo Study (Athletic Performance). *Pedaling Test.* According to the results shown in Figure 4, each subject had a

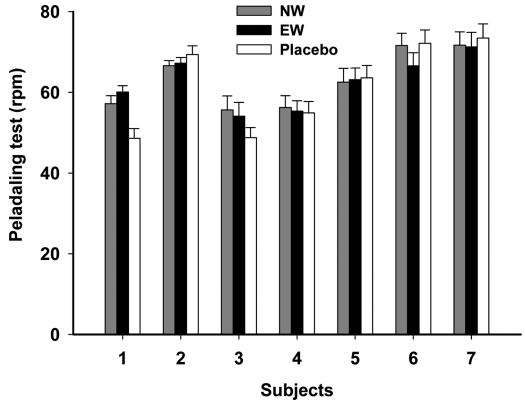

Figure 4. Pedaling test on a cycle ergometer in revolutions per minute (rpm). Values are mean (n = 48 ± SE, 8 cycles of 30 s each and 6 measurements were performed every 5 s). NW, unpasteurized natural watermelon juice; EW, enriched unpasteurized watermelon juice. No significant differences were found between tests for each of the substances supplied.

constant and particular frequency throughout the pedaling test. Revolutions per minute (rpm) were between 48 and 73. According to Lucía et al.[35] trained cyclists generally pedal at 90−100 rpm, and this high pedaling cadence (>80 rpm) causes less muscle fatigue.[36] However, the cadence is more efficient at speeds of 50−60 rpm.[37] Participants were students of sport sciences, but not trained cyclists. Therefore, the speeds reached during the test were less than those of professional cyclists. However, the main objective was to determine athletic performance after ingestion of each substance. No significant differences were observed when the rpm and intake of different drinks were compared, although two subjects (1 and 3) with a lower placebo pedaling speed had slightly increased speeds with the watermelon juices. This could show that citrulline is more efficient in those people who practice moderate physical activity than in professional atheletes.

Heart Rate. Figure 5 shows the heart rate of subjects at the beginning and end of the test and for 1 and 3 min of recovery. Mean heart rates throughout the experimental protocol followed a similar pattern across trials, independent of beverage. The initial average heart rate was 106 beats per min (bpm) and increased to 170 bpm during exercise. After 1 and 3 min of recovery, the rate decreased to 148 and 138 bpm. Although no significant difference was found, a trend of greater heart rate reduction after 1 and 3 min of recovery was observed when natural or enriched watermelon juices were ingested.

Perceived Exertion and Muscle Soreness. Perceived exertion was focused on "what" the individual was feeling during exercise, using the Borg scale. Mean perceived exertion was between 18.75 and 19.33 (Figure 6). These values denote a maximal exercise test on a cycle ergometer, as 20 is the maximum value on the scale for perceived exertion. No differences in ratings of perceived exertion were observed in each test or between beverages provided. This indicates that the effort for the subjects was the same in all tests regardless of the beverage ingested.

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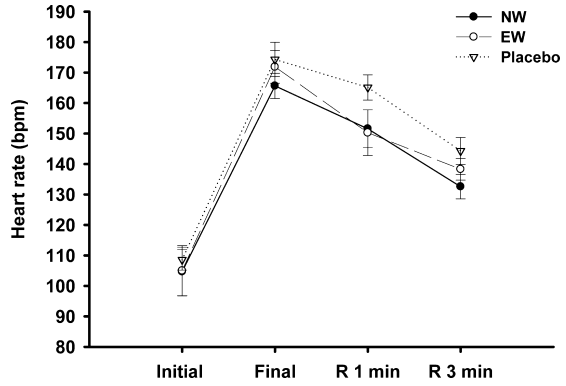

Figure 5. Heart rate in beats per minute (bpm), at the start (initial) and at the end of physical effort (final) and after 1 and 3 min of recovery (R 1 and R 3). NW, unpasteurized natural watermelon juice; EW, enriched unpasteurized watermelon juice. Values are mean (n = 7 ± SE subjects). No significant differences were found between tests for each of the substances supplied.

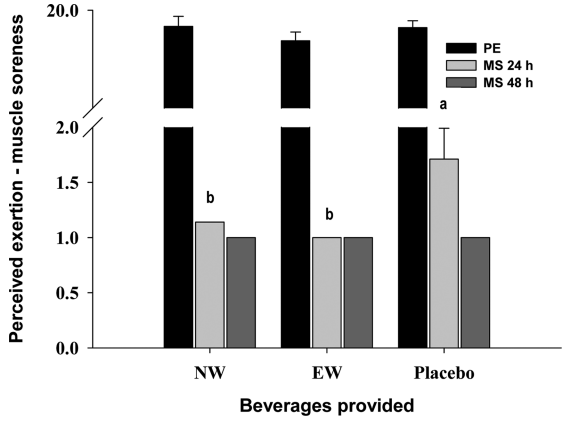

Figure 6. Perceived exertion (PE, scale of 6−20) and muscle soreness (MS, scale 1−5) after 24 and 48 h of performed exercise tests according to beverage. NW, unpasteurized natural watermelon juice; EW, enriched unpasteurized watermelon juice. Values are mean (n = 7 subjects ± SE). The analysis of variance showed significant differences only in MS 24 h. The same letter at each time indicates no statistical difference according to the LSD test at $p \leq 0.05$.

Muscle soreness after 24 h of exercise was significantly greater when placebo was supplied. This indicates that subjects felt more muscle soreness when they performed physical exercise with placebo than with either watermelon juice. However, NW and EW juice did not differ from each other, indicating that 1.17 g of L-citrulline was sufficient amino acid to help to reduce the physical soreness. After 48 h, the muscle soreness was reduced to 1 in all treatments, even in placebo drinks, with no significant differences. These results partially agree with those reported by Perez-Guisado and Jakeman,[12] where citrulline malate supplementation (8 g) reduced muscle soreness 24 and even 48 h anaerobic postexercise.

Some studies have found that supplementation of citrulline reduces fatigue, stimulates hepatic ureogenesis, and promotes the renal reabsorption of bicarbonates.[38] These metabolic actions have a protective effect against acidosis and ammonia poisoning, explaining the antifatigue properties of citrulline malate. Bendahan et al.[39] demonstrated that citrulline malate ingestion reduces the sensation of fatigue, increases the rate of oxidative ATP production during exercise by 34%, and increases the rate of phosphocreatine recovery after exercise by 20%, indicating a larger contribution of oxidative ATP synthesis to energy production. According to our results, supplying watermelon juice enriched or not in L-citrulline significantly reduced muscular soreness 24 h after exercise when compared with placebo. Takeda et al.[40] indicated that citrulline supplementation prolongs the period until exhaustion in swimming exercise and that this effect involves inhibition of blood ammonia accumulation when the intensity of exercise is comparable to maximal lactate steady state. These authors suggest that citrulline suplementation would be very helpful for individuals performing high-intensity exercise.

Blood Lactate Concentration. Blood lactate levels (data not shown) before starting an intensive activity were in the normal range for blood lactate, from 0.5 to 2.2 mmol/L,[41] with no statistical differences between treatments. Blood lactate concentrations were slightly elevated from basal levels with the onset of exercise, increasing to 14.43 mmol/L after 2 min of exercise, values similar to those reported by Leicht et al.,[42] who obtained a lactate concentration of 14.8 ± 2.9 mmol/L in anaerobic test with a bicycle ergometer for 30 s. Concentrations increased after 4 min for the three treatments (17.40 mmol/L) and gradually decreased following recovery, regardless of the beverage ingested. According to these results blood lactate concentration is associated with an increase in exercise intensity, and this agrees with other published works.[43]

In this experiment, no significant differences were observed between the drinks supplied and blood lactate concentration obtained. Although bood lactate concentration is an indicator of the intensity of anaerobic work done,[44] there are arguments over its suitability as lactic acid accumulates in nonactive muscles during exercise[45] and muscle fatigue is due to an increase in hydrogen ions (lower pH) in the muscle tissue, not the lactate itself.[43,46] Citrulline is not thought to regulate pH, so it is not surprising that no differences were found in this experiment. However, authors have found that supplementation with this amino acid helps improve athletic performance and achieve faster recovery.[9,38,39] Giannesini et al.[47] demonstrated that citrulline malate supplementation in healthy rats has an ergogenic effect associated with an improvement of muscular contraction efficiency.

In conclusion, this paper demonstrates the possible "functionality" of watermelon juice rich in natural L-citrulline. The in vitro study showed a greater L-citrulline bioavailability when contained in a natural matrix such as nonpasteurized watermelon juice, and this juice, enriched or not in L-citrulline, helped to reduce muscle soreness. Future research should be focused on the minimal concentrations of citrulline required for reduction of muscle fatigue and other health benefits in stress, athletic performance, and cardiovascular disease. This amino acid could be supplied as watermelon juice or as products enriched in citrulline from watermelon extraction. In both case, it is important to choose watermelon cultivars rich in this amino acid. Functional compound contents in fruits and vegetables play a key role in the design of new natural and functional products (beverages, juices, energy bars, etc.) by the food industry instead of synthetic compounds from pharmaceutical industry.

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Notes

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