Modification of fatigue indicators using citrulline malate for high performance endurance athletes

Key words: Citrulline malate, high exercise, endurance training, sport, fatigue, lactate.

Palabras clave: Malato de citrulina, ejercicio intensidad alta, entrenamiento de resistencia, deporte, fatiga, lactato.

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JA López-Cabral,* A Rivera-Cisneros,** H Rodríguez-Camacho,* JM Sánchez-González,*** I Serna-Sánchez,* M Trejo-Trejo**

* Centro de Medicina y Ciencias Aplicadas al Deporte CODE Jalisco.
** División Ciencias de la Salud, Universidad de Guanajuato.
*** Academia Mexicana de Cirugía.

Corresponding author:
JA López-Cabral
George Santayana 4963. Jardines Universidad 45110. Zapopan, Jalisco, México. E-mail: cabral_lopez@hotmail.com

Abstract

High intensity physical exercise favors accumulation of lactate in muscles and bloodstream, leading to impaired muscle function and exercise performance. This study evaluated the effect of citrulline malate (CM) on blood lactate concentration and perception of fatigue after muscular effort in high performance athletes. Seventy-two high-performance athletes who gave written informed consent were randomly assigned to CM 3 g/day, CM 6 g/day or placebo for 13 days. Blood lactate measurements were performed every training day. Fatigue was assessed through an auto-administered questionnaire at baseline and after 6 and 13 days. Main criterion was the percentage of blood lactate recovery 30 minutes after training. The mean percentage of lactate recovery 30 minutes after training was 89.3 ± 1.1% in the CM3 and 97.9 ± 1.3% in the CM6 group, becoming significantly greater than placebo at day 5 with CM3 and at day 3 with CM6. Lactate variations were stable or decreased in the 2 active groups, whereas they increased in the placebo group. At day 13, there was no fatigue perceived in 87.8% (CM6), 71.3% (CM3) and 15.9% (placebo) athletes. Citrulline malate enhances lactate and fatigue recovery of high performance endurance athletes, allowing a faster recovery and training adaptation without deletarious effects.

Resumen

Hacer ejercicio físico con una intensidad alta favorece la acumulación de lactato en los músculos y circulación sanguínea, lo cual afecta a la función muscular y el rendimiento deportivo. Este estudio evaluó el efecto del malato de citrulina (MC) en la concentración de lactato en sangre y la percepción de la fatiga después de un esfuerzo muscular en deportistas de alto rendimiento. A 72 deportistas de alto rendimiento que dieron su consentimiento informado por escrito se les asignó al azar a 3 g/día de MC, 6 g/día de MC o placebo durante 13 días. Las mediciones de lactato en sangre se llevaron a cabo todos los días de entrenamiento. La fatiga se evaluó mediante un cuestionario autoadministrado al inicio del estudio y después de seis y 13 días. El criterio principal fue el porcentaje de recuperación de lactato en sangre 30 minutos después del entrenamiento. El porcentaje medio de recuperación de lactato 30 minutos después del entrenamiento fue 89.3 ± 1.1% en el grupo MC3 y 97.9 ± 1.3% en el grupo MC6, llegando a ser significativamente mayor que el placebo en el día 5 con MC3 y en el día 3 con MC6. Las variaciones de lactato se mantuvieron estables o disminuyeron en los dos grupos activos, mientras que aumentaron en el grupo de placebo. En el día 13, no se percibió fatiga en 87.8% (MC6), 71.3% (MC3) ni en 15.9% (placebo) de los deportistas. El malato de citrulina mejora la recuperación de lactato y la fatiga de los deportistas con un entrenamiento de alta resistencia, lo que permite una recuperación más rápida y una adaptación al entrenamiento sin efectos nocivos.

Introduction

Overtraining syndrome frequently occurs in athletes who train beyond the body's ability to recover. Without adequate rest and recovery, long and hard training regimens can backfire, and actually decrease physical performance. During physical exercise, when the demand of energy is higher than the maximum aerobic capacity, the anaerobic metabolism compensates leading to lactic acid production. Once formed, lactic acid quickly dissociates and diffuses in the blood stream as lactate. As the intensity of physical exercise increases and the anaerobic cellular reactions become predominant, changes occur in specific metabolites associated to the muscle contractile machinery ($H+$, ATP, ADP and inorganic phosphate). These changes together with decrease in the sarcoplasmic reticulum Ca^{2+} release definitely culminate in the inhibition of skeletal muscles contractility and reduction of performance known as fatigue.[1,2] In these conditions, the lactate production is faster than its clearance and the large amount of lactate produced accumulates in the muscles and blood stream. Even if the importance of removing the accumulated lactate is under continuing debate, it has been recognized that elevated levels of skeletal muscle and blood lactate are associated with impaired muscle function and exercise performance.[3-5]

During high intensity exercise, blood concentration of ammonia, another metabolite, also increases. Ammonia is produced during the reactions of ATP re-synthesis and is eliminated through the cycle of urea, responsible of ammonia detoxification to urea in the liver. High blood concentrations of ammonia are associated to muscle exhaustion. Ammonia, in fact, facilitates the production of lactate by activating the phosphofructokynase enzyme and prevents the oxidative metabolism of pyruvate hindering the supply of ATP to skeletal muscle.[6]

Citrulline malate (CM - Reactimol® CONCORDIA/BIOCODEX) is a mixture of citrulline, one of non-essential amino acids, which is involved in the urea cycle, and malate, a tricarboxylic acid cycle (TCA) intermediate. It is usually prescribed in the treatment of asthenia, at a daily dose of 3 g. Several body of evidence in humans and rats has indicated that CM treatment significantly reduces the sensation of fatigue and improves aerobic muscle function in subjects suffering from asthenia.[7]

Perez-Guisado *et al*[8] demonstrated that a single dose of CM conferred a benefit in pain control and performance to athletes engaged in high-intensity anaerobic exercises which increase lactate, ammonia, and acidosis.

The present study aimed to evaluate the effect of 3 and 6 g/day CM on blood lactate concentration recovery and the perception of fatigue after muscular effort in high performance athletes (pentathlon, athletics and speed skating).

Materials and methods

Subjects and study design. The trial was designed as a randomized, double-blind, placebo-controlled, parallel-groups study and took place at CODE Jalisco, Guadalajara (Mexico) in April 2011. Eligible subjects were healthy volunteer athletes of both genders, aged 13 to 20 years. They had to belong to Jalisco CODE teams (pentathlon, athletics and speed skating) with a minimum of two years of high performance training. Pregnancy or breast feeding, declared allergy to CM or use of CM or other anti-asthenic products within 1 month before the study were exclusion criteria. The study was approved by the Jalisco Health Department Ethics Committee, and written informed consent was obtained from all participants and their family before study initiation. Subjects were randomly assigned to receive CM 3 g/day, CM 6 g/day or placebo for 13 days. Randomization was stratified by sport. Commercially available CM was diluted in 1L of commercial lemon-flavour beverage used for hydration of athletes. Subjects assigned to placebo received the beverage only. Beverages containing CM were indistinguishable from those without CM,

nor colour, nor smell, nor taste. The drinks were prepared by a staff not involved in the study conduct. The study lasted two weeks (13 days), with a 1-hour training session every day except the last day of first week (Day 7) considered a rest day. The study criteria included blood lactate levels, an auto-administered fatigue questionnaire, body weight and heart rate, and adverse event. Blood lactate measurements were performed every training day before training (Baseline Level – BL), immediately after training (LES), then 5 minutes (LES_5) and 30 minutes (LES_{30}) after training, using a commercial digital lactate analyzer (Lactate Scout®, EKF Diagnostic). The questionnaire used to define the subjective level of muscle fatigue as perceived by athletes was a variant of the 26-points questionnaire described by Brun *et al.*[9] The number of questions was reduced to 12 in order to reduce the time of athlete's application and maintain their attention focused. Athletes filled the questionnaire at the end of the training week before study initiation (Day 0), Day 6 and Day 13 of the study, in the interval of time LES_5 – LES_{30}, answering at each question with no (absence of muscle fatigue); occasionally (occasional tiredness); always (presence of muscle fatigue); results were expressed as a percentage of each type of answer over the 12 questions. Body weight and heart rate were measured before and immediately after training at baseline and Day 13.

The primary efficacy criterion was the percentage of blood lactate recovery 30 minutes after training, defined as (LES – LES_{30}) / (LES – BL) x 100, where LES, LES_{30} and BL represent the maximum level of blood lactate registered at the end of the training, the level of blood lactate at 30 minutes after the training phase and at baseline (before training), respectively. Secondary criteria were the percentage of responders determined as subjects achieving an 80% or higher level of recovery of blood lactate level 30 minutes after training, changes over time in lactate levels, changes in rates of subjective fatigue levels, and safety.

Statistical methods. Sample size was determined using a relevant difference of 20% between placebo and CM in blood lactate recovery, with a mean effect size of 11.8%, a variance of 23.5, an alpha risk of 0.05, and a power of 0.80. The homogeneity on the basal blood lactate level in the three groups of study was assessed using an analysis of variance. The percentage of recovery was determined each day of the trial (except the resting day) and results expressed as mean ± standard error of the mean (SEM) for each study group. Percentages of recovery, changes in lactate levels over time and changes in fatigue were compared between groups using an analysis of variance for repeated measurements model. The percentage of responders was compared between groups using a χ^2 test. The primary efficacy criterion analysis was planned on the Intention-to-Treat (ITT) population, i.e., subjects allocated to treatment, and on the Per Protocol analysis (PP) i.e., subjects who completed the study without major deviations to the protocol Minitab® Statistical Software, version 15.0 (Minitab Inc. Pennsylvania) was used for analyses.

Results

Seventy-two subjects were enrolled. All completed the study without major deviations to the protocol and were included in the ITT population, which was identical to the PP population. Twenty-five athletes were allocated to receive CM 3 g/day, 24 to CM 6 g/day and 23 to placebo. Demographics, baseline characteristics and baseline levels of lactate were evenly distributed between the 3 groups (table I). The mean level of lactate at baseline was over the accepted normal range (0.5 – 2.2 mmol/L).

Percentage of recovery of blood lactate level 30 minutes after a training session (figure 1). The overall mean percentage of recovery of blood lactate level 30 minutes after training was 89.5 ± 1.4% in the placebo group, 89.3 ± 1.1% in the CM 3 g group and 97.9 ± 1.3% in the CM 6 g. The difference in blood lactate recovery for subjects treated

Table I. Demographics and baseline characteristics.

	Citrulline Malate 3 g/day n = 25	Citrulline Malate 6 g/day n = 24	Placebo n = 23
Age (years)*	16.9 ± 2.2	17.3 ± 2.3	17.2 ± 2.2
Male n (%)	17 (68.0)	14 (58.3)	15 (65.2)
Sport n (%)			
Pentathlon	8 (32,00)	8 (33,33)	7 (30,43)
Athletics (Coach A)	7 (28,00)	7 (29,17)	7 (30,43)
Athletics (Coach B)	7 (28,00)	6 (25,00)	7 (30,43)
Speed-skating	3 (12,00)	3 (12,50)	2 (8,70)
Lactate level (mmol/L)**	2.6 ± 0.1	2.8 ± 0.1	2.8 ± 0.1

* Mean ± standard deviation. ** Mean ± standard error of the mean.

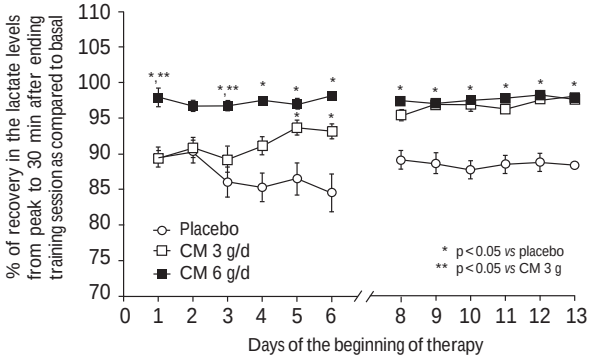

Figure 1. Percentage of recovery of lactate levels 30 minutes after the end of training compared to basal levels over time. Percentage of recovery of blood lactate 30 minutes after training is defined as (LES – LES_{30}) / (LES – BL) x 100, where LES represents the maximum level of blood lactate registered at the end of the training, LES_{30} the level of blood lactate 30 minutes after the training phase and BL the lactate baseline level (before training).
Data are shown as mean ± standard error of the mean (SEM).
CM = Citrulline malate.
* p < 0.05 compared to the placebo.
** p < 0.05 *versus* the lower dose of citrulline malate 3 g.

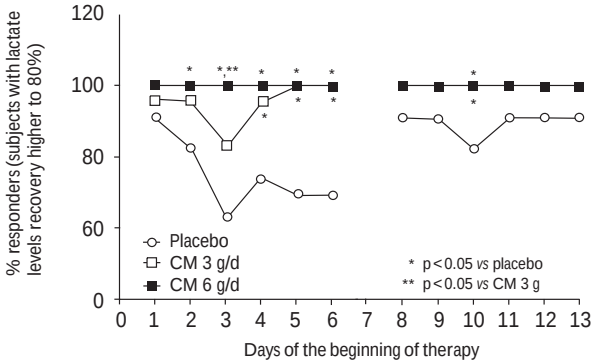

Figure 2. Frequency of subjects reaching at least 80% recovery. During the 2nd week, D8 to D13, the 2 citrulline malate curves are superimposed.
Data are shown as the percentage of each group. CM = Citrulline malate.
* p < 0.05 compared to the placebo.
** p < 0.05 *versus* the lower dose of citrulline malate 3 g.

with CM 6 g and placebo started to be statistically significant since the 1st day and confirmed from the 3rd day up to the end of the treatment period. In the CM 3 g group, the difference from placebo became significant at Day 5.

Percentage of responders to CM therapy (figure 2). During the first week of study, the percentage of responders in the placebo group progressively dropped from 82.6% to 63.6% during the first three days of training, then stabilized to about 70%. In the group of athletes treated with CM 6 g, 100% of subjects reached a percentage of recovery higher than 80% and this value statistically differed from that observed in placebo group starting from Day 2. In the CM 3 g group, the percentage of responders exceeded 80% and became statistically different from the placebo group at Day 4. On Day 3, in the placebo as in the

CM 3 g groups the percentage of responders had a negative peak which was consistent with the largest training load of the week. In the second week of study, the percentage of responders in the placebo group increased and remained stable to about 90%, except at Day 10 in which training intensity was again maximal. In the same week, the totality of athletes treated with CM reported a percentage of lactate recovery higher than 80%, so responded to therapy, without differences between the two dose groups.

Changes over time in lactate levels at baseline, at peak at the end of training, and during the recovery phase (figure 3). Basal lactate levels in CM 6 g were statistically different from those in placebo group since Day 2 up to the end of the treatment. In the CM 3 g group, significant differences appeared from Day 8 (figure 3A). At the end of the training, when the peak of blood lactate concentration was reached (LES), lactate levels were statistically different from those observed in the placebo group starting from Day 2 in the CM 6 g group and Day 4 in the CM 3 g group (figure 3B). At Day 8, after the resting day, the difference was statistically significant from placebo for the CM 6 g group. At baseline as well as at LES, lactate blood concentrations tended to slightly increase over time or remain stable in placebo group, whereas athletes treated with CM showed a lactate recovery trend during the first week and mostly during the second week of study.

Five minutes after the end of training (LES_5), the values of lactate concentrations and the general trend of curves in the three groups of study were

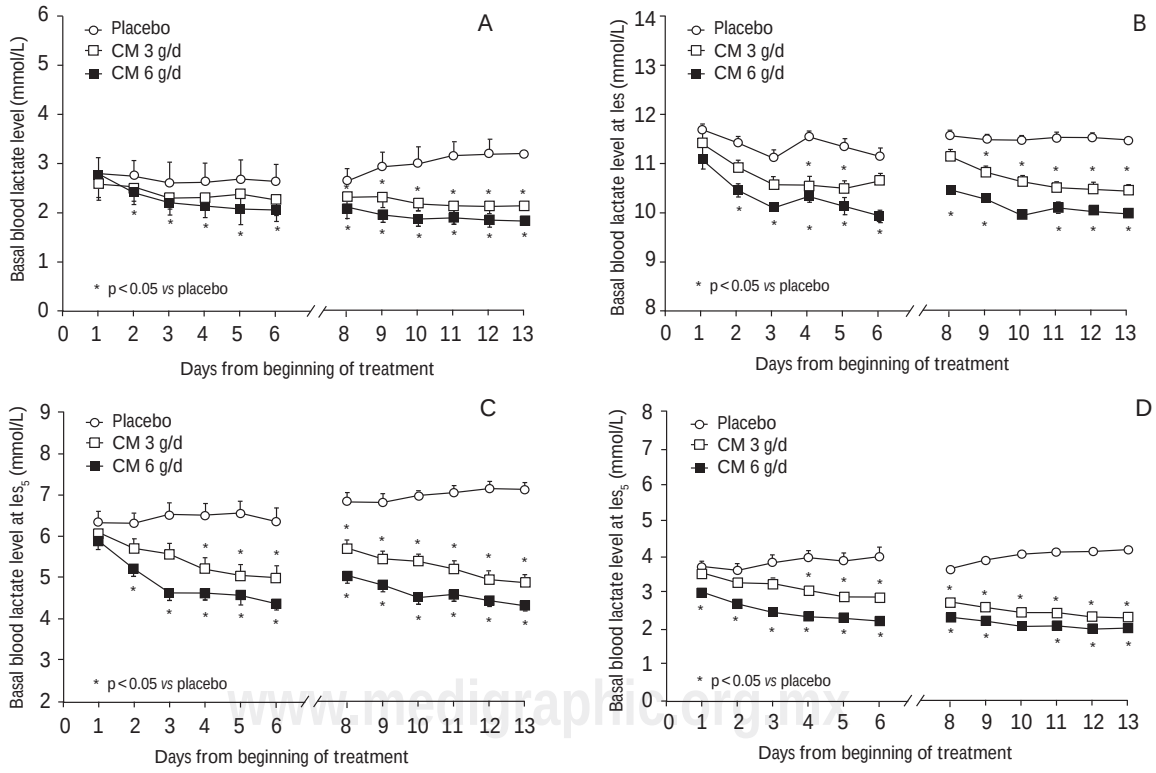

Figure 3. Changes over time in blood lactate levels during the 4 phases of monitoring: A) basal, B) end of training; C) recovery 5 min after training, and D) recovery 30 min after training.
Data are shown as mean ± standard error of the mean (SEM). CM = Citrulline malate.
* p < 0.05 compared to the placebo.

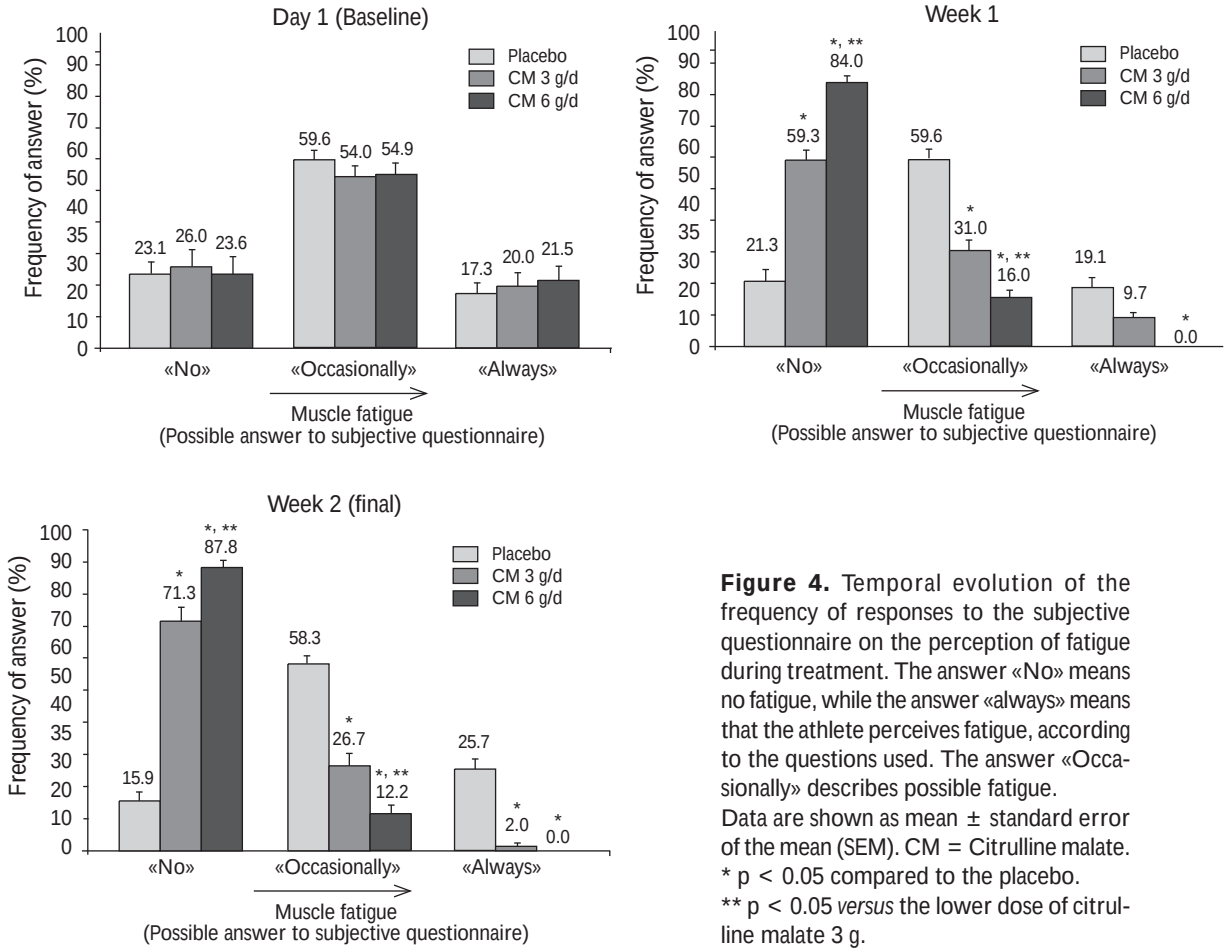

Figure 4. Temporal evolution of the frequency of responses to the subjective questionnaire on the perception of fatigue during treatment. The answer «No» means no fatigue, while the answer «always» means that the athlete perceives fatigue, according to the questions used. The answer «Occasionally» describes possible fatigue.
Data are shown as mean ± standard error of the mean (SEM). CM = Citrulline malate.
* $p < 0.05$ compared to the placebo.
** $p < 0.05$ *versus* the lower dose of citrulline malate 3 g.

very similar to those reported at LES *(figure 3C)*. The LES_{30} differed significantly between the 2 CM groups and the placebo, with differences starting from the 1st day for the CM 6 g group and from the 4th day for the CM 3 g (figure 3D). These differences persisted until the end of the study and also after washout resting day (Day 7).

Fatigue level assessed by questionnaire (figure 4). At baseline, the 3 groups had a similar level of perceived fatigue. After one week of treatment, the perceived fatigue decreased in the 2 active groups whereas it remained stable in the placebo group. Both doses of CM reduced the perception of fatigue during treatment. This effect appears to be dose dependent in the first week and be similar in the second week.

Safety. No adverse event was reported during the study. No dehydration occurred. Monitoring of weight and heart rate throughout the study showed no changes.

Discussion

The present study shows that CM allows a greater recovery of blood lactate and of fatigue after muscular effort in high performance athletes. The methodology used for the trial (randomised, double-blind, placebo-controlled) is adequate to warrant the validity of statistical results. The stratification scheme prevents analyse results with sport as cofactor. The study population, teenagers, represents the majority of high-level athletes,

which allows extrapolate the observed results to any endurance sportsman.

The mean basal levels of blood lactate concentration were similar in the three study groups and were higher than the normal range. This may be due to the high training to which athletes were submitted during the two weeks preceding the study; this intensive training aimed to guarantee blood lactate levels high enough to well appreciate potential CM effects. The blood lactate concentration has been chosen as evaluation criterion because it represents one of the most often measured parameters during clinical exercise testing and a rise in its value is considered an indicator of intense muscular activity.[10]

CM demonstrated its efficacy on the main study efficacy criterion, blood lactate recovery 30 min after training. This was supported also by the effects obtained on the percentages of responders and perceived fatigue. The study allowed also showing that the expected results are obtained quicker with 6 g daily, although the 2 dosages have the same efficacy after 2 weeks.

Athletes treated with placebo showed a lactate recovery rate from peak and a percentage of responders in the second week of treatment slightly higher than those reported in the first week. This has been considered physiological and interpreted as a consequence of the metabolic adaptation which occurs during intense muscle exercise.[11-13] The same difference in values between the first and the second week of study is less obvious in the two CM treated groups where the effects of CM are dominant over the physiological adaptation.

Lactate concentration variations from baseline to peak and during recovery phase, in the two weeks of study, showed that CM is efficient in holding lactate concentration stable or even decreasing, whereas in the placebo group blood lactate accumulates over time. The CM efficiency is evident at any monitoring point (baseline, LES, LES_5 and LES_{30}). Overall, if comparing the results obtained in the two weeks, the CM effect on lactate recovery at baseline as well as at peak is less variable and more pronounced during the second week. This is due to the mechanism of adaptation in the metabolic response of the working muscles during the first week training. The beneficial effects exerted by muscular activity on removal of lactate overproduction during endurance training are gathered from blood lactate levels as measured at Day 8 of peak. This is the day following the resting day and the corresponding values of lactate concentrations are higher than those registered on Day 6, confirming that physical exercise also contributes to stimulate lactate recovery. The physiological mechanisms underlying the CM effect on lactate recovery observed in this study could be hypothesized on the basis of results coming from previous investigations. CM administrated in association with physical exercises resulted in a significant reduction in the sensation of fatigue and increase in the rate of oxidative ATP production during exercise.[7] These effects have been ascribed to malate, a TCA intermediate, which could affect aerobic ATP production through anaplerotic reactions. In another study, CM administration has been associated to an increased rate of ammonia clearance.[14] Citrulline is involved in the urea cycle and allows detoxification of ammonia, which concentration increases during intense physical exercises with potential risks due to its cellular toxicity.

CM, whereas given at recommended dosage or higher dosage, has also shown to be well tolerated, without adverse effects in this teenager population.

Conclusions

This randomized, double-blind, placebo-controlled trial demonstrates that a supplementation of citrulline malate enhances lactate clearance and fatigue recovery of high performance endurance athletes. It becomes relevant to design strategies that clears blood lactate after high intensity exercise bouts, as this enables a faster recovery of the subject and may support subsequent high-intensity exercise, leading

to greater overload and consequently enhanced training adaptation, without deleterious effect.

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