



Molecular and Cellular Pharmacology

Citrulline malate supplementation increases muscle efficiency in rat skeletal muscle

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ABSTRACT

Citrulline malate (CM; CAS 54940-97-5, Stimol®) is known to limit the deleterious effect of asthenic state on muscle function, but its effect under healthy condition remains poorly documented. The aim of this longitudinal double-blind study was to investigate the effect of oral ingestion of CM on muscle mechanical performance and bioenergetics in normal rat. Gastrocnemius muscle function was investigated strictly non-invasively using nuclear magnetic resonance techniques. A standardized rest-stimulation- (5.7 min of repeated isometric contractions electrically induced by transcutaneous stimulation at a frequency of 3.3 Hz) recovery-protocol was performed twice, i.e., before (t_0-24 h) and after (t_0+48 h) CM (3 g/kg/day) or vehicle treatment. CM supplementation did not affect PCr/ATP ratio, [PCr], [Pi], [ATP] and intracellular pH at rest. During the stimulation period, it led to a 23% enhancement of specific force production that was associated to significant decrease in both PCr (28%) and oxidative (32%) costs of contraction, but had no effect on the time-courses of phosphorylated compounds and intracellular pH. Furthermore, both the rate of PCr resynthesis during the post-stimulation period ($VPCr_{rec}$) and the oxidative ATP synthesis capacity (Q_{max}) remained unaffected by CM treatment. These data demonstrate that CM supplementation under healthy condition has an ergogenic effect associated to an improvement of muscular contraction efficiency.

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1. Introduction

On the basis of experiments conducted *in vivo* and *in vitro*, it has been well established that oral administration of citrulline malate (CM; CAS 54940-97-5, Stimol®) limited the deleterious effects of asthenic state on muscle performance. For example, an increased force output in finger flexor muscles and a reduced fatigue sensation have been reported in men complaining of asthenia and submitted to a CM treatment (Bendahan et al., 2002). In endotoxemic rats, a model of asthenia in which muscle performance is reduced, CM administration has been reported to improve running endurance in treadmill tests (Verleye et al., 1995) and to limit the decreased electrically-evoked force-generating capacity *in vitro* in isolated epitrochlearis muscle (Goubel et al., 1997) and *in situ* in gastrocnemius muscles (Giannesini et al., 2009). These beneficial effects of CM could actually be linked to the fact that both malate and citrulline intervene at the muscle energy metabolism level. Malate is an intermediate of tricarboxylic acid cycle (TCA) and its supplementation could enhance

energy production (Bendahan et al., 2002; Wagenmakers, 1998). Besides, citrulline has been shown to accelerate the clearance of plasma ammonium and lactate, and might be involved in the elimination of muscle metabolism by-products thereby contributing to the improvement of muscle function (Briand et al., 1992; Verleye et al., 1995). These hypotheses have been reinforced on the basis of ³¹P-MRS measurements showing that CM supplementation promotes aerobic energy production in asthenic patients (Bendahan et al., 2002) and normalizes energy metabolism in endotoxemic rats (Giannesini et al., 2009).

Besides the positive effects of CM supplementation in asthenic patients and endotoxemic rats, the corresponding effects on healthy muscle have been scarcely reported. A study conducted in humans has recently shown that CM enhanced muscle resistance to fatigue and reduced muscle soreness during anaerobic exercise (Perez-Guisado and Jakeman, 2010) but the bioenergetics underpinnings have still to be documented.

The aim of this double-blind placebo-controlled work was to investigate *in situ* the effects of an oral administration of CM on skeletal muscle function in healthy rats. Longitudinal investigations were performed strictly non-invasively in electrically stimulated gastrocnemius muscle. ¹H-magnetic resonance imaging (MRI), ³¹Phosphorus spectroscopy (³¹P-MRS) and mechanical performance recording were used in order to characterize CM administration from functional and metabolic points of view.

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2. Materials and methods

2.1. Animal care and feeding

Twenty-four virus-free male Wistar rats (Charles River Laboratories, L'Arbresle, France) weighing 300–325 g were used for these experiments according to the guidelines of the National Research Council Guide for the care and use of laboratory animals, and the French Law on the Protection of Animals. Rats were housed in an environmentally controlled facility (12–12 h light–dark cycle, 22 °C), and received water and standard food *ad libitum* until the time of experiment.

2.2. Animal preparation

Animals were randomly assigned to one of the two groups. The first group (CM: $n = 12$) orally received CM treatment (1 g/kg) three times a day during 48 h, the last dose being administered 1 h before the MR experiment. The second group (vehicle: $n = 12$) received equivalent volumes of vehicle and served as control.

For each animal, MR investigation of muscle function was performed twice i.e. before ($t_0 - 24$ h) and immediately after the oral treatment (at $t_0 + 48$ h).

Animals were initially anesthetized in an induction chamber with 4% isoflurane (Forene®; Abbott France, Rungis, France) mixed in 33% O₂ (0.5 l/min) and 66% N₂O (1 l/min). Once the right lower hindlimb has been shaved, electrode cream for electromyogram was applied at knee and heel levels in order to optimize electrical stimulation. Anesthetized animal was placed supine in a home-built cradle, which has been designed for the strictly non-invasive functional and metabolic investigation of rat gastrocnemius muscle (Giannesini et al., 2005). Briefly, this cradle integrates a hydraulic ergometer and two rod-shaped transcutaneous electrodes connected to an electrical stimulator (Type 215/T; Hugo Sachs Elektronik - Harvard Apparatus GmbH, March-Hugstetten, Germany). Both electrodes are integrated in the cradle so that when the rat is placed inside, one electrode is located above the knee and the other at the heel level. The foot was positioned on the pedal of the ergometer and the hindlimb was immobilized in the cradle. In that position, the lower hindlimb was centered inside a 30-mm-diameter ¹H-MR Helmholtz imaging coil and the belly of the gastrocnemius muscle was located above an elliptic (10 × 16 mm) ³¹P-MRS surface coil. The optimal length of the gastrocnemius muscle was adjusted at rest by rotating the pedal in order to modify the angle between the foot and the lower hindlimb. Throughout the experiment, anesthesia was maintained by gas inhalation with a facemask continuously supplied with 2.5% isoflurane in 33% O₂ (0.4 l/min) and 66% N₂O (0.8 l/min) by an open-circuit gas anesthesia machine (Isotec 3; Ohmeda Medical, Herts, UK). Corneas were protected from drying by application of ophthalmic cream. Animal body temperature was maintained using an electric heating blanket (Prang + Partner AG, Pfungen, Switzerland) in a feedback loop with a temperature control unit (ref 507137; Harvard Apparatus, Holliston, Massachusetts, USA) connected to a rectal probe (ref 507145; Harvard Apparatus).

2.3. Stimulation protocol and force measurement

Muscle contractions were electrically induced with square-wave pulses (6–8 mA, 1 ms duration) throughout a stimulation protocol consisted in 5.7 min of repeated isometric contractions at a frequency of 3.3 Hz. Electrical signal coming out from the pressure transducer was amplified (reference: 13-4515-50, Gould, USA), converted to a digital signal and processed on a personal computer using ATS software (Sysma, Aix-en-Provence, France). Isometric force production was calculated by integrating isometric tension (in N) relative to time, and was expressed as tension-time integral (in Ns).

2.4. MR acquisition

MR data were acquired in a 4.7-Tesla horizontal superconducting magnet (47/30 Biospec Avance; Bruker, Germany). Five consecutive non-contiguous axial imaging slices (2 mm thickness, spaced 1 mm) were selected across the lower hindlimb. Multiecho images of these slices (8 echoes, 16 ms spaced; 1000 ms repetition time; 4 cm field of view; 256 × 128 acquisition matrix; 4.57 min total acquisition time) were recorded at rest. ³¹P-MR spectra (16 accumulations; 1.8 s repetition time; 8 kHz spectral width) from the gastrocnemius muscle region were continuously acquired in 28.5-s blocks throughout the experimental protocol, i.e., 5.7 min of rest, 5.7 min of stimulation and 16.6 min of recovery. MR data acquisition was gated to muscle stimulation in order to reduce potential motion artifacts due to contraction.

2.5. MR data processing

MR data were processed using a proprietary software developed using IDL (Interactive Data Language, Research System Inc., Boulder, Colorado, USA). Cross-sectional area (CSA, in mm²) of gastrocnemius muscle was measured in MR images by manually outlining regions of interest with a custom-written image analysis program, excluding fat and bone. Gastrocnemius volume (in mm³) was calculated as the sum of the four volumes included between the five consecutive slices.

Relative concentrations of phosphorylated compounds were calculated by a time-domain fitting routine using the AMARES-MRUI Fortran code (Vanhamme et al., 1997). Signal area were corrected for magnetic saturation effects using fully relaxed spectra collected at rest with a repetition time of 20 s. Absolute concentrations of phosphorylated compounds were expressed relative to a resting ATP concentration (5.1 mM) determined from HPLC assays in extracts of freeze-clamped gastrocnemius muscle samples (Giannesini et al., 2007). Intramuscular pH (pH_i) was calculated from the chemical shift of P_i relative to PCr (Arnold et al., 1984). Time-points for the time course of phosphorylated metabolite concentrations and pH_i were assigned to the midpoint of the acquisition interval.

2.6. Calculations

The initial rate of PCr degradation ($V_{PCr, start}$, in mM/min) at the start of the stimulation period was calculated as the product of k_{stim} (the pseudo-first-order rate-constant of PCr degradation) and $[PCr]_{cons}$ (the amount of PCr consumed at the end of the stimulation period). The constant k_{stim} was determined by fitting the PCr time-course during this period to a single exponential curve described by the equation: $[PCr]_t = [PCr]_{end} + [PCr]_{cons} e^{-kt}$, where $[PCr]_{end}$ is the concentration of PCr measured at the end of the stimulation period (Foley and Meyer, 1993). Similarly, the initial rate of PCr resynthesis ($V_{PCr, rec}$, in mM/min) at the start of the post-stimulation period was the product of k_{rec} and $[PCr]_{cons}$ (McCully et al., 1994). The constant k_{rec} was determined by fitting the PCr time-dependent changes during this period to a single exponential curve described by the equation: $[PCr]_t = [PCr]_{rest} - [PCr]_{cons} e^{-kt}$, where $[PCr]_{rest}$ is the concentration of PCr measured at rest. The rate of PCr degradation was scaled to the specific force production in order to obtain the PCr cost of contraction (in μmol/N*s) as previously described (Foley and Meyer, 1993). Considering that $V_{PCr, rec}$ can be used to estimate the rate of oxidative ATP production at the end of the stimulation period (Boska, 1991), oxidative cost was measured at this time as the ratio between $V_{PCr, rec}$ and specific force production.

The maximal oxidative ATP synthesis capacity (Q_{max} , in mM/min) was calculated during the post-stimulation recovery period using the initial rate of PCr resynthesis ($V_{PCr, rec}$): $Q_{max} = V_{PCr, rec} (1 + K_m/[ADP]_{end})$ (Kemp and Radda, 1994), in which K_m (the [ADP] at half-maximal oxidation rate) was 50 μM in rat skeletal muscle (Thompson et al., 1995) and $[ADP]_{end}$ is the concentration of free cytosolic ADP at the end of the stimulation period. $[ADP]_{end}$ was calculated from $[PCr]$, $[ATP]$ and pH_i

using the creatine kinase equilibrium constant ($K = 1.67109 \text{ M}^{-1}$) (Roth and Weiner, 1991).

2.7. Statistical analysis

The overall time-courses of variables changing with respect to time during the stimulation period (isometric force production, metabolite concentrations, and pH_i) were compared using ANOVAs with repeated-measurements (JMP software, SAS Institute Inc., Cary, North Carolina, USA). The between-group comparisons were performed using one-way ANOVA. Two-way ANOVA were performed in order to investigate the effects of treatment in each group. Other variables were compared with paired (treatment effect) or unpaired (group effect) Student's *t*-test. Values are means \pm S.E.M. *P* values of less than 0.05 were considered as significant.

3. Results

3.1. Force measurements

Before treatment, the time-course of specific force during the 5.7-min stimulation period did not differ between vehicle and CM groups ($P = 0.86$) (Fig. 1). For each group, specific force decreased progressively throughout the stimulation period as a sign of fatigue development. At the end of the stimulation period, the extent of this relative decrease was similar in both groups (Table 1).

Vehicle treatment did affect neither the time-course ($P = 0.17$; Fig. 1) nor the extent of force reduction measured at the end of the stimulation period (Table 1). On the contrary, considering the curve integral, i.e., the total specific force produced during the whole stimulation period, a significant ($P = 0.02$) increase (averaging 23%) was measured as a result of the CM group treatment (Table 1). However, the extent of force reduction measured at the end of the stimulation period was unchanged in CM group, hence indicating that CM had no effect on muscle fatigability (Table 1).

3.2. Energy metabolism

Before treatment, PCr/ATP ratio, [PCr], $[\text{P}_i]$, [ATP] and pH_i did not differ between groups at rest (Table 1). As illustrated in Fig. 2, time-courses in [PCr] ($P = 0.89$; Fig. 2A), $[\text{P}_i]$ ($P = 0.29$; Fig. 2B), [ATP] ($P = 0.71$; Fig. 2C) and pH_i ($P = 0.76$; Fig. 2D) were similar in both groups. At the onset of the stimulation period, [PCr] was rapidly consumed (Fig. 1A) and the corresponding rate ($\text{VPCr}_{\text{start}}$) was similar in both groups (Table 1). After 1.5 min of stimulation, [PCr] reached a steady-state that was maintained until the end of the stimulation period. At this stage, [PCr] was $6.2 \pm 0.5 \text{ mM}$ and $5.9 \pm 0.3 \text{ mM}$ in the vehicle and CM group respectively (Fig. 2A). At the start of the post-stimulation recovery period, VPCr_{rec} illustrated similar PCr time-

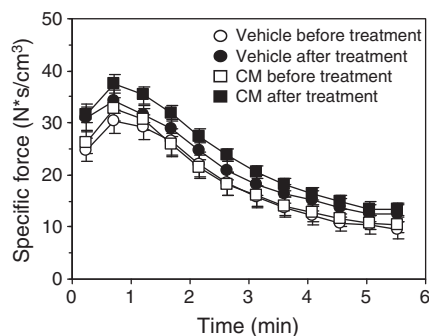


Fig. 1. Changes in specific isometric force during 5.7 min of repeated isometric contractions induced electrically at 3.3 Hz in rat gastrocnemius muscle. Values are means \pm S.E.M.

Table 1
Mechanical performance and energy metabolism in rat gastrocnemius muscle.

	Vehicle group		CM group	
	Before treatment	After treatment	Before treatment	After treatment
Rest				
[PCr]/[ATP]	2.96 ± 0.10	3.00 ± 0.07	3.07 ± 0.08	3.10 ± 0.07
[PCr] (mM)	15.1 ± 0.5	15.4 ± 0.4	15.7 ± 0.4	15.9 ± 0.3
$[\text{P}_i]$ (mM)	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	0.8 ± 0.1
[ATP] (mM)	5.2 ± 0.3	5.0 ± 0.2	5.1 ± 0.2	5.1 ± 0.3
pH_i	7.09 ± 0.01	7.07 ± 0.02	7.09 ± 0.02	7.09 ± 0.02
Initial phase of the stimulation period				
$\text{VPCr}_{\text{start}}$ (mM/min)	20.0 ± 1.1	21.5 ± 1.1	21.4 ± 1.0	19.0 ± 1.0
PCr_{cost} ($\mu\text{mol}/\text{N}^*\text{s}$)	41.7 ± 4.3	37.8 ± 6.0	41.4 ± 3.5	29.9 ± 2.7^a
End of the stimulation period				
Ox_{cost} ($\mu\text{mol}/\text{N}^*\text{s}$)	21.5 ± 3.6	18.0 ± 3.7	19.0 ± 2.6	13.0 ± 1.5^a
ΔPCr (% of basal value)	40.7 ± 2.6	37.5 ± 2.2	37.4 ± 2.1	39.3 ± 2.0
pH_i	6.42 ± 0.04	6.43 ± 0.03	6.39 ± 0.03	6.40 ± 0.02
ΔpH (pH units)	0.67 ± 0.04	0.64 ± 0.04	0.70 ± 0.03	0.69 ± 0.02
Total force production (Ns/cm^3) ^b	213 ± 22	247 ± 20	220 ± 20	271 ± 11^a
Force reduction (% of start value)	37.9 ± 5.6	39.1 ± 3.8	40.1 ± 4.4	43.3 ± 4.9
Post-stimulation recovery period				
VPCr_{rec} (mM/min)	3.2 ± 0.3	3.6 ± 0.3	3.5 ± 0.3	3.3 ± 0.2
Q_{max} (mM/min)	19.7 ± 1.6	18.2 ± 1.7	19.2 ± 1.7	17.9 ± 0.9

Values are means \pm S.E.M. Before treatment, no difference was found between vehicle and CM group, hence demonstrating the high reliability of the corresponding measurements. $\text{VPCr}_{\text{start}}$, initial rate of PCr degradation at the start of the stimulation period; VPCr_{rec} , initial rate of PCr resynthesis at the start of the post-stimulation recovery period; PCr_{cost} , PCr cost of contraction; Ox_{cost} , oxidative cost of contraction; Q_{max} , oxidative ATP synthesis capacity.

^a Significantly different from pre-treatment value within the group ($P < 0.05$).

^b Specific force production during the whole stimulation period.

dependent changes in both groups. (Table 1). The time course of $[\text{P}_i]$ exhibited an initial phase of rapid and massive accumulation followed by a steady state (Fig. 2B). At the end of the stimulation period, $[\text{P}_i]$ amounted to $11.9 \pm 0.5 \text{ mM}$ and $12.5 \pm 0.4 \text{ mM}$ in the vehicle and CM group respectively. [ATP] slightly decreased during the stimulation period under both conditions (Fig. 2C). At the end of the stimulation period, the corresponding concentration was similar in the vehicle ($3.3 \pm 0.3 \text{ mM}$) and the CM-treated group ($3.5 \pm 0.3 \text{ mM}$). For each group, pH_i fell rapidly in the early stage of the stimulation period and reached a minimum value after 3 min of stimulation (Fig. 2D). Afterward, pH_i recovered slightly and was similar in the vehicle (6.42 ± 0.04) and CM-treated group (6.39 ± 0.03) at the end of the stimulation period. Overall, the similarity between mechanical and metabolic measurements performed in both groups before the treatment period highlights the large measurements reliability as previously reported (Giannesini et al., 2010).

The effect of oral treatment was investigated within each group. Vehicle and CM administration did disturb neither variables at rest (PCr/ATP ratio, [PCr], $[\text{P}_i]$, [ATP] and pH_i ; Table 1), nor the time-courses in [PCr] ($P = 0.51$ for vehicle and $P = 0.71$ for CM; Fig. 2A), $[\text{P}_i]$ ($P = 0.32$ for vehicle and $P = 0.26$ for CM; Fig. 2B), [ATP] ($P = 0.95$ for vehicle and $P = 0.31$ for CM; Fig. 2C) and pH_i ($P = 0.93$ for vehicle and $P = 0.33$ for CM; Fig. 2D) during the stimulation period. Overall, the extents of PCr and pH_i changes measured at the end of the stimulation period as well as the end-stimulation pH_i were affected neither by the CM treatment nor by the vehicle supplementation (Table 1).

3.3. Energy cost of contraction and oxidative capacity

Before treatment, no differences of Q_{max} , PCr_{cost} and Ox_{cost} were found between both groups (Table 1). Vehicle treatment did not affect these variables (Table 1). On the contrary, CM supplementation did significantly decrease both PCr_{cost} (28% decrease) and Ox_{cost} (32%, decrease) thereby illustrating an improved muscular efficiency. The

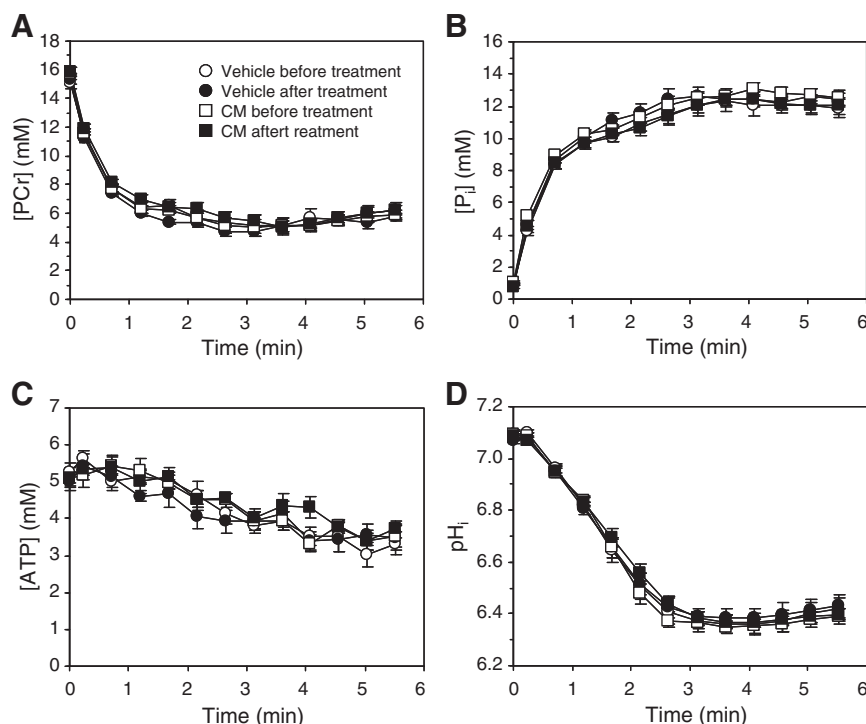


Fig. 2. [PCr] (A), [P_i] (B), [ATP] (C) and pH_i (D) changes during 5.7 min of repeated isometric contractions induced electrically at 3.3 Hz in rat gastrocnemius muscle. The first time point (t = 0) indicates the resting value. Values are means ± S.E.M.

maximal rate of oxidative production was not modified in the CM-treated group.

4. Discussion

This noninvasive study demonstrated that oral administration of CM enhanced muscle force production and decreased energy cost of contraction in healthy rats. It must be underlined that mechanical and metabolic measurements performed before the treatment sessions were highly similar in both experimental groups, hence demonstrating the very high reliability of the corresponding quantitative measurements. On that basis, the effect of treatment was investigated longitudinally within each group.

Our findings are in line with previous studies conducted in asthenic weakened muscle showing that CM treatment was related to an improved force generating capacity (Bendahan et al., 2002; Giannesini et al., 2009; Verleye et al., 1995). While previous results have indicated that CM partially restored muscle performance toward a normal level under pathological conditions, the present results clearly showed that, under normal conditions, CM-treatment can significantly improve force production in electrically stimulated healthy rat muscle. However, this improvement was not associated to any change in muscle fatigability, which is in contrast with previous experiments reporting that CM supplementation could limit the deleterious effects of endotoxins on muscle fatigability in isolated rat muscle electrically-stimulated (Goubel et al., 1997). Overall, our data further supports the beneficial effect of CM on muscle force production and demonstrate its ergogenic effect in healthy muscle.

Although the beneficial effects of CM supplementation on muscular performance have been established in both humans and animals, the exact corresponding mechanisms remain to be clarified. It has been suggested that these beneficial effects would be related to the involvement of malate in muscle energetics (Bendahan et al., 2002; Briand et al., 1992; Verleye et al., 1995; Wagenmakers, 1998). Malate is a TCA intermediate that could affect mitochondrial ATP production through anaplerotic reaction, i.e., reactions replenishing the TCA cycle

intermediates pool (Gibala et al., 2000; Sahlin et al., 1990). It can be dehydrogenated in the TCA cycle to form oxaloacetate, the concentration of which plays a crucial role in the control of aerobic ATP production. Interestingly, malate shows the largest relative and absolute changes during exercise, typically accounting for 350% of the net increase in the total pool size (Gibala et al., 1998). Measurements of metabolic changes in exercising muscle have demonstrated that asthenic patients could reach higher rates of aerobic ATP production as a result of CM treatment (Bendahan et al., 2002). Accordingly, it has been proposed that the limitation of TCA intermediates supply in contracting muscle would decrease the flux through the TCA cycle, thereby contributing to muscle fatigue development (Sahlin et al., 1990; Wagenmakers, 1998). In the present study, the rate of mitochondrial ATP production during the 6-min stimulation was inferred from the initial rate of PCr resynthesis during the post-stimulation recovery period ($V_{PCr_{rec}}$), as previously described (Kemp and Radda, 1994; Paganini et al., 1997). It is noteworthy that $V_{PCr_{rec}}$ was not modified in the CM treated group, hence indicating that the improvement in muscle performance cannot be due to any malate-induced increase in mitochondrial ATP production during the stimulation period.

Interestingly, we found that CM treatment significantly decreased both PCr and oxidative costs of contraction, by 28% and 32%, respectively. Intramuscular PCr is under the control of the creatine kinase (CK), which reversibly transfers high energy phosphate from PCr to ADP to form ATP. A large part of the CK activity is ensured by cytosolic CK (90–95% in fast-twitch fibers and 70% in slow twitch fibers) which is coupled to glycolysis, and the rest is due to mitochondrial CK (Wallimann et al., 1992). In the early stage of muscle contraction, the PCr-CK system is commonly considered as a temporal energy buffer because the rapid net breakdown in PCr (which is the only source of energy contributing to ATP regeneration at this stage of muscle activity) allows to maintain the ATP pool unchanged although ATP demand can significantly increase i.e. more than 100-fold (Sahlin et al., 1998; Wallimann et al., 1992). With continuation of muscular activity, glycolysis and oxidative phosphorylation progressively carry out ATP regeneration so that the PCr reaches a steady state. Then, the PCr-CK

system functions as a spatial energy buffer, and the pool of PCr behaves as a shuttle for the transport of high-energy phosphates between the sites of production and utilization of ATP (Meyer et al., 1984; Wallimann et al., 1992). It is therefore possible that the decreased energy costs of contraction related to CM treatment are due to an improvement of PCr-CK system leading to an optimization of energy transport and/or utilization to produce force.

The origin of CM-induced energy cost decrease remains to be elucidated. Apart from malate, citrulline could also be considered as an actor of the force improvement recorded in the CM-treated group. During repeated contractions, ATP is used for both contractile and non-contractile processes. The latter process is related to ions and metabolites transport and has been reported to represent 20 to 50% of the total ATP demand according to the type of activity (Hogan et al., 1998; Juel, 1996). CM might be involved in the elimination of muscle metabolism by-products thereby contributing to the improvement of muscle function. Citrulline has indeed been shown to accelerate the clearance of plasmatic ammonium and lactate (Briand et al., 1992; Verleye et al., 1995). Further, it has been reported in human that acute CM intake prior to a cycling session raises plasma L-arginine concentration and arginine-derived metabolites such as nitrite, creatinine, ornithine and urea, probably in relation to an increased utilization of branched chain amino acids (Sureda et al., 2010). It is then legitimate to hypothesize that the facilitation of by-products elimination by citrulline reduces ATP consumption by non-contractile process, hence decreasing the global energy cost of contraction.

Considering the reduced oxidative cost of contraction reported as a result of the CM treatment one could expect an adaptive mechanism related to an increased glycolytic contribution thereby keeping the total energy cost constant. Changes in pH_i during muscle activity are caused by intramyoplasmic accumulation of protons associated to anaerobic ATP production; Indeed, when coupled to ATP hydrolysis, glycolytic ATP production is linked to a net proton generation with a stoichiometry of 1.5 moles ATP per proton, because the degradation of each mole of glycosyl unit generates 3 moles of ATP that, when hydrolyzed by cell ATPase, causes the net yield of 2 moles of protons (Hochachka and Mommsen, 1983). Considering the similar changes in pH (resulting from H^+ accumulation) and PCr (related to H^+ consumption) during the stimulation period, we can rule out an increased glycolytic flux as a compensatory mechanism of the reduced oxidative cost.

In conclusion, CM supplementation under healthy condition produces a net increase in force production during a fatiguing bout of exercise in situ. This ergogenic effect is associated to an improvement in energy cost of contraction that could be explained by a reduced ATP consumption by non-contractile process due to the known role of citrulline in by-products elimination.

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