European Journal of Pharmacology 602 (2009) 143–147

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, Gastrointestimal and Urogenital Pharmacology

Beneficial effects of citrulline malate on skeletal muscle function in endotoxemic rat

Benoît Giannesini [a,*], Marguerite Izquierdo [a], Yann Le Fur [a], Patrick J. Cozzone [a], Marc Verleye [b], Marie-Emmanuelle Le Guern [b], Jean-Marie Gillardin [b], David Bendahan [a]

[a] Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS 6612, Faculté de Médecine de Marseille, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France

[b] Biocodex, Centre de Recherche, Zac de Mercières, 60200 Compiègne, France

ARTICLE INFO

Article history:
Received 22 July 2008
Received in revised form 21 October 2008
Accepted 10 November 2008
Available online 17 November 2008

Keywords:
Stimol®
Fatigue
Muscle weakness
Energy metabolism
Non invasive nuclear magnetic resonance

ABSTRACT

Although citrulline malate (CM; CAS 54940-97-5, Stimol®) is used against fatigue states, its anti-asthenic effect remains poorly documented. The objective of this double-blind study was to evaluate the effect of oral ingestion of CM on a rat model of asthenia, using in situ ^{31}Phosphorus magnetic resonance spectroscopy (^{31}P-MRS). Muscle weakness was induced by intraperitoneal injections of *Klebsiella pneumoniae* endotoxin (lipopolysaccharides at 3 mg/kg) at t_0 and t_0+24 h. For each animal, muscle function was investigated strictly non-invasively before (t_0−24 h) and during (t_0+48 h) endotoxemia, through a standardized rest-stimulation-recovery protocol. The transcutaneous electrical stimulation protocol consisted of 5.7 min of repeated isometric contractions at a frequency of 3.3 Hz, and force production was measured with an ergometer. CM supplementation in endotoxemic animals prevented the basal phosphocreatine/ATP ratio reduction and normalized the intracellular pH (pH_i) time-course during muscular activity as a sign of an effect at the muscle energetics level. In addition, CM treatment avoided the endotoxemia-induced decline in developed force. These results demonstrate the efficiency of CM for limiting skeletal muscle dysfunction in rats treated with bacterial endotoxin.

1. Introduction

Asthenia, more commonly known as pathological fatigue, is linked to an altered skeletal muscle performance in many infectious and metabolic diseases. Citrulline malate (CM; CAS 54940-97-5, Stimol®) is usually prescribed as an anti-asthenic treatment and studies performed in animal models have shown that CM ingestion reduces muscle fatigability and weakness. More particularly, it has been shown in endotoxemic rats (a model of muscle weakness) that CM treatment improves the running endurance in treadmill tests (Verleye et al., 1995) and increases the electrically-evoked force-generating capacity in isolated *epitrochlearis* muscle (Goubel et al., 1997). These beneficial effects have also been documented in humans (Bendahan et al., 2002).

Considering the potential role of muscle energetics in muscle fatigue (Allen et al., 2008; Allen and Westerblad, 2001; Sahlin et al., 1998) and given that fatigue reduction and improved muscle performance have been reported in asthenic patients and treated animals as a result of CM administration, one could hypothesize an effect of CM on muscle energetics. Fatigue is actually a complex mechanism (Allen et al., 2008; Fitts, 1994). Potential factors involved in its etiology fall into two broad categories: central factors, which would disturb neuromuscular transmission between the central nervous system and muscle membrane, and peripheral factors, which would lead to alteration within the muscle. During the last decades, the relative contributions of central and peripheral factors in fatigue development have been subject to controversy. However, it seems now established that fatigue would have, at least for a significant part, a peripheral origin, central factors contributing modestly (Kent-Braun, 1999; Westerblad et al., 1998). Regarding the metabolic origin of peripheral fatigue, intramuscular accumulation of by-products of ATP hydrolysis and limitation in ATP availability have been proposed, among others, as potential causative factors of fatigue development (Allen and Westerblad, 2001; Sahlin et al., 1998). In that respect, the beneficial effect of CM on mechanical performance might be linked to a direct contribution of both malate and citrulline at the muscle energy metabolism level as previously suggested (Goubel et al., 1997; Meneguello et al., 2003). On the one hand, malate is an intermediate of the tricarboxylic acid cycle (TCA) and its supplementation would enhance energy production (Wagenmakers, 1998). On the other hand, citrulline is involved in the urea cycle and it has been shown to accelerate the clearance of plasma ammonium and lactate (Briand et al., 1992). However, the isolated effect of citrulline on

* Corresponding author. Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS 6612, Faculté de Médecine de Marseille, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France. Tel.: +33 4 91 32 44 70; fax: +33 4 91 25 65 39.
E-mail address: benoit.giannesini@univmed.fr (B. Giannesini).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.11.015

muscle function is controversial given that the time to exhaustion following oral citrulline supplementation increases in rats (Meneguello et al., 2003) but decreases in humans (Hickner et al., 2006).

The aim of this double-blind study was to investigate *in vivo* the effect of oral ingestion of CM on muscle energetics and mechanical performance in a rat model of muscle weakness. Given the established beneficial effects of CM on muscle fatigue, we hypothesized that CM treatment can modify muscle energetics under endotoxemia. Muscle weakness was induced by intraperitoneal injections of bacterial endotoxins as done previously (Goubel et al., 1997; Verleye et al., 1995), and investigations were performed using ^{31}P-MRS. The novelty of our approach was to repeat investigations of skeletal muscle function twice in the same animals before and during treatment, using an original experimental setup (Giannesini et al., 2005).

2. Materials and methods

2.1. Animal care and feeding

Nineteen virus-free male Wistar rats (Charles River Laboratories, L'Arbresle, France) weighing 300–325 g were used for these experiments, in agreement with the French guidelines for animal care and with the approval of the local Ethics Committee. 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. Experimental design

Animals were randomly assigned to two groups. For each group, animals received two intraperitoneal injections of a lipopolysaccharides (LPS) suspension at 3 mg/kg body weight, at t_0 and at t_0+24 h. For this purpose, lyophilized LPS from *Klebsiella pneumoniae* (ref L4268; Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) was dissolved in a sterile physiological saline (0.9% NaCl w/v). The first group (CM: n=9) orally received CM treatment (1 g/kg) three times a day during 48 h after the first LPS injection. The last dose was administered 1 h before the muscle function investigation. The second group (Vehicle: n=10) received equivalent volumes of vehicle.

2.3. Animal preparation

For each animal, muscle function was investigated at two times, at t_0−24 h (before treatment) and at t_0+48 h (during treatment). Basal body temperature and body weight were measured at rest immediately before each investigation.

Rats were initially anaesthetized in an induction chamber with 4% isoflurane (Forene®; Abbott France, Rungis, France) mixed in 33% oxygen (O_2; 0.5 L/min) and 66% Nitrous oxide (N_2O; 1 L/min). Once the right lower hindlimb was shaved, electrode cream for electromyogram was applied at knee and heel levels in order to optimize electrical stimulation. Anaesthetized rat was placed supine in a home-built cradle especially designed for the strictly non-invasive functional investigation of the right gastrocnemius muscle (Giannesini et al., 2005). This cradle integrates a hydraulic ergometer and two rod-shaped transcutaneous electrodes (located above the knee and under the heel respectively) connected to an electrical stimulator (Stimulator I series, Hugo Sachs Elektronik, Harvard Apparatus, USA). The foot was positioned on the ergometer pedal and the hindlimb was immobilized in the cradle. In this position, the belly of the gastrocnemius muscle was located above an elliptic (10×16 mm) ^{31}P-MRS surface coil. The pedal position was adjusted in order to modify the angle between the foot and the lower hindlimb so that the gastrocnemius muscle was passively stretched at rest and produced a maximum isometric twitch tension in response to supramaximal square wave pulses (6–8 mA, 1 ms duration).

Throughout the experiment, anesthesia was maintained by gas inhalation with a facemask continuously supplied with 2.5% isoflurane in 33% O_2 (0.4 L/min) and 66% N_2O (0.8 L/min). The facemask was connected to an open-circuit gas anesthesia machine (Isotec 3; Ohmeda Medical, Herts, UK). Exhaled and excess gases were removed through a canister filled with activated charcoal mounted on an electrical pump extractor (Equipement Vétérinaire Minerve, Esternay, France). Corneas were protected from drying by application of ophthalmic cream (Lacrigel®; Europhta, Monaco). During anesthesia, animal body temperature was maintained through a feedback loop including an electrical heating blanket (Prang+Partner AG, Pfungen, Switzerland), a temperature control unit (ref 507137; Harvard Apparatus, Holliston, Massachusetts, USA) and a rectal probe (ref 507145; Harvard Apparatus).

2.4. Stimulation protocol and force measurement

Muscle contractions were electrically induced with square-wave pulses (6–8 mA, 1 ms duration). The 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 for each 14.25 s period of stimulation by integrating isometric tension (N) with respect to time (s).

2.5. MR data acquisition data processing

Investigations were performed in a 4.7 Tesla horizontal superconducting magnet (47/30 Biospec Avance, Brüker, Germany). ^{31}P-MR spectra (16 accumulations; 1.8 s repetition time; 8 kHz spectral width, 512 data points) 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 post-stimulation recovery. MR data acquisition was gated to muscle stimulation in order to reduce potential motion artifacts due to contraction.

MR data were processed using a proprietary software developed using IDL (Interactive Data Language, Research System Inc., Boulder, Colorado, USA). Relative concentrations of phosphorylated compounds were obtained by a time-domain fitting routine using the AMARES-MRUI Fortran code (Vanhamme et al., 1997). Signal areas were corrected for magnetic saturation 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). We calculated pH_i from the chemical shift difference between P_i and phosphocreatine (PCr) peaks (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. The rate constant (k_{rec}) of PCr resynthesis during the post-stimulation period was determined by fitting the PCr time-dependent changes during this period to a single exponential curve described by the equation: $[PCr]_t=[PCr]_{rest}-[PCr]_{cons}\ e^{-krec*t}$, where $[PCr]_{rest}$ and $[PCr]_{cons}$ are respectively the concentration of PCr measured at rest and the difference between $[PCr]_{rest}$ and the PCr concentration measured at end of the stimulation period.

2.6. Statistical analysis

For variables changing with respect to time during the stimulation period (isometric force, metabolite concentrations and pH_i), the effect of LPS treatment on the overall time-course was analyzed with repeated-measurements ANOVAs using JMP software (SAS Institute

Table 1
Body temperature and body mass

	Vehicle before treatment	Vehicle+LPS	CM before treatment	CM+LPS
Body temperature (°C)	36.5±0.1	37.2±0.1[a]	36.7±0.1	37.2±0.1[b]
Body mass (g)	306±4	287±5[a]	322±7	296±5[b]

Values are means±S.E.M.
[a] $P<0.05$ versus vehicle before treatment.
[b] $P<0.05$ versus CM before treatment.

Table 2
Energy metabolism in rat gastrocnemius muscle

	Vehicle before treatment	Vehicle+LPS	CM before treatment	CM+LPS
Basal pH_i	7.04±0.01	7.04±0.01	7.05±0.01	7.04±0.01
Basal [PCr]/[ATP]	3.68±0.06	3.22±0.09[a]	3.51±0.12	3.20±0.12
k_{rec}, in min^{-1}	0.35±0.02	0.43±0.03[a]	0.36±0.02	0.35±0.03

Values are means±S.E.M. k_{rec}, rate constant of PCr resynthesis during the post-stimulation period.
[a] $P<0.05$ versus vehicle before treatment.

Inc., Cary, North Carolina, USA). Other variables were compared with Student's *t*-test. Values are means±S.E.M. In all statistical analyses, the 0.05 level of significance was used.

3. Results

3.1. Clinical outcomes

Endotoxin treatment induced a number of pathological features such as decreased sensitivity to touch, piloerection, behavioral depression, lethargy and diarrhea. Body temperature and body mass did not differ between both groups before treatment (Table 1). LPS injections were associated to a significant increase in body temperature (+ 0.7±0.1 °C and+0.4±0.1 °C in vehicle and CM group, respectively) and a reduction of body mass (−6.1±0.6% and −8.3± 0.7% in vehicle and CM group, respectively).

3.2. Muscle force measurements

Before the endotoxin injections, one-way repeated-measurements ANOVA indicated that the time-course of force production did not differ between both groups ($P=0.74$) throughout the whole stimulation period (Fig. 1). For each group, force decreased throughout the stimulation period as a sign of fatigue. At the end of the stimulation period, the force output did not differ between groups, reaching 59.1± 6.1% and 55.1±6.3% for vehicle and CM group respectively. In both groups, LPS injections did not alter the extent of relative force at the end of the stimulation period. However, two-way repeated-measurements ANOVA indicated that the time-course of force production throughout the stimulation period was reduced in the vehicle group ($P=0.025$) whereas it was not altered in the CM group ($P=0.054$).

3.3. Muscle energy metabolism

Before endotoxemia, pH_i and PCr/ATP ratio at rest did not differ between groups (Table 2). During the stimulation period, there were no differences between both groups regarding the time-courses in [PCr] ($P=0.372$; Fig. 2A), [ATP] ($P=0.690$; Fig. 2B) and pH_i ($P=0.533$; Fig. 2C). PCr was rapidly consumed at the onset of the stimulation period and reached a steady-state after 1.5 min of stimulation (Fig. 2A). This level was maintained until the end of the stimulation period, when PCr levels were 45.8±3.6% and 45.6±2.4% of their basal value for the vehicle and CM groups respectively. In addition, the rate constant of PCr recovery (k_{rec}) was similar between both groups (Table 2). At the same time, [ATP] decreased slightly throughout the stimulation period (Fig. 2B). For each group, pH_i fell rapidly in the early stage of the stimulation period and reached a minimum value 3 min after the onset of stimulation (Fig. 2C). Then, pH_i slowly increased and reached, at end

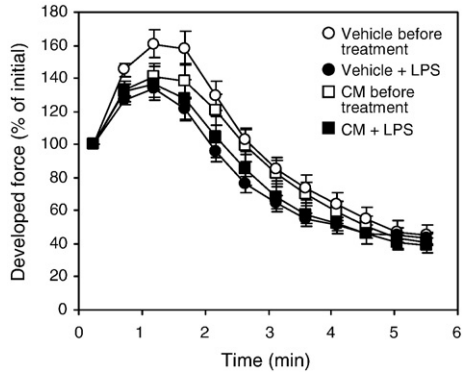

Fig. 1. Changes in relative isometric force during 5.7 min of repeated isometric contractions induced electrically at 3.3 Hz. Values are means±S.E.M.

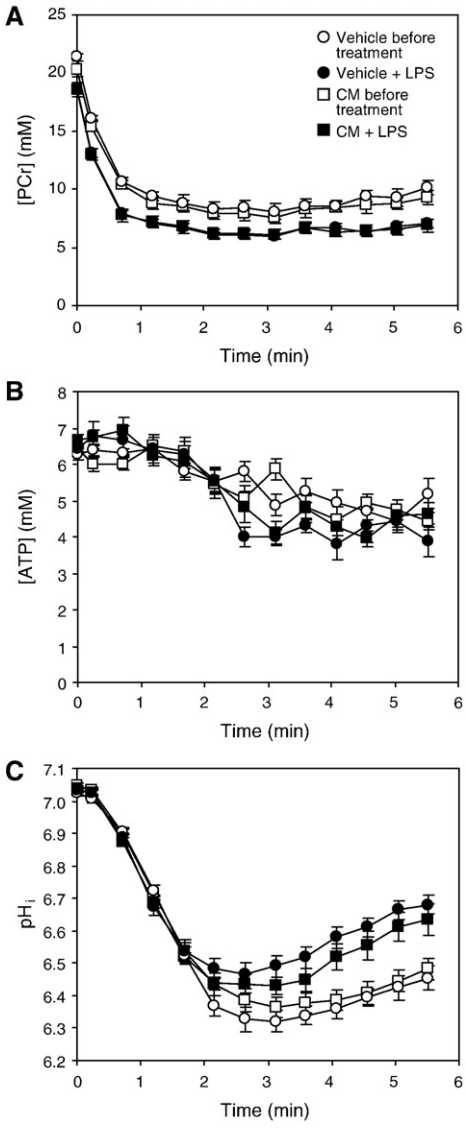

Fig. 2. Changes in [PCr] (A), [ATP] (B) and intracellular pH (C) during 5.7 min of repeated isometric contractions induced electrically at 3.3 Hz. Values are means±S.E.M.

of the stimulation period, a value of 6.45 ± 0.03 in the vehicle group and 6.48 ± 0.04 in the CM group.

Endotoxin injections did not disturb the basal pH_i value whatever the group but led to a significant reduction in the PCr/ATP ratio in the vehicle group (Table 2). On the contrary, the basal PCr/ATP ratio remained unchanged when the LPS treatment was accompanied by an oral administration of CM. Throughout the stimulation period, endotoxemia did not affect the [ATP] time-course whatever the group (P=0.153 and P=0.501 for vehicle and CM groups respectively) (Fig. 2B). On the contrary, the [PCr] time-course was significantly altered by LPS injections in both groups (P=0.0015 and P=0.011 for vehicle and CM groups respectively) (Fig. 2A). At the end of the stimulation period, PCr levels were 37.2 ± 1.2% and 37.1 ± 2.1% in the vehicle and CM group respectively. In addition, k_{rec} was reduced in the vehicle group but remained unchanged in CM-administrated animals (Table 2). Endotoxemia strongly affected the pH_i time-course in the vehicle group (P=0.0005; Fig. 2C). In this group, the pH_i recovery recorded during the second part of the stimulation session was enhanced when compared to the pre-endotoxemia time-course so that the end of the stimulation value was 6.68 ± 0.03. On the contrary, the pH_i kinetics was not affected by endotoxemia in CM animals (P=0.142) and the end-of-stimulation pH_i value was 6.63 ± 0.05.

4. Discussion

The present work reported for the first time results related to repeated investigations of muscle function in contracting rat gastrocnemius muscle using ^{31}P-MRS. We showed that CM supplementation in endotoxemic rats prevented the basal PCr/ATP ratio reduction and normalized the pH_i time-course during muscular activity as a sign of an effect at the muscle energetics level. In addition, CM treatment avoided the endotoxemia-induced decline in developed force.

4.1. CM effect in resting muscle

The increased body temperature and the body mass reduction we reported in the vehicle group as a result of endotoxemia is comparable with results of experiments conducted in animal models of sepsis and endotoxemia (Angeras et al., 1991; Brealey et al., 2004; Mizobata et al., 1995; Verleye et al., 1995). It is noteworthy that CM supplementation had no protective effect on these parameters, as previously reported in endotoxemic rats (Verleye et al., 1995).

In agreement with previous studies, we measured a significant reduction in basal PCr/ATP ratio resulting from LPS injections in the vehicle group (Giannesini et al., 2007; Jacobs et al., 1991; Jacobs et al., 1988; Lara et al., 1998; Mizobata et al., 1995). This alteration in basal energy metabolism could be due to a failure in oxygen supply. Cellular hypoxia and abnormal microvascular control of oxygenation have indeed been reported in endotoxemic rats (Anning et al., 1999; Sair et al., 1996). However, similarly to previous in vivo ^{31}P-MRS studies in septic rats (Jacobs et al., 1991; Jacobs et al., 1988; Mizobata et al., 1995), we did not measure at rest any intracellular acidosis, a marker of cellular hypoxia. Thus, we can dismiss an impaired oxygen supply as an accounting factor of the reduced PCr/ATP ratio.

On the basis of ^{31}P-MRS experiments in rat gastrocnemius muscle, the PCr/ATP reduction at rest has also been related to an impaired muscle oxidative activity which would ultimately limit energy supply (Jacobs et al., 1991). Muscle oxidative activity can be assessed through the rate constant of PCr resynthesis during the post stimulation period (k_{rec}), since this constant has been demonstrated to linearly depend on oxidative capacity (Paganini et al., 1997; Roussel et al., 2000). In the present study, we showed that k_{rec} was increased in LPS-injected vehicle animals as previously described (Giannesini et al., 2007; Mizobata et al., 1995). This data is in agreement with the results of a study showing that oxygen consumption by a flow-controlled canine hindlimb preparation is increased early after systemic administration of endotoxin, hence suggesting an increased mitochondrial respiration (Broadie et al., 1980). Increased k_{rec} thereby excludes any reduction of mitochondrial activity as a cause of basal PCr/ATP reduction. Rather than a simple enhancement in oxidative phosphorylation activation, this increased rate constant could reflect, as previously suggested, an upward shift of the feedback loop controlling mitochondrial activity (Giannesini et al., 2007).

Interestingly, CM treatment prevented the endotoxin-induced reduction in basal PCr/ATP ratio, hence suggesting a protective effect of CM on basal energy metabolism. Given that k_{rec} was not affected by endotoxemia in this group, it is conceivable that CM corrects the impaired control of oxidative function through a mechanism which has still to be determined.

4.2. CM effect in contracting muscle

CM treatment prevented the marked force decline, which we observed in the vehicle animals treated with LPS. This result obtained *in vivo* further confirms previous results obtained in isolated muscle and during running sessions (Goubel et al., 1997; Verleye et al., 1995). Indeed, it has been reported that CM administration limits the decrease in electrically-evoked force-generating capacity in isolated epitrochlearis (Goubel et al., 1997) and improves running endurance on treadmill tests (Verleye et al., 1995).

However, the mechanisms accounting for the deleterious effects of LPS on muscle function and the opposite effects exerted by CM administration remain to be clarified. Yet, one can hypothesize that the CM supplementation beneficial effects could be linked to the involvement of malate and/or citrulline at the muscle energy metabolism level (Goubel et al., 1997; Meneguello et al., 2003). Energy metabolism impairment is involved in the development of fatigue given the central role of ATP supply for contractile and non contractile processes related to muscle contraction (Allen et al., 1997; Westerblad et al., 1998). Besides, any increase in energy supply would improve muscle function (Allen et al., 1997; Westerblad et al., 1998).

Malate is a TCA intermediate and its supply can affect oxidative ATP production through anaplerotic reactions (Gibala et al., 2000) allowing the replenishment of the TCA intermediates pool (Sahlin et al., 1990). Studies in humans and animals have shown that the total TCA intermediate pool increases very quickly and several fold at the start of exercise in skeletal muscle (Gibala et al., 1997). If the TCA intermediates pool would not increase, the flux through the TCA cycle would decrease, thereby contributing to muscle fatigue development as previously suggested (Sahlin et al., 1990; Wagenmakers, 1998). Interestingly, among the TCA intermediates, malate shows the largest relative and absolute change during exercise likely in order to allow high rates of ATP production through the TCA cycle (Sahlin et al., 1990; Wagenmakers, 1998). However, we clearly showed that CM supplementation did not affect muscle oxidative activity thereby indicating that, in LPS-treated animals, malate supply did not accelerate the TCA cycle energy production. Then, the limitation of force decline in CM group cannot be linked to any increase in oxidative ATP supply.

Citrulline is involved in the pathway of nitric oxide (NO) synthesis, which is linked to the urea cycle metabolism via the synthesis of L-citrulline from L-arginine. Oral ingestion of citrulline is known to increase NO production (Hayashi et al., 2005) and interestingly, an enhanced NO production has been shown to accelerate glucose uptake into skeletal muscle cells during exercise (Kingwell et al., 2002). Such an uptake could affect muscle energy metabolism and explain the metabolic events we observed in the CM-treated animals under endotoxemia. In the vehicle group, endotoxemia reduced acidosis during the stimulation period. Yet, the pH drop during muscular contraction is linked to an increased glycolytic activity (Hochachka and Mommsen, 1983). Then, considering that glycolytic activity depends on available glycogen content, we could hypothesize that the reduced acidosis in vehicle would be linked to a decreased

intramuscular glycogen store due to endotoxemia as previously suggested (Giannesini et al., 2007). Indeed, in endotoxemic animals, a raised epinephrine production would enhance sarcolemmal Na^+-K^+-ATPase activity thereby accelerating the basal glycolytic flux and leading to a glycogen store reduction (L'Her and Sebert, 2004; Levy et al., 2005; Virkamaki and Yki-Jarvinen, 1994).

On the contrary, in the CM-treated group, endotoxemia did not affect the time-course of pH_i. This normalizing effect of CM on pH_i indicates that CM would protect skeletal muscle from basal glycogen depletion due to endotoxemia. The corresponding mechanism might be linked to a limitation of the sarcolemmal Na^+-K^+-ATPase activity. Another possibility would be that CM supplementation would increase glucose uptake, thereby leading to an increased glycolytic activity and a corresponding larger stimulation-induced acidosis.

4.3. Conclusion

CM supplementation prevents the decline in muscle performance and normalizes energy metabolism in LPS-treated animals thereby illustrating the inhibition of the deleterious effect of endotoxemia on muscle function. Although the exact mechanism has still to be elucidated, our results demonstrate that CM administration efficiently limits muscle dysfunction under asthenia.

Acknowledgments

This work was supported by grants from CNRS (UMR 6612), the French Interdisciplinary Small Animal Imaging Program (CEA-CNRS) and the ACI "Plates-Formes d'Explorations Fonctionnelles Thématisées" Program.

References

Allen, D.G., Lamb, G.D., Westerblad, H., 2008. Skeletal muscle fatigue: cellular mechanisms. Physiol. Rev. 88, 287–332.
Allen, D.G., Lannergren, J., Westerblad, H., 1997. The role of ATP in the regulation of intracellular Ca2+ release in single fibres of mouse skeletal muscle. J. Physiol. 498 (Pt 3), 587–600.
Allen, D.G., Westerblad, H., 2001. Role of phosphate and calcium stores in muscle fatigue. J. Physiol. 536, 657–665.
Angeras, U., Hall-Angeras, M., Wagner, K.R., James, H., Hasselgren, P.O., Fischer, J.E., 1991. Tissue metabolite levels in different types of skeletal muscle during sepsis. Metabolism 40, 1147–1151.
Anning, P.B., Sair, M., Winlove, C.P., Evans, T.W., 1999. Abnormal tissue oxygenation and cardiovascular changes in endotoxemia. Am. J. Respir. Crit. Care Med. 159, 1710–1715.
Arnold, D.L., Matthews, P.M., Radda, G.K., 1984. Metabolic recovery after exercise and the assessment of mitochondrial function in vivo in human skeletal muscle by means of 31P NMR. Magn. Reson. Med. 1, 307–315.
Bendahan, D., Mattei, J.P., Ghattas, B., Confort-Gouny, S., Le Guern, M.E., Cozzone, P.J., 2002. Citrulline/malate promotes aerobic energy production in human exercising muscle. Br. J. Sports Med. 36, 282–289.
Brealey, D., Karyampudi, S., Jacques, T.S., Novelli, M., Stidwill, R., Taylor, V., Smolenski, R.T., Singer, M., 2004. Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286, R491–R497.
Briand, J., Blehaut, H., Calvayrac, R., Laval-Martin, D., 1992. Use of a microbial model for the determination of drug effects on cell metabolism and energetics: study of citrulline-malate. Biopharm. Drug Dispos. 13, 1–22.
Broadie, T.A., Homer, L., Herman, C.M., 1980. Effect of endotoxin on oxygen consumption by a flow-controlled canine hind-limb preparation. Surgery 88, 566–574.
Fitts, R.H., 1994. Cellular mechanisms of muscle fatigue. Physiol. Rev. 74, 49–94.
Giannesini, B., Izquierdo, M., Dalmasso, C., Fur, Y.L., Cozzone, P.J., Verleye, M., Guern, M.E., Gillardin, J.M., Bendahan, D., 2007. Endotoxemia causes a paradoxical intracellular pH recovery in exercising rat skeletal muscle. Muscle Nerve 36, 505–514.
Giannesini, B., Izquierdo, M., Le Fur, Y., Cozzone, P.J., Fingerle, J., Himber, J., Kunnecke, B., Von Kienlin, M., Bendahan, D., 2005. New experimental setup for studying strictly noninvasively skeletal muscle function in rat using 1H-magnetic resonance (MR) imaging and 31P-MR spectroscopy. Magn. Reson. Med. 54, 1058–1064.
Gibala, M.J., Tarnopolsky, M.A., Graham, T.E., 1997. Tricarboxylic acid cycle intermediates in human muscle at rest and during prolonged cycling. Am. J. Physiol. 272, E239–244.
Gibala, M.J., Young, M.E., Taegtmeyer, H., 2000. Anaplerosis of the citric acid cycle: role in energy metabolism of heart and skeletal muscle. Acta Physiol. Scand. 168, 657–665.
Goubel, F., Vanhoutte, C., Allaf, O., Verleye, M., Gillardin, J.M., 1997. Citrulline malate limits increase in muscle fatigue induced by bacterial endotoxins. Can. J. Physiol. Pharmacol. 75, 205–207.
Hayashi, T., Juliet, P.A., Matsui-Hirai, H., Miyazaki, A., Fukatsu, A., Funami, J., Iguchi, A., Ignarro, L.J., 2005. L-Citrulline and L-arginine supplementation retards the progression of high-cholesterol-diet-induced atherosclerosis in rabbits. Proc. Natl. Acad. Sci. U S A. 102, 13681–13686.
Hickner, R.C., Tanner, C.J., Evans, C.A., Clark, P.D., Haddock, A., Fortune, C., Geddis, H., Waugh, W., McCammon, M., 2006. L-citrulline reduces time to exhaustion and insulin response to a graded exercise test. Med. Sci. Sports Exerc. 38, 660–666.
Hochachka, P.W., Mommsen, T.P., 1983. Protons and anaerobiosis. Science 219, 1391–1397.
Jacobs, D.O., Kobayashi, T., Imagire, J., Grant, C., Kesselly, B., Wilmore, D.W., 1991. Sepsis alters skeletal muscle energetics and membrane function. Surgery 110, 318–325.
Jacobs, D.O., Maris, J., Fried, R., Settle, R.G., Rolandelli, R.R., Koruda, M.J., Chance, B., Rombeau, J.L., 1988. In vivo phosphorus 31 magnetic resonance spectroscopy of rat hind limb skeletal muscle during sepsis. Arch. Surg. 123, 1425–1428.
Kent-Braun, J., 1999. Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort [In Process Citation]. Eur. J. Appl. Physiol. 80, 57–63.
Kingwell, B.A., Formosa, M., Muhlmann, M., Bradley, S.J., McConell, G.K., 2002. Nitric oxide synthase inhibition reduces glucose uptake during exercise in individuals with type 2 diabetes more than in control subjects. Diabetes 51, 2572–2580.
L'Her, E., Sebert, P., 2004. Effects of dichloroacetate and ubiquinone infusions on glycolysis activity and thermal sensitivity during sepsis. J. Lab. Clin. Med. 143, 352–357.
Lara, T.M., Wong, M.S., Rounds, J., Robinson, M.K., Wilmore, D.W., Jacobs, D.O., 1998. Skeletal muscle phosphocreatine depletion depresses myocellular energy status during sepsis. Arch. Surg. 133, 1316–1321.
Levy, B., Gibot, S., Franck, P., Cravoisy, A., Bollaert, P.E., 2005. Relation between muscle Na^+K^+ ATPase activity and raised lactate concentrations in septic shock: a prospective study. Lancet 365, 871–875.
Meneguello, M.O., Mendonca, J.R., Lancha Jr., A.H., Costa Rosa, L.F., 2003. Effect of arginine, ornithine and citrulline supplementation upon performance and metabolism of trained rats. Cell Biochem. Funct. 21, 85–91.
Mizobata, Y., Prechek, D., Rounds, J.D., Robinson, V., Wilmore, D.W., Jacobs, D.O., 1995. The duration of infection modifies mitochondrial oxidative capacity in rat skeletal muscle. J. Surg. Res. 59, 165–173.
Paganini, A.T., Foley, J.M., Meyer, R.A., 1997. Linear dependence of muscle phosphocreatine kinetics on oxidative capacity. Am. J. Physiol. 272, C501–510.
Roussel, M., Bendahan, D., Mattei, J.P., Le Fur, Y., Cozzone, P.J., 2000. 31P magnetic resonance spectroscopy study of phosphocreatine recovery kinetics in skeletal muscle: the issue of intersubject variability. Biochim. Biophys. Acta 1457, 18–26.
Sahlin, K., Katz, A., Broberg, S., 1990. Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. Am. J. Physiol. 259, C834–841.
Sahlin, K., Tonkonogi, M., Soderlund, K., 1998. Energy supply and muscle fatigue in humans. Acta Physiol. Scand. 162, 261–266.
Sair, M., Etherington, P.J., Curzen, N.P., Winlove, C.P., Evans, T.W., 1996. Tissue oxygenation and perfusion in endotoxemia. Am. J. Physiol. Heart Circ. Physiol. 271, H1620–1625.
Vanhamme, L., van den Boogaart, A., Van Huffel, S., 1997. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J. Magn. Reson. 129, 35–43.
Verleye, M., Heulard, I., Stephens, J.R., Levy, R.H., Gillardin, J.M., 1995. Effects of citrulline malate on bacterial lipopolysaccharide induced endotoxemia in rats. Arzneimittelforschung 45, 712–715.
Virkamaki, A., Yki-Jarvinen, H., 1994. Mechanisms of insulin resistance during acute endotoxemia. Endocrinology 134, 2072–2078.
Wagenmakers, A.J., 1998. Muscle amino acid metabolism at rest and during exercise: role in human physiology and metabolism. Exerc. Sport Sci. Rev. 26, 287–314.
Westerblad, H., Allen, D.G., Bruton, J.D., Andrade, F.H., Lannergren, J., 1998. Mechanisms underlying the reduction of isometric force in skeletal muscle fatigue. Acta Physiol. Scand. 162, 253–260.