ABSTRACT

Dietary L-citrulline malate (CM) consumption has been suggested to improve skeletal muscle metabolism and/or contractile efficiency, which would be expected to predispose exercising individuals to greater fatigue resistance. The purpose of this study was to examine the effects of CM supplementation on acid-base balance and high-intensity exercise performance. In a double-blind, placebo-controlled, crossover study, ten well-trained males consumed either 12 g of CM (in 400ml) or lemon sugar-free cordial (Placebo [PL]) 60 min prior to completion of two exercise trials. Each trial consisted of subjects performing ten (x 15 s) maximal cycle sprints (with 30 s rest intervals) followed by 5 min recovery before completing a cycle time-to-exhaustion test (TTE) at 100% of individual peak power (PP). Significant increases in plasma concentrations of citrulline (8.8-fold), ornithine (3.9-fold) and glutamine (1.3 fold) were observed 60 min after supplementation in the CM trial only ($p<0.05$) and none of the subjects experienced gastrointestinal side-effects during testing. Significantly higher exercise heart rates (HR) were observed in CM condition (*vs.* PL) although no between trial differences in performance related variables [TTE: (120±61s CM *vs.* 113±50 s PL), PP or mean power, power fatigue index: 36±16% CM *vs.* 28±18% PL)], subjective RPE or measures of acid-base balance (pH, lactate, bicarbonate, base-excess) were observed ($P > 0.05$). This study demonstrated that acute supplementation of 12 g CM does not provide acute ergogenic benefits using the protocol implemented in this study in well-trained males.

Keywords: acid-base balance; high-intensity exercise, fatigue

INTRODUCTION

Citrulline (CIT) is a non-essential amino-acid (AA), first isolated from watermelon [citrullus vulgaris]. In both clinical and applied exercise fields, supplements containing CIT have been ingested in isolation or as the salt of other anions such as malate (a tricarboxylic acid cycle intermediate), forming L-Citrulline-malate (CM). Previous studies have shown that CM supplementation (under brand name, Stimol ®) can enhance muscle performance in humans with asthenia after acute disease (11, 13) and limit increases in muscle fatigue in rats treated with endotoxins (17). Chronic CM (6g/d x 15 days) supplementation has been shown to enhance skeletal muscle power output in conjunction with a lower pH-to-power ratio and elicit greater oxidative energy turnover (4) in humans, while oral administration (over 48 h) of CM enhanced muscle force production and lowered ATP cost of muscle force production in healthy rats (16). Additionally, acute ingestion of CM (8g) one hour before exercise has been shown to enhance both upper- (22) and lower-body exercise capacity (32) in addition to relieving muscle soreness (22).

Conversely, acute ingestion of CIT (6 g) taken 1 to 2 h prior to exercise has been shown to be ineffective in improving the total number of upperbody (chest press) repetitions or treadmill time to exhaustion (12). Furthermore, a reduction in treadmill time has been observed following CIT ingestion (3-9 g taken 3 to 24 h prior to testing) (19). On the available evidence, it would appear that a combination of dosage, timing and interactive effects of CM (*vs.* CIT alone) may account for observed differences in study findings. Despite an increasing number of studies investigating CIT or CM on exercise performance, limited research has been carried out to investigate its proposed ergogenic effects. Since data suggests that short-term CM supplementation might improve skeletal muscle metabolism and/or contractile efficiency, targeted areas of investigation would appear merited in the ability of CM to promote greater fatigue resistance. It is known that high-intensity exercise results in the accumulation of glycolytic metabolites during times of limited oxygen availability to the working cell (9, 28). As the rate of glycolysis is increased, so too does the acidity of the working cells and this is primarily caused by hydrogen ion (H+) accumulation (3, 5) which in turn, can lead to significant impairments to exercise performance at high intensities (23). By facilitating greater ammonium clearance through the urea cycle, CM ingestion is purported to reduce accumulation of lactate through malate-induced metabolic shuttling and subsequent aerobic utilisation/gluconeogenesis (22). Additional ergogenic benefits of L-citrulline are thought to reside in its ability to increase systemic L-arginine concentrations (2, 25) by avoiding catabolism along the intestinal-renal axis and possibly by enhancing arginine bio-availability (26).

Since nitric oxide (NO) is produced by the conversion of arginine into CIT (by one of the three isoforms of nitric-oxide synthase; NOS), and is thought to depend largely on extracellular arginine availability (33), it is feasible to suggest that supplementation with CM or CIT alone could represent a novel way to improve NO bioavailability and therefore blood-flow and possibly oxygen delivery to the exercising muscle. Using a short-term CIT supplementation protocol (6g/d x 7 days), enhanced endurance performance and faster overall $\dot{V}O_2$ kinetics have been observed (2). Furthermore, lower end-exercise blood lactate and ammonia concentration (29), as well as a lower rate of muscle PCr degradation has been observed in rats (15). With the above in mind, it is feasible that CM supplementation may increase the proportional O_2 delivery to muscle microvasculature and attenuate muscle fatigue (via greater metabolite clearance) during high-intensity exercise. However, very few randomised controlled placebo studies are available to ascertain its ergogenic effectiveness on a well-trained athletic population (32).

The aims of the current study were to (a) explore the possible mechanistic effects of acute CM consumption from changes in acid-base balance (b) whether or not short-term high-intensity power and time to exhaustion could be improved in well-trained subjects.

METHODS

Experimental approach to the problem

A randomised double-blind placebo controlled cross-over design was implemented with each subject asked to report to the laboratory on three occasions with each trial was separated by 7 days (Figure 1). The first visit served as a habituation trial to experimental conditions in addition to determination of $\dot{V}O_{2\,max}$. This was achieved using a discontinuous (3 min exercise, 2 min recovery) incremental ramp-test to volitional exhaustion on a calibrated cycle ergometer (SRM Ergometer, SRM Training system, Jülich, Germany). Following a 3 min warm-up at 150 W, work-rate was increased by 30W for each consecutive stage until volitional exhaustion. Breath-by-breath pulmonary gas exchange (K4b^2, Cosmed, Rome, Italy) and heart rate (HR) (RS800, Polar Electro, Kempele, Finland) data were collected continuously during this incremental test. Gas analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated with a 3-liter syringe (Hans

Rudolph, Kansas City, MO). The subjects' rating of perceived exertion (RPE CR10, Borg et al., 1985) was collected at the end of each exercise stage. Individual $\dot{V}O_{2\,max}$ was identified as the highest 30 s mean value attained before subjects volitional exhaustion. Preferred pedal cadence, ergometer seat and handlebar height were recorded for each individual during this first visit, and reproduced for all subsequent trials. Following completion of the ramp-test, subjects were given a 30 min break before being habituated to the main exercise trials. This was done by asking the subjects to complete 50% of the exercise component contained within the main experimental trials (all other experimental conditions/testing points were maintained). Following completion of this incremental test and habituation, subjects were randomly assigned in a cross-over design to either 'test (CM)' or 'placebo (PL)' group for the remaining two experimental trials.

Insert table 1 approx. here

Subjects

Ten healthy well-trained men volunteered to participate in this study. Subject characteristics and training history are displayed in table 1. At time of testing, none of the subjects were smokers, were injury free and not on current medication or dietary supplements. After explanation of experimental procedures, all subjects gave their written informed consent before study commencement which was approved by the UCL Institutional Research Ethics Committee. The subjects were fully familiarised with the laboratory exercise testing procedures and most had previously participated in studies employing cycle ergometry.

Insert figure 1 approx. here

Procedures

In the 24 h prior to the first trial, subjects were asked to record all dietary and fluid-intake using a provided food diary. This was photocopied and returned to subjects whom were then asked to replicate this dietary intake before all remaining trials. Additionally, subjects were asked to refrain from caffeine and alcohol intake 6 h and 24 h before each trial and to avoid strenuous exercise in the 24 h preceding each testing session. On the morning of trials, subjects were asked to consume 500 ml of water upon waking to standardise hydration status, arrive at the laboratory in a rested state and at least 2 h

postprandial. All trials were performed at the same time of day (± 1 h) and laboratory environmental conditions were kept constant (21°C, 45-55% RH).

Upon entry to the laboratory, subjects rested quietly in a supine position for 15 min before a blood sample was obtained by venipuncture from an antecubital vein and collected into two vacutainer tubes (Becton Dickinson, Oxford, UK). Blood samples collected in K_3EDTA vacutainers (4-mL) were kept at room temperature and samples used for calculation of changes in plasma volume. All plasma derived analytes were subsequently corrected following analysis (14). Additionally, blood (7 mL) was collected into sterile lithium-heparin vacutainers (Becton Dickinson, Oxford, UK) and immediately placed on ice before being centrifuged (1,500g x 10 min, 4°C) within 10 min of collection. Plasma was aspirated into appropriate aliquots and stored at -80°C until future analysis for plasma amino-acids (described below). Capillary blood samples were also taken at this time point for analysis of circulating blood lactate (Lactate Pro™, Arkway, Japan), blood gases and acid-base balance ($CG8^+$ cartridge, i-STAT® system, Abbott Laboratories, Illinois, USA).

Subjects were then provided with either a test (12 g L-Citrulline-Malate dissolved in 400 ml water) or placebo drink (400 ml of sugar-free lemon squash) to consume over a 15 min period. The timing and dose of the drink provision was elected following previous research which has shown peak citrulline (CIT) levels to occur after 0.72 ± 0.03 h (20). The subjects were not aware of the experimental hypotheses to be tested but were informed that the purpose of the study was to compare two sports beverages on aspects of cycling performance. The personnel administering the tests were not aware of the type of beverage being consumed by subjects and the test drinks were similar in appearance and smell. Following consumption, subjects rested quietly in the laboratory for a further 45 min during which time ratings of gastrointestinal (GI) discomfort (bloatedness, nausea, reflux) were taken at 15 min intervals using a Likert (ranging from 6 = *none* to 20 = *very, very strong*) scale. At the end of this time period, subjects were fitted with a HR monitor before transitioning onto the cycle ergometer.

Repeated high-intensity sprints

Following a set 2 min warm-up at 150W, subjects were asked to perform 10 x 15 s maximal effort sprints on the cycle ergometer (SRM Training system, Jülich, Germany) interspersed with 30 s of active recovery (cadence at 60 rpm; 30 W). Verbal encouragement was provided throughout each sprint and subjects were

instructed to generate the highest possible power (P) to ensure maximal effort. P, HR and cadence were recorded continuously to an adjoining computer and post-test analysis was carried out using associated software (SRM, Schoberer Rad Messtechnik,Welldorf, Germany; accuracy = 0.5% in freewheel conditions). Subject RPE (taken at end of each sprint), GI discomfort (end of 2^{nd}, 6^{th}, 10^{th} sprint) and capillary blood samples were taken throughout the exercise protocol. HR was taken at the end of each 15 s sprint. Peak power (PP) was calculated as the highest power achieved for each sprint and mean power (MP) was determined as ± 5 s from the point of this PP value. Power fatigue index was calculated as the percentage difference between power at the end of the test and PP (10).

Exercise time to exhaustion (TTE)

Following a 5 min passive rest (remaining seated on the cycle ergometer); subjects were asked to perform an all-out cycle to exhaustion test. This test has been previously shown to be a reliable exercise protocol that can be used for nutritional interventions designed to affect intracellular and extracellular pH changes (24). Cycling resistance was set at the same power achieved during the final (3 min) stage of the discontinuous incremental ($\dot{V}O_{2\,max}$) exercise test performed in the first laboratory visit. If the subject failed to complete all 3 min of the final stage during the incremental test, power was estimated as the power output in the last fully completed stage plus half the increment in power of the following stage (1). Throughout this TTE trial, subjects were given verbal encouragement to cycle as long as possible and to keep within ± 5 rpm of their normal self-selected cadence (recorded during $\dot{V}O_{2\,max}$ test). Throughout all cycling trials, subjects were blinded from HR, time and power data but feedback on cadence was reinforced verbally to ensure maximal effort. Termination of the test occurred when the subjects were unable to sustain set cadence following a 5 s warning. MP, speed, distance, work and TTE were subsequently recorded. Between trial reliability for this test performed on well trained individuals in our lab was <5%, similar to values shown previously (24). After completion of the exercise protocol, a capillary blood sample was taken whilst the subjects remained on the bike for recovery measurements (5 min) before transfer to an adjoining bed where they remained in a resting supine position (25 min passive recovery). During this time period, further capillary blood samples, HR and ratings of GI discomfort were obtained.

Blood analysis

Circulating levels of plasma citrulline, ornithine, and glutamine levels were determined using high performance liquid chromatrography with mass spectrometry detection (LC-MS). Briefly, a known concentration of stable isotope labelled amino-acid (Cambridge Isotopes, Boston, USA) was added to the plasma for later determination of recovery. Plasma samples were then de-proteinized by the addition of cold acetonitrile (Fisher, Loughborough, UK) and the samples centrifuged (13,000 rpm, 10 min, 4°C) to remove bulk protein. The supernatant was then separated using a Dionex Ultimate 3000 HPLC system (Dionex, Surrey, UK) fitted with a Pinnacle DB biphenyl column (1.9µm, 50 x 21.mm; Thames Restek, Saunderton, UK) at a flow rate of 0.4ml/min using a gradient mobile phase of water:acetonitrile with 0.1% formic acid (10% acetonitrile rising to 90% over 6 min); and measured using a Orbitrap XL (Thermo Fisher, Loughborough, UK) mass spectrometer in positive ion mode. Sample concentrations were determined by comparison to standard curves and corrected for recovery using the added stable isotopes. The reproducibility of the LC-MS/MS approach for measuring amino-acids (AA) and AA metabolites has been found to be high (average CV = 9.5%) based on repeated measurements (19).

Statistical Analyses

All data are presented as mean ± standard deviation and statistical analyses were performed using SPSS v 20. Following testing for normality of distribution by the Kolmogorov-Smirnov test, data were analysed using a two-way repeated measures ANOVA (time and treatment were the within subject factors) with Bonferroni post-hoc test for multiple comparisons to identify the difference between treatments (CM *vs.* PL) at respective time points. For within group comparisons, a one-way ANOVA was used. Paired t tests were used to compare variables between the time-to-exhaustion trials. RPE and GI discomfort were analysed using the Wilcoxon signed rank test. Significance level was set at $p<0.05$ for all analyses.

Insert table 2 & 3 approx. here

RESULTS

Plasma amino acids

Oral citrulline-malate (CM: 12g) consumption led to an acute increase in plasma citrulline concentrations (peak 343±41 μM) compared to values at baseline (39±12 μM) in test drink (CM) condition ($F(1,9)$ = 435, $p<0.001$, $\eta p^2 = 0.98$); Figure 2a). No change in citrulline concentration from baseline values (39±11 μM) was observed following PL consumption. The increased citrulline concentrations after CM supplementation were accompanied by pre-exercise increases (approx. 60 min after consumption) in plasma glutamine (501±177 μM *vs.* 399±50 μM at baseline; Figure 2b), with pre-exercise values greater than those observed in PL condition (P=0.05). Significant increases in pre-exercise plasma ornithine (9.5±3.1 μM) were also observed compared to values at baseline (2.4±1.6 μM; P<0.001). Plasma citrulline and ornithine concentrations remained elevated 30 min post exercise (~115 min from initial consumption) in CM condition (P<0.001; Figure 2a & 2c). Plasma ornithine and glutamine concentrations did not change from baseline levels at any time following PL consumption.

Insert figure 2 approx. here

Acid-base balance

Changes in acid-base balance are shown in Table 2. As expected, significant decreases in blood pH, PCO_2, TCO_2, HCO_3 and base-excess were observed immediately following exercise (P<0.001). Conversely, increases in blood PO_2 and lactate were observed following exercise (P<0.001). Compared to baseline, pH, PCO_2, TCO_2, HCO_3, base-excess and lactate were still not fully recovered 30 min post exercise (P<0.001). No significant differences in any marker representative of acid-base balance were observed between CM and PL conditions during testing.

Subjective RPE and GI discomfort

Subjective RPE were taken before exercise, immediately after sprints 2, 4, 6, 8, 10 and before and after the TTE trial. Repeated sprints resulted in a progressive increase in RPE scores in both trials across time (P<0.001). Higher RPE ratings were reported for CM compared to PL for sprint 2 (Z = -2.3, P=0.02) although a main effect for treatment did not reach statistical significance. No differences in RPE ratings were observed before or after the TTE exercise trial between conditions.

Insert figures 3 & 4 approx. here

Increased ratings of GI discomfort were noted after the second sprint and these continued to the end of the TTE trial and into the first 5 min of recovery (Figure 3). No between treatment effects were observed following supplementation or during exercise. A trend for increased ratings of GI discomfort appeared in the CM trial (*vs.* PL) during the first 5 min of recovery (Z = -1.90, P=0.06). No between treatment effects were observed for any other time point and no GI disturbances were reported by subjects on test days or the day following each trial.

Exercise performance

Repeated high intensity exercise

Significant effects for time ($F(1.74,13.8) = 57.2$, $p<0.001$, $\eta p^2 = 0.87$) and treatment ($F(1,9) = 10.6$, $p=0.01$, $\eta p^2 = 0.57$) were observed for mean heart HR responses during the repeated sprints trials (Figure 4). HR data were significantly higher during CM condition (*vs.* PL) from the second sprint onwards ($t(9) = 3.0$, P=0.01). Peak power (PP) and mean power (MP) data during the ten repeated sprints are shown in Figure 5a & 5b, respectively. As expected, a significant effect of time was observed in both trials as power declined from sprint one to ten ($F(1.6,14.1) = 22.9$, $p<0.001$, $\eta p^2 = 0.72$). Percentage fatigue index (FI) calculated from PP data were 29±12% and 26±15% for CM and PL beverages respectively. In turn, FI calculated from MP data were 36±16% and 28±18% for CM and PL respectively with comparisons for MPO between trials failing to reach significance ($t(9) = 0.24$, P>0.05). No significant treatment effect was observed for power output ($F(1,9) = 0.13$, $p>0.05$, $\eta p^2 = 0.14$. With the exception of PP during sprint 3 (CM lower than PL; P<0.05) and MP during sprint 1 (CM higher than PL; P<0.05), no other differences were observed at any time point between conditions.

Insert figure 5 approx. here

Time to exhaustion

Higher mean HR values (166±7 bpm) were observed in the CM condition than in the PL condition (160±7 bpm); $t(9) = 3.01$, P=0.01 during the TTE trial (Table 3). Analysis of TTE trial data showed that 50% of subjects increased cycling time to exhaustion following supplementation with CM. No between treatment differences were observed (CM *vs.* PL) for TTE (120±61 s *vs.* 113±50 s; $t(9) = 0.72$, p=0.48).

Furthermore, no between treatment effects were observed for MP, average speed, cadence, distance or work achieved; all p>0.05, Table 3.

Insert table 3 approx. here

DISCUSSION

To our knowledge, few studies have investigated whether supplementation with CM could enhance high-intensity performance in well trained subjects (32). The principle finding of this randomized double-blind placebo controlled crossover study was that acute consumption of CM (12g) did not attenuate fatigue induced by repeated high-intensity cycling or prolong time to exhaustion.

Previous findings have shown CM supplementation to enhance human skeletal muscle power output in conjunction with a greater oxidative energy turnover and a lower pH to power ratio (3), improve muscle contractile efficiency in rats (15), prevent the decline in muscle force production rats with endotoxemia (14) and facilitate greater ammonium clearance (28). With the above in mind, a repeated sprint protocol followed by an exercise capacity test was chosen in an attempt to best capture any purported ergogenic effects. However, the results showed that the acute dosage protocol chosen in this study did not affect the power fatigue index of ten repeated maximal cycling sprints. Furthermore, no changes in either PP or MP were observed between the CM or PL conditions. This data differs from previous research which showed that a single dose of CM (8g) was capable of increasing work capacity in high-intensity anaerobic exercises with short rest times, by an average of 19% (21). However, a bench-press protocol was used in the latter study which utilises a smaller muscle mass and may have different cardiovascular and metabolic demands to our chosen experimental approach.

In the current study, a high intensity short-duration exercise stimulus was employed in an attempt to elicit significant disturbances in acid-base balance. While a decrease in blood pH and base-excess were elicited with corresponding increased levels of blood lactate, no between treatment effects (*vs*. PL) were observed. This is in contrast to previous studies showing positive changes in acid-base balance following CM supplementation (6, 30). It is possible that differences in supplementation protocol and/or training status (recreational *vs.* well trained) may account for these discrepancies in findings. CM consumption over 3 days has been previously shown to increase bicarbonate levels and base-excess in humans (7) with

authors speculating that this overall rise in bicarbonate (linked to probable increase in urinary ammonia excretion) could explain better fatigue resistance and delay in metabolic acidosis. Further exploration of optimal dosage and dose duration (acute *vs*. 3-5 days) is therefore required to determine any possible effects CM has on fatigue resistance and muscle buffering capacity.

Previous work has shown L-citrulline (CIT) supplementation to have a negative effect on time to exhaustion when consumed over 24 h (9g) or three hours (3g) before exercise (18). In contrast, seven days of CIT (6g/d) has been shown to improve O_2 uptake kinetics (by lowering the $\dot{V}O_2$ mean response time), increase total amount of work completed (7%) and improve tolerance (12%) to severe-intensity exercise in recreationally active men with authors suggesting an improvement in O_2 delivery or enhanced oxidative metabolism (1). In the present study, no between treatment (CM *vs.* PL) differences were observed for TTE (120±61s *vs.* 113±50s) or mechanical work achieved during the TTE trials. Since one of the purported benefits of exogenous citrulline supplementation lies in its potential ability to enhance blood flow (via direct and indirect effects on NOS) and the extent to which NO bioavailability is influenced by CIT may in turn depend on duration of supplementation period (1), these conflicting findings might be a function of supplementation regime (duration and type of CIT dose used). It should be noted that 50% of subjects in the present study did show an overall improvement in exercise capacity during TTE trial and in MPO during the repeated sprint trial. While, the data suggests that acute citrulline supplementation in the form of CM (not as CIT alone) fails to influence high-intensity exercise performance at the group level, to rule out its effect as an ergogenic aid may be premature. Although all subjects in the present study were from a well-trained background, heterogeneity did exist in the type of exercise they routinely carried out. Therefore, one cannot discount the possibility of individual 'responder' type benefits and this should be explored more fully. Such trials should aim to employ a randomised double-blind placebo controlled cross-over design as used in the present work and ideally, with larger subject numbers.

Data did show that higher exercise HR's were observed in the CM trial. These occurred despite similar cycling frequencies and completed work in both CM and PL conditions. Previous data has shown subjects to consistently report higher subjective RPE ratings during exercise following acute CIT ingestion (19) although no between trial differences in subject RPE ratings were observed in the present data suggesting

that observed HR increases were not a function of increased work intensity. In a recent study investigating the functional properties of watermelon juice, no difference was observed in exercise HR in untrained subjects whom consumed either PL or CIT enriched beverages before an incremental cycle protocol (29). However, a trend of greater HR reduction was observed during exercise recovery in the latter study. In line with the possible CIT inducing effects on blood-flow and increased tissue perfusion, the present results require further exploration.

Other interesting aspects of this study showed that acute CM supplementation was accompanied by pre-exercise increases in plasma glutamine (501±177 μM *vs.* 399±50 μM), ornithine (9.5±3.1 μM *vs.* 2.4±1.6 μM) and as expected, citruilline (343±41 μM *vs.* 39±12 μM) concentrations. These findings complement previous data showing that CM is capable of eliciting increases in blood levels of other AA involved in the urea cycle (7) and better use of branch-chained amino-acids during prolonged exercise (26), although this was not a focus of the present investigation. Concerns of adverse GI side effects can often limit the practical applicability of AA ingestion. Negative side effects such as diarrhoea, GI discomfort and nausea have been frequently observed after arginine supplementation (17). Indeed, in a recent study, about 15% of participants reported feelings of stomach discomfort following CM supplementation (8g) prior to performing a resistance exercise protocol (21). However, data in this study suggests that a higher dose of CM (12g) taken approx. 60 min prior to high-intensity exercise poses no adverse effects to GI comfort before, during or after maximal exercise suggesting that this supplement is well-tolerated.

PRACTICAL APPLICATIONS

In the current study, an acute dose of CM was provided to subjects approximately 60 min before the beginning of exercise. Data showed that CM was well tolerated during exercise but did not appear to affect aspects of acid-base balance, was ineffective in enhancing exercise capacity or in attenuating power output declines during repeated high-intensity exercise. Our findings at group level, do not justify the use of a single dose (12 g) of CM in improving repeated sprint ability or endurance capacity in well trained cyclists. Practitioners interested in utilising CM as a supplement for acute benefits in a well-trained population should consider other alternatives and/or assess if longer term supplementation protocols could offer possible ergogenic effects.

Conflict of interest

No conflict of interest

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Tables

Table 1 Subject performance characteristics. Data are mean ± SD.

Age (y)	23.5 ± 3.7
Height (m)	1.81 ± 0.1
Body mass (kg)	80.7 ± 10.4
Body fat (%)	13.0 ± 2.5
Max heart rate (bpm)	190 ± 5
$\dot{V}O_{2\,max}$ ($ml.kg^{-1}.min^{-1}$)	58.1 ± 10.3
Peak power (W)	1000 ± 114*
Training days (per week)	5.5 ± 1.0
Training (hours per week)	12.0 ± 4.0

*Data collected from 10 s 'all out' cycling sprint on SRM ergometer. Subjects consisted of endurance trained rowers, cyclists and triathletes (n = 8), a semi-professional footballer (n = 1) and mixed martial arts athlete (n = 1).

Table 2 Blood (capillary) measures before and after (+ 75 min) drink consumption, immediately (0 min) and 30 min following exercise. Data are mean ± SD. * indicates time-dependant differences from baseline within each group (P<0.001). *P*CO_2: partial pressure of carbon dioxide; *P*O_2: partial pressure of oxygen; TCO_2: total carbon dioxide; HCO_3: bicarbonate; BE_{ecf} : base excess.

	Supplementation period		Post Exercise	
Variable	Before	+75 min	0 min	30 min
pH				
CM	7.44 ± 0.02	7.42 ± 0.02	7.12 ± 0.06*	7.37 ± 0.03*
Placebo	7.42 ± 0.01	7.42 ± 0.03	7.14 ± 0.07*	7.36 ± 0.03*
PCO_2 (kPa)				
CM	5.22 ± 0.23	5.53 ± 0.31*	4.14 ± 0.46*	4.41 ± 0.29*
Placebo	5.33 ± 0.21	5.55 ± 0.39*	4.17 ± 0.42*	4.47 ± 0.41*
PO_2 (kPa)				
CM	9.85 ± 1.20	9.42 ± 2.00	12.51 ± 1.35*	9.99 ± 1.53
Placebo	9.86 ± 1.04	9.04 ± 0.79	12.93 ± 0.81*	9.54 ± 1.14
TCO_2 (mmol/L)				
CM	27.8 ± 1.9	28.1 ± 1.5	11.2 ± 2.1*	20.1 ± 2.2*
Placebo	27.4 ± 1.0	28.1 ± 0.9	11.8 ± 2.8*	20.3 ± 2.1*
HCO_3 (mmol/L)				
CM	26.6 ± 1.7	26.9 ± 1.3	10.3 ± 2.0*	19.1 ± 2.0*
Placebo	26.2 ± 1.0	26.8 ± 1.0	10.8 ± 2.7*	19.1 ± 2.0*
BE_{ecf} (mmol/L)				
CM	2.6 ± 2.0	2.4 ± 1.4	-18.8 ± 2.8*	-6.1 ± 2.5*
Placebo	2.7 ± 1.2	2.1 ± 1.4	-18.4 ± 3.8*	-6.3 ± 2.2*
Glucose (mmol/L)				
CM	6.0 ± 0.7	5.6 ± 0.5	7.8 ± 1.1*	6.0 ± 1.3
Placebo	6.2 ± 1.0	5.7 ± 0.6	7.7 ± 0.7*	5.5 ± 0.8
Lactate (mg/dL)				
CM	0.9 ± 0.2	0.9 ± 0.2	12.9 ± 1.0*	9.6 ± 4.1*
Placebo	0.9 ± 0.1	1.0 ± 0.6	13.2 ± 1.7*	8.0 ± 3.1*

Table 3: Physical performance data (time to exhaustion). Data are mean (SD). # indicates between treatment differences (P<0.05).

	CM	Placebo
Mean Power (W)	356 (58)	357 (58)
Speed ($km.h^{-1}$)	32.3 (1.1)	32.3 (0.9)
Distance (km)	1.09 (0.5)	1.03 (0.6)
Work (kJ)	44 (26)	41 (22)
Average HR (bpm)	166 (8)	160 (7) #
RPE (0-10)	9.1 (1.1)	9.5 (0.7)

Figure legends

Figure 1: Study protocol. TTE: Time to exhaustion trial. RPE: rating of perceived exertion. GI: Gastrointestinal discomfort.

Figure 2. Mean (±SD) plasma concentrations of L-Citrulline (A), L-Glutamine (B) and L-Ornithine (C) at rest, 60 min after CM or PL consumption and after exercise. Citrulline: time x treatment interaction, $P<0.0001$; time effect, $P<0.0001$, treatment effect, $P<0.0001$; Glutamine: treatment effect, $P<0.05$; Ornithine: time x treatment interaction, $P<0.0001$; time effect, $P<0.0001$, treatment effect, $P<0.0001$.

Symbols δ and # indicate significant differences compared to PL ($P<0.001$ and $P<0.05$); * indicates time-dependant differences from baseline within each treatment group ($P<0.001$).

Figure 3: Mean (±SD) subjective ratings of GI discomfort following supplementation, during exercise and recovery. Data ranging from 6 = none to 20 = very, very strong. * indicate time-dependent differences from baseline (pre) within each treatment group ($P<0.05$). No between treatment effects observed.

Figure 4: Mean (±SD) HR data during repeated sprint exercise. # indicates differences compared to PL ($P<0.05$); * indicates time-dependant differences from baseline within each condition ($P<0.001$).

Figure 5: Mean (±SD) peak power (A) and mean power (B) achieved by subjects during sprints. # indicates differences compared to PL ($P<0.05$); * time-dependant differences from sprint 1 within each condition ($P<0.05$). Figure 5c shows individual and group mean (filled black circles) for TTE.

Figure 1

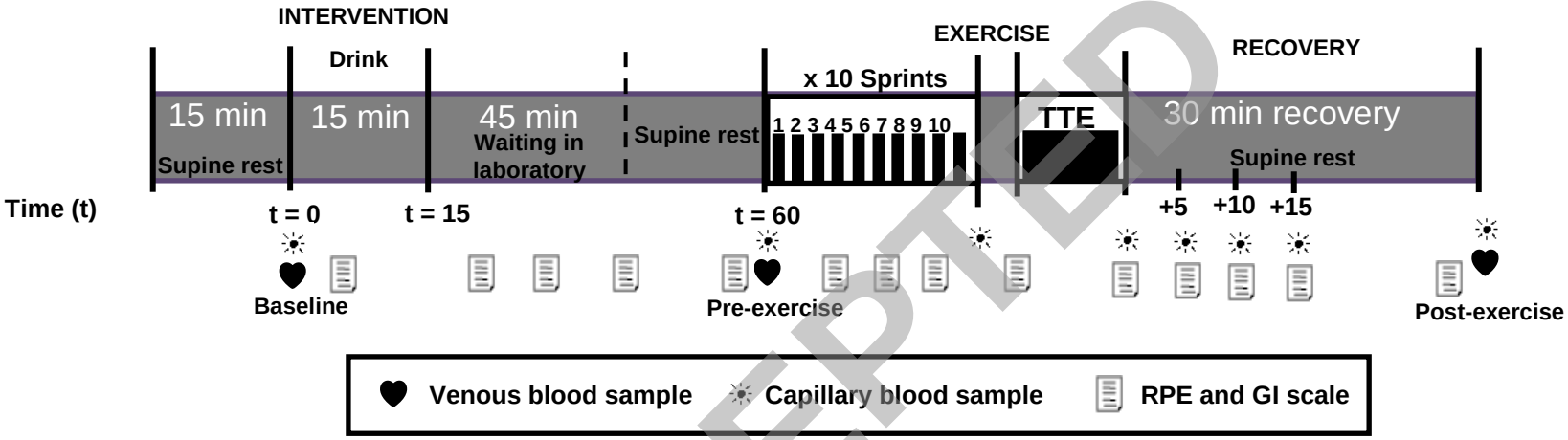

Figure 2

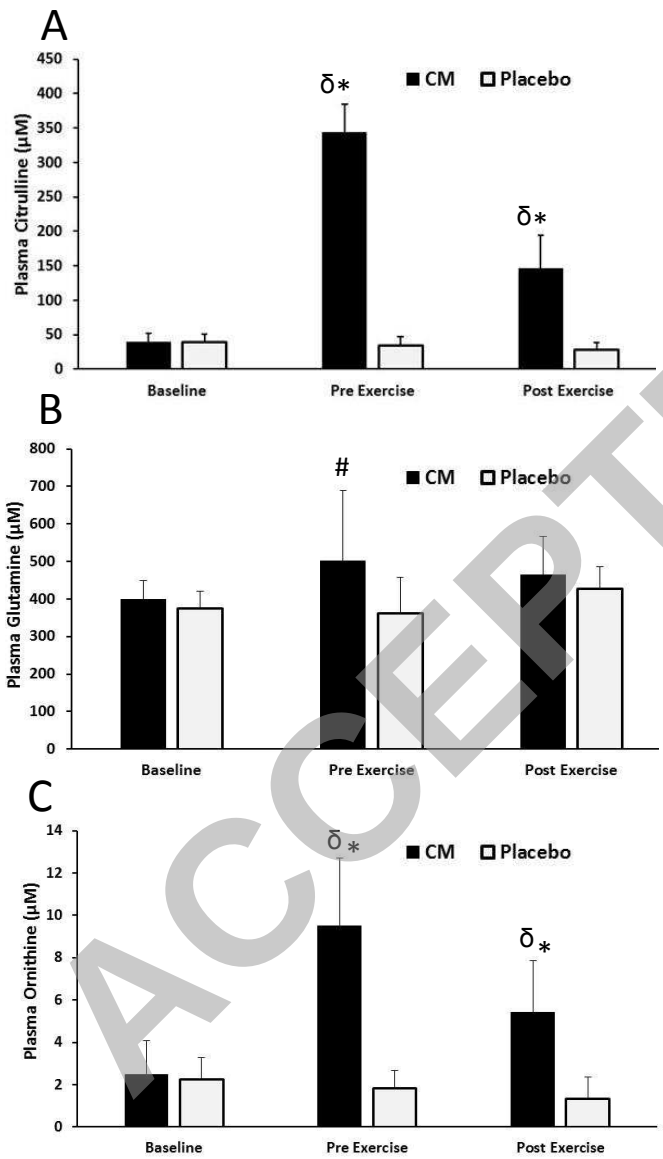

Figure 3

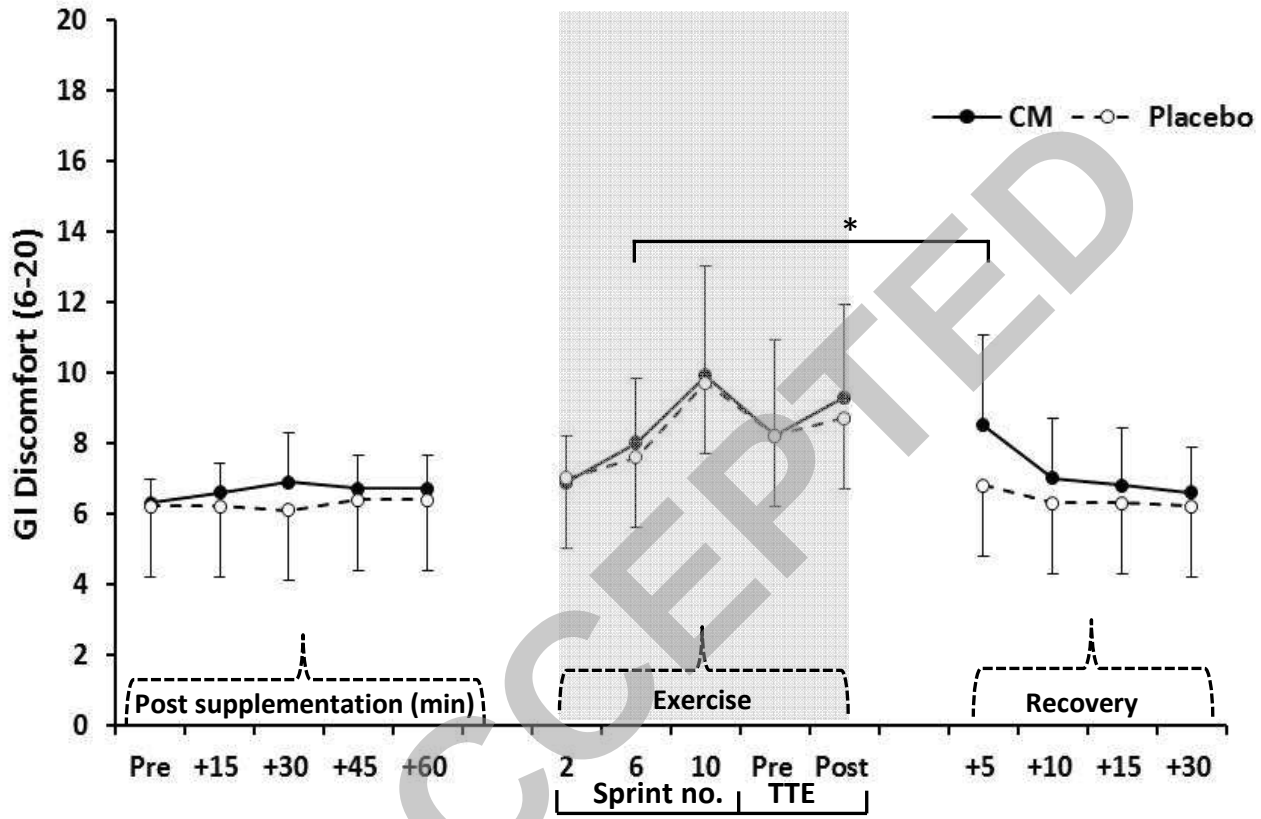

Figure 4

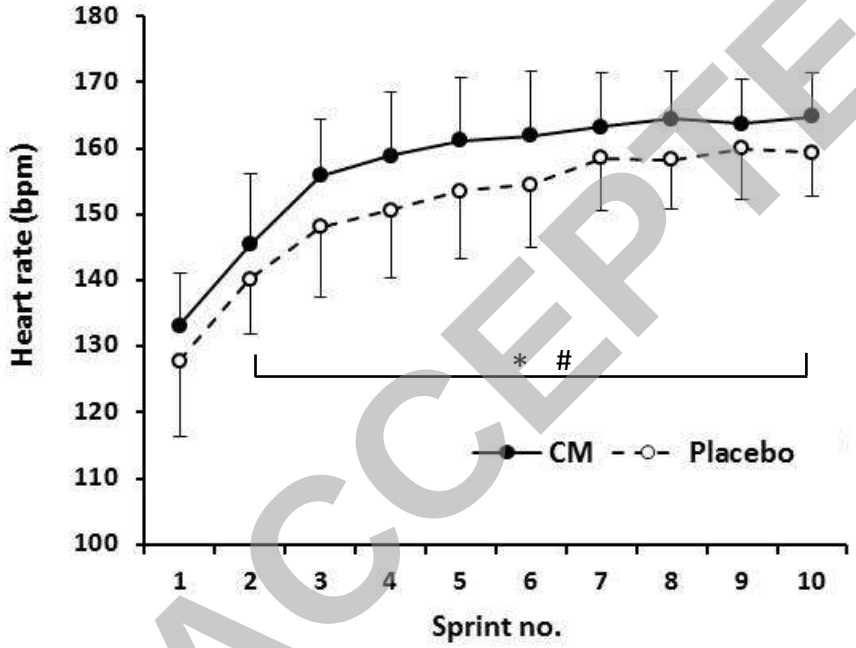

Figure 5