

R- 27238

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Running title: Citrulline malate and athletic performance

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Abstract

The purpose of the present study was to determine the effects of a single dose of citrulline malate (CM) on the performance of flat barbell bench presses as an anaerobic exercise and in terms of decreasing muscle soreness after exercise. 41 men performed 2 consecutive pectoral training session protocols (16 sets). The study was performed as randomized, double blind, two-period crossover design. 8 g of CM was used in one of the 2 training sessions and a placebo in the other. The subjects' resistance was tested using the repetitions to fatigue test, at 80% of their predetermined 1 repetition maximum (RM), in the 8 sets of flat barbell bench presses during the pectoral training session (S1-4 and S1'-4'). The p-value was 0.05. The number of repetitions showed a significant increase from placebo treatment to CM treatment from the third set evaluated ($p < 0.0001$). This increase was positively correlated with the number of sets, achieving 52.92% more repetitions and the 100% of response in the last set (S4'). A significant decrease of 40% in muscle soreness at 24 h and 48 h after the pectoral training session and a higher percentage response than 90% was achieved with CM supplementation. The only side effect reported was a feeling of stomach discomfort in 14.63% of the subjects. We conclude that the use of CM might be useful to increase athletic performance in high intensity anaerobic exercises with short rest times and to relieve post-exercise muscle soreness. Thus, athletes undergoing intensive preparation involving a high level of training or in competitive events might profit from CM.

Keywords: anaerobic exercise, ergogenic aids, lactate, sport performance, sport supplements, weight training.

INTRODUCTION

A nutritional supplement that enhances exercise capacity is said to have an ergogenic effect. The proposed or advertised ergogenic effect of many supplements is based on a presumptive metabolic pathway and may not necessarily translate to quantifiable changes in a variable as broadly defined as exercise performance. In Spain, citrulline malate (CM) is a popular sports supplement in spite of the fact that it is a pharmaceutical drug only authorized for the treatment of asthenia ("Stimol") of which recommend dose is 1g three times a day. Nevertheless, it is said that the best consumption pattern as an ergogenic aid is to intake a single dose of 4-10g of CM just 1hour before the sport session. Whether CM intake really improves performance has not been appropriately established, although sportsmen seeking higher performance and fast recovery after intensive training, seem convinced that it does. For that reason we found CM as a fascinating research subject and we decided to investigate the presumptive metabolic pathway of CM and to establish whether CM really improves exercise performance or not.

The accumulation of lactic acid has for long been considered an essential element in the phenomena of muscular fatigue (10). More recently several studies have shown the close connection between the accumulation of ammonia in blood and tissue, and the blockage of cellular energetic processes (3,11). Moreover, nitric oxide (NO) regulates many physiological functions of skeletal muscle (13). In connection with these concepts, the potential use of CM as an ergogenic aid should be based upon three hypothetical mechanisms of action:

1. The excess availability of citrulline, one of the amino acids of the ureogenesis cycle enables, via the mass action law, acceleration of the rotation of this cycle and thereby facilitates the clearance of ammonium. Ammonium is a very important fatigue factor since its intracellular

accumulation stimulates glycolysis, while blocking the aerobic utilization of pyruvate but also its recycling in the direction of neoglucogenesis. This results in deviation of energy metabolism towards the exclusive formation of lactate (14).

2. Malate, a metabolic intermediate of the krebs cycle, is capable of behaving as metabolic shuttle between cytoplasm and mitochondria, enables bypassing of blockade of the oxidative pathway induced by ammonia and hence limits accumulation of lactic acid by reorienting it towards pyruvate genesis and its aerobic utilization or neoglucogenesis. Accumulation of lactate and resultant acidosis lead secondarily to the blockage of glycolysis (14).

3. NO regulates many physiological functions of skeletal muscle including glucose uptake and oxidation, mitochondriogenesis, contractile functions,, blood flow and fatty acid oxidation, as well as muscle repair through satellite cell activation and the release of myotrophic factors (13).

NO is synthesized by nitric oxide synthase, which utilizes L-arginine as substrate and produces L-citrulline as the second reaction product. L-arginine can be synthesized from L-citrulline providing a recycling pathway for the conversion of L-citrulline to NO via L-arginine (9).

Moreover, comparing oral citrulline and oral arginine intakes, oral citrulline is a more efficient way of increasing body arginine levels (6,12)

The activity and excellent safety/acceptability of CM for the asthenia have been confirmed in two double-blind placebo-controlled trials (5,7). Nevertheless, few studies have examined the ergogenic potential of CM to enhance resistance exercise performance in humans. Though the precise mechanism of action is, as yet, unknown the purported ergogenic property of CM is probably linked to the following known actions:

1. In a microbial model, malate accelerated ammonium clearance and citrulline facilitated lactate metabolism. These results suggest a synergistic action of the complex (2).

2. In both animal and human models, results showed that CM stimulates hepatic ureogenesis and favours renal bicarbonate reabsorption. These metabolic actions confer a protective effect against acidosis and ammonia poisoning and may explain the fatigue-resistant property of CM in humans (4).

As a supplement, CM has been proven to be effective in athletic performance in very few studies:

1. In a rat model: resistance to fatigue (it was quantified by measuring tension production during repetitive electrical stimulation of the isolated epitrochlearis muscle) was improved after treatment with CM (8).

2. In humans, the effect of supplementation with six grams of CM a day for 15 days found that CM led to a significant reduction in the sensation of fatigue, a 34% increase in the rate of oxidative ATP production during exercise (finger flexions-recovery protocol) and a 20% increase in the rate of phosphocreatine recovery after exercise. They concluded that CM acts to promote aerobic energy production (1).

On the assumption that CM increases NO production and reduces the muscular fatigue through the reduction of lactic acid and ammonia in blood and tissue and that anaerobic exercise produces high levels of such metabolic wastes, we hypothesized that CM might be an ergogenic aid for an anaerobic exercise such the flat barbell bench press. The purpose of the present study was to determine the effect of a single dose of CM (8g) on:

1. Performance in an anaerobic exercise of a high intensity comprising repeated sets of flat barbell bench press

2. Muscle soreness measured 24 and 48h following the high intensity exercise bout.

METHODS

Experimental Approach to the Problem

This study was conducted in 6 gyms of Andalusia (the south of Spain). The subjects participated in 1 familiarization session and 2 identical testing sessions. During the familiarization session 1 repetition maximum (1RM) strength test in weight training for the flat barbell bench press was determined. Participants ingested CM (8g) or placebo (two-period crossover design) 1 hour before the testing work out. The subjects' resistance was tested using the reps-to-fatigue test, at 80% of their predetermined 1 repetition maximum (RM), in the 8 sets of flat barbell bench presses during the pectoral workout session (4 sets at the beginning of the pectoral workout and 4 sets at the end). The effect of CM on muscle soreness 24 and 48 hours after the pectoral workout was tested using a self-reported soreness score (scale from 1 to 5).

During the familiarization session (the week before the study period), informed consent statements were signed, medical and exercise history forms were completed and 1 repetition maximum (1RM) strength test in weight training for the flat barbell bench press was determined. The subjects participated in 1 familiarization session and 2 identical testing sessions.

The training program consisted of 5 workouts per week (from Monday to Friday) distributed as follows: Monday for chest, Tuesday for back, Wednesday for legs, Thursday for shoulders and Friday for arms. Saturday and Sunday were resting days. Subjects rested for 48 hours before the testing day (Monday). The training program was the same during the two-week study period (the same weight, exercises, sets and reps). The length of wash-out between treatments was 1 week. The pectoral workout protocol (figure 1) comprised 16 sets in the following order: 4 sets of flat barbell bench presses (80% 1RM weight for the flat bench press),

4 sets of incline barbell bench presses (80% 1RM weight for the flat bench press), 4 sets of incline flyes (60% 1RM weight for the flat bench press) and 4 sets of flat barbell bench presses (80% 1RM weight for the flat bench press). The speed of each rep was 3-4 seconds (1-2 for the positive and 1-2 for the negative). Workout sessions were always at the same time for each person but not necessarily between subjects. All subjects were required to perform each exercise to the point at which they reached muscular failure at the last repetition of each set. Subjects were instructed to rest for 1 minute between sets and for 2 minutes between each exercise. All workouts were completed at the participant's own training facility.

Prior to testing, subjects were instructed to eat the same food in the same order over the two week study period and not to consume caffeinated beverages on the testing days and during the 2 days beforehand.

Subjects

In order to participate in this study, subjects had to sign statements indicating that they: were currently training >3 hours per week with a program that included the bench press and pectoral exercises; agree to follow a predetermined workout program; refrain from participating in any sporting activity throughout the entire study; have not used anabolic steroids either now or in the past; have not ingested creatine, HMB, thermogenics, or ergogenic levels of any nutritional supplements for an 8-week period and have not taken any nutritional supplements or non-prescription drugs during the study; do not have any existing medical conditions that would compromise their participation in the study; and avoid any changes in their usual diet.

This study was carried out throughout February-March in 41 healthy men, whose mean weight was 81.12 ± 17.43 Kg and mean age was 29.80 ± 7.64 years. They were recruited from 6 gyms. These gyms are located in 3 cities of the south of Spain (Cordoba, Granada and Almeria). All subjects were free from chronic diseases, were not regularly taking prescription

medications, were not taking any ergogenic aids and had never taken CM supplements before. Moreover, all the subjects had participated in weight-training workouts over at least the previous 6 months and were physically active and thoroughly familiar with the pectoral workout protocol. Subjects had been training during the previous 6 months for an average of 6 ± 2 hours/week and 4 ± 2 workouts/week. The study was approved by the University of Cordoba human research ethics committee, and written informed consent was obtained from all participants before data collection.

Procedures

The study was performed using a randomized, double blind, two-period crossover design. In a double-blind manner, subjects were randomly assigned a 200 ml CM beverage [80 ml CM solution (8g CM: 8 Stimol sachets from “Laboratorios Pérez Giménez”, Spain), 20 ml lemon juice, 10g powdered sugar, 60 mg sodium saccharine and tap potable water until complete 200 ml of solution) or a 200 ml placebo beverage that was similar in appearance, smell, consistency and taste (40 ml lemon juice, 10g powdered sugar, 60 mg sodium saccharine and tap potable water until complete 200ml of solution). Both beverages were shaken until fully dissolved and served in disposable white plastic glasses. Subjects drank the beverage one hour before the testing work out. Subjects were instructed to report any possible side effects to the researchers as well as their compliance with the training and supplementation protocols. The investigator who made the beverages and the investigator who evaluated the subjects were different.

On the test day (Monday), 1 hour after consuming the beverage, subjects performed the reps-to-fatigue test at 80% of their predetermined 1RM, for the 8 sets of flat barbell bench presses (S1-4: 4 sets at the beginning of the pectoral workout; S1'-4': 4 sets at the end of the pectoral

workout). 16 measurements were taken for each subject (8 for the CM session day and 8 for the placebo session day).

The subjects self-reported their soreness 24 h and 48 h after the pectoral day testing on a scale from 1 to 5 as follows:

1. "no soreness"
2. "minimal soreness with no impact on immediate training"
3. "medium soreness with minimal impact on immediate training"
4. "high soreness with negative impact on immediate training"
5. "maximum soreness with physical disability for immediate training"

Statistical Analyses

The number of repetitions for each set and the score for muscle soreness are expressed as mean \pm standard error (Table 1). The incremental percentage for reps with CM compared with the placebo is expressed as Δ % and the decremental percentage for muscle soreness with CM compared with the placebo is expressed as ∇ %.

For quantitative variables (number of reps), difference between placebo and CM in the number of repetitions performed on the sets of flat barbell bench presses (S1-4: 4 sets at the beginning of the pectoral workout; S1'-4': 4 sets at the end of the pectoral workout) was analysed by within-group factorial two-way ANOVA. Previously, the Kolmogorov-Smirnov and Shapiro-Wilk test were done for testing normality and the F-Snedecor test for the assumption of homoscedasticity. The effect of CM on muscle soreness score (24 and 48 hours after the pectoral workout), was analyzed by Wilcoxon Signed-Rank Test. The P value for responders and non-responders for the number of repetitions for each set and the score for muscle soreness was calculated by using a Fisher's exact test for a 2 x 2 contingency

table (Table 2). SPSS (version 12.0) was the statistical software package employed for all analyses. Significance was accepted at a probability of $p < \leq 0.05$.

RESULTS

The ingestion of citrulline malate was tolerated well by the majority of subjects with only six subjects (15%) reporting stomach discomfort as a side effect following the ingestion of CM.

The mean change in the number of repetitions and muscle soreness scores are shown in Table 1. The individual data, indicating the variability of 'responders' and 'non-responders' to CM is illustrated in Table 2. The number of repetitions showed a significant increase from placebo treatment to CM treatment from the third set evaluated. This increase was positively correlated with the number of sets inside each group (group of sets 1-4 and group of sets 1'-4'), achieving 18% extra repetitions for S4 and 53% extra repetitions (Table 1) for the last set (S4'). The percentage of responders was 61% for S4 and 100% for the last set (Table 2).

Compared to placebo, the muscle soreness score at 24 and 48h post-exercise was significantly lower in the CM trial, achieving equal decremental percentage value of 40% at each time point (Table 1). The percentage of responders at 24 and 48h post-exercise (Table 2) was significantly equal (97.56% versus 90.24 %).

Graphical individual responses are represented by bar graphs (Figures 2-9).

DISCUSSION

CM is reported to lead to a significant reduction in the sensation of fatigue by increasing the rate of oxidative ATP production during exercise and the rate of phosphocreatine recovery after exercise (1). Moreover, many Spanish sportsmen seeking higher performance and fast recovery after intensive training session, seem convinced that a single dose (4-10g) of CM 1 hour before the sport session is an effective ergogenic aid. The results from the present study are consistent with the known capacity of CM as a buffer to acidosis (4), hyperammonemia

(2,4) and lactate accumulation (2). The results of the present study indicate that a single dose of CM may confer a training and/or performance benefit to athletes engaged in high intensity anaerobic exercise carried out consecutively, such as weight training or sprint sessions that significantly engage anaerobic metabolism and result in increased lactate, ammonia and acidosis. For these same reasons, it is likely that CM supplementation would be less effective in enhancing the performance of short aerobic exercises sessions, or anaerobic sessions with sufficient rest time or high enough intensity, where lower levels of acidosis, lactate and ammonium production would occur. The ability of CM to buffer the effect of exercise-induced increase in acidosis, lactate and ammonium may also part explain our observation of a reduction in muscle soreness 24 and 48h post-exercise. Thus, we can give scientific arguments to support the belief of many Spanish sportsmen founded on experience: the potential use of CM as an ergogenic aid.

PRACTICAL APPLICATIONS

In light of these results, the use of CM might be useful to increase athletic performance in high intensity anaerobic exercises with short rest times and to relieve post-exercise muscle soreness. CM might be also very useful to athletes undergoing intensive preparation involving a high level of training or in competitive events. Nevertheless, further research is required to determine which sports or activities might be enhanced with CM.

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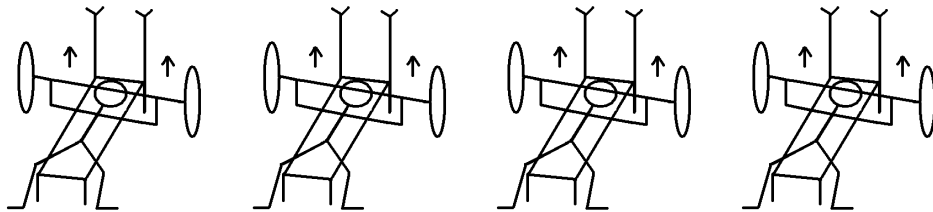
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FIGURE LEGENDS

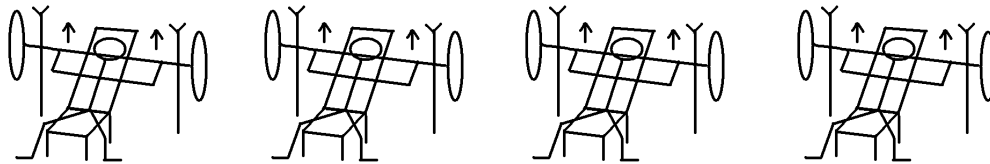
Figure 1. Pectoral workout protocol

Objective: To count the number of reps (flat barbell bench press) of each set with the 80% of their predetermined 1RM weight for this exercise



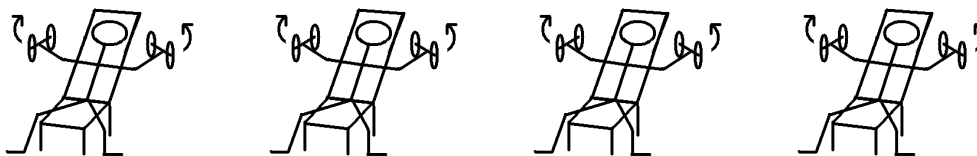
S1 (1 min rest) → **S2** (1 min rest) → **S3** (1 min rest) → **S4** (2 min rest) →

Objective: to perform each set (incline barbell bench press) to muscular failure with the same weight than the exercise before.



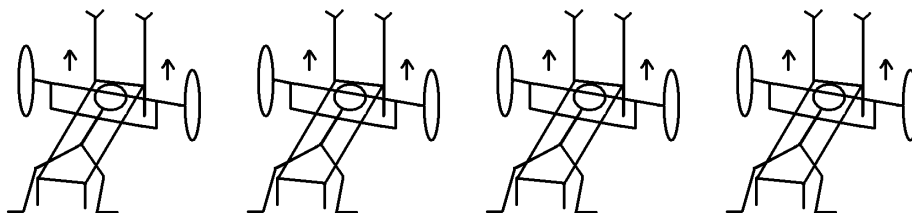
S5 (1 min rest) → **S6** (1 min rest) → **S7** (1 min rest) → **S8** (2 min rest) →

Objective: to perform each set (incline flyes) to muscular failure with the 60% 1RM weight for the flat barbell bench press



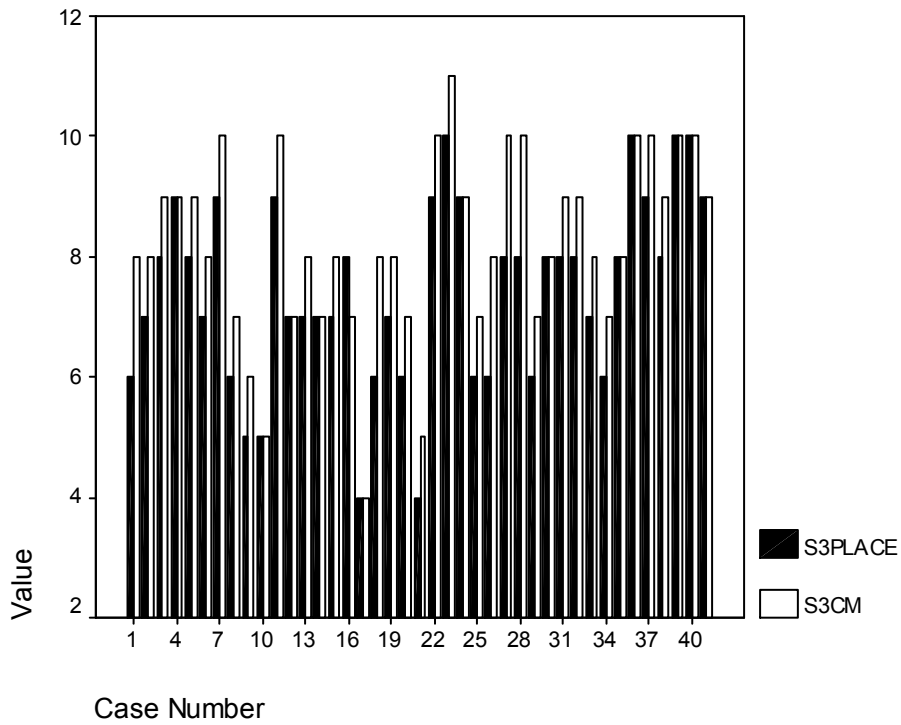
S9(1 min rest) → **S10** (1 min rest) → **S11** (1 min rest) → **S12** (2min rest) →

Objective: To count the number of reps of each set with the 80% of their predetermined 1RM weight for this exercise



S1' (1 min rest) → **S2'** (1 min rest) → **S3'** (1 min rest) → **S4'** (final)

Figure 2: Response for set 3



*S = Set
 *Place = Placebo
 *CM = Citrulline Malate

Figure 3: Response for set 4

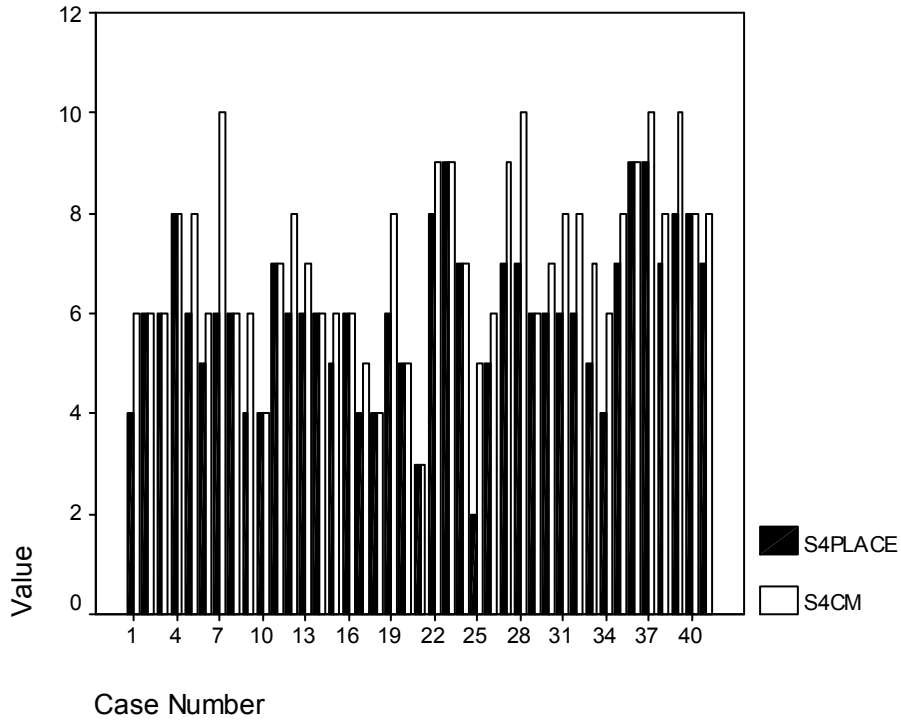


Figure 4: Response for set 1'

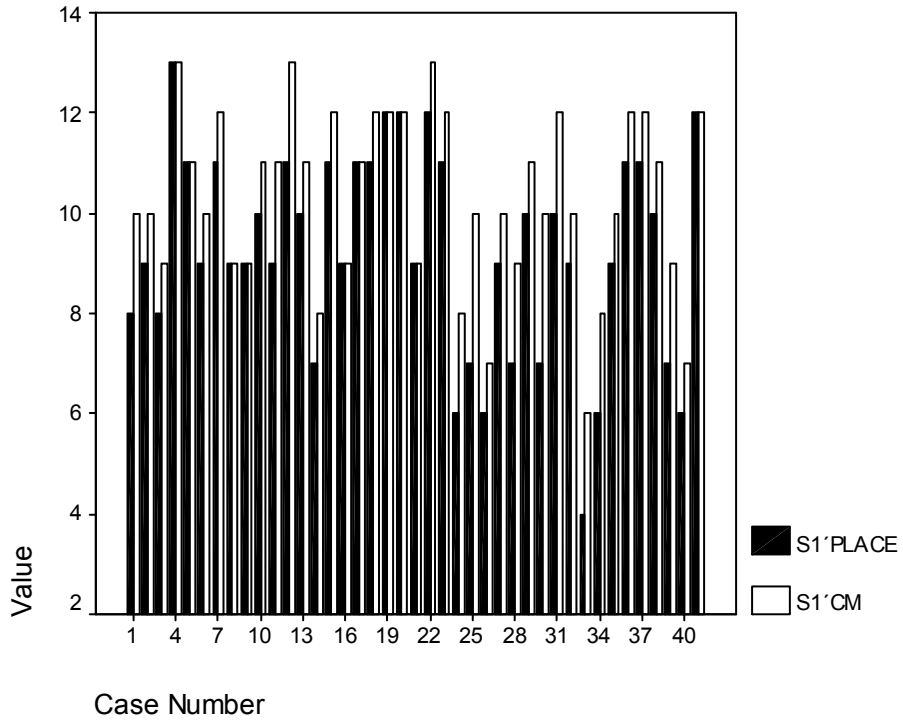


Figure 5: Response for set 2'

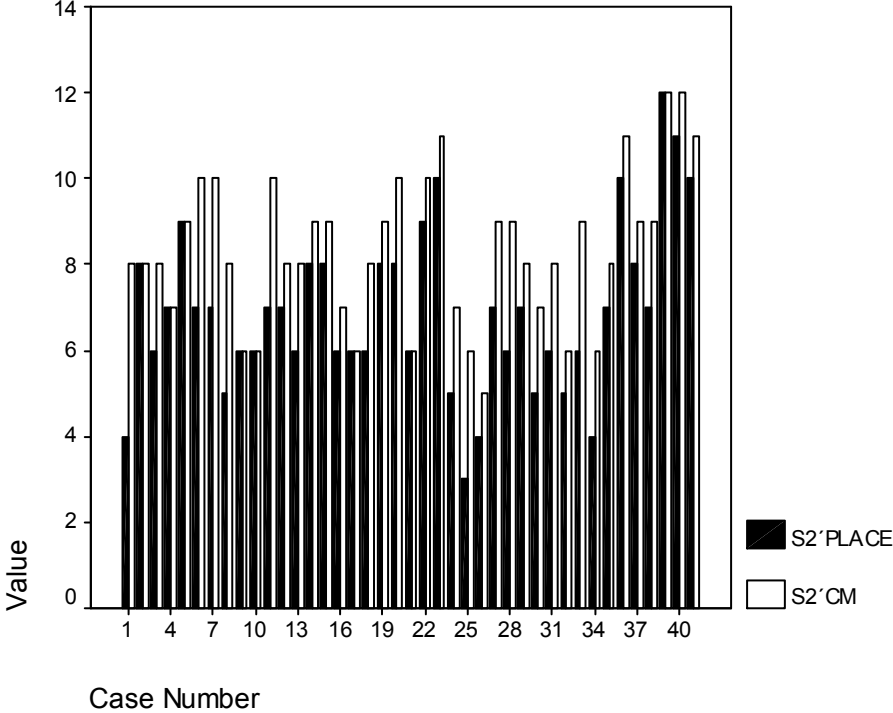


Figure 6: Response for set 3'

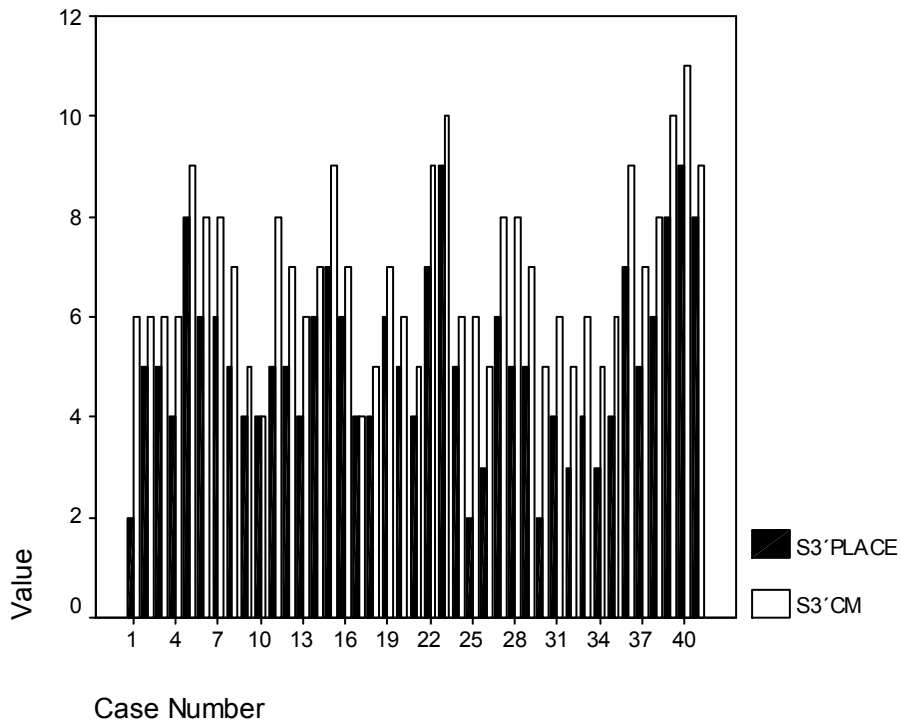


Figure 7: Response for set 4'

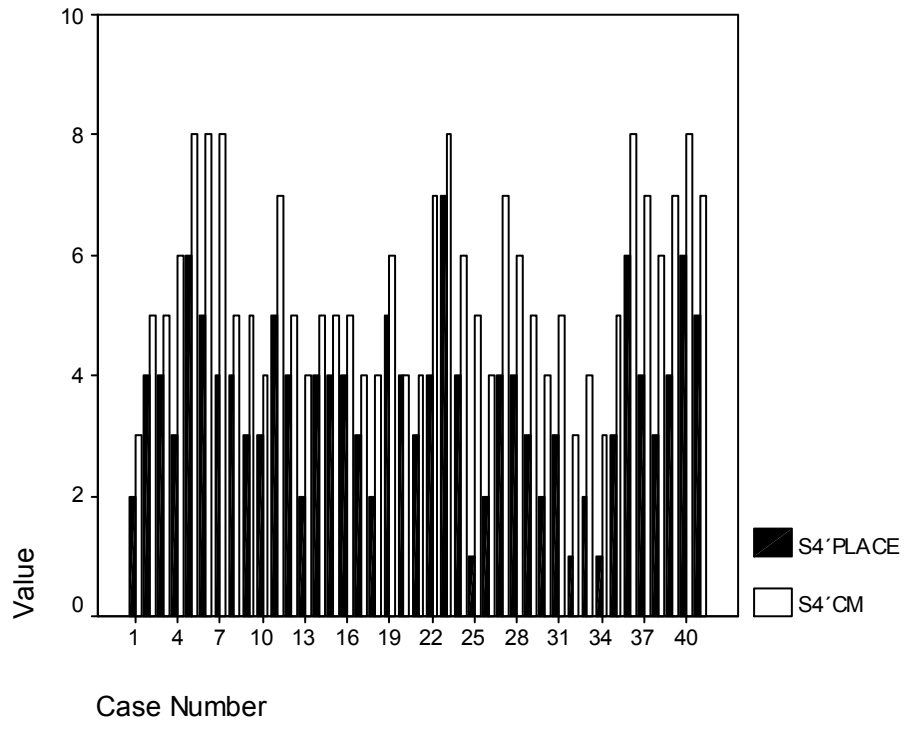


Figure 8: Response for muscle soreness at 24 h

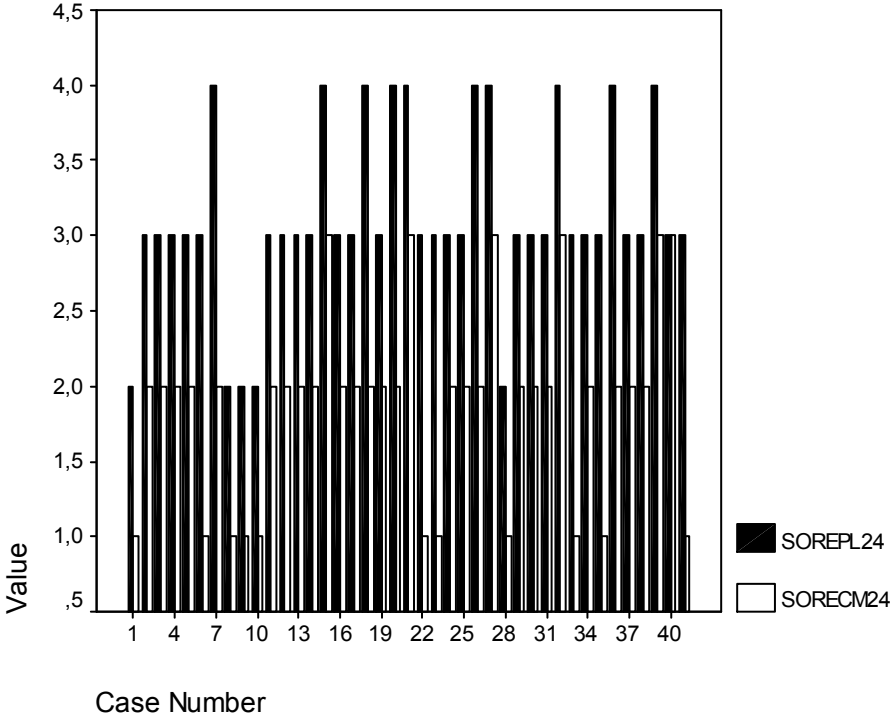


Figure 9: Response for muscle soreness at 48 h

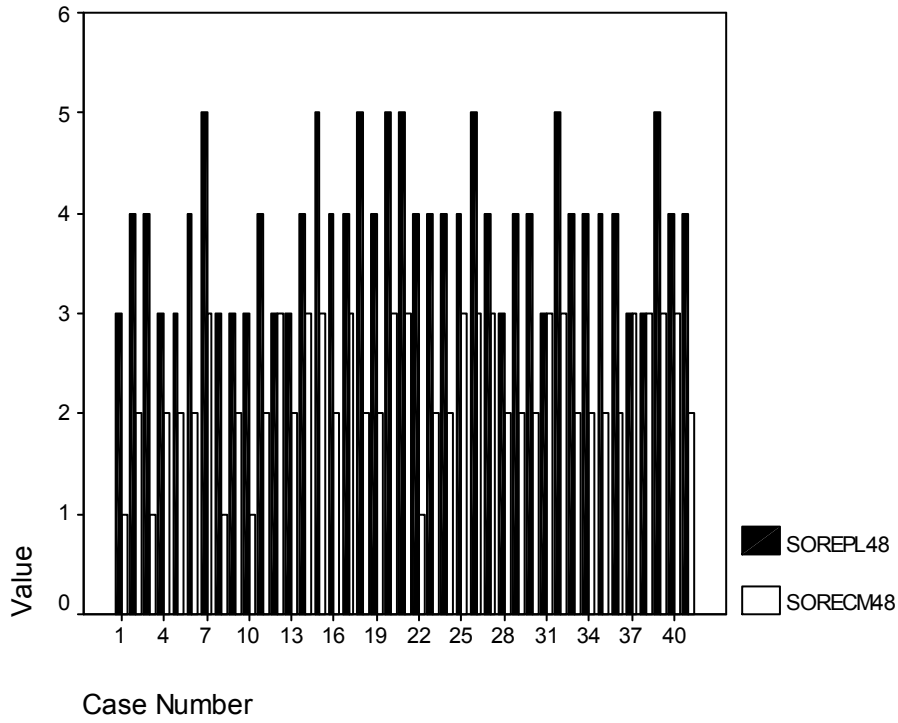


TABLE 1. CHANGES IN THE NUMBER OF REPS ACHIEVED WHEN PERFORMING FLAT BARBELL BENCH PRESSES AND IN THE SCORES ASSIGNED FOR MUSCLE SORENESS

Number of sets	Reps with Placebo*	Reps with CM*	Δ%	P	Range
Set 1	12.27± 0.45	12.39± 0.49	0.97	0.1334	-0.28 to 0.04
Set 2	9.51± 1.63	9.71±1.54	2.10	0.0583	-0.40 to 0.01
Set 3	7.44±1.58	8.22±1.56	10.48	< 0.0001	-1.00 to -0.56
Set 4	6.00±1.61	7.05±1.73	17.50	< 0.0001	-1.38 to -0.72
Set 1'	9.24± 2.08	10.32±1.75	11.69	< 0.0001	-1.33 to -0.81
Set 2'	6.90±1.95	8.37±1.76	21.30	< 0.0001	-1.80 to -1.12
Set 3'	5.12±1.78	6.88±1.71	34.38	< 0.0001	-2.03 to -1.48
Set 4'	3.59±1.40	5.49±1.53	52.92	< 0.0001	-2.18 to -1.62
Muscle soreness	Score with Placebo*	Score with CM*	∇%	P	Range
24 h after workout	3.12±0.60	1.88±0.64	39.74	< 0.0001	1.09 to 1.40
48 h after workout	3.90±0.70	2.27±0.67	41.79	< 0.0001	1.39 to 1.88

* Data are expressed as mean ± standard error. Δ= incremental; ∇ = decremental. Sample size=41 men. Testing was done at 80% of their predetermined 1RM for flat barbell bench presses. Sets 1-4 are performed consecutively at the beginning of the pectoral workout protocol (made up of 16 sets) and Sets 1'-4' at the end of the aforementioned protocol. The range represents the 95% confidence interval of the difference between placebo and CM scores.

TABLE 2. Percentage of Response to the CM for each set and muscle soreness

Number of sets	Responders	Non-responders	To make worse	P
Set 1	8 (19.51%)	30 (73.17%)	3 (5.88%)	0.1935
Set 2	10 (24.39%)	26 (63.41%)	5 (12.20%)	0.1635
Set 3	27 (65.85 %)	13 (31.71%)	1 (2.44%)	0.0001
Set 4	25 (60,97%)	16 (39.02%)	0 (0%)	0.0001
Set 1'	31 (75.60%)	10 (24.40%)	0 (0%)	0.0001
Set 2'	35 (85.37%)	6 (14.63%)	0 (0%)	0.0001
Set 3'	39 (95.12%)	2 (4.88%)	0 (0%)	0.0001
Set 4'	41 (100%)	0 (0%)	0 (0%)	0.0001
Muscle soreness	Responders	Non-responders	To make worse	P
24 h after workout	40 (97.56%)	1 (2.44%)	0 (0%)	0.0001
48 h after workout	37 (90.24%)	4 (9.76%)	0 (0%)	0.0001

*P= P value of the Fisher test