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Effects of Carnitine with and without Glutamine Supplementation on Markers of Muscle Damage and Muscle Soreness among Football Players: A Randomized Controlled Clinical Trial

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Abstract

Background: Exercise-induced muscle damage can affect exercise performance. The purpose of this study is to examine the effects of Carnitine and Glutamine supplementation on markers of muscle damage and muscle soreness after physical exertion on football players.**Materials and Methods:** Twenty eight healthy male football players aged 21.1±0.7 were recruited in a double blind, randomized, placebo-controlled clinical trial on 3 weeks of supplementation. Before supplementation protocol, each participant had to run on a treadmill for 30 minutes at 75% VO2max. Participants were randomly divided into 4 groups: L-Carnitine, L- Glutamine, L-Carnitine plus L- Glutamine and placebo. Blood samples were obtained pre-exercise and immediately after exercise. Muscle soreness was assessed on both occasions and two days after each exercise.**Results:** L-Carnitine and L-Glutamine supplementation for 21 days significantly decreased Creatine Kinase activity as a marker of muscle's damage before (P=0.014) and after exercise (P=0.047), and muscle soreness two days after physical exertion (P=0.057). However, Lactate Dehydrogenase activity was affected by Carnitine supplementation after exercise.**Conclusion:** Chronic oral supplementation of Carnitine and Glutamine before exercise can reduce chemical markers of muscle tissue damage after exercise. In addition, these supplements may reduce muscle pain after exercise and optimize the processes of muscle tissue repair.**[GMJ. 2014;3(4):207-15]**

Key Words: Carnitine; Glutamine; Dietary Supplement; Creatine Kinase; Exercise.

Introduction

Professional football players have to train or compete several days a week and this intensive exercise program may not allow adequate time for muscle recovery, therefore the risk of muscle damage may increase [1]. Symptoms of muscle damage after exercise include muscle pain, prolonged decreased muscle strength and increased muscle protein

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in blood [2].

In muscle damage, cell membranes are damaged and some cellular proteins and enzymes will be leaked into the bloodstream. Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST)andMyoglobin are the most important enzymes and proteins that can be found in bloodstream [1]. In a study on 28 football players, results revealed that after a football match, activity of CK, LDH and Myoglobin significantly increased [3].

Exercise-induced muscle damage affects exercise performance. Muscle's function is influenced due to a decrease in peak muscle power and strength for some time after injury [4]. Marcora *et al*. have shown that exercise-induced muscle damage can significantly reduce running speed by 2 percent in runners [4]. Accordingly, researchers are experimenting with compounds, such as Carbohydrates, Vitamin C, Carnitine and Amino acids to reduce the severity of muscle damage and speed up the rehabilitation of muscle after injury.

Carnitine is a vitamin-like chemical that is structurally similar to Amino acids. Over half of the daily requirement of Carnitine is found in a balanced diet, which includes meat, poultry, fish and some dairy products. The remainder is synthesized from Methionine and Lysine [5]. Although the main role of this compound in body is long-chain fatty acid transport into mitochondria, other roles that include antioxidant activities are also considered [5]. Recently the specific effects of Carnitine have been presented in reducing the indicators of tissue damage after moderate exercise and its role in muscle tissue repair [6]. Some studies indicate that the effect of L-Carnitine L-Tartarat(LCLT) on reducing the effects of hypoxia after endurance exercise, reducing tissue damage and decreasing muscle soreness [7,8].

A second substance thought to be effective in the prevention and treatment of muscle damage is Glutamine. It is indicated that damage from exercise changes the availability of Glutamine for immune cells.Physical stress and prolonged exercise correlate with the reduction of plasma levels of Glutamine[9]. Therefore it is hypothesized that Glutamine supplementation may enhance plasma levels of Glutamine and can be effective in ameliorating muscle ache and muscle damage after intensive exercise training and competition [9]. Nosaka *et al*.found that Amino acid supplementation (consisting 8 essential Amino acids and Arginine, Glutamine, Proline and Histidine) on recovery days had some positive effects on muscle soreness and plasma CK response [10].

It is hypothesized that Glutamine and Carnitine supplementation may reduce markers of muscle damage in football players. Thus, the aim of this study is to evaluate the effects of

Table 1. Subject characteristics* (N=7).

	CRN	GLU	CRN+GLU	PLA	P-Value
Age (years)	20.7 ± 0.7	21.2 ± 0.6	21.3 ± 0.7	21.2 ± 0.9	0.56
Height (cm)	175 ± 7.1	173 ± 5.8	173 ± 4.2	171.7 ± 6.2	0.78
Weight (kg)	64.9± 4.4	61.5± 7.6	59.9± 6.2	65.2± 7.1	0.35
BMI(kg/m²)	21.2 ± 1.4	20.5 ± 1.9	19.9 ± 1.6	22.2 ± 2.8	0.23
Body fat (%)	12.6 ± 3.1	16.3 ± 3	12.8 ± 3.1	15.5 ± 7.1	0.30
VO2max (ml/kg/min)	44 ± 5.3	45.7 ± 5.8	45.7 ± 2.36	42.1 ± 7.8	0.60
Training history (years)	4.4 ± 0.4	4.3 ± 0.6	4.6 ± 0.5	4.7 ± 0.7	0.55
Current football training (hr/week)	6.9 ± 1.9	6.9 ± 2.1	7.4 ± 2.5	6.5 ± 1.5	0.88

CRN: Carnitine supplementation group; GLU: Glutamine supplementation group;
CRN + GLU: Carnitine and Glutamine supplementation group; PLA: Control group.
*Data are presented as mean ±SD.

Carnitine and Glutamine supplementation on markers of muscle damage and muscle soreness in football players.

Materials and Methods

Subjects

Twenty eight healthy football players whose background is summarized in table 1, were enrolled in this double-blind, randomized, placebo-controlled trial. Since, in this study it was important that athletes havethe same training history and current football training per week (as mentioned in table 1), we recruited subjects from the same team club. Prior to the study, subjects had averaged 10 hours/week training for the past 6 months. Individuals who were current smokers, had a history of medical illness, took medication for a chronic condition, took any form of nutritional supplementations, weighed less than 40 kg, donated blood 1 month prior to the study, or had a blood hemoglobin level below 12.0 g/dL, were excluded from the experiments. Good health was also assessed by physical examination, blood pressure, blood chemistry panel (Glucose, Hemoglobin, Aspartate Aminotransferase, Alanine Aminotransferase, Creatine Phosphokinase and Triacylglycerols), complete blood count and urine analysis. The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran, approved this study (IRCT138809282890N1). All subjects were aware of the content of the study and if they agreed to participate, written informed consent was obtained.

The study design and timeline are shown in figure 1.

Experimental Design

We followed the players during off-season training and enrolled them in the study. One week prior to the experiment, all subjects visited the laboratory to become familiar with the environment and to perform the Bruce Treadmill Test in order to determine their maximal oxygen consumption (VO2 max) [11].

After this initial assessment, subjects were instructed to maintain their normal diet and sleep pattern for 1 week prior to each experimental phase. Subjects recorded food intake for three days before each experimental phase by using food record questionnaire. These records were analyzed with software (Food Processor II; Nutrition System, Iran). One day before each experimental day, the subjects finished dinner by 08:00 PM and then fasted overnight. Dinner before the experimental day consisted of the same food for all subjects and each athlete was prescribed their dietary intake based on attaining a minimum of 6 g Carbohydrate/kg body mass (BM)/day and 1.5 g protein/kg BM/day and achieving estimated energy requirements [12].

Next day at 7:30 a.m., blood pressure and heart rate were measured and blood samples were collected. Subjects were fed with the similar breakfast of 450 calories (70% Carbohydrates, 15% fat, and 15% protein).All subjects were only allowed to drink water during the experiment. Studies were conducted in a quiet, temperature and humidity-controlled environment. The trials took place in the Physical Fitness Assessment and Improvement Center at the National Olympic Academy of the Islamic Republic of Iran.

At 10:00 a.m., the subjects were asked to subjectively rate their muscle soreness. After a 10 minute warm-up (five minute run at 50% VO2max and five minutes stretching), the subjects ran on the treadmill for 30 minutes at 75% VO2max. This exercise intensity was

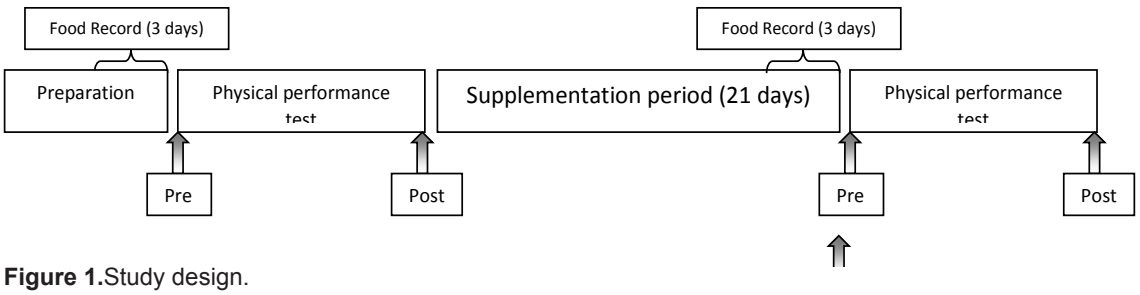

Figure 1. Study design.

chosen based on previous study [11,13]. Heart rate was continuously monitored (Polar Electro, Finland) [11]. Within 5 minutes of completion of the 30 minute treadmill run at 75% VO2 max, blood samples were collected and subjects were asked to subjectively score their muscle soreness. After the exercise protocol, all subjects were allowed to drink water and consume the prescribed snack within 30 minutes after training. This snack is similar and has 400 Calories (70% carbohydrates, and 15% protein). This snack is based on attaining one gram Carbohydrate per kilogram body weight [12]. After this phase, subjects were randomly assigned into the placebo (PLA) or supplements (CRN, GLU, CRN-GLU) groups.

Muscle soreness was assessed again 2 days after the experimental protocol. After the experimental protocol, subjects received supplementation according to the supplementation protocol for 3 weeks. On day 19, subjects recorded food consumed for three days. On day 22, at the end of the supplementation protocol, all experimental procedures were repeated.

Supplementation Protocol

After phase 1 of the experiment, subjects were randomly allocated into four groups to receive the following interventions for 3 weeks before the experimental day in a double-blind fashion:

- CRN group: Four capsules of elemental L-Carnitine per day [2 grams of L-Carnitine (Shahrdarou Inc., Iran)].
- GLU group: Four capsules of L-Glutamine per day [2 grams of L-Glutamine(Karen Nutrilife Co., Iran)].
- CRN-GLU group: Four capsules of L-Carnitine + L-Glutamine per day (2 grams Glutamine + 2 grams Carnitine).
- PLA group: Four capsules of Maltodextrine as placebo per day [2 grams of Maltodextrine(Karen Nutrilife Co., Iran)].

All capsules were identical in appearance. The capsules were packed in bottle and each bottle held sufficient capsules to 21 days of intervention. The method of randomization (sequentially-numbered containers by the pharmacy department) was used to ensure adequate concealment of allocation in all groups. This study was double blinded for researchers and patients.

Subjects were instructed to consume one capsule at breakfast, 1 at lunch, 1 at afternoon snack and 1 at dinner. Subjects were asked to keep a capsule record to ensure compliance with supplementation. Supplementation commenced on the first day after phase 1. Dosing instructions were provided with each patient pack.

The doses of interventions were based on previous human studies. The 2g dose of L-Carnitine per day was chosen to maximize plasma Carnitine concentrations without exceeding the renal threshold for Carnitine [6]. The dose of 2g L-Glutamine per day is based on a previous study on Glutamine supplementation [14].

Perceived Muscle Soreness

Perceived muscle soreness was assessed using a 10-cm-long linear visual analog scale with labels that corresponded to no pain and extreme pain at either end [6]. Subjects marked their level of subjective pain in leg muscles using a vertical line along the continuum, and the distance was reported as the raw score [6,15]. Muscle soreness was assessed at rest before the exercise workout, immediately after workout and two days after the exercise workout.

Blood Collection

Morning blood samples were obtained after a 12-hour overnight fast on each experimental day and immediately after the experimental protocol. A 10-mL blood sample was taken from Antecubital vein. Blood was collected in two 5-mL tubes. Tube 1 contained Ethylendiaminetetracetic Acid (EDTA). These samples were untouched at room temperature for two hours before measuring hematocrit and hemoglobin concentration. Tube 2 was used for serum separation. These tubes were left in standing position for 15 minutes and then centrifuged at 3000g for 10 min to obtain serum. Then, serum was transferred to appropriate containers, stored at -20 ˚C, and analyzed in less than two weeks. A standard laboratory kit (Pars Azmun Inc.; Tehran, Iran) and an auto analyzer(Alcyon 300; Abbott) measured serum CK, LDH and AST activity.

Statistical Analyses

Values are presented as mean ± SD. Two-way analysis of variance was used to evaluate the significance of difference among groups.

P values were two-tailed, and those less than 0.05 were considered statistically significant.

Results

The baseline characteristic of subjects is presented in Table 1. There were no significant differences (P>0.05) and it seems that the groups were comparable.

Dietary intake of participants based on results of 3-days diet records before and after intervention, is shown in table 2. As shown in table 2, dietary intake of energy, protein, fat and carbohydrate are similar between four groups, before and after intervention.

All subjects verbally confirmed that they were not taking any dietary supplements or anti-inflammatory agents prior to the study. Of 28 subjects who met screening criteria, none dropped out of the study, and all completed the entire protocol as described.

Blood Biochemistry

Exercise had effects on markers of muscle damage before and after treatment as shown in table 3.

As expected, exercise intensity at 75% of maximum oxygen consumption for half an hour was associated with a significant increase in muscle damage indices in blood.

However, comparing index values at rest (before exercise) and after exercise in two phases revealed that activity of CK before and after exercise, in CRN and CRN-GLU groups significantly decreased as compared to pre-phase intervention (P<0.05). In addition, LDH activity in CRN group was not statistically significant but approachedsignificance (P=0.069).

Table 2. Energy and Macronutrient Intake Assessed Third Prior the Main Trials*.

Group	Index	Before Intervention	After Intervention	P-value †
CRN	Energy (kcal/d)	3857 ± 359	3587 ± 336	0.19
	Protein (g/d)	131 ± 47	121 ± 33	0.70
	Carbohydrate (g/d)	600 ± 77	565 ± 48	0.29
	Fat (g/d)	102 ± 19	91 ± 14	0.14
GLU	Energy (kcal/d)	3642 ± 449	3500 ± 480	0.39
	Protein (g/d)	178 ± 51	167 ± 40	0.47
	Carbohydrate (g/d)	528 ± 95	509 ± 105	0.46
	Fat (g/d)	89 ± 13	86 ± 12	0.66
CRN + GLU	Energy (kcal/d)	3300 ± 259	3167 ± 625	0.49
	Protein (g/d)	124 ± 35	123 ± 38	0.91
	Carbohydrate (g/d)	490 ± 27	462 ± 78	0.38
	Fat (g/d)	92 ± 18	90 ± 21	0.76
PLA	Energy (kcal/d)	3535 ± 805	3735 ± 563	0.35
	Protein (g/d)	159 ± 39	182 ± 27	0.15
	Carbohydrate (g/d)	535 ± 135	551 ± 99	0.64
	Fat (g/d)	82 ± 21	87 ± 22	0.49

CRN: Carnitine supplementation group.
GLU: Glutamine supplementation group.
CRN + GLU: Carnitine and Glutamine supplementation group.
PLA: Control group.
*Data are presented as mean ±SD.
†Paired t Test

Muscle Perceived Soreness

Upon intervening in two phases among four groups, baseline muscle soreness levels increased significantly compared to pre-exercise trials (Table 4).

However, two days after exercise trials, this index decreased. When compared to the baseline and pre-exercise, it is still noticeable.

Comparing this index data between pre and post intervention among these groups, results indicate that in all treatment groups (CRN, CRN-GLU and GLU), the level of muscle soreness decreasedtwo days after exercise (P=0.057).

Table 3.Effects of Glutamine with and Without Carnitine on Biochemical Parameters*

	CRN	GLU	CRN+GLU	PLA	P-value
LDH† before test phase 1	310±54.7	277.4±39	261.3±51.2	306.6±43.8	0.193
LDH† after test phase 1	381.9±78.7	332.7±88.6	298.4±46.4	343.3±59	0.198
LDH† before test phase 2	278.1±43	260.6±42.5	266±35.6	313.3±42	0.098
LDH† after test phase 2	333.7±45	290±54	329±27	354.4±43	0.069 ‡
CK† before test phase 1	221.6±74.7	152.3±41.3	144.9±42	199.8±56.5	0.044 ‡
CK† after test phase 1	250.7±81.6	177.9±6.34	177.3±35	229.3±68.2	0.095
CKb before test phase 2	191±55.2	162.1±34.4	122.6±12.8	192.4±50.3	0.014 ‡
CK† after test phase 2	211.6±59	174.4±35.4	146.3±21.9	204.7±54.7	0.047 ‡
AST† before test phase 1	25±6.4	25.9±11.3	28.4±13.8	23.4±2.2	0.799
AST† after test phase 1	35.3±4.9	29.6±10	40.4±17.4	27.4±3.9	0.117
AST† before test phase 2	27.9±4.8	23.9±3.1	26.3±9.8	22±2.4	0.268
AST† after test phase 2	33.1±6.1	30.9±6.6	30.1±8	25.6±2.2	0.158

CRN: Carnitine supplementation group.
GLU: Glutamine supplementation group.
CRN + GLU: Carnitine and Glutamine supplementation group.
PLA: Control group.
LDH, Lactate dehydrogenase; CK, Creatine kinase; AST, Aspartate aminotransferase.
*Data are presented as mean ±SD.
†U/L: Unit per Liter
‡P < 0.05, Significantly Difference Between Groups (Two-Way Analysis of Variance).

Table 4.Changes in Muscle Soreness Before (Pre), Immediately After (Post) and 2 Day (2D) After Exercise Before and After Intervention*

		CRN	GLU	CRN+GLU	PLA	P-value
Before intervention (Centimeter)	Pre	0.04±0.08	0.03±0.08	0.1±0.09	0.03±0.05	0.449
	Post	6.1±0.9	5.3±1.1	5.3±1.8	4.9±1	0.307
	2D	2.7±0.7	2.3±0.72	2.06±0.9	2.6±0.7	0.346
After intervention (Centimeter)	Pre	0.01±0.04	0.01±0.04	0.03±0.5	0.03±0.5	0.861
	Post	5.2±0.8	5±1.17	4.3±1.13	5.1±1.2	0.403
	2D	2.1±0.5	1.8±0.5	1.9±0.62	2.7±0.9	0.057

CRN: Carnitine supplementation group.
GLU: Glutamine supplementation group.
CRN + GLU: Carnitine and Glutamine supplementation group.
PLA: Control group.
*Data are presented as mean ±SD.

Discussion

To the best of researcher's knowledge, this study is the first to evaluate the effects of Carnitine and Glutamineon biological markers of muscle damage after exercise in young male football players.

Researchers showed that football players on three weeks of supplementation of Carnitine alone or with Glutamine had a reduction on indices of muscle damage (CK and LDH) before and after exercise.

In our study, in response to exercise intensity at 75% Vo2max for 30 minutes compared to the base state in all groups before and after intervention, serum CK, LDH and AST increased. An increased activity of these cytosolic enzymes after exercise indicates damage to the membrane of muscle cells causing leakage of these enzymes into the circulation [6]. As mentioned in table 3, serum CK activity before and after exercise in CRN and CRN-GLU, showed lower increase.In addition, LDH activity after exercise in CRNshowed lower increase. This reduced LDH and CK activity at rest and before exercise is indicative of the fact that Carnitine supplementation may protect cell membrane in intense exercise.This finding is in agreement with other studies [6,8,16] and indicates reducing the activity of CK after doing exercise and receiving Carnitine supplement. Since in recipients of Glutamine supplement the same effects could not be observed, it seems that changes in the recipients of Carnitine-Glutamine would be the results of Carnitine ingestion.

The researchers suggest that lowerincrease in these enzymescould relate to reduced antioxidant damage, resulting from Carnitine and Glutamine supplementation [6,17,18]. Based on previous research findings, during intense exercise, lipid peroxidation and increased permeability of cell membranes may cause leakage of some intracellular compounds such as CK in the blood stream [5].L-Carnitine has some antioxidant effects that inhibit the accumulation of the end product of lipid peroxidation in the blood [5]. Carnitine also acts as a chelator when bound to iron, which has an important role as a free radical and reduces its cytosolic level [5]. Thus, reduction of CK and LDH in the blood indicates decreased muscle damage during exercise. This may be as a result of enhanced cellular membrane integrity and hence a reduced leakage of these enzymes into the blood [19]. Also, Sugino *et al*. demonstrated that after administration of one gram Carnitine for eight days, no alteration was observed in the activity of CK, LDH and AST. The researcher proposed that an increased dosage and duration of Carnitine supplementation will be needed [20].

However, there are few studies availableregarding Glutamine and its effects on the aforementioned indices. In rats, after twenty one days of Glutamine (1 g/kg) and Alanine (0.61 g/kg) supplementations, the activity of CK was lower than control group; but LDH activity is not affected by supplementation therapy [21]. In a studyon human subjects, after a four-week administration of Glutaminesupplementation, CK activity declined [22]. The difference between results of this study,Cruzat *et al*. and Tajariet *et al*. may be related to the supplementation protocol. In the present study, subjects received only two grams Glutamine per day for 21 days. Therefore, to make decisions about the effects of Glutamine on CK, AST and LDH,further studies are required.

In our study, individual's subjective perception of muscle soreness significantly increased immediately two days after exercise. It is noteworthy two days after exercise, in all supplementation groups, perceived muscle soreness decreased. This finding is in agreement with previous studiesin whichCarnitine supplementation has a beneficial effect on muscle damage indicatorssuch as CK, LDHand muscle soreness[5,6,16].

Some limitations of our study need to be addressed. The principal limitation of this study is the relatively small sample size in our study which restricts the precision of the estimates. The power of our study for CK and AST is less than 80 percent. In spite of this limitation, supplementtherapy has considerable effect on indices of muscle damage. Future studieswith a larger sample size are needed to support or refute our findings. Another limitation is that we used 30-minute treadmill running at 75%

of VO2max as an exercise intervention.
We suggest that future study use simulate soccer matches.
Strengths of the study are its focus on athletes who share the same training background. In addition, since athletes usually fortify their diets with several supplements [23], in this study we used a combination of two popular dietary supplements (Carnitine and Glutamine).

Conclusion

The major finding of our study is that Carnitine supplementation can beneficially affect markers of muscle damage and muscle soreness after exercise.In summary, the present results demonstrated that 21 consecutive days of oral supplementation with 2 grams of L-Carnitine or L-Carnitine plus L-Glutamine could reduce CK and LDH activity as markers of muscle damage caused by exercise and may decrease muscle damage. In addition, Carnitine and Glutamine supplementation possibly decrease muscle soreness, but the mechanisms remain unknown. Future research should assess the effects of dose and timing of Glutamine and Carnitine supplementation on trained athletes.

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