Study Type: Interventional

Actual Enrollment: 205 participants

Allocation: Randomized

Intervention Model: Parallel Assignment

Masking: Double (Participant, Investigator)

Primary Purpose: Treatment

Official Title: Effects of Carnitine Supplementation on Exercise-Induced Muscle Damage: A Randomized Controlled Trial

Actual Study Start Date: May 9, 2025

Actual Primary Completion Date: May 15, 2025

Actual Study Completion Date: June 5, 2025

METHOD

Participants

Initially, 30 participants volunteered to take part in the study. However, participants numbered 17, 18 and 30 withdrew before the pre-test assessments. Participant number 25 withdrew after the second set of the high-intensity interval exercise protocol during the pre-test session, and participant number 12 withdrew before the post-test measurements. Therefore, the study was completed with 25 participants (mean age = 20.48 ± 1.61 years; mean training age = 9.04 ± 3.78 years). Participants were informed about the study protocol before the study began and were informed that they could withdraw from the study at any time during any part of the study. Participants who had clinically significant circulatory, respiratory, digestive, urinary, and nervous system disorders, who had a history of any muscle damage within the last week before the study day, using "cold and flu" medications and vitamins/foods/supplements containing antioxidants, who took food and supplement products with anti-inflammatory effects, or who used any commercially available supplements/ food/ nutritional products (creatine, protein drinks, amino acids, vitamins, etc.) for performance enhancement were not included in the study. Participants were instructed to avoid unusual exercises and resistance exercises (especially exercises involving eccentric contractions) for 1 week prior to the study protocol to prevent any muscle damage. Participants were randomly assigned to receive either 3 g/day of L-Carnitine (Group LC; n=13) or a placebo (Group PLS (CHO); n=12) for 21 days. The experimental design process for conducting the study is presented in figure 1. Before the study, participants were asked to fill out a questionnaire regarding their eating habits, medical history and supplement use (Supplementary File 2). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Istanbul Medipol University Clinical Research Ethics Committee (approval number/date: E-95961207-604.01.01-2244/30 March 2023). This research was supported by Kutahya Dumlupınar University Scientific Research Projects Coordination Office under grant number #2024-24.

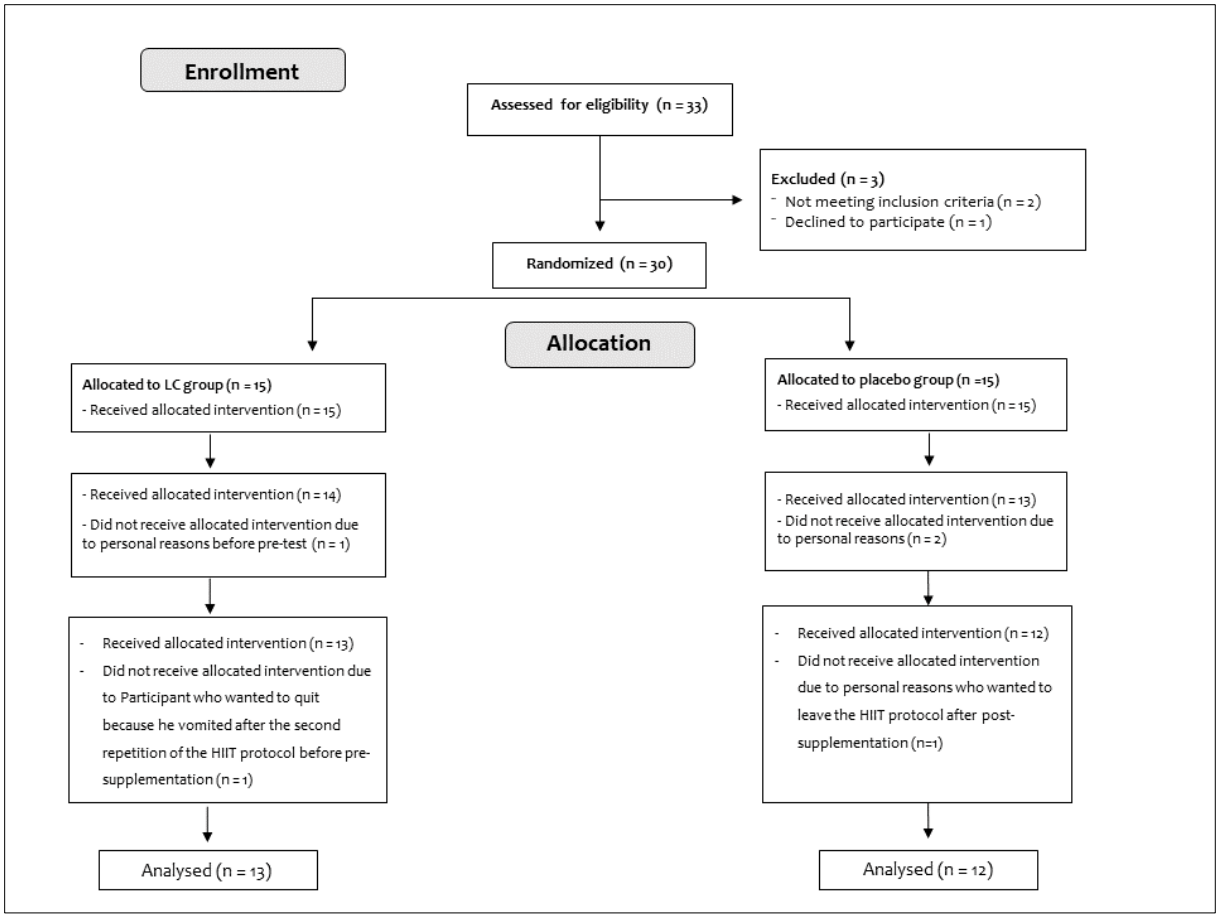

Figure 1. Consolidated standards of reporting trials (CONSORT) flow diagram of study participation from enrollment to analysis.

LC: L-Carnitine

Study Design

This randomized, double-blind, placebo-controlled study was conducted to investigate the effects of L-carnitine supplementation on markers of muscle damage and delayed onset muscle soreness following high-intensity interval training in trained athletes. The study was reported in accordance with CONSORT guidelines for randomized controlled trials. The exercise protocol, anthropometric measurements (and supplement protocol of the study were carried out by the researchers at the Exercise Laboratory of Istanbul Sabahattin Zaim University Faculty of Sports Sciences. The study consisted of two stages. On the first day of the visit (Figure 2), the participants' height (Tarti Telescopic Height Meter) and weight (Tanita MC 780 ST Black) measurements were determined. In the next stage, before the supplementation period, resting venous blood samples of the participants were taken from the antecubital vein by a specialist nurse working at Istanbul Sabahattin Zaim University Health Unit. Following the

blood collection process, the randomization processes of the participants were carried out. For this purpose, written numbers from 1 to 24 were placed inside a box in a way that the participants could not see, and the participants were asked to choose one of the numbers at random, accompanied by an independent observer other than the researcher. This process was recorded by the observer both on the survey form and digitally. The second day of the visit took place after a 21-day reinforcement period (Figure 2), blood samples were collected from the participants by a healthcare professional at rest, 2 h, 24 h, 48 h and 72 h after exercise, and were centrifuged by the researcher and stored at -20 degrees.

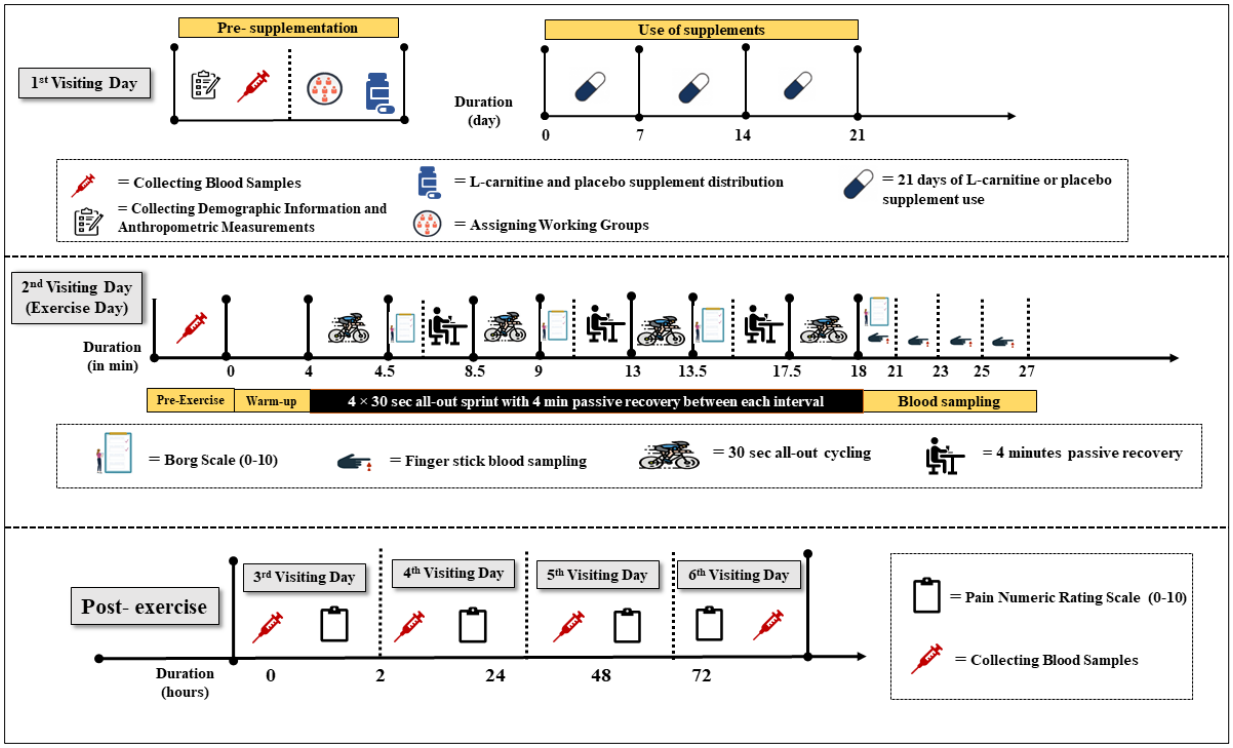

Figure 2. Experimental Design Scheme for conducting the study

High-Intensity Interval Training Protocol

During the pre- and post-supplementation period, participants performed a high-intensity interval training protocol to exhaustion on a Wingate-based cycle ergometer (Monark 894E) at a workload of 0.75 g/kg watts, consisting of 30-s bouts of work divided by 4-min recovery intervals (Figure 2). Blood samples were taken from the fingertip to measure blood lactate levels immediately before the test and at the 1st, 3rd, 5th, 7th, 9th and 11th minutes after the end of the test. A lactate analyzer (LactateScout (+)) was used to measure blood lactate levels. In addition, participants were asked to rate the feeling of fatigue they felt on the Borg Scale

(BorgScale 6-20) to determine the exercise intensity level at the end of each HIIT set (Figure 2).

Supplementation Protocol

Although the optimal dose and form of L-carnitine administration are unclear, in a review article examining the pharmacokinetics of L-carnitine, the Alsuntangled Group (2020) and Evan and Fornasini (2003) reported that L-carnitine would be relatively ineffective in increasing plasma levels unless doses of 2–4 g were used daily due to its low bioavailability [36,37]. Additionally, pharmacokinetic data support the administration of multiple daily doses of LC. Therefore, in our study, participants in group LC were given 3 g/day of supplementation (1 g LC capsules three times daily at 8-hour intervals) for 3 weeks (SepeNatural, L-Carnitine L-Tartrate, China). The product contains L-carnitine (% 68), L-Tartaric acid (% 31) and a very small amount of chloride. This carnitine dose was chosen to maximize plasma carnitine concentrations without exceeding the renal threshold for carnitine [29]. The PLS group was given powdered sugar at the same rates and intervals (1 g CHO capsules three times daily at 8-hour intervals). The usage instructions for each participant were written inside the numbered boxes. The capsules containing L-carnitine and placebo to be given to the participants were filled manually by the researcher using a precision scale (Neck; MH 3000 Model, 0.01 Gr sensitivity). All capsules belonging to the PLS and LC groups had the same appearance, color and dimension. During the study, the researcher made reminders every 8 hours via WhatsApp to monitor the participants' use of carnitine and placebo, and additional precautions were taken with alarms set by the participants every 8 hours. The products used in each session were taken after a full stomach. Additionally, no adverse effects related to the supplement were reported by the participants in this study.

Determination of Muscle Pain Sensation

Muscle pain sensation in the leg extensor and leg flexor muscles was determined using the Numbered Pain Rating Scale (NPRS), one of the most widely used scales due to its simplicity and practicality [38-40]. The NPRS is a scoring method where a score of zero represents no pain and a score of 10 represents maximum pain. While the NPRS has been validated on numerous patients as a measurement tool for pain intensity [41], its use as a screening tool has not yet been tested [41]. In the application, the patient is asked to rate the severity of their pain. This score is used as the pain score. In this study, we classified the scale scores as mild (1-3), moderate (4-6), or severe (7-10) as previously described [39,42]. Following 21 days of supplementation, participants were asked to quantify their pain sensations using the NPRS

during blood sample collection at 2, 24, 48, and 72 hours after the HIIT protocol. Feedback from participants was recorded on the questionnaire by the supervisor.

Blood Sampling and Analysis

Total carnitine blood sample was collected from the antecubital vein at rest before the 21-day LC supplementation period by a specialist nurse at Istanbul Sabahattin Zaim University Health Unit, as well as immediately before HIIT and at 2 hours, 24 hours, 48 hours and 72 hours after HIIT following the 21 days of supplementation (CK,LDH,). The collected blood samples were kept at room temperature for approximately 30 minutes and then centrifuged at +4°C, 3000 rpm, for 20 minutes using a centrifugal machine (Hitachi Koki Himac CR22N High-Speed Refrigerated Centrifuge, cCS Aus model/913350C2). After the centrifugation process, the transparent liquid remaining in the tube was transferred to 2 ml Eppendorf tubes, then stored at -20 C and analyzed in the laboratory. In addition, following 21 days of supplementation period, blood lactate levels were determined from the fingertip immediately before the HIIT test and at the 1st, 3rd, 5th, 7th, 9th and 11th minutes after the end of the test.

Human Total Carnitine Measurement from Serum

Total carnitine levels in serum samples were measured using a Human Total Carnitine ELISA kit (Bioassay Technology Laboratory, Shanghai, China). Absorbance readings were obtained with a Chromate 4300 microplate reader (Awareness Technology, Inc., Martin Hwy., Palm City, USA). Results were expressed in nmol/ml. Data were calculated using linear regression, with absorbance measured at 450 nm.

Measurement of CRP, CK, LDH and Uric Acid

Serum CK and LDH were analyzed on a fully automatic analyzer (Roche Cobas Integra 400 Plus, Roche Diagnostics GmbH, Mannheim, Germany) using ROCHE kits (Mannheim, Germany).

Statistical Data Analysis

Data obtained using a double-blind placebo control group experimental design was analyzed using SPSS 25.0 statistical software. The analysis process began by assessing whether the data exhibited a normal distribution. Normality of the data was assessed using the Shapiro–Wilk test, as well as skewness and kurtosis values. Homogeneity of variances was examined using Levene's test. A two-way repeated measures ANOVA (condition × time) was performed to

determine the effects of L-Carnitine (LC) and placebo (PLS) supplementation on BMI, body fat percentage, lean body mass, resting heart rate, blood lactate concentration, delayed onset muscle soreness (DOMS), and muscle damage markers (CK, LDH) across different time points. When significant main or interaction effects were found, pairwise comparisons were conducted using the Bonferroni post-hoc test. All data are presented as mean ± standard deviation (SD). Statistical significance was accepted at $p < 0.05$.