

ผลของการเสริมแอล-คาร์นิทีนที่มีต่อความปวดระบมของกล้ามเนื้อภายหลังจากฝึก พลัยโอเมตริก

The effect of L-carnitine supplement on delayed onset of muscle soreness after plyometric training

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ABSTRACT

The purpose of this research aimed to investigate the effect of L-carnitine supplement on delayed onset of muscle soreness after plyometric training. The subjects were 10 physically trained male amateur football players, 18 to 22 years of age. A double-blinded, crossover, placebo-controlled design was used. The subjects were supplemented for two trials, either 2 g/d of L-carnitine or placebo. The two 21-day trials were interspersed with a 14-day washout period before the other counterbalanced trial was initiated. On day 18 of each trial, the subjects performed nine sets of plyometric exercise, composed of double leg jumps, single leg hops and drop jumps. Soreness sensation and maximum voluntary isometric contraction force (MVIC) of knee extension were evaluated at baseline and at 24, 48, 72 and 96 hours after the training. Blood samples for serum creatine kinase (CK) determination were obtained at baseline and at 24 and 48 hours after the training. Two g/d of L-carnitine supplementation revealed a trend for a beneficial effect after the plyometric training on soreness sensation of calf muscles ($p = 0.06$). However, no difference of such effect and MVIC was observed in front thigh muscles, in comparison with placebo. These results support the role of L-carnitine in alleviating muscle damage after plyometric training.

Keywords: creatine kinase; DOMS; L-carnitine; muscle strength; plyometric training

บทคัดย่อ

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมแอล-คาร์นิทีนที่มีต่อความปวดระบมของกล้ามเนื้อภายหลังจากฝึกพลัยโอเมตริก ในอาสาสมัครนักฟุตบอลสมัครเล่นเพศชายที่ผ่านการฝึกร่างกายอายุ 18 ถึง 22 ปี จำนวน 10 คน โดยทำการศึกษาแบบ double-blinded, crossover, placebo-controlled โดยจัดให้ได้รับทั้ง แอล-คาร์นิทีน 2 กรัม/วัน และ สารหลอก ชนิดละ 21 วันสลับกันโดยเว้นไม่ได้รับสิ่งทดลอง 14 วันระหว่างการได้รับสิ่งทดลองแต่ละชนิด ในวันที่ 18 ของการได้รับสิ่งทดลองแต่ละชนิด อาสาสมัครทำการฝึกพลัยโอเมตริก 3 ท่า ได้แก่ double leg jumps, single leg hops และ drop jumps รวม 9 เซต เซตละ 10 ครั้ง โดยก่อนฝึกและหลังการฝึก 24, 48, 72 และ 96 ชั่วโมงทำการวัดความปวดระบมกล้ามเนื้อ แรงสูงสุดแบบ isometric (MVIC) ของกล้ามเนื้อที่ใช้ในการเหยียดเข่า และ วัดระดับ creatine kinase (CK) ก่อนการฝึก และหลังการฝึก

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24 และ 48 ชั่วโมง ผลการศึกษาพบว่า การเสริมแอล-คาร์นิทีนที่มีแนวโน้มช่วยให้กล้ามเนื้อที่มีความปวดระบม น้อยกว่า ($p=0.06$) แต่กลับไม่พบความแตกต่างของความปวดระบมและ MVIC ในกล้ามเนื้อต้นขาด้านหน้าเมื่อ เทียบกับการได้รับสารหลอก ผลการศึกษาคั้งนี้สนับสนุนบทบาทของแอล-คาร์นิทีนที่ช่วยให้ความเสียหายของ กล้ามเนื้อที่เกิดจากการฝึกพลัยโอเมตริกบรรเทาลงได้

คำสำคัญ: ครีเอทีนไคเนส อาการปวดระบมกล้ามเนื้อหลังออกกำลังกาย แอล-คาร์นิทีน ความแข็งแรงของ กล้ามเนื้อ การฝึกพลัยโอเมตริก

Introduction

L-carnitine (L-3-hydroxytrimethylammoniobutanoate) can be synthesized in mammals from the essential amino acids lysine and methionine. Primary dietary sources of L-carnitine are red meat and dairy products (Kraemer *et al.*, 2008). L-carnitine is long known to be a substance which facilitates long-chain fatty acid transport across outer and inner membrane and enter into the matrix of mitochondria for subsequent energy production. The carnitine-mediated entry process is the rate-limiting step for oxidation of fatty acids in mitochondria (Lehner and Quiroga, 2016). The effect of L-carnitine on exercise performance, especially its role on increasing fat oxidation, glycogen sparing effect and delay fatigue, was studied extensively before the end of the 20th century (Stephens *et al.*, 2007). To some extent, scientific interest in the ergogenic effect of L-carnitine declined for some period of time because it became apparent that L-carnitine feeding does not alter fuel metabolism during exercise (Wächter *et al.*, 2002; Stephens *et al.*, 2007). A different paradigm for L-carnitine's role on exercise performance has shifted as a result of more recent investigations. L-carnitine supplementation has been theorized to have the ability to facilitate exercise recovery and reduce hypoxic stress (Kraemer *et al.*, 2005). In 1996 Giamberardino *et al.* demonstrated that delayed onset of muscle soreness (DOMS) and serum

creatine kinase (CK) during recovery from step-test was attenuated in subjects that supplemented with 3 g of L-carnitine per day. Their findings indicated that L-carnitine supplementation may have a favorable effect on exercise recovery partly via protecting tissues from ischemia and accelerating the regeneration process by vasodilatation mechanisms (Giamberardino *et al.*, 1996). Volek *et al.* also found that administering 2 g of L-carnitine per day has a positive beneficial effect on biochemical markers related to muscle tissue disruption after squat exercise (Volek *et al.*, 2002). They favor the vascular compartment as the target for the beneficial effect of L-carnitine on exercise recovery responses (Volek *et al.*, 2002; Volek *et al.*, 2008). They explained that L-carnitine is able to reduce chemical tissue damage caused by exercise-induced hypoxia, reducing muscle soreness, and speeding up recovery after exercise (Kraemer *et al.*, 2008). Recently, they examined the effect of L-carnitine supplementation in healthy and recreationally active persons. They also found significant beneficial effects on biochemical markers of purine metabolism (i.e., hypoxanthine, xanthine oxidase), free radical formation (malondialdehyde), muscle tissue disruption (myoglobin, creatine kinase), and muscle soreness after physical exertion (Ho *et al.*, 2010). In addition, the most recent research published the beneficial effects of L-carnitine (12 mg per

kg body weight) in Labrador retrievers. It was found to prevent exercise-induced muscle damage and reduced oxidative stress during strenuous exercise (Varney *et al.*, 2017).

Nowadays plyometric training has been used as a powerful functional training program for developing muscle strength, speed and explosive power. Many sports require these abilities for successful performance. Therefore, plyometric training is used extensively by coaches and athletes (Theanthong *et al.*, 2012). Plyometric training, which involves a stretch shortening cycle with high intensity eccentric muscle contraction, has been known to induce muscle damage and DOMS (Davies *et al.*, 2015). DOMS commonly reaches a peak after 24-48 hours and usually lasts no longer than 5 days (Giamberardino *et al.*, 1996; Lees and

Graham-Smith, 1996). During this period, the unfamiliar soreness related to muscle damage causes the athletes to refrain from effective training. Any regimens which could facilitate the recovery from DOMS and help the athlete return to effective training will be useful and desirable. L-carnitine has been demonstrated in healthy subjects to have beneficial effects on muscle soreness and recovery after exercise (Giamberardino *et al.*, 1996; Volek *et al.*, 2002; Kraemer *et al.*, 2008; Volek *et al.*, 2008; Ho *et al.*, 2010; Varney *et al.*, 2017). Thus, based on previous studies, it would be hypothesized that L-carnitine may have beneficial effects on DOMS related parameters i.e. soreness sensation, MVIC and serum CK especially after plyometric training in amateur football players.

Materials and Methods

Subjects and study design

Ten male amateur football players, aged 18-22 years, were examined. They were physically trained and practiced football regularly at least three times per week, one to two hours a day for more than three years. They had no musculo-skeletal injury of the lower limbs. Their anthropometric data were as follows: mean height 171.04 ± 2.2 cm; mean body weight 68.1 ± 2.26 kg. All subjects were fully informed about the research design, experimental procedure, associated benefits and possible risks of the investigation prior to giving their written consent and commencing the experiment. During the experiment, the subjects were instructed to have their usual activities except intense eccentric contraction activities and forceful collisions during contact sports.

This study involved a double-blinded, crossover, placebo-controlled design that examined the effects of L-carnitine on parameters related to DOMS after plyometric training. The crossover design was used for eliminating the inter-individual variations such as body weight for training load, plyometric training skill, physical performance, proportion of muscle fiber type, inherited serum CK level, fatigue tolerance, and subjective expression of soreness. Moreover AB/BA format was used for balancing the carry over effect by assigning five subjects into each trial at a time. Double-blinded technique was also used to minimize psychological effects and biases.

The study consisted of two 21-day supplementation periods interspersed with one 14-day washout period. For each supplementation period, subjects were supplemented with either 2 g L-carnitine per day or placebo. Subjects were asked to refrain from any nutritional supplements

during the study. On day17 of each supplementation period, blood samples were collected for serum CK determination and subjects were tested for soreness sensation and MVIC during knee extension. On day18 of the period, subjects performed plyometric training. After plyometric training, serum CK was determined at 24 and 48 hours; soreness sensation and MVIC was followed up at 24, 48, 72 and 96 hours. Washout period for 14 days was allowed to eliminate the carry over effect of either the supplement or the training (Volek *et al.*, 2002). After washout period, the second supplementation period took place. The same group of subjects consumed another supplement that they had not yet received for another 21-day supplementation period and performed exactly the same procedures as the first supplementation period.

Supplementation protocol

Subjects were provided four capsules containing either 500 mg L-carnitine (YAINTHAI Co.Ltd., Nonthaburi, THAILAND) or corn flour as placebo. Both types of capsules had the same external appearance. The subjects were instructed to consume two capsules after breakfast and two capsules after dinner. A total dose of 2 g of L-carnitine per day or placebo were consumed. This dose of L-carnitine was chosen to maximize plasma L-carnitine concentrations (Harper *et al.*, 1988). To ensure compliance of supplementation, researcher reminded the subjects to take the supplementation via telephone call, social network or direct contact every day. Supplementation continued for 21 days during each supplementation period. No supplementation was given during washout period.

Plyometric training

Before challenging the subjects with plyometric training on day 18 in each supplementation period, a standardized 15 minutes warm-up was performed. Warm-up consisted of 5 minutes of jogging around 70% of maximum heart rate; two sets of angling; two sets of high knee; two sets of butt kick (distance for angling, high knee and butt kick were 5 meters alternated with 15 meters-dash), 1 minute rest between sets. The last warm-up activity was two sets of 20-meter dash, one minute and thirty seconds rest between sets. After warm-up, subjects performed plyometric training which consisted of three activities. The first and second activities were two sets of double leg jumps and four sets of single leg hops, 10 continuous repetitions each. Subjects were allowed to rest for one minute and thirty seconds between sets. The last activity was three sets of drop jump, 10 trials each, 6 seconds rest between trials and 3 minutes rest between sets. Strong verbal encouragement was provided during plyometric training to motivate subjects to perform a maximal effort. The plyometric training program used in this study is described in detail elsewhere (Theanthong *et al.*, 2012).

Soreness sensation assessment

The soreness sensation of front thigh muscles were assessed when the subject sat on a chair and relaxed his front thigh muscles so that his knees were bent about 90 degrees in starting position. Then the subject extended his right knee to 180 degree in final position. The soreness sensation of calf muscle group was assessed when the subject stood on both legs and performed the calf raise action. Pain-rating

scale (Cole, 2002) was used to classify the intensity of soreness sensation. The scale ranged from 0 to 10, 0 representing no soreness at all and 10 representing the worst soreness.

MVIC measurement

Knee extension MVIC measurement of right leg was performed using the Biodex System 3 pro dynamometer (Biodex Medical Systems, Inc., Shirley, New York, USA). The subject sat on the Biodex System chair with the subject's knee axis of rotation was aligned directly to the dynamometer shaft. The knee attachment was adjusted so that it is proximal to medial malleoli then secured with strap. The subject's lower leg was assisted to move to the desired knee angle at 90 degree flexion. The subject's shoulder, waist and thigh were then stabilized with inelastic straps to prevent compensation movement. The subject was encouraged by the evaluator to exert his maximal effort on knee extension for 5 seconds. The maximum force generated over the two trials was recorded.

Serum CK determination

Four milliliters of blood sample was collected using VACUETTE® Z Serum Clot Activator Tube from antecubital vein for serum CK determination. Blood collection was performed on day 17, 19 and 20 of the supplementation period, 24 hours prior to plyometric training, and at 24 and 48 hours after the training. The subjects were asked to refrain from any other strenuous exercise for at least 24 hours before blood collection. Within 2 hours after the blood collection, the blood samples were kept and transferred in an ice box to a laboratory of Nakhon Pathom hospital for subsequent analysis. The activity of CK was analyzed using a colorimetric reaction. When

serum was added to the reaction mix, the CK in the sample facilitated conversion of ADP to ATP. The ATP produced was then detected by a coupled enzymatic reaction of hexokinase in which the ATP was used to produce glucose-6-phosphate. Glucose-6-phosphate was then used by a third enzyme (glucose-6-phosphate dehydrogenase) to produce NADPH from NADP⁺. NADPH production, which corresponded to CK activity, was then monitored by the absorbance change at 340 nm (Roche Diagnostics GmbH, 2012).

Statistical analysis

Mean \pm SE were calculated for soreness sensation, MVIC, and serum CK activity. Paired t-test was used to evaluate the mean difference of the variables among the L-carnitine treated and placebo trials. One way analysis of variance with repeated measures followed by the LSD method was tested for the changes of the variables within each trial at different time points. The criteria for significant difference was set at $p < 0.05$.

Results and Discussion

Figure 1 showed the calf muscle soreness sensation of the L-carnitine treated and the placebo trials. The soreness sensation of the placebo trial increased significantly and peaked (2.9 ± 0.75) at 24 hours, and then returned to baseline at 72 hours after plyometric training. Whereas that of L-carnitine treated trial (1.1 ± 0.58) did not significantly change. Among the two trials, the soreness sensation of L-carnitine treated compared with placebo trial was, on average, only 38% at 24 hours and 47% at 48 hours after plyometric training. Anyhow, these differences did not reach statistical significance ($p = 0.06$ and 0.19 , respectively).

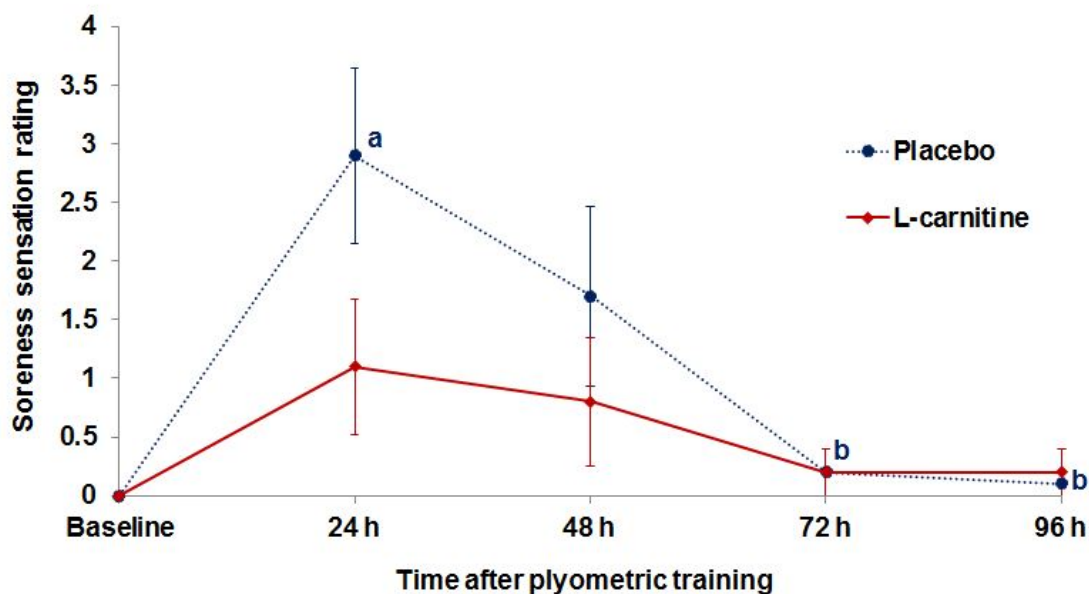


Figure 1 Calf muscles soreness sensation before and after plyometric training of placebo and L-carnitine treated trials (mean \pm SE)

^aSignificantly different from baseline; ^bSignificantly different from 24 hours after plyometric training

Figure 2 showed the soreness sensation of front thigh muscles among placebo and L-carnitine treated trials. In contrast to calf muscle, soreness sensation of front thigh muscles among the placebo and L-carnitine treated trials were similar across time point. That is, soreness sensations were significantly higher above baseline in both trials (5.0 ± 0.39 and 4.7 ± 0.59 , respectively) at 24 hours and remained high score (4.8 ± 0.53 and 4.7 ± 0.65 , respectively) through 48 hours after the training. However,

soreness sensations of both trials declined gradually and reached the baseline value at 96 hours after training. These results were corresponded, in reverse manner, to MVIC as shown in Figure 3. That is, the MVIC at 24 hours were significantly lower than baseline in both placebo and L-carnitine treated trials (193.90 ± 10.85 vs. 224.80 ± 7.47 Nm and 196.40 ± 6.30 vs. 226.20 ± 10.02 Nm, respectively) and then gradually recovered to the baseline level at 96 hours after the training.

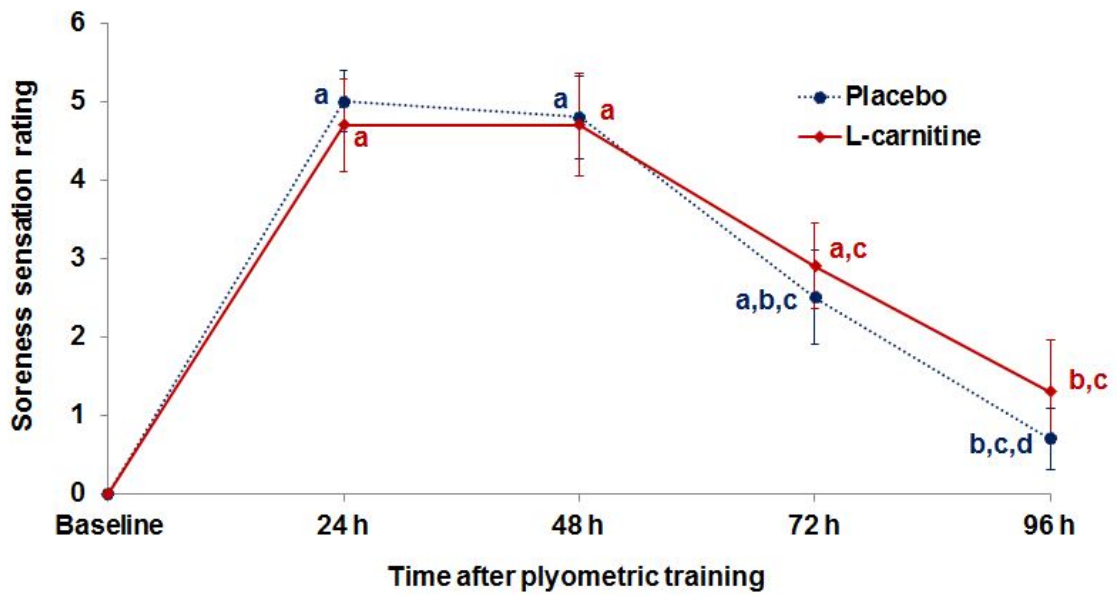


Figure 2 Front thigh muscles soreness sensation before and after plyometric training of placebo and L-carnitine treated trials (mean \pm SE)

^aSignificantly different from baseline; ^bSignificantly different from 24 hours after plyometric training;

^cSignificantly different from 48 hours after plyometric training; ^dSignificantly different from 72 hours after plyometric training

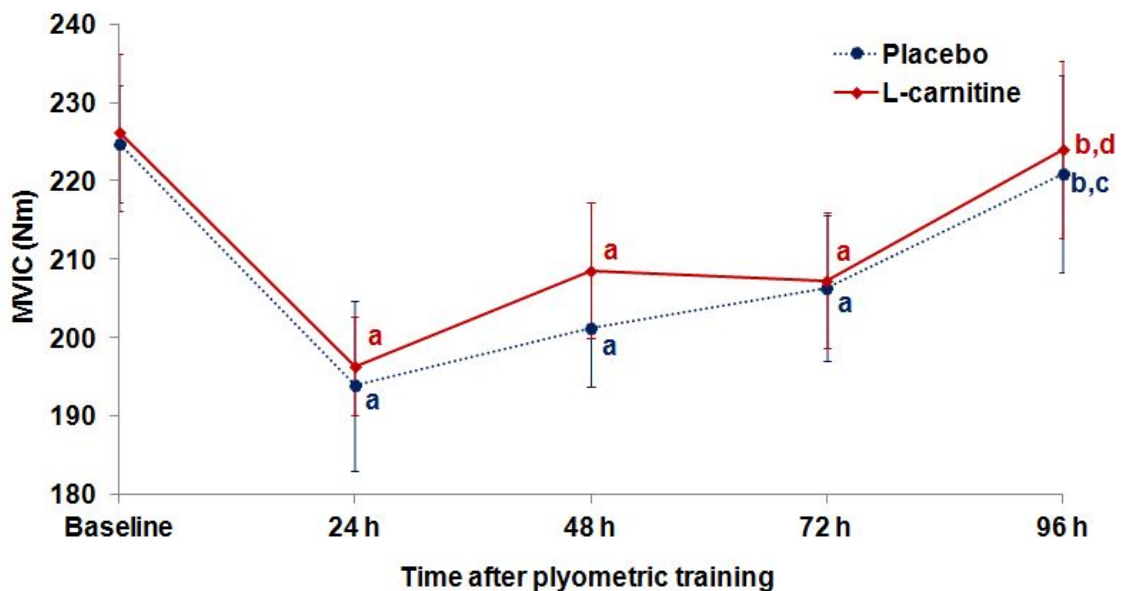


Figure 3 Maximum voluntary isometric contraction (MVIC) of the front thigh muscles before and after plyometric training of placebo and L-carnitine treated trials (mean \pm SE)

^aSignificantly different from baseline; ^bSignificantly different from 24 hours after plyometric training;

^cSignificantly different from 48 hours after plyometric training; ^dSignificantly different from 72 hours after plyometric training

At 24 hours after the plyometric training, the soreness sensation pattern of the calf muscles was different from the front thigh muscles. Firstly, a trend toward significant difference of soreness sensation of calf muscles was found between placebo and L-carnitine treated trials (2.9 ± 0.75 vs. 1.1 ± 0.58 , respectively, $p = 0.06$). On the other hand, the soreness sensation of the front thigh muscles was found to be similar between placebo and L-carnitine treated trials (5.0 ± 0.39 vs. 4.7 ± 0.59 , respectively).

Secondly, soreness sensation started to decline gradually at 24 hours in calf muscles, whereas, in the front thigh muscles soreness sensation lasted until 48 hours and began to decline afterwards. These differences might result from a) different degrees of mechanical force that stress on both muscle groups during eccentric-concentric contraction with maximal exertion of the plyometric training and b) different proportion of muscle fiber types in both muscle groups, that is, front thigh muscles composed of more fast fibers than slow fibers but calf muscle composed of more slow fibers than fast fibers (Edgerton *et al.*, 1975; Behm *et al.*, 2002).

High mechanical force developed during plyometric training caused rupture of the structural proteins of muscle fibers and associated connective tissue (Davies *et al.*, 2015). Many researchers speculated that the damaged sarcolemma resulted in an influx of calcium ion from interstitium into muscle cell, due to both Na-K-ATPase and Ca^{2+} -ATPase pump dysfunction which in turn inhibited cellular respiration (Giamberardino *et al.*, 1996; Baird *et al.*, 2012). In addition, the damaged sarcolemma

also resulted in an efflux of intracellular components into interstitium and blood circulation, which in turn activate nociceptors via histamine, kinins and potassium that produced from phagocytosis process and cellular necrosis. The impairment of energy metabolism made the cell more susceptible to stress resulting in further degree of muscle damage (Giamberardino *et al.*, 1996). In addition, Kraemer *et al.* proposed that exercise induced hypoxia resulted in mismatch between ATP supply and demand. Consequently ATP dependent calcium pumps malfunctioned and the breakdown of ATP was catalyzed further to AMP and finally to superoxide radical which worsen the tissue, both muscle and capillary endothelial cell, disruption (Kraemer *et al.*, 2005). These proposed cascade events were responsible for the causes of muscle pain and DOMS after the plyometric training.

The trend of less soreness sensation of the calf muscles in L-carnitine treated trial found in this study could be in part due to the vasodilation effect property of L-carnitine (Dubelaar *et al.*, 1991; Hülsmann and Dubelaar, 1992; Kraemer *et al.*, 2008; Volek *et al.*, 2008) and high proportion of slow muscle fiber of calf muscles (Edgerton *et al.*, 1975; Behm *et al.*, 2002). This vasodilation property might play an important role in increasing oxygen supply especially to high capillary density areas such as vascular bed of slow muscle i.e. calf muscles. The cascade events resulting from cellular hypoxia during plyometric training might occur in a lesser extent in this well oxygenated area. Moreover, activity of oxidative enzyme in slow fiber was much higher than in fast fiber (Edgerton *et al.*, 1975; Cebašek *et al.*, 2005).

The impairment of energy metabolism from cellular hypoxia and its consequences could be alleviated and resulting in less free radical destruction and tissue disruption. Therefore, less soreness sensation of the calf muscles in L-carnitine treated trial was found. The different situation occurred in the front thigh muscles during plyometric training, that is, the front thigh muscles which are composed of a high proportion of fast fiber with low capillary density received less benefit from vasodilation property of L-carnitine. Therefore, the front thigh muscles still prone to exercise-induced hypoxia. The consequences of hypoxia such as mismatch between ATP supply and demand, ATP dependent calcium pump malfunction and superoxide radical formation still existed to a certain degree in the L-carnitine treated trial. Thus prominent differences among the L-carnitine treated and the placebo trial was not observed in fast fiber dominated front thigh muscles.

The soreness sensation of the front thigh muscles was found to be higher and sustained longer than the calf muscles after plyometric training. It might be because of the predominance of fast muscle fiber, i.e. in front thigh muscles, which was recruited at high intensity load and its higher anaerobic capabilities. In addition, a greater recruitment of fast muscle fibers in high intensity eccentric exercise has been found to make the muscle more susceptible to disruption compared to slow muscle fibers (Magal *et al.*, 2010). Furthermore, high intensity exercise did elicit larger decline in performance and slower recovery (Baird *et al.*, 2012). Because of high intensity load on the front thigh muscles and its fast fiber property, the mechanic and metabolic disturbance from plyometric training resulted in a higher soreness sensation and slower recovery with less contribution from L-carnitine.

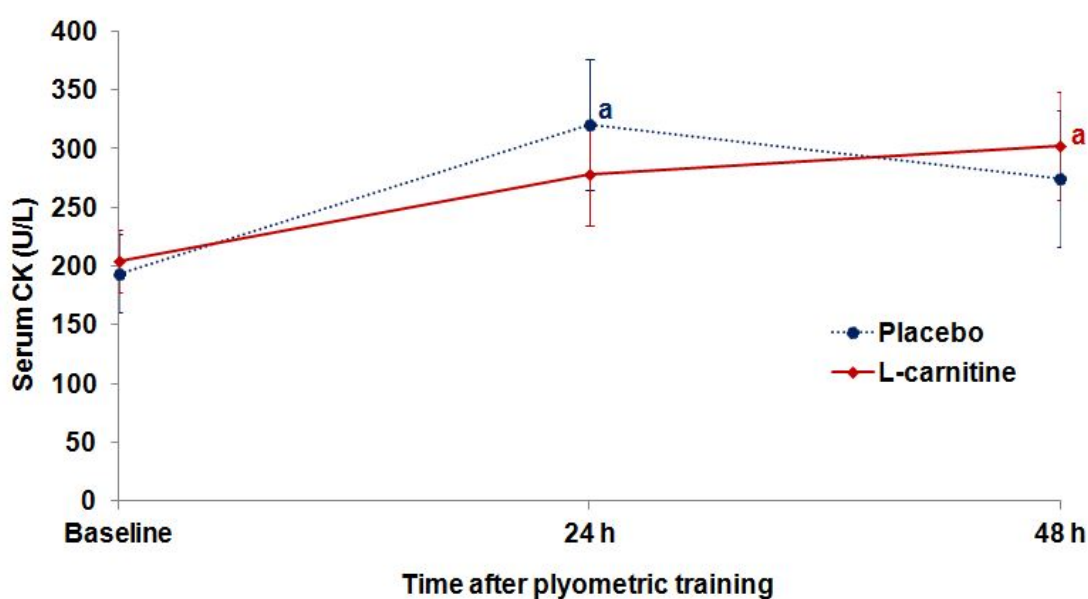


Figure 4 Serum creatine kinase (CK) before and after plyometric training of placebo and L-carnitine treated trials (mean \pm SE)

^aSignificantly different from baseline

Loss of myofibrillar proteins from the muscle cell into the blood may occur at several stages along the cascade events, which may result from exercise-related muscle damage (Baird *et al.*, 2012). In this study, serum CK was then inspected at 24 and 48 hours after the training compared to the baseline data as shown in Figure 4. The plyometric training caused elevated serum CK over the baseline value at 24 hours after the training, which was significantly higher in placebo trial (65.3% higher) but not in L-carnitine treated trial (36.2% higher). The average serum CK of placebo trial was 42.3 unit/L higher than L-carnitine treated trial (320.00 ± 55.78 vs. 277.70 ± 43.09 unit/L, respectively). However, this difference did not reach a significant level ($p = 0.19$). At 48 hours after the training, serum CK in placebo trial declined to the level which was not different from the baseline value. Magal *et al.* found a biphasic pattern of serum CK that increased until 23 hours after exercise and declined weakly at 47 hours before increasing again and peaking 95 hours after exercise. This biphasic response might be related to the time line of inflammation (Magal *et al.*, 2010). Baird *et al.* described that intensive exercise initiated an immune response resulting in acute and delayed leukocytosis. The delayed proinflammatory response may in part be related to the serum CK response observed after exercise-induced muscle damage, due to leucocytes infiltrating and destabilizing the cell membrane during the process of repair (Baird *et al.*, 2012). Unfortunately, the biphasic pattern, if any, could not be revealed in this study because serum CK was monitored only at 24 and 48 hours after exercise.

Different patterns of increased serum CK after plyometric training of the two trials were observed. Therefore, the area under the curve of time versus serum CK of the L-carnitine treated trial and the placebo trial were then calculated. We found that the area under the curve of the L-carnitine treated trial was 20% less than that of placebo trial. Although the magnitude of eccentric contractions involved in the exercise activity and the subsequent extent of muscle disruption was an important factor that determined serum CK response, serum CK levels alone may not provide a fully accurate reflection of structural damage to muscle cells (Magal *et al.*, 2010). In addition, smaller size of calf muscles, compared to front thigh muscles, might be one reason, in particular, to release not much amount of CK to blood circulation and made a little contribution to a significant difference in serum CK among placebo and L-carnitine treated trial.

The elevated serum CK in placebo trial seems to correspond to the trend of higher soreness sensation of calf muscles compared to L-carnitine treated trial after the training at 24 hours time point (Figure 1) but there was not enough statistical meaning to confirm this relationship. Anyhow, the continuous series of change, both mechanically and metabolically, within the muscle resulting from the plyometric training in this study as discussed above existed to a different degree and the effect of L-carnitine was partly evidenced in slow fiber.

Conclusion

The data from this study does not clearly reveal the effect of L-carnitine supplement on DOMS after plyometric training. We found only a trend of beneficial effect of L-carnitine in

reducing soreness sensation of slow fiber dominated calf muscle and serum CK level.

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