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Effect of L-carnitine on Bone Strength: An Experimental Study

L-karnitinin Kemik Gücü Üzerindeki Etkisi: Deneysel Çalışma

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Abstract

Objective: Decreased bone density and strength increase the risk of fracture and thus the rates of morbidity and mortality. L-carnitine (LC) is a supplement commonly used by individuals who participate in sports, especially to increase muscle mass. The aim of this study was to investigate the effect of LC on bone strength.
Materials and Methods: In our main study 10 male Wistar rats per group, a tendon injury model was applied by clamping the left Achilles tendons of rats except the control group. LC (100 mg/kg/day) was administered intraperitoneally every day for 5 weeks in the pre-LC group and for 4 weeks in the post-LC group. LC was not given to the control group. Apart from to our main study, remaining right os femurs of rats were extracted at the end of the experiment to test bone strength to reduce the number of animals used in scientific research. 3-D bending test was performed on the extracted bones using a Zwick Roell Z0.5 mechanical testing machine.
Results: There were no significant differences among the groups in the parameters and measurements used to evaluate bone strength ($p>0.05$).
Conclusion: LC supplementation had no beneficial or detrimental effects on bone strength in healthy subjects.
Keywords: Femur biomechanics, bone strength, L-carnitine, rat, 3-D bending test

Öz

Amaç: Azalan kemik yoğunluğu ve dayanıklılığı, kırık riskini dolayısıyla morbidite ve mortalite oranını artırmaktadır. L-karnitin (LK), özellikle spor yapan bireylerin kas kitlesini artırmak için yaygın olarak kullandığı takviyelerden biridir. Bu çalışmanın amacı, LK'nin kemik dayanıklılığı üzerine etkisini araştırmaktır.
Gereç ve Yöntem: Her grupta 10 erkek Wistar sıçan bulunan ana çalışmamızda kontrol grubu dışındaki sıçanların sol Achilles tendonlarına klemple tendon injury modeli uygulanmıştır. Pre-LC grubuna 5 hafta, post-LC grubuna 4 hafta boyunca her gün LC (100 mg/kg/gün) intraperitoneal olarak uygulandı. Kontrol grubuna ise LC verilmedi. Ana çalışmamız yanında bilimsel araştırmalarda kullanılan hayvan sayısını azaltmak amacıyla deney sonunda sıçanların sağ os femurları kemik dayanıklılığını test etmek için çıkarıldı. Çıkarılan kemiklere ZwickRoell Z0.5 mekanik test cihazı ile 3-D bükme testi uygulandı.
Bulgular: Kemik dayanıklılığını değerlendirmek için kullanılan parametreler ve ölçümler bakımından gruplar arasında istatistiksel olarak anlamlı bir fark bulunmadı ($p>0,05$).
Sonuç: LC takviyesinin sağlıklı deneklerin kemik dayanıklılığı üzerine yararlı ya da zararlı bir etkisi yoktur.
Anahtar kelimeler: Femur biyomekaniği, kemik dayanıklılığı, L-karnitin, sıçan, 3-D bükme testi

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Introduction

Bone structure deteriorates due to some metabolic reasons and with advancing age, leading to bone fractures. With ageing, the balance between bone formation and destruction is disturbed and osteoblastic activity decreases compared to osteoclastic activity, which leads to loss of bone mass (1). Oxidative stress and mitochondrial dysfunction are closely related to bone strength. A large amount of energy, thus mitochondrial function, is required for the maintenance of bone mass and osteoblastic activity required for bone formation (2). Mitochondrial dysfunction causes excessive production of reactive oxygen species (ROS) (3). L-carnitine (LC), which is an essential cofactor in lipid metabolism, also has antioxidant properties. LC plays an important role in the transport of long-chain fatty acids from the inner mitochondrial membrane to the mitochondrial matrix. Thus, it contributes to energy production through beta oxidation of long-chain fatty acids and prevents oxidative stress (4). Although some studies have suggested that glucose is the main energy source for bone formation, some of them have revealed that osteoblastic cells provide their energy needs mostly by fatty acid oxidation (40-80%) (5,6). A recent *in vitro* study showed that LC increased protein production and metabolic activity of porcine osteoblast cells (7).

Bone strength, which determines bone quality, is related to the size, shape and content of bone. Changes in bone quality directly affect the biomechanical properties of bone. Three-point bending test performed to determine bone quality helps to determine the biomechanical performance of bone in research (8).

Non-pharmacological supportive treatments are sought to increase bone density and strength in order to prevent fractures. There is high tendency to use of supported treatments for well being. However, this unproven belief leads to a considerable economic cost and irrelevant drug use. Most supplements, need more attention of scientific research. The increased popularity of LC supplements is an example of this trend, as people seek natural ways to enhance their well-being. Therefore, the aim of this study is to investigate whether LC supplementation increases bone strength using biomechanical parameters.

Materials and Methods

Our study was carried out in Aydın Adnan Menderes University Faculty of Medicine Experimental Animal Production and Experimental Research Laboratory. The both tendon injury and bone strenght studies were approved by the Aydın Adnan Menderes University for Animal Experiments Local Ethics Committee (approval no: 64583101/2020/100, date: 28.10.2020).

This study was mainly planned to investigate whether LC has a healing power on tendon injury. In order to reducethe use of animals in medicine and to help future studies, the remained right os femurs of the sacrificed rats were taken from three groups to test the bone strength. Thirty male Wistar albino rats weighing 400-530 g at 36 weeks of age were divided into three groups as control group, pre-LC group and post-LC group (n=10). Control group rats were not subjected to any treatment during the study. Pre-LC group and post-LC group rats were injected intraperitoneally with ketamine hydrochloride and xylazine at a dose of 90 mg/kg+10 mg and clamped (non-invasive-ischemic) local tendon injury was performed under anaesthesia. In the pre-LC group rats, 100 mg/kg LC was administered intraperitoneally every day for a total of 5 weeks, starting one week before the injury and continuing for 4 weeks after tendon injury. Post-LC group rats were administered 100 mg/kg LC intraperitoneally every day for a total of 4 weeks after tendon injury. Throughout the study, the weight of the animals was measured and recorded consistently weekly, always on the same day. The right os femurs of the rats sacrificed on the 30^{th} day of tendon injury were dissected from the surrounding tissues and frozen at -20 °C in gauze soaked with saline. The bones were thawed at room temperature before mechanical testing. Then, the lengths of the bones (L) were measured and the midpoint of the bone was marked (Figure 1a, b). The midpoint was determined as the loading point for the three-point bending test. Before the mechanical test, cranio-caudal (Ext_{CrCau}) and medio-lateral (Ext_{ML}) periosteal external diameters were measured (Figure 1c). In the three-point bending test, Zwick Roell Z0.5 mechanical testing machine was used in the Agricultural Biotechnology and Food Safety Application and Research Center of Aydın Adnan Menderes University. For the mechanical test, the support points (span length) were determined as 15 mm, preload as 2N, and strain rate as 1 mm/d. A cranio-caudal load was applied from the midpoint of the bone (Figure 2). After the test, medio-lateral (Int_{ML}) and cranio-caudal (Int_{CrCau}) endosteal internal diameters were measured from the fractured bones. Using the endosteal and periosteal diameters, the cross-sectional moment of inertia of the bones and the two-way corticomedullary index (CMI) were calculated according to the following formula. CMI (%): [(Diaphysis diameter - Medullary canal diameter) / Diaphysis diameter]x100. The stiffness value of the bone was calculated using the force-deformation curve (Figure 3) obtained after mechanical testing. Using the stiffness, moment of inertia (I), bone diameter and distance between the support points, ultimate strength and elastic modulus were calculated by using the formulas specified in the references (9-14).

Statistical Analysis

In the statistical analysis of all the data obtained, normal distribution was checked with the Shapiro-Wilk test. One-Way ANOVA was performed for normally distributed values and Kruskal-Wallis intergroup comparison was performed for non-normally distributed values. In One-Way ANOVA test, Levene's test results for homogenous values were checked with the post-hoc Bonferoni test. For non-homogeneous values, Welch's test results were checked with post-hoc Tamhane test.

Results

The average percentage of weight gain between the 1st and 5th week of the groups was 4.48% in the control group, 2.60% in the pre-LC group and 2.51% in the post-LC group (p>0.05).

The results of bone morphometric measurements and 3-D bending test and the data obtained by using formulae are as presented (Table 1). Accordingly, it was determined that there was no statistically significant difference between the measured and formula calculated parameters between the groups (p>0.05). When the diameter values were analysed, it was noted that the medio-lateral diameter measurement values were larger than the cranio-caudal diameter measurement values in all three groups. This indicates that the corpus section of the bone has an elliptical geometry in the medio-lateral direction. In addition, in contrast to the diameter values, the CMI values were larger in the cranio-caudal direction in all three groups. Although fracture force (Fmax), strength and elastic modulus values were lower in the pre-LC group compared to the other groups, no statistically significant difference was found between the groups.

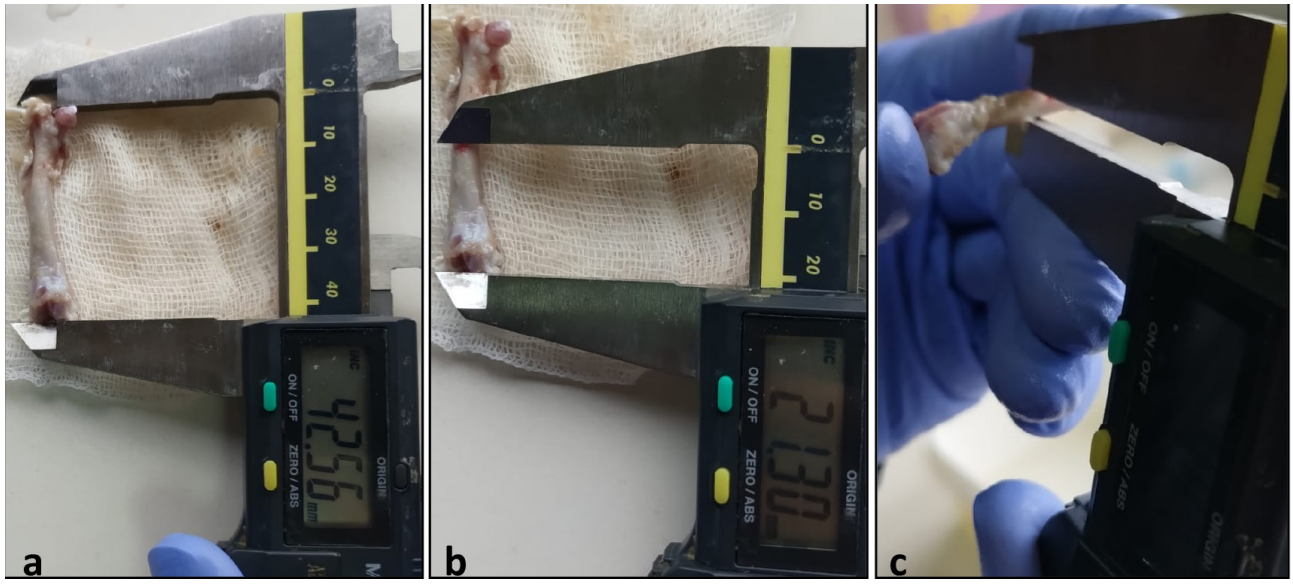

Figure 1. Measurement of the length of the bone (a), marking the midpoint of the bone (b), external cranio-caudal diameter measurement (c)

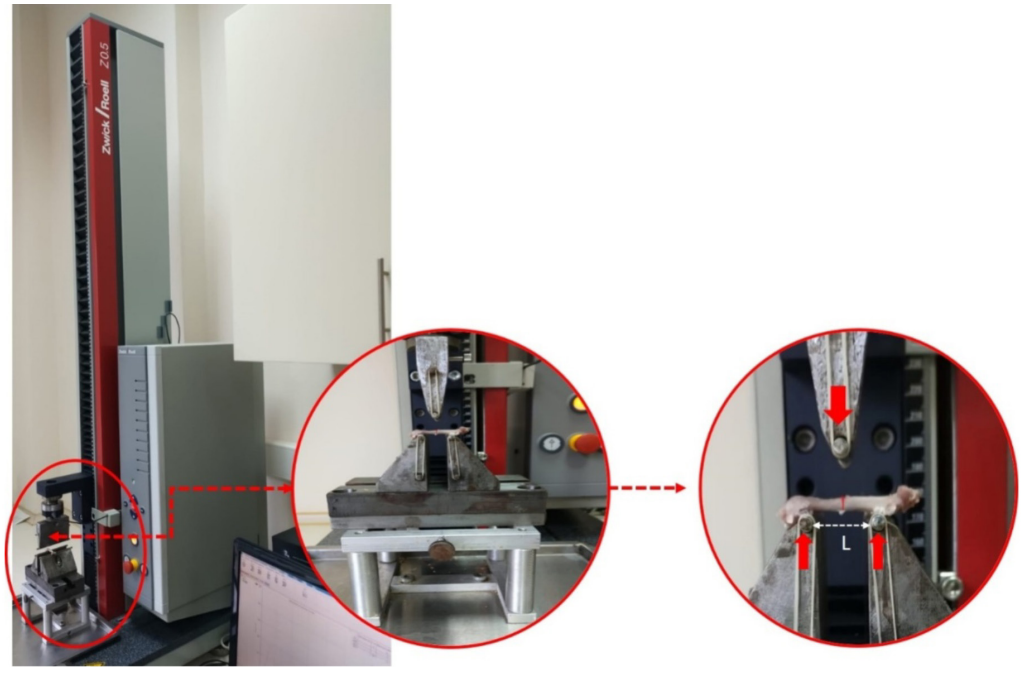

Figure 2. Three-point bone bending test with zwickroell z0.5 mechanical testing machine

Discussion

This study examined the impact of LC, a popular supplement among individuals seeking to enhance muscle strength for professional or recreational sports, on bone strenght and body metabolism. The widespread belief in the efficacy of dietary supplements for promoting well-being incurs significant economic costs.

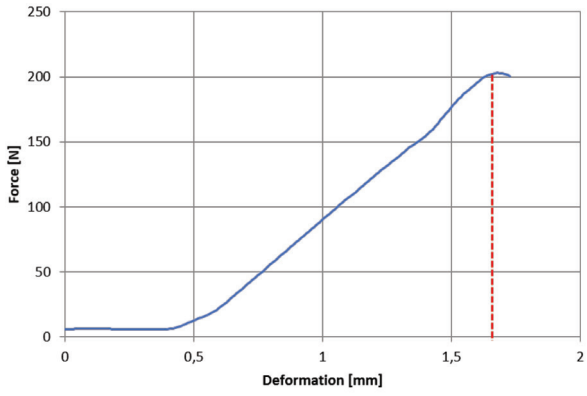

Figure 3. Force-deformation curve N/mm

In order to investigate the effect of LC on body metabolism and for adjustment of LC doses, weekly weight monitoring of the rats was performed. According to the data obtained, the groups receiving LC supplements gained less weight than the control group. In addition, the least weight gain was found in the post-LC group. Although LC supplementation was started in the pre-LC group one week before the tendon injury, it was found that the weight gain was higher in the rats compared to the post-LC group, which started LC supplementation after tendon injury. According to this result, we believe that LC shows its effect on metabolism mostly in inflammation. However, more experimental studies are needed to clarify the issue.

Many studies are showing that LC increases fracture healing and bone formation. Colucci et al. (15) showed that LC and isovaleryl LC, a derivative of LC, can prevent osteoporosis by stimulating osteoblastic activity in their *in vitro* studies based on the fact that a high energy requirement is necessary for osteoblastic activity. Terruzzi et al. (16) the results of another *in vitro* study investigating the effect of LC on osteoblastic activity in human trabecular bones revealed that LC decreased ROS production resulting from mitochondrial activity in osteoblasts and stimulated osteogenesis. Based on these results, Terruzzi et al.

Table 1. Morphometric and 3-D bending test data of the bones in all groups

		Control mean ± SD (min-max)	Pre-LC mean ± SD (min-max)	Post-LC mean ± SD (min-max)	p-value
Measured values	Ext_{ML} (mm)	4.83±0.29 (4.48-5.40)	4.84±0.29 (4.22-5.30)	4.99±0.28 (4.46-5.44)	0.387
	Ext_{CrCau} (mm)	3.82±0.27 (3.28-4.19)	3.92±0.29 (3.44-4.44)	3.75±0.26 (3.35-4.18)	0.404
	Int_{ML} (mm)	3.04±0.32 (2.56-3.41)	3.01±0.47 (2.47-3.96)	2.99±0.4 (2.48-3.52)	0.956
	Int_{CrCau} (mm)	2.02±0.23 (1.60-2.47)	2.33±0.39 (1.77-2.89)	2.17±0.29 (1.71-2.60)	0.288
	L (mm)	41.80±1.30 (39.63-43.38)	41.68±0.66 (40.90-42.53)	41.11±1.55 (38.96-43.79)	0.422
	Fmax (N)	209.00±34.27 (147.00-258.00)	192.10±20.00 (165.30-230.00)	206.00±49.51 (119.00-294.00)	0.553
Calculated values	CMI_{ML}	0.37±0.08 (0.27-0.52)	0.38±0.08 (0.25-0.51)	0.40±0.06 (0.30-0.50)	0.607
	CMI_{CrCau}	0.47±0.06 (0.31-0.53)	0.43±0.08 (0.29-0.53)	0.42±0.08 (0.32-0.55)	0.322
	I (mm^4)	12.22±3.28 (7.19-18.03)	12.80±3.28 (8.58-20.06)	11.61±3.15 (6.99-18.49)	0.718
	Stiffness (N/mm)	172.96±27.75 (102.19-196.86)	179.11±8.82 166.66-191.07	172.40±8.46 (156.95-188.22)	0.342
	Strenght (MPa)	128.29±31.73 (76.30-179.10)	114.60±22.52 (73.90-159.60)	129.14±33.84 (80.10-186.80)	0.481
	ElasticModulus (MPa)	1046.12±284.80 (743.00-1625.31)	1041.66±245.22 (664.58-1382.05)	1104.47±262.63 (659.93-1671.18)	0.840

SD: Standard deviation, min-max: Minimum-maximum, LC: L-carnitine, Ext_{CrCau}: External cranio-caudal diameter, Ext_{ML}: External medio-lateral diameter, Int_{CrCau}: Internal cranio-caudal diameter, Int_{ML}: Internal medio-lateral diameter, L: Bone length, Fmax: Fracture force, CMI_{ML}: Corticomedullary index medio-lateral, CMI_{CrCau}: Corticomedullary index cranio-caudal, I: Moment of inertia

(16) recommended LC supplementation as a candidate molecule to prevent osteoporosis. Aydin et al. (17) examined the effect of LC on fracture healing by creating an experimental fracture model in the femur bones of rats with or without ovariectomy. The results of bone density measurements of the os femurs of rats receiving low and high dose LC supplementation for thirty days showed that 100 mg/kg LC accelerated bone healing by reducing inflammation (17). Ghany et al. (18) examined the effect of amlodipine and LC supplementation on bone turnover after ovariectomy in their experimental studies on rats. In their study, alkaline phosphatase and osteocalcin markers, which are markers of bone turnover, were evaluated by biochemical analysis of blood samples obtained from rats and histomorphology of os femur metaphysis was analysed. The results of the study showed that the combination of amlodipine and LC was more effective than amlodipine and LC supplementation alone. The search for an active substance (molecule) without side effects to stimulate the bone formation mechanism for the treatment of osteoporosis continues today. In an experimental study conducted for this purpose, it was concluded that LC administration prevented dexamethasone-induced osteoporosis by reducing oxidative stress and apoptosis in bone tissue (19). In adult male rats, LC prevented the decrease in cortical thickness of the femur bone due to hyperthyroid-induced osteoporosis and showed a healing effect on histological and immunohistochemical changes (20).

In the selection of experimental animals in bone tissue studies, factors such as easy accessibility to the animal, easy maintenance and feeding, as well as bone turnover (bone formation-destruction) times are taken into consideration, but most rats are preferred as experimental animals. In our study, three-point bending test was performed on the right os femurs of rats to investigate the effect of LC on bone strenght. In our study, it was determined that the bone strength parameters and morphometric measurement values of the bone were not significantly different amongst the experimental groups. The slope of the force deformation curve gives the stiffness value. However, to interpret the strength of the bone per unit area, elastic modulus and strength values should be evaluated. In our study, it was determined that the results of these two parameters were not significantly different between the experimental groups.

Prodinger et al. (21) evaluated the mechanical test parameters in the long bones of rats weighing <400 gr and >400 gr in their study. As a result of the study, it was observed that the Fmax values of the os femurs of the group with weight (>400 gr) were compatible with the Fmax values in the present study. Hooshmand et al. (22) in the study, on the effect of LC supplementation on bone reported that LC slowed bone loss and improved bone microstructural properties by reducing bone turnover. In their study, LC and *ad libitum* nutrition were applied for 8 weeks. In addition, micro computed tomography (microCT) and bone density were analysed and trabecular bone change was demonstrated. In this case, it may not be sufficient for the effect of the 5-weeks application on the mechanical properties of the bone in this study. However, to explain the effect on trabecular tissue, it can be revealed by examining with microCT, density or histopathology. Since these methods were not applied, these deficiencies can be expressed as limitations of the study.

Conclusion

Nowadays, LC is included in many support products offered for healthy living as asupplement. In this study, it was determined that the bone biomechanical properties evaluated after LC supplementation did not differ from the control group. Based on this result, we believe that when used by healthy subjects, it will not have any positive or negative effect on bone strength. Further large-scale experimental studies are needed for the use of LC to increase bone formation in diseased models.

Ethics

Ethics Committee Approval: The study were approved by the Aydın Adnan Menderes University for Animal Experiments Local Ethics Committee (approval no: 64583101/2020/100, date: 28.10.2020).

Informed Consent: When this study is performed on animals, no informed consent is required.

Authors Contributions:

Surgical and Medical Practices: Z.S.K., Concept: Z.S.K., B.D., Design: Z.S.K., B.D., Data Collection or Processing: Z.S.K., F.S.K., B.D., Analysis or Interpretation: Z.S.K., F.S.K., B.D., Literature Search: Z.S.K., F.S.K., B.D., Writing: Z.S.K., F.S.K., B.D.

Conflict of Interest: No conflicting interest are declared by authors.

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