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L-Carnitine enhances exercise endurance capacity by promoting muscle oxidative metabolism in mice

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ABSTRACT

L-Carnitine (LC), the bioactive form of carnitine, has been shown to play a key role in muscle fuel metabolism during exercise, resulting in increased fatty acid oxidation and energy expenditure. However, whether LC contributes to improved endurance exercise performance remains controversial. This study was designed to investigate the effects of LC administration on endurance capacity and energy metabolism in mice during treadmill exercise. Male C57BL/6 mice were divided into two groups (sedentary and exercise) and received daily oral administration of LC (150 mg/kg) or vehicle with a high-fat diet for 3 weeks. During the experimental period, all animals were trained three times a week on a motorized treadmill, and the total running time until exhaustion was used as the index of endurance capacity. LC administration induced a significant increase in maximum running time with a reduction of body fat compared with the control group when mice were subjected to programmed exercise. The serum levels of triglyceride, non-esterified fatty acid, and urea nitrogen were significantly lower in the LC group than the corresponding levels in the control group, while serum ketone body levels were higher in the LC group. Muscle glycogen content of LC administered-mice was higher than that of control mice, concomitant with reduced triglyceride content. Importantly, muscle mRNA and protein expressions revealed enhanced fatty acid uptake and oxidative metabolism and increased mitochondrial biogenesis by LC administration. These results suggest that LC administration promotes fat oxidation and mitochondrial biogenesis while sparing stored glycogen in skeletal muscle during prolonged exercise, resulting in enhanced endurance capacity.

1. Introduction

It is well established that enhancing fatty acid oxidation is an important metabolic factor to improve endurance capacity in humans and rodents [1,2]. Fatty acid oxidation in muscle mitochondria followed by aerobic reactions of the TCA cycle is adequate to generate a large proportion of the ATP required for muscular contraction during prolonged exercise [3]; thus, enhanced fatty acid oxidation during endurance exercise reduces carbohydrate consumption as an energy source and suppresses lactate production, resulting in improved endurance capacity. In this respect, much research has been performed to improve endurance capacity by nutritional regimens that promote fatty acid oxidation [4–6].

L-Carnitine (LC) is essential for the transport of long-chain fatty acids from the cytosol into the mitochondrial matrix, where long-chain fatty acids are oxidized, resulting in the production of ATP. Recently, it has been suggested that LC plays a key role in muscle fuel metabolism during exercise, resulting in increased fatty acid oxidation and energy expenditure [7]. In addition, a mouse model with systemic carnitine deficiency exhibits a mitochondrial abnormality in muscle and the heart [8], and LC directly improves the fatigue characteristics of muscles enriched in type I fibers in vitro [9]. These results suggest that LC could contribute to improve endurance exercise performance, but it remains controversial. Indeed, there is some evidence showing that LC supplementation improves endurance capacity with increased oxygen uptake or fatty acid oxidation [10–12], however others did not observe the positive influences of LC supplementation on endurance capacity and energy metabolism during exercise [13–15]. Most recently,

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Pandareesh and Anand [11] reported that the supplementation of LC with fat increased the exhaustive swimming time and reduced glycogen consumption in the liver and muscle in rats, but they did not observe changes in skeletal muscle oxidative metabolism. Thus, we here investigated the effects of LC on endurance capacity and related physiological metabolism using the treadmill system, which has the distinct advantage over other forms of exercise, including swimming, in that the total amount of external work done by the experimental animal can be calculated easily [16]. This study may provide a better understanding of LC in controlling energy metabolism and endurance capacity.

2. Materials and methods

2.1. Animals and diet

The care and treatment of experimental animals conformed to a protocol approved by the Institutional Animal Care and Use Committee of Korea University (Seoul, Korea). Male C57BL/6 mice (10-week-old) (Orient Bio Inc., Seongnam-Si, Korea) were housed in standard cages under controlled conditions of temperature (22 ± 0.5 °C), humidity (50%), and lighting (light from 0900 to 2100 h). During a 1-week adaptation period, all mice were fed a control diet and were subjected to running exercise three times (velocity of 10 m/min on a 0° inclination for 15 min with shock grid OFF, followed by 10 min with shock grid ON) to acclimate to the treadmill. At the end of the adaptation period, all animals were subjected to an endurance test for the measurement of their baseline values on the running time to exhaustion. To minimize individual variations on endurance capacity baseline, we selected 30 mice with the closest value to the mean baseline from the original 40 mice [5]. Finally, selected mice were divided into two groups (sedentary and exercise) and fed a high-fat diet (HFD, 45% fat from calories). LC (99.8%, provided by Immunotech, Cheonan, Korea) was dissolved in distilled water and administered orally to mice (150 mg/kg body weight) once daily for 3 weeks with vehicle. At the end of study, the mice were fasted for 4 h, ran for 25 min according to the endurance protocol, and were then immediately euthanized by an overdose of avertin (2,2,2-tribromoethanol). Blood was collected by cardiac puncture, and skeletal muscle was collected for further analysis.

2.2. Exercise training and endurance protocol

During the experimental period, all animals were trained three times a week on a motorized treadmill (Mirae-STCorp, Daejeon, Korea). Training was performed for a total 15 min (10 min at 10 m/min, then an increase of 1 m/min every minute for 5 min) on a 10° inclination with shock grid ON. Mice were encouraged to run with the use of an electric grid placed at the end of the treadmill (0.97 mA, 1 Hz).

Endurance capacity was determined 1 and 3 weeks after baseline measurement by placing animals on an individual treadmill at room temperature. The exercise regimen was started with shock grid ON and 10° inclination at 10 m/min for 10 min before the speed was increased by 1 m/min up to 25 m/min (15 min with increase speed) and then held at 25 m/min until exhaustion. Based on previous studies that measured treadmill endurance capacity [17–19], the mice were defined as exhausted if they sustained the shock grid three times for more than 2 s or remained on the shock grid for five consecutive seconds. At the moment of exhaustion, the mouse was removed from the treadmill. The total running time until exhaustion was recorded and used as the index of endurance capacity.

2.3. Biochemical parameters

Following euthanasia, serum was separated by centrifugation at 3000 g for 20 min at 4 °C. Serum samples were used for determination of triglyceride (TG), glucose, non-esterified fatty acids (NEFA), ketone body, and urea nitrogen using commercial kits as specified by the manufacturer. For the TG measurement in muscle, tissue saponification in ethanolic KOH and neutralization with $MgCl_2$ were performed as previously described [20]. Glycerol content was determined by enzymatic colorimetric methods using a commercially available kit. The glycogen content in muscle was measured as glucose residues after enzymatic hydrolysis of tissue samples using a commercial kit.

2.4. Immunoblot analysis

Antibodies were obtained from the following sources: AMPKα, phospho-AMPKα Thr172, Nrf1 (Cell Signaling Technology, Danvers, MA, USA), CPT1ββand β-actin (Santa Cruz Biotechnology, Dallas, TX, USA), UCP2, PGC1α, TFAM and OPA1 (Abcam, Cambridge, UK). Conventional immunoblotting procedures were used to detect the target proteins: Gastrocnemius muscle tissues were collected to extract protein using RIPA buffer (Cell Signaling Technology). Tissue lysates were then cleared by centrifugation at 15,000 × g for 20 min. Total protein concentration was determined by Bradford assay. Equal amounts of protein were separated on 12% SDS/PAGE and the proteins were transferred to polyvinylidene difluoride membranes. The membranes were then blocked for 30 min in a PBS solution containing 3% BSA and 0.1% Tween-20 and then probed with primary antibody overnight in 0.5% BSA, 0.1% Tween-20 in PBS. After washing, membranes were incubated for 1 h with horseradish peroxidase-linked secondary antibodies (Sigma—Aldrich, St. Louis, MO, USA) in PBS solution containing 0.5% BSA and 0.1% Tween-20 in PBS. Finally, after three 10 min washes in 0.1% PBS/Tween-20, proteins were visualized by ImageQuant LAS 4000 (General Electric, Pittsburgh, PA, USA). Band intensities were quantified with ImageJ software (National Institute of Health, NIH Version v1.32j).

2.5. Immunofluorescence staining

Gastrocnemius muscles were extracted and saturated in 30% sucrose-PBS overnight. Frozen sections (5 μm) were cut in a cryostat on microscope slides, washed in PBS and permeabilized in PBS with 0.1% Triton X-100 for 10 min. Blocking was performed using a PBS with 5% normal goat serum for 30 min at room temperature, followed by incubation with OPA1 antibody (Abcam) diluted 1:100 at 4 °C overnight. Three consecutive washes with PBS for 5 min each were followed by sequential incubation with Alexa Fluor 488 goat anti-rabbit IgG secondary antibody (1:200) at room temperature for 1 h. The slides were washed three times with PBS and mounted using anti-fade mounting medium. Images were captured under the confocal microscope (Carl Zeiss AG; Oberkochen, Germany).

2.6. Real-time Q-PCR

The gastrocnemius muscle was homogenized in 1 mL of TRIzol reagent, and then total RNA was isolated according to the TRIzol protocol. Total RNA was reverse transcribed to cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster, CA) as described in the manufacturer protocol. cDNA was used as a template for the relative quantitation for the selected target genes with predesigned TaqMan gene expression assay kits. Each 20 μL reaction contained 100 ng cDNA, 2 × TaqMan Gene

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