



EFFECTS OF L- CARNITINE; ON SKELETAL MUSCLE OF RABBIT

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INTRODUCTION

Skeletal muscle can be excited chemically, electrically and mechanically to produce action because of contractile proteins actin and myosin. Performance of the muscle gradually declines when muscle is used repeatedly at its maximum force; this decline of performance is called muscle fatigue.¹ It is reflected by reduced force of contraction in graphical recording. Many compounds are used to increase the muscle performance and L-carnitine is one of them.

L-carnitine is found in nearly all cells of the body.² The most important biological function of L-carnitine is in the transport of fatty acids into the mitochondria.³ L-carnitine, converts the fats into energy. It enhances the fatty acid oxidation for production of energy thus delays the skeletal muscle fatigue.⁴

The specific objectives of study were to determine

ABSTRACT... L-carnitine (L-car) is a trimethylated amino acid required for the transformation of long chain free fatty acids into acylcarnitine and their subsequent transport into the mitochondrial matrix. It enhances the fatty acid oxidation for energy thus delaying the skeletal muscle fatigue. **Objectives:** Objectives of this study were to determine effect of L-carnitine supplementation on skeletal muscle lactate after fatigue, to observe effect of L-carnitine supplementation on force of contraction in skeletal muscle and to estimate the time to onset of fatigue after L-carnitine supplementation. **Animals & treatment:** In control group, the rabbit sedated and soleus muscle was excised, Homogenized and centrifuged. Supernatant collected, was mixed with reagents of lactate test kit. Absorbance of light was read with the help of spectrophotometer and readings were noted. In experimental group, L-carnitine was given in a dose of 80mg / kg. The period of administration was two weeks. Soleus muscle was excised after giving the last dose of L-carnitine. Lactate level was measured by using lactate test kit (Randox[®]). Readings for lactate measurement were noted after fatigue in both groups. **Results:** Statistically significant results ($p < 0.05$) were found in all three variables. Data was analyzed by applying "t" test in IBM SPSS Statistics - 20 soft ware. **Conclusion:** L -carnitine delays the onset of fatigue of skeletal muscle. This was the stated alternative hypothesis.

Key words: L-carnitine, rabbit, lactic acid, lactate, skeletal muscle, soleus muscle, skeletal muscle fatigue.

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effect of L-carnitine supplementation on skeletal muscle lactate after fatigue, to observe effect of L-carnitine supplementation on force of contraction in skeletal muscle and to estimate the time to onset of fatigue after L-carnitine supplementation.

MATERIAL AND METHODS

Setting

The study was conducted in Physiology Department of Dow Medical College, a unit of Dow University of Health Sciences Karachi.

Duration of study

07 April 2007 to 06-April 2008.

Study Design

Quasi Experimental study.

Chemicals and drugs

L- Carnitine was used for manipulation. It was

procured from local laboratory. All other used chemicals and solvents made up of international/ local laboratories were taken from Institute (DUHS) laboratory.

Animal and Treatment

Adult male Rabbits bred *Lepus Albino* body weight ranging from 1.50 – 1.60 Kg were obtained. They were housed in individual wire floored cage under controlled conditions on a 12 h light / 12 h in dark cycle. The room temperature was kept at 28–30° C .They were fed on normal grass of the animal house of the University.

The animals were divided into two groups of thirty rabbits.

Group A (Control) – Control animals received the normal diet and was without manipulation.

Group B (Study/ Experimental) – These animals were also kept on normal grass but received the L-Carnitine as intervention to undergo experiment.

Sampling technique

Non probability, convenient sampling method was performed.

Sample size Total 60 rabbits were procured and placed in two categories of control and study groups of 30 rabbits respectively.

Selection Criteria

Healthy male rabbits aged one year and Weight ranged between 1 kg to 1.5 Kg.

PROCEDURE

The animals in group A (control) were sedated with Ketamine (Calypsol® R/G Ltd Hungry) 25-50mg / Kg and Xylazine (Xylaz® Farvet, Holland) 5 mg/kg dose IM.⁵ Animal was kept in supine position, the calcaneous bone and tendon Achilles were palpated superficially. After conforming that there was no response on painful stimulus, a longitudinal incision was made with size 22 surgical blade on the medial aspect of the rabbit's leg, skin flaps were everted and gastrocnemius muscle was identified. The belly of gastrocnemius was everted with the help of muscle retractor. Tendo Achilles was followed up to the soleus muscle. The soleus

muscle was separated from the underlying plantar muscle and overlying gastrocnemius muscle and was removed carefully with minimum handling by giving incision on its origin and the tendon side. The rabbit's soleus muscle contains 98% of (type I) slow muscle fibers with high mitochondrial contents.⁶ The muscle was immediately put into the tissue bath containing freshly prepared Tyrode's solution (Pharmacy lab Dow Medical College Karachi) containing (mm) NaCl 121, KCl 5, MgCl₂ 0.5, Na₂HPO₄ 0.4, CaCl₂ 1.8, EDTA 0.1 , NaHCO₃ 24 and glucose 5.5.⁷ The Hemostasis was secured and skin was closed by giving interrupted sutures of silk size 3/0 (Ethicon®, Johnson and Johnson).The rabbit was shifted to the cage after procedure. Muscle was fixed in the muscle holder MLA013 (AD instruments®). The isolated muscle was placed in the upper chamber of the muscle holder. A beaker was built-in with muscle holder which was filled with freshly prepared Tyrode's solution. A silicon tube on the side of the holder connected to an oxygen source, to create a humid environment around for the muscle. The muscle holder also contained built in stimulating electrodes for the stimulation of muscle. Holder was connected with the teaching force transducer MLT0210 and ML4818 Power Lab®15 T(AD Instruments®). Calibration of force transducer and zeroing was done.

After zeroing of the force transducer, the record was started for up to 5 second and then stopped. The known weight of 5 grams was hanged on force transducer and recorded for 5 seconds. Reading of weight was entered in the unit conversion table and the value of N was calculated by Labtutor unit convertor. The record was selected by wave form cursor and the value dragged into the calibration panel as point 1. Again 10 gram weight was hanged on force transducer and same procedure adopted for point 2 reading. Apply button was clicked. Labtutor calibrated the force from mV to N. Tetanic stimuli were given at the interval of 20 ms at frequency of 50Hz. The rate of pulses was set at 1700 pulses /sec continuously till the muscle fatigued. The graph was observed on the Power Lab® labtutor software. The digital record was saved on the Power Lab® system for

data acquisition. Time to onset of fatigue was measured and recorded. The muscle was frozen immediately by applying liquid nitrogen⁸ (Farco gas supply Karachi). Muscle was crushed with mortar and pestle and then was immediately put in to a beaker containing measured amount of distilled water. Beaker was placed at the stand on which homogenizer was fixed. Homogenization of muscle was done at selected speed of 15000 rpm. The homogenate was put into a centrifuge tube, centrifuged in a centrifuge machine⁹ at 10,000 rpm till the supernatant formed. The supernatant was taken for the spectrophotometric lactate measurement with Randox[®] Kit (LC2389).

Reading for lactate measurement ranged from 23.00 to 23.04mmol/kg was taken as base line level of lactate.

In group B the L-carnitine (Natrol[®] Inc Chatsworth, CA 91311 Made in USA) achi) in a dose of 80mg/kg¹⁰ was dissolved in 10 ml of distilled water in clean glass beaker. It was administered through a nasogastric tube sized 10 G into the stomach of the rabbit. This calculated dose was given for two weeks. During this period, the rabbits were on normal (Alfalfa hay) grass diet and were housed in separate cages. The soleus muscle was removed on the day at 3.5 hours of administration of the last dose, by adopting the same procedure as described above. The graphical recording and analysis on the Chart software of Power Lab[®] computerized system were carried out (Figure 1 & 2).

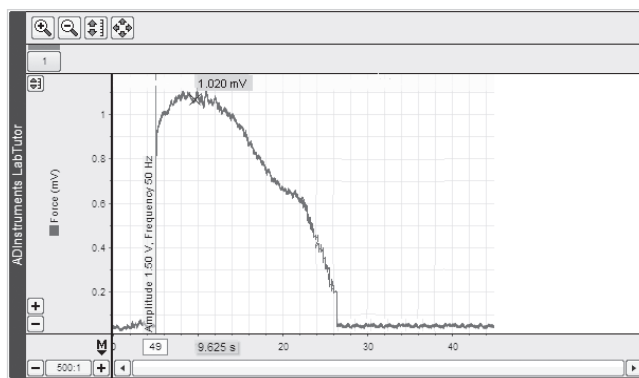


Figure 1. Decline of force and onset of fatigue in control Group.

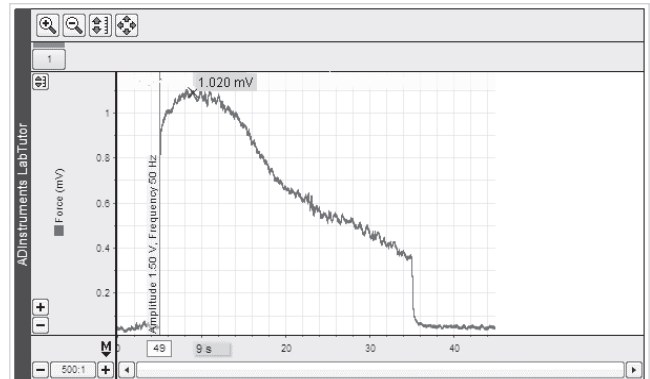


Figure 2. Decline of force and onset of fatigue in Experimental Group.

Data was processed on SPSS-20 soft ware. Results were tabulated and respective interpretation of data was done. Quantitative response variables like lactate level were presented by Mean \pm Standard deviation. "T" test was applied to compare the difference of means based on differences of lactate concentrations before and after the onset of skeletal muscle fatigue and between the control and L carnitine ingested groups. Statistical significance was taken at $p < 0.005$.

RESULTS

Three study variables, muscle lactate level, Percentage decline in force of contraction of muscle and time to onset of muscle fatigue were analyzed and compared.

The mean lactate level after fatigue in control group was 23.73 ± 0.52 mmol/kg while the mean lactate level in experimental group with L-carnitine supplementation after fatigue, value was 23.35 ± 0.36 mmol/kg. The difference was significant ($p < 0.05$) (Fig: 3). In control group the mean percentage decline of force in skeletal muscle of rabbit during the stimulation till fatigue was $70.74 \pm 3.12\%$. While in experimental L-carnitine supplemented group mean percentage decline in force of contraction of skeletal muscle was $57.57 \pm 6.15\%$. The difference was significant ($p = 0.00$) (Figure 4). In control group the mean time to onset of fatigue was 28.75 ± 1.97 seconds, whereas in experimental L-carnitine supplemented group the mean time to onset of muscle fatigue was 32.81 ± 2.45 seconds. The difference was

significant ($p = 0.00$) (Fig: 5).

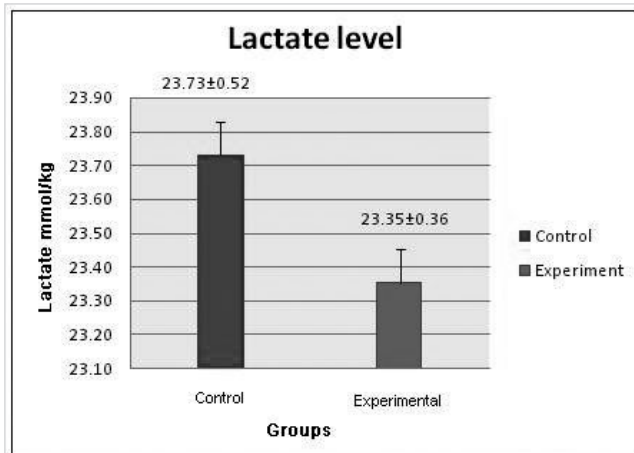


Figure 3. Lactate Level

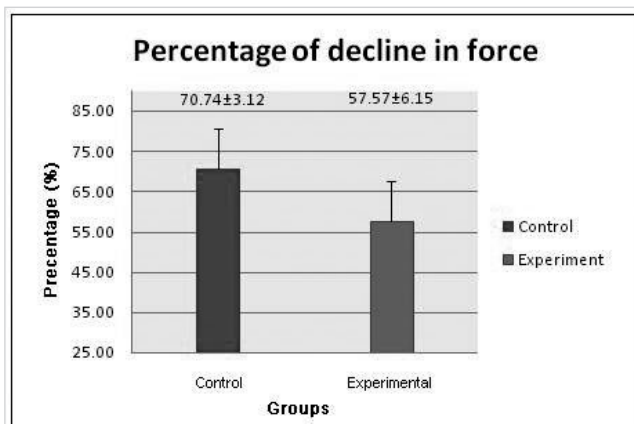


Figure 4. Percentage of decline in force

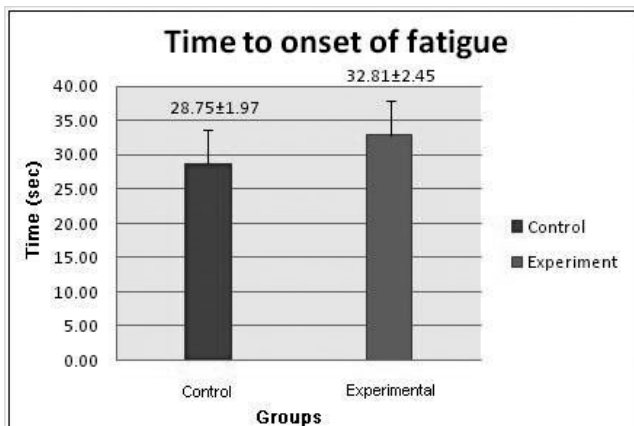


Figure 5. Time of onset of fatigue

DISCUSSIONS

We analysed lactate accumulation in skeletal muscle during exercise and found significant results. A study carried out by Stephens et al¹¹ to

compare the L-carnitine in strict vegetarians and in non vegetarians, found a reduced carnitine level in vegetarians. Another study conducted by Etzioni et al¹² observed the low carnitine level in vegetarians than non vegetarians. Similar results were observed by Wall et al¹³ in their study on human volunteers and found lower level (44%) in the carnitine muscle lactate in ingested group compared to control group following exercise.

During exercise, the glycogen stores depletes specially during sprint exercises muscle fatigue occurs rapidly. The muscle used in this form of exercise contains mostly white type fibers which uses glycogen for ATP production by glycolysis. The fatigue occurs late in that muscle which contains mostly red muscle fibers. The red fibers are fatigue resistant. During endurance exercises the muscle uses glycogen for energy but energy also be provided by fatty acids through beta oxidation. Fatigue is a multi-factorial phenomenon. One of the causes is accumulation of lactic acid in skeletal muscle. Lactic acid is produced when skeletal muscle uses glycogen during exercise. L-carnitine reduces the lactate accumulation in the skeletal muscle by reducing the use of glycogen for ATP production thus it has glycogen sparing effect.

In our study in control group, the mean decline of force in skeletal muscle of rabbit during the stimulation till fatigue was $71.3 \pm 0.45329\%$, while the mean decline of force in skeletal muscle of rabbit in experimental (L-Carnitine ingested group) was $59.09 \pm 7.37411\%$. In control group the maximum (17/30 frequencies) were having readings of 71 and 71.5 %, while in experimental group these level highest readings were found in only two (6.66%) readings. This difference was statistically significant ($p < 0.05$). The result was comparable with studies conducted by Dutta and Ray et al¹⁴ and Brass et al¹⁵, showing that L-carnitine delays the onset of fatigue and decline of force during tetanic stimulation of skeletal muscle.

The last variable under study was observing time of onset of fatigue in seconds. In control group,

the skeletal muscle showed an increase in decline of force upon continuous stimulation. So the time of onset of fatigue was normal. But in case of L carnitine (L-Car) ingested group; the decline of force was decreased. In control group the time of onset of fatigue was 28.75 ± 0.36 seconds while in experimental group it was 32.80 ± 0.44 seconds. This difference was visual prove of causation of L-carnitine.

In control group the readings of this variable was highest in 22 readings (73.33%) with in time interval of 26 – 30 seconds. In experiment group, the frequency of observations of the time of fatigue (in seconds) in skeletal muscle were maximum (26 readings 86.66 %) with in time interval of > 30 seconds ($P < 0.05$).

CONCLUSION

Our observations concluded that L-carnitine significantly reduces lactate accumulation, decline in percentage of force of contraction and increased time to onset of fatigue in exercising skeletal muscle of experimental group of rabbits. Thus our study suggests the delaying effect of L-carnitine on skeletal muscle fatigue.

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“People may hear your words,
but they feel your attitude.”

John C. Maxwell



AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Dr. M. Khalid Siddiqui	Original research work, concept and design	
2	Dr. Sikander Adil Mughal	Critical revision of article especially results & discussion	
3	Dr. M. Saeed Siddiqui	Statistical analysis of data, cross check of completed work	
4	Dr. Atif Hayat	Data arraying and experiment supervision	