EFFECTS OF SINGLE DOSE OF CITRULLINE MALATE ON PERFORMANCE IN COLLEGIATE MALE ATHLETES

EFFECTELE UNEI SINGURE DOZE DE CITRULLIN MALAT ASUPRA PERFORMANȚEI SPORTIVILOR DE SEX MASCULIN

Nishant Sharma[1], Gaurav Shori[2], Deepti Soni Jaipuriar[3]

Keywords: VO_2 max, fatigue, blood lactate threshold, physical work, work capacity

Cuvinte cheie: VO_2 max, oboseală, lactat din sânge, antrenament fizic, capacitate de efort

Abstract

Objective. To study the effects of single dose of citrulline malate on performance in collegiate male athletes.

Study Design. Same subject pre post quasi-experimental design. **Study Setting.** Male Fitness Center. **Participants.** 20 male collegiate athletes (age 20-28 years) were selected based on inclusion and exclusion criteria. **Intervention.** Subjects were randomly divided into two groups. After pre test measurements, group A (n=10) ingested citrulline malate beverage and group B (n=10) consumed placebo drink (glucon D lemon flavor). One hour later, subjects performed pushups to fatigue and immediately, post test measurements were documented. After a wash out period of one week, crossing over was done. Group A consumed placebo beverage while group B had citrulline malate and same procedure was performed.

Outcome Measures. Pre and post measurement of blood lactate, vertical jump height (VJH) and VO_2 max was done. **Results.** Blood lactate was significantly lower in citrulline group (55.92%) as compared to placebo (105.30%). VJH was significantly higher in citrulline group (5.86%) as compared to placebo group (0.75%). Reduction in VO_2 max in citrulline group (17.61%) was significantly lesser when compared with placebo (38.17%).

Conclusion. Ingestion of single dose of citrulline malate significantly improves performance in collegiate male athletes.

Rezumat

Obiective. Studierea efectelor administrării unei singure doze de citrulin malat asupra performanței sportivilor de sex masculin.

Design-ul studiului. Studiu pre-post cvasi experimental. **Locul de desfășurare.** Male Fitness Center.

Participanți. 20 de sportivi de sex masculin (între 20-28 de ani) au fost selectați pe baza unor criterii de incluziune și excluziune. **Intervenție.** Subiecții au fost divizați aleatoriu în două grupuri. După evaluările inițiale subiecții din grupul A (n=10) au ingerat o soluție de citrullin malat iar subiecții din grupul B (n=10) au consumat o băutură placebo (glucoză cu gust de lămâie). După o oră, subiecții au efectuat flotări până la apariția oboselii și imediat după, s-au efectuat evaluările finale. După o pauză de o săptămână, s-a realizat același studiu pe grupele inversate. Grupul A a consumat bautura placebo iar grupul B a consumat soluția de citrulin malat, urmându-se aceeași procedură de administrare și evaluare. **Teste de evaluare.** S-au efectuat valuări pre și posttest pentru lactatul din sânge, săritura în înălțime pe verticală și VO_2 max.

Rezultate. Lactatul din sânge a fost semnificativ mai mic la grupul care a consumat citrulin malat (55.92%), comparativ cu grupul care a consumat băutură placebo (105.30%). Săritura în înălțime a fost mai înaltă la grupul care a consumat citrulin malat (5.86%), comparativ cu grupul placebo (0.75%). Reducerea VO_2 max la grupul care a consumat citrulin malat (17.61%) a fost semnificativ mai mică decât la grupul placebo (38.17%).

Concluzii. Ingestia unei singure doze de citrulin malat îmbunătățește semnificativ performanța sportivilor de sex masculin.

[1] Postgraduate Student (MPT-Orthopaedics, Department of Physiotherapy, I.T.S Paramedical College, Delhi-Meerut Road, Muradnagar, Ghaziabad, U.P, India

[2] Assistant Professor, Department of Physiotherapy, I.T.S Paramedical College, Delhi-Meerut Road, Muradnagar, Ghaziabad, U.P, India

[3] Lecturer, Department of Biochemistry, I.T.S Center of Dental Sciences and Research, Delhi-Meerut Road, Muradnagar, Ghaziabad, U.P, India

Corresponding author: Email: gauravshori@its.edu.in, Ph: +91-9999797466, Fax: 01232-260765, 225380

Introduction

Nutritional and biochemical supplements are continually introduced into sport and physical fitness. As the use of these nutritional supplements continues to increase, so does the need to investigate their effect on human performance. [1] For many years arginine was the explosive, new product in the sports supplement industry. Supplementation was supposed to increase nitric oxide production leading to increased blood flow to muscle tissue thereby increasing nutrient delivery, growth and strength. Current evidence demonstrates no benefit to oral L-arginine supplementation however, which has naturally led to the development of new products to attempt to accomplish the same goals. One of these supplements is citrulline malate.

Citrulline malate (CM) is a mixture of citrulline, an amino acid involved in the urea cycle, and malate, a tricarboxylic acid cycle intermediate. Circulating levels of citrulline is directly related to endogenous arginine synthesis, possibly to an even greater degree than supplementing with straight arginine. CM has been shown to significantly increase aerobic capacity, ATP production and phosphocreatine recovery after training, therefore reducing lactate and providing substrate for the aerobic energy production pathway. Early studies have also shown an antiaesthenic (resistance to muscle fatigue) effect. Studies indicate that CM is involved in three physiological roles: 1) stimulates nitric oxide; 2) removes toxins; and 3) reduces lactic acid and ammonia. Therefore, citrulline malate may be useful for all athletes in maintaining energy levels, improving recovery, enhancing exercise performance and fatigue resistance. [2]

Citrulline malate supplementation is a relatively recent and growing area of research. It carries potential beneficial effects with high intensity exercise including anaerobic sprints and resistance training. Limited research has been done till now to see the ergogenic effects of single dose of citrulline malate. Although, past studies have claimed numerous effects of CM on athletic performance, however, majority of their claims were rejected owing to its methodological limitations. Therefore, this research aims towards studying the effects of single dose of pure form of citrulline malate on performance in collegiate male athletes.

Material and Method

20 male collegiate athletes (age 20-28 years) were selected based on inclusion (no abnormal findings in ECG, LFT values within normal range, normal heamogram) and exclusion criteria (any musculoskeletal, cardio- pulmonary, neurological complications and systemic disorders in past 6 months diagnosed by physicians, consumption of any pre-workout supplement in last 6 months, involvement in other heavy training workout). Subjects were randomly divided into group A (n=10) and group B (n=10). The study was approved by local ethical committee and a duly signed consent form was obtained from all the participants. Before commencement, a brief explanation about the procedure was given to all the subjects.

Procedure

The subjects underwent LFT, ECG and complete blood haemogram to rule out any abnormality related to liver and blood.

Testing for Blood Lactate

All the standard precautions were taken before taking blood sample. Pre test measurement of blood lactate were obtained and documented with the help of portable lactate analyzer in millimoles/litre.

Testing for Anaerobic Power

Testing for anaerobic power was done using vertical jump height. Prior to the vertical jump height, the subjects were lead through an 8-10 minute dynamic warm-up which consisted of squats, lunges, quad stretches. The subject chalked the end of his finger tips and stands side onto the wall, keeping both feet remaining on the ground, reached up as high as possible with one hand and marked the wall with the tips of the fingers (M1). The subject from the static position jumps as high as possible and marked the wall with the chalk on fingers (M2). The therapist measured and record the distance between M1 and M2. The player repeated the test 3

times. The therapist recorded the best of the 3 distance in centimeters and used this value to assess the player's performance. [3]

Testing for Aerobic Power

Testing of aerobic power was done using Queen's College Step Test. Following 5-7 minutes warm up, subject undertook the step test, which was performed on a stool of 16.25 inches (41.3 cm) height for a total duration of 3 minute. The metronome was used to monitor the stepping cadence, which was set at 90 beats per minute (complete 24 bilateral steps) for males. After completion of test, the subjects remained standing while the carotid pulse was measured for 15 seconds, 5-20 seconds into recovery. [4]

This 15 second pulse rate was converted into beats per minute and the following equation was used to predict the maximum oxygen uptake capacity:

$$PVO_2 \text{ max (ml/kg/min)} = 111.332 (0.426 \text{ pulse rate in beats/min})$$

The experimental group i.e. group A (n=10) ingested 200 ml Primaforce TM citrulline malate beverage (8 gram, and filter water until complete 200 ml of solution) and control group i.e. group B (n=10) ingested placebo beverage (8 gram Glucon D lemon flavour, and filter water until complete 200 ml of solution). Both beverages were shaken until fully dissolved and served in disposable white plastic glasses. Subjects drank the beverages one hour before the testing work out. Subjects were instructed to report any possible side effects or discomfort to the researcher as well as their compliance with the performance of activities during the study.

Testing Work Out

The testing workout involved standard pushups to fatigue test. Each participant performed standard push up positioning themselves with their hands directly under the shoulder, pointed forward, head up, back straight, using the toes as pivot point. The participant began in the down position, with elbows bent and the chest touching a 3.75 inch (9.5 cm/ plastic cup, which was centered directly below the sternum. They then raised the upper body and straightened the arms without locking the elbows; then lowered back down to touch the cup with the chest. The up and down movement of the push up was coordinated by the beat of the metronome.

The metronome was set to 60 beats per minute, as this was found to be a reliable cadence, according to Kim et al (2002) and Kravitz et al (2003). With each beat, there was a movement, either an upward push of the body to straight arms or the lowering of the body to the point that the chest touched the plastic cup. This continued until fatigue, or the point at which the participant could no longer maintain the exercise cadence with metronome beat or proper form. [5]

After the testing work out, post test measurements were taken to check the blood lactate, VO_2 max and vertical jump height. A wash out phase of one week was kept. After wash out period of one week, crossing over was done. Pre tests measurements were repeated before group

A ingested with placebo beverage (8 grams Glucon D lemon flavour, and filter water until complete 200 ml of solution) and group B ingested with Primaforce^{TM} citrulline malate beverage (8 gram citrulline malate, and filter water until complete 200 ml of solution). After one hour of the same, the testing workout and post test measurements were done.

Results

A total of 20 subjects were divided into two groups A (experimental group) and B (control group) with their mean age (22.45±1.95), (22.45±1.95); height (171.30±4.84), (171.30±4.84); and weight (68.28±4.02), (68.28±4.02) respectively.

Baseline Data

To analyze the difference between pre lactate, pre VJH and pre VO_2 max, independent t-test was used. Results reflected insignificant difference between the two groups with pre lactate (p=0.785), pre VJH (p=0.167) and pre VO_2 max (p=0.958). (Table 1)

Table 1: Representing pre-test measurements of group A and group B

	Group A (Mean ± SD)	Group B (Mean ± SD)	P value
Pre Lactate	6.33 ± 0.98	6.41 ± 0.97	0.785
Pre VJH	41.80 ± 5.50	39.75 ± 3.41	0.167
Pre VO_2	46.73 ± 5.29	46.65 ± 4.60	0.958

Between group analysis

The mean differences of lactate (gained scores) for group A (experimental group) and group B (control group) are (3.54±1.00) and (6.75±0.90) respectively. (Table 2 and Figure 1)

Table 2: Representing gained scores (pre-test, post-test difference in scores) of lactate

	Group A (mean ± SD)	Group B (mean ± SD)	P value
Difference lactate	3.54 ± 1.00	6.75 ± 0.90	0.0001

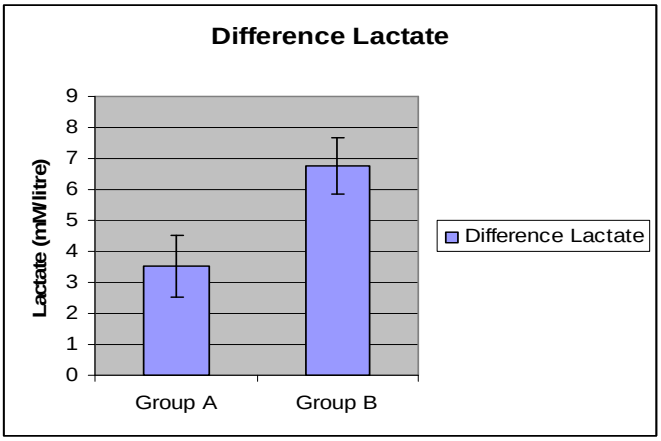

Figure 1: Representing gained scores (pre-test, post-test difference in scores) of lactate

The mean differences of VJH (gained scores) for group A (experimental group) and group B (control group) are (2.45±1.60) and (0.30±0.57) respectively. (Table 3 and Figure 2)

Table 3: Representing gained scores (pre-test, post-test difference in scores) of VJH

	Group A (mean ± SD)	Group B (mean ± SD)	P value
Difference VJH	2.45 ± 1.60	0.30 ± 0.57	0.0001

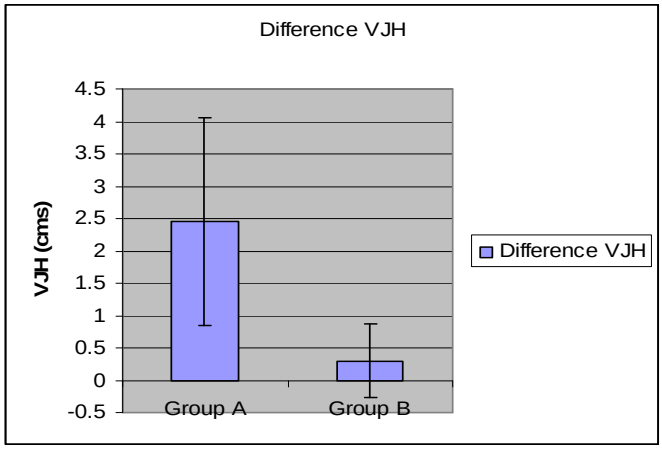

Figure 2: Representing gained scores (pre-test, post-test difference in scores) of VJH

The mean difference of VO_2 max (gained scores) for group A (experimental group), and group B (control group) are (8.23±2.60) and (17.81±2.85) respectively. (Table 4 and Figure 3)

Table 4: Representing gained scores (pre-test, post-test difference in scores) of VO_2 max

	Group A (mean + SD)	Group B (mean + SD)	P value
Difference VO_2 max	8.23 ± 2.60	17.81± 2.85	0.0001

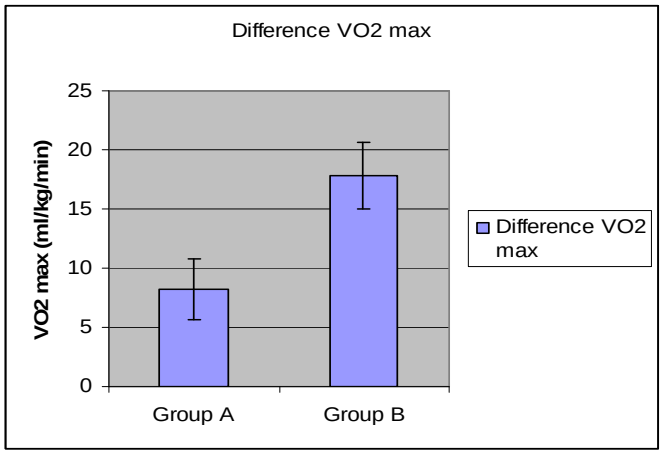

Figure 3: Representing gained scores (pre-test, post-test difference in scores) of VO_2 max

Significant differences were observed in levels of lactate, vertical jump height and VO_2 max in group A (experimental group) as compared with group B (control group).

Discussion

Citrulline, one of the non-essential amino acids, is a component of the urea cycle, which is involved in the detoxification of ammonia in the liver, along with arginine and ornithine.

Supplementation with a mixture of citrulline, arginine and ornithine is known to suppress the increased accumulation of ammonia during exercise. [6]

Perez Guisado et al hypothesized that CM fights fatigue through accelerating the clearance of NH_4^+. Asserting that supplementation with CM drives the urea cycle by accelerating the rotation of the cycle. Callis et al reported a pH buffering effect of CM that supports Perez Guisado et al hypothesis about CM removing excess NH_4^+ from our system. [7, 8]

The main feature of citrulline is to be taken up by the kidney and metabolized into arginine. [9] This is confirmed by the large increase of plasma arginine after citrulline administration. [10] CM supplementation also increases blood arginine levels (better than

actually supplementing with arginine). This then leads to an increase in nitric oxide (NO) production which produces vasodilatation. Increase blood flow would also serve to clear metabolic waste and flush more oxygen and nutrients towards working muscles allowing for more muscular work to occur (i.e. decrease in fatigue). [7] Not only will NO expand blood vessels, but different NO levels are used to regulate the expansion. [11]

Endothelial NO synthase (eNOS) uses the amino acid arginine as a substrate to synthesize NO. When released from endothelium cells, NO can dilate arteries to increase blood flow, help maintain endothelial elasticity, prevent platelets from adhering to artery walls, mediate erection through smooth muscles relaxation and increase capacity for exercise. [12, 13, 14, 15, 16]

Nitric oxide regulates many physiological functions of skeletal muscle including glucose uptake and oxidation, mitochondriogenesis, contractile functions, blood flow and fatty acid oxidation, as well as muscle repair through satellite cell activation and the release of myotrophic factors. Arginine can be synthesized from citrulline providing a recycling pathway for the conversion of citrulline to NO via arginine. Increase in arginine levels in turn, increases phosphocreatine (PCr) levels in the muscle cells which serves as a high energy compound formed from creatine. [17, 18]

Creatine as an amino acid derivative synthesized from arginine, glycine, and methionine in the kidneys, liver, and pancreas. About 90-95% of the body's creatine is found in skeletal muscle. Of this, approximately one-third is free creatine whereas two-thirds exist as Phosphocreatine (PCr). The uptake from circulation is an active process facilitated by a Na^+-dependent transporter against a concentration gradient. PCr serves a major role in energy metabolism. When energy demands increases, PCr donates its phosphate to ADP to produce ATP. The ATP-PCr system can provide energy at high rates, but only for a few (10-15) seconds before the PCr store is emptied. Thus, creatine is involved in temporal energy buffering, and also in spatial energy buffering, proton buffering and glycolysis regulation. Because PCr is a limiting factor in maintaining ATP re-synthesis during maximal short term exercise, an increased PCr concentration should theoretically increases the energy reserve for such exercise. [19, 20, 21, 22, 23, 24]

Documented research findings support present study results which reflect that blood lactate increased (105.30%) in placebo group and (55.92%) in citrulline group, which was significantly lower in citrulline group as compared to placebo group. Moreover, there is incremental increase of vertical jump height (VJH) in citrulline group (5.86%), which was found to be significantly higher when compared to placebo group (0.75%).

On the other hand, malate also serves to increase the ergogenicity in CM supplementation. Malate is the tri-carboxylic acid (TCA) cycle intermediate that shows the largest change in concentration during exercise. The TCA cycle is a key metabolic pathway for the oxidative production of protons for the electron transport system. Increased available malate could potentially increase the $NADH^+ + H^+$ ion production of the TCA cycle, which could increase ATP turn over. Malate is also a key carrier of protons across the mitochondrial membrane in the malate-aspartate-shuttle. Thus, increases in available malate could potentially increase transport of protons into the mitochondria to feed the electron transport chain, which could also positively influence ATP turnover. [25, 26, 27, 28]

VO_2 max decreased for citrulline (17.61%) as well as for placebo group (38.17%) but the reduction in value was significantly lesser for citrulline supplementation group. Malate-aspartate-shuttle enables bypassing of blockade of the oxidative pathway induced by ammonia and hence limits accumulation of lactate by reorienting it towards pyruvategenesis and its aerobic utilization or gluconeogenesis. More malate leads to more ATP production, which is energy currency of the body required for all physiological functions.

Conclusion

Although human DNA does not code for citrulline directly, several proteins contain citrulline as a result of a posttranslational modification. These citrulline residues are generated by a family of enzymes called peptidylarginine deiminases (PADs), which convert arginine into citrulline in a process called citrullination or deimination. Citrulline in the form of citrulline malate is sold as a performance enhancing athletic dietary supplement, which was shown to reduce muscle fatigue in a preliminary clinical trial.

It is thus concluded from present study that ingestion of single dose of citrulline malate significantly improves performance in collegiate male athletes.

Acknowledgement

I would like to acknowledge, Ruchika Gupta, Lecturer, Department of Physiotherapy, I.T.S Paramedical College, for extending help in data analysis and interpretation.

References

[1] Becque MD, Lochmann JD, Melrose DR (1999), Effects of oral creatine supplementation on muscular strength and body composition. *Med Sci Sports Ex;* 32(3): 654-658.

[2] Briand J, Blehaut H, Calvayrac R, Laval MD (1992), Use of a microbial model for the determination of drug effects on cell metabolism and energetics: study of citrulline malate. *Biopharm Drug Dipos*; 13(1): 1-22.

[3] Changela PK, Bhatt S (2012), The correlational study of the vertical jump test and wintage gate cycle test as a method to assess anaerobic power in high school basketball players. *International Journal of Scientific Research Publications;* 2(6): 2250-3153.

[4] Chatterjee S, Chatterjee P, Mukherjee PS, Bandyopadhyay A (2004), Validity of queen's college step test for use with young men. *Br J Sports Med;* 38: 289-291.

[5] Dean JA, Foster C, Thompson N (1987), A simplified method of assessing muscular strength. *Medicine and Science in Sport and Exercise;* 19: 63.

[6] Meneguello MO, Mendonca JR, Lancha AH, Costa Rosa LF (2003), Effect of arginine, ornithine and citrulline supplementation upon performance and metabolism of trained rats. *Cell Biochem Funct;* 21: 85-91.

[7] Guisado P, Joaquin, Jakeman, Philip M (2010), Citrulline malate enhances athletic anaerobic performance and relieves muscle soreness. *Journal of Strength and Conditioning Research;* 24(5): 1215-1222.

[8] Vanuxem P, Vanuxem D, Fomaris E, Bernasconi P (1986), The role of lactate and ammonium in fatigue. *Gazette Medicale;* 7: 62-72.

[9] Morris SM (2006), Arginine: beyond protein. *Am J Clin Nutr;* 83: 508-512.

[10] Moinard C, Nicolis I, Neveux N, Darqug S, Benazeth S, Cynober L (2008), Dose-ranging effects of citrulline administration on plasma amino acids and hormonal patterns in healthy subjects: the citrudose pharmacokinetic study. *British Journal of Nutrition;* 99: 855-862.

[11] Sholevar C, Criscuolo CL (2009), The production and effects of L-arginine on the human body.

[12] Maxwell AJ, Ho HV, Le CQ, Lin PS, Bernstein D, Cooke JP (2001), L-arginine enhances aerobic capacity in association with augmented nitric oxide production. *J Appl Physiol;* 90(30): 933-938.

[13] Hayashi T et al (2005), L-citrulline and L-arginine supplementation retards the progression of high cholesterol diet induced atherosclerosis in rabbits. *Proc Natl Acad Sci;* 102: 1366-1368.

[14] Walker JB (1979), Creatine: biosynthesis, regulation and function. *Adv Relat Areas Mol Biol;* 50: 177-242.

[15] Guimbal C, Kilimann MW (1993), A Na^+ dependent creatine transporter in rabbit brain, muscle, heart and kidney. cDNA cloning and functional expression. *J. Biol Chem;* 268: 8418-8421.

[16] Greenhaff PL (2001), The creatine-phosphocreatine system: there's more than one song in its repertoire. *J Physiol;* 537: 657.

[17] Ptrovic V et al (2008), Antioxidative defence alterations in skeletal muscle during prolonged

acclimation to cold: role of L-arginine/NO producing pathway. *The Journal of Experimental Biology;* 211: 114-120.
[18] Harris RC, Soderlund K, Hultman E (1992), Elevation of creatine in resting and Exercised muscle of normal subjects by creatine supplementation. *Clinc Sci(lond);* 83: 367-374.
[19] Jones DP, Borsheim E, Wolfe RR (2004), Potential ergogenic effects of arginine and creatine supplementation. *The Journal of Nutrition.*
[20] Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL (1996), Muscle creatine loading in men. *J Appl Physiol;* 81: 232-237.
[21] Wug, Meininger CJ (2002), Regulation of nitric oxide synthesis by dietary factors. *Annu Rev Nutr;* 22: 61-86.
[22] Kinlay S et al (2001), Endothelium derived nitric oxide regulates arterial elasticity in human arteries in vivo. *Hypertension;* 38(5): 1049-1053.
[23] Preli RB, Klein KP, Herrington DM (2002), Vascular effects of dietary L-arginine supplementation. *Atherosclerosis;* 16(1): 1-15.
[24] Mills TM, Pollock DM, Lewis RW, Branam HS, Wingard CJ (2001), Endothelium-1 introduced vasoconstriction is inhibited during erection in rats. *Am J Physiol Regul Integr Comp Physiol;* 281(2): 476-483.
[25] Gibala MJ et al (1998), Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. *American Journal of Physiology;* 275: 35-42.
[26] Brown AC, McCrae HS, Turner NS (2004), Tricarboxylic-acid-cycle intermediates and cycle endurance capacity. *International Journal of Sports Nutrition and Exercise;* 14(6): 720-729.
[27] Sahlin K, Katz A, Broberg S (1990), Tricarboxylic acid cycle in human muscle during prolonged exercise. *American Journal of Physiology;* 259: 34-41.
[28] Wu JL, Wu QP, Huang JM, Chen R, Cai M, Tan JB (2007), Effects of L-malate on physical stamina and activities of enzymes related to the malate-aspartate shuttle in liver of mice. *Physiological Research;* 56(2): 213-220.