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The Effect of Choline Supplementation on the Level of Plasma Free Fatty Acids and Beta-Hydroxybutyrate during a Session of Prolonged exercise

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ABSTRACT

The purpose of the present research is to study the effect of choline supplementation on plasma free fatty acids and beta-hydroxybutyrate as well as lipid metabolism during a session of prolonged exercise among elite male triathletes (with an average of 21.44±2.83 of age, 54.71±5.34 kilograms of weight, and 71.25±4.36 mlkg $^{-1}$min^{-1} of VO_2 max). Nine elite triathletes performed two 120-minute sessions of running on treadmill with an intensity of 59-64% VO_2 max in the form of a translational research with a single-blind design. The subjects took placeboes one hour before the exercise in the first session and took Choline Bitatrate supplements one hour before the exercise in the second session. Blood sampling was done before and after exercises in order to measure plasma free fatty acids and beta-hydroxybutyrate using the colorimetric method. Data were analyzed using repeated analysis of variance at $P \leq 0.05$ significance level.

A comparison between the results of the exercise sessions showed that the level of plasma free fatty acids at the end of the exercise with choline supplements was significantly lower than that of the exercise with placeboes, and that the level of plasma beta-hydroxybutyrate at the at the end of the exercise with choline supplements was significantly higher than that of the activity with placeboes. It was thus concluded that choline supplementation accelerates the lipid oxidation process during prolonged sports exercises through increasing the uptake of fatty acids and increasing plasma beta-hydroxybutyrate.

Keywords: Choline supplement, free fatty acids, beta-hydroxybutyrate, prolonged exercise, triathlon.

INTRODUCTION

Fat stores are the major energy stores of the body and the total amount of energy stored as triglyceride is more than 60 times greater than the energy stored as glycogen [3]. It has been shown that the increase in the ability of fatty acid oxidation during endurance exercise will delay the release of glycogen from muscles and liver and will decrease blood sugar; as a result, it will delay the occurrence of fatigue and will prolong the activity [3]. Any exercise strategy and dietary manipulation that can increase the oxidation of lipids and decrease the utilization of carbohydrates can be helpful for endurance athletes [4]. It has been shown that the contribution of fats and carbohydrates in the energy production process depends on factors such as exercise intensity, exercise duration, physical fitness, VO_2 max, gender, and the type of diet [4]. Regarding the ideal intensity for fat oxidation, it has been confirmed that the maximum lipid oxidation occurs between 59 and 64 percent VO_2 max in trained individuals and between 47 and 52 percent VO_2 max in untrained individuals [4]. Nowadays, various supplements are used in endurance and ultra-endurance sports in order to enhance performance and delay fatigue. Among these supplements, there is a compound called choline which has positive neuromuscular, metabolic, mental, and structural effects on the body of mammals [5],and it has recently been classified as a basic nutrient for humans by the Food and Nutrition Board of the Institute of Medicine (USA)[6]. Considering the fact that this compound is the precursor molecule for the synthesis of neurotransmitter acetylcholine, the effect of choline on athletic performance has been confirmed in many studies with an emphasis on neuromuscular fatigue mechanisms [1, 7, 8, 9, & and 10]. But choline is at the same time the most important lipotropic compoundin the body of mammals [6]. Lipotropic compounds are substances that help the breakdown of fat during metabolism in the body; they promote the export of fat from the liver and are essential for maintaining a healthy liver and for burning the exported fat [11]. It is observed in previous studies that the effect of choline as a lipotropic substance on the process of lipid metabolism during sports exercise has been less paid attention to and that limited number of studies has examined the effect of long-term choline supplementation on fat and lipid metabolism indices in animals and non-athletes [12, 13, & 14]. Of these indices one can mention the changes in the level of plasma free fatty acids and beta-hydroxybutyrate as a result of long-term choline supplementation. The research of Nobuko and Sachan (2000) showed that long-term supplementation of a combination of caffeine, carnitine, and choline in rats increased the uptake of free fatty acids by muscle cells through the effect of choline on the level of permeability of the plasma membrane of these cells which increases triglyceride stores in muscle cells and decrease the level of triglyceride and plasma free fatty acids at the end of the period of choline supplementation and these changes were followed by a decrease in body fat indices such as subcutaneous fat [14]. Moreover, in other studies, Daily and Sachan (1995) and Nobuko and Sachan (2003) showed that long-term choline supplementation in humans will increase muscle carnitine stores and the level of plasma beta-hydroxybutyrate at the end of the period and considering the key role of carnitine in the process of mitochondrial oxidation of lipids, they considered these changes as increasing fat oxidation, but because these changes were not followed by the decrease of respiratory exchange ratio, they came to the conclusion that choline

supplementation will increase the capacity of incomplete oxidation of lipids. The reason for such a conclusion was the presence of acetyl groups due to beta-oxidation of lipids as beta-hydroxybutyrate in plasma [12 & 13]. But the effect of choline supplementation on the indices of lipid metabolism in a session of prolonged exercise is an issue that has not yet been dealt with.
Thus, the present study aims to consider the effect of a prolonged exercise session – whose intensity and duration are ideal for maximum fat oxidation – on the changes in plasma free fatty acids and beta-hydroxybutyrate at the end of this exercise in elite triathletes and thus to assess the effect of choline supplementation on lipid metabolism.

MATERIALS AND METHODS

Subjects

Nine male triathletes of Zanjan Province with regular exercise during the past three years and with championship at the provincial, national, Asian, and International levels participated in the research as voluntary samples. The general and physiological characteristics of the subjects are presented in table 1.

Table 1 – General and physiological characteristics of the studied sample ($n = 9$)

General and Physiological Characteristics	Mean ± Standard Deviation
Age (Years)	21.44 ± 2.83
Weight (Kilograms)	54.71 ± 5.34
Body Fat (%)	12.43 ± 3.64
Body Mass Index (Kg/M^2)	22.67 ± 1.96
Maximum Heart Rate (Number)	195 ± 5.81
VO_2 max ($ml\ kg^{-1}\ min^{-1}$)	71.25 ± 4.36

Figure 1 – A chart of the research design

Time	During Three Days before Exercise	Exactly before the Beginning of Exercise	120 Minutes after Exercise	Exactly after the End of Exercise
1st Week	Recording the Diet	Blood Sampling (Pretest)	Prolonged Exercise	Blood Sampling (Posttest 1)
2nd Week	Repeating the Recorded Diet	Blood Sampling (Pretest)	Prolonged Exercise	Blood Sampling (Posttest 1)

Research Design

This design was conducted as a single-blind design translational research. This research design entails the administration of two separate prolonged exercise sessions with a defined intensity and duration. The prolonged exercise of interest involved 120 minutes of running on a treadmill with the intensity of 59-64% VO_2 max (equivalent to 70-75% maximum heart rate). This is the most appropriate exercise intensity for maximum fat oxidation [4]. There was an interval of one week between the two exercise sessions and a three-day rest was considered for the subjects before performing the exercisesin the first and second week. An hour before the beginning of the first exercise session, 250 ml of placebo (orange juice) was given to subject and an hour before the beginning of the exercise in the second week, Chorine Bitatrate Supplement (a product of Life Extension Inc., USA) was mixed with 250 ml of orange juice up to 0.05 grams for each kilogram weight of subjects. Blood samples were taken from the subject's exactly at the beginning and the end of both exercise sessions in order to measure the level of plasma free fatty acids and beta-hydroxybutyrate.

Measurement of Physiological Characteristics of the Subjects

The VO_2 max of the subjects was measured using the treadmill test one week before performing the first endurance exercise. The maximum heart rate of the subjects was measured at the end of the Bruce test and at the point of extreme fatigue. A treadmill (COSMED T170, Italy) was used for the treadmill test and the maximum heart rate was recorded using the heart rate indicator of this device. A body composition measurement device (Fortex 6100/XL, China) was used for measuring subjects' body mass index and body fat percentage.

Diet Control

The subjects were asked in a briefing session to record their diet with placebo during the 72 hours before exercise and then they were asked to repeat the recorded diet with choline supplements. It must be noted that the subjects did not take any other nutrient after having placebos in the first exercise and supplements in the second, nor did they eat anything during performing the exercises.

Blood Sampling

In order to measure the level of plasma free fatty acids and beta-hydroxybutyrate, blood samples were taken from the subjects in both exercise groups (exercise with placebo and exercise with choline supplement) as pretest (exactly before exercise) and posttest (exactly after exercise). Thus, 5 ml of venous blood was taken from the right hand of the subjects in the seated position by a laboratory technician; then, the samples were poured into hemolysis tubes and were transported to the laboratory for separating the serum from the clot. In the laboratory, the hemolysis tubes were centrifuged at 400 rpm for 4 minutes and the formed serum was moved into two microtubes with a volume of 2 ml and were transferred to the temperature of 20 degrees centigrade to prevent it from the *freeze and thaw* process which will decrease the accuracy and validity of the analysis of samples. Colorimetric assay was used for measuring plasma free fatty acids and beta-hydroxybutyrate by means of Biovision kits (Biovision Research Products, USA). Measurements related to plasma free fatty acids (cat≠k612-100) were done at 570 nm wavelength and measurements of beta-hydroxybutyrate (cat≠k632-100) were done at 450 nm wavelength.

Statistical Analysis

Descriptive statistics were used for summarizing the collected data and gaining a better understanding of the population. Further, repeated analysis of variance was applied in order to examine the existence of a significant relationship between the means of the groups under study for each of the variables. In case there was a significant relationship between the groups ($\mathbf{P \leq 0.05}$), LSD post hoc test would be used for testing research hypotheses. Moreover, $\mathbf{P \leq 0.05}$ was set for rejecting or accepting the hypotheses and all the statistical calculations were done using SPSS 18 software.

RESULTS

To study the effect of choline supplementation on the level of plasma free fatty acids and beta-hydroxybutyrate during a prolonged exercise session, the level of these two indices were measured at the beginning and the end of the two separate exercises with placebos and choline

supplements. The level of plasma free fatty acids and beta-hydroxybutyrate of the subjects under different research conditions are presented in table 2 and diagrams 1 and 2.Comparing the level of plasma free fatty acids in the exercise with placebo at the pretest and posttest revealed that plasma free fatty acids increased significantly after 120 minutes of exercise($\mathbf{P \leq 0.05}$). However, in the exercise with choline supplement, the level of plasma free fatty acids did not show any significant difference between the pretest and posttest ($\mathbf{P \leq 0.05}$; table 2 and diagram 1). Comparing the level of plasma free fatty acids of the posttest of the two groups revealed that the level of plasma free fatty acids was significantly lower in the exercise with choline supplement group ($\mathbf{P \leq 0.05}$; table 2 and diagram 1). In the exercise with placebo group and the exercise with choline supplement group, the level of plasma beta-hydroxybutyrate in the posttests did not show any significant difference as compared to the values from the pretests (table 2 and diagram 2). However, comparing the two groups it was observed that the level of plasma beta-hydroxybutyrate was significantly higher in the pretest and posttest of the exercise with choline supplement group as compared to the exercise with placebo group ($\mathbf{P \leq 0.05}$; table 2 and diagram 2).

Table 2 – Comparison of the level of plasma free fatty acids and beta-hydroxybutyrate of male elite triathletes under different conditions of prolonged exercise ($n = 9$)

Conditions Variables	Exercise with Placebo		Exercise with Choline Supplement					
	Pretest	Posttest	Pretest	Posttest	P1	P2	P3	P4
Plasma Free Fatty Acids (mmol/L)	0.877±0.530	5.43±2.69	2.38±2.99	2.77±2.34	0.001*	0.804	0.194	0.012*
Plasma Beta-Hydroxybutyrate	0.475±0.120	0.564±0.205	0.904±0.349	1.156±0.568	0.270	0.151	*0.028	*0.031

*Notes: P1 = comparing the pretest and posttest in exercise with placebo; P2 = comparing the pretest and posttest in exercise with choline supplement; P3 = Comparing the pretests of exercise with placebo and exercise with choline supplement; P4 = Comparing the posttests of exercise with placebo and exercise with choline supplement. * Significance at $P \leq 0.05$ level.*

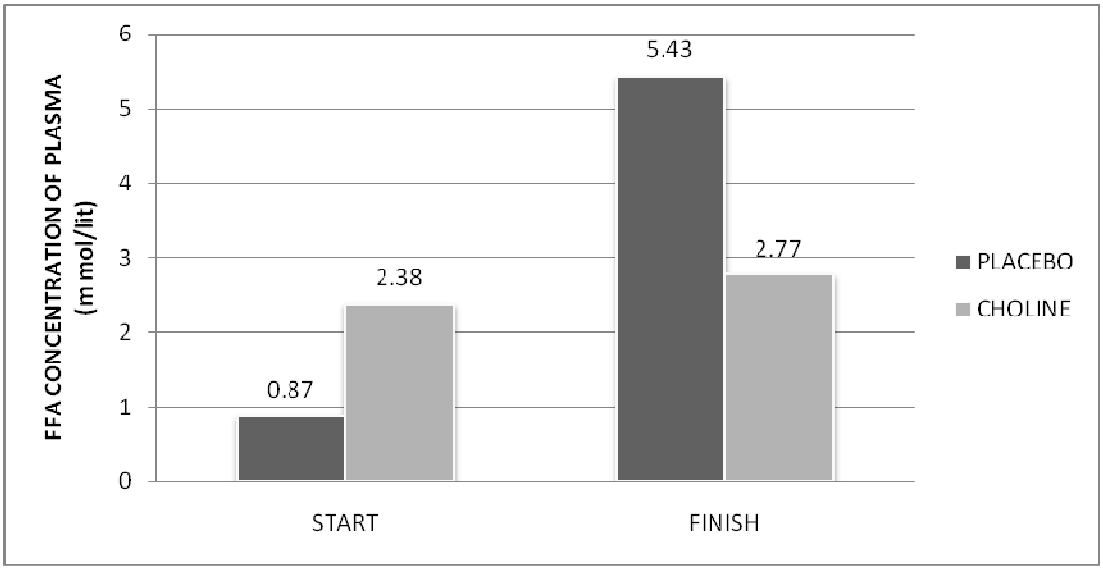

Diagram 1 - A comparison of the level of plasma free fatty acids of male elite triathletes under different conditions of prolonged exercise ($n = 9$)

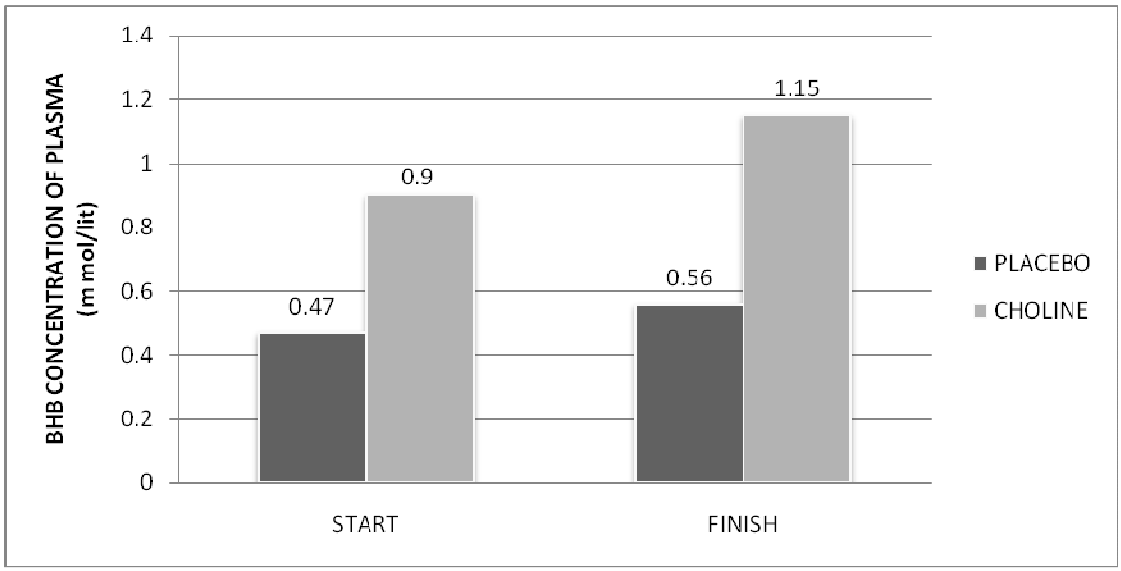

Diagram 2 - A comparison of the level of plasma beta-hydroxybutyrate of male elite triathletes under different conditions of prolonged exercise ($n = 9$)

DISCUSSION AND CONCLUSION

Choline supplementation at the beginning of a 120-minute exercise session with proper intensity for lipid oxidation which was performed by a group of elite triathletes led to a significant increase in plasma free fatty acids at the end of the exercise session (Table 2 and Diagram 1). The increase in plasma free fatty acid was reported by Wolfe et al. (1990) at the end of 30 minutes of running exercise with moderateintensity [15], by Romijn et al. (1993) at the end of 120-minute exercises with 25% and 65% VO_2 max on a cycle ergometer [16],by Achten et al. (2002) at the end of running exercise with an intensity of 45% VO_2 max[17], and by Mourtzakis et al. (2006) at the end of a three-hour run with an intensity of 44% VO_2 max [18]. These findings are consistent with the results of the present research. On the other hand, this finding is inconsistent with another finding by Romijn and colleagues who reported an increase in availability of free fatty acids in low-to-moderate intensities and its decrease in high intensities (85% VO_2 max) [16]. Fatty acids are the primary energy sources during resting and performing sports exercises with low-to-medium intensity and one of the most important sources of fatty acids utilized during sports exercises are plasma free fatty acids. The main cause of fat oxidation during sports exercises with medium intensity is the increase in availability of plasma free fatty acids and this increase is the result of increased lipolysis in adipose tissue, decreased saturation of fatty acids, and increased blood flow to adipose tissue [4]. It has been reported that active skeletal muscles consume 80-90% of fatty acids taken up from the blood [19]. Thus, the significant increase in plasma free fatty acids at the end of exercise with placebo which was designed for ideal lipid oxidation indicates the increase in lipid oxidation in this exercise.

But the level of plasma free fatty acids did not show any significant increase in the posttest of exercise with choline supplement in comparison with the pretest (Table 2 and Diagram 1). No study has been carried out on the effect of choline supplementation on plasma free fatty acids

during a session of sports exercise, but Sachan and Nobuko (2000) showed that the level of plasma free fatty acids decreased significantly in rats after a four-week period of taking a combination of choline, caffeine, and carnitine and they associated this issue with the positive effect of increased plasma choline on permeability of the membrane of skeletal muscles in comparison to fatty acids and as a result, there will be greater uptake of these substances by muscle cells [14]. Similarly, in exercise with choline supplement in the present research, considering the higher concentration of plasma free fatty acids in the pretest of this exercise in comparison with other exercises (table 2 and diagram 1), the increase in plasma choline (not mentioned here) will probably increase the permeability of plasma membrane of muscles cells that are involved in the exercise to plasma free fatty acids and as a result it will increase the level of uptake of free fatty acids by muscle cells and consequently will prevent the increase of plasma concentration in the posttest exercise in comparison with the pretest. Comparing the two exercises, the level of plasma free fatty acids in the pretest of exercise with choline supplement was significantly higher than the exercise with placebo (table 2 and diagram 1). Hence, it appears that choline supplement has increased the availability of free fatty acids in the beginning of exercise. The possible mechanism for this effect of choline can be attributed to its lipotropic quality which increases the release of lipids from the liver, the mobility of free fatty acids, and their transfer to energy generation parts [11]. However, the level of plasma free fatty acids at the end of exercise with choline supplement was significantly lower than that of the exercise with placebo and as was mentioned earlier, this issue can be attributed to the effect of choline on the permeability of muscle cell membranes to free fatty acids and their greater absorption and consequently to the increase in their oxidation capacity. The significant increase in the level of plasma beta-hydroxybutyratein the posttest of this exercise can confirm this issue. The level of plasma beta-hydroxybutyrate in the posttest of the exercise with placebo only insignificantly increased in comparison with the pretest of this exercise (table 2 and diagram 2). This finding is inconsistent with the findings of Bordin et al. (1992) and Carlson et al. (1971) [20 & 21]. In the research carried out by Bordin et al. (2003), 90 minutes of treadmill exercise with an intensity of 50-60% VO_2 max led to a significant increase in the level of plasma beta-hydroxybutyrate in non-athlete subjects [20]. The difference in the fitness of subjects may be the reason for the inconsistency between the results of the present research and the research of Bordin. In the exercise with choline supplement also the level of plasma beta-hydroxybutyrate insignificantly increased in the posttest in comparison with the pretest of this exercise (table 2 and diagram 2). But there has been no research regarding the effect of choline supplementation before performing a session of physical exercise on the level of plasma beta-hydroxybutyrate. Yet, considering the research studies carried out by Sachan and Nobuko (2003) on the effect of long-term choline supplementation on the increase of plasma beta-hydroxybutyratein female non-athletes [13], this issue was expected to be realized. Comparing the two exercises, it was observed that the values of plasma beta-hydroxybutyrate in the pretest and the posttest of the exercise with choline supplement were significantly higher than those of the activity with placebo (table 2 and diagram 2). As we mentioned, there has previously been no research on the effect of choline supplementation on plasma beta-hydroxybutyrate changes during a session of physical exercise. Nonetheless, Sachan and Nobuko (2003) confirmed the effect of a 5-week period of taking a combination of choline, caffeine, and carnitine on the level of plasma beta-hydroxybutyrate in female non-athletes and considered the increase in its plasma concentration as an indication of the increase in the process of incomplete fat oxidation in the body and the transformation of acetyl coenzyme A produced by beta-oxidation of fatty acids to acetoacetate

and finally to beta-hydroxybutyrate [13]. Since in case of a decrease in the carbohydrates of the liver and muscles, as in endurance sports exercises, ketone bodies are a suitable energy source for tissues such as skeletal muscles, the brain, heart muscle, and kidneys, they can thus help maintaining blood glucose [2]. Hence, the high level of plasma beta-hydroxybutyrate at the end of exercise with choline supplement in comparison with the other exercise can indicate an increase in fat oxidation and a decrease in the consumption of blood sugar and maintaining body carbohydrate sources.

Considering the results of the present research we can conclude that choline supplementation before a prolonged exercise will increase fat oxidation capacity through increasing the availability and uptake of plasma free fatty acids; the increase in fat oxidation can be observed in this exercise as the increase in the level of plasma beta-hydroxybutyrate. Thus, we can say that choline can save carbohydrate sources in the body during performing prolonged exercises by increasing fat oxidation.

REFERENCES

[1]. Azad A, Qarakhanloo R, & Gaeeni AA, *Journal of Research in Sports Sciences*, summer of **2005**, 7.
[2]. Maughan R, Gleeson M, &Greenhaff PL, Oxford Medical Publications, **1997**.
[3]. Horowitz FJ & Klein S, *American Journal of Clinical Nutrition*, **2000**, Vol. 72, 2, 558-563.
[4]. Juul A, Asker E, *J Nutrition*, **2004**, 20, 7-8, 716-727.
[5]. Zeisel SH, *Ann Rev Nutr* **1994**, 14, 269-271.
[6]. Yao ZM, Vance DE, *J of Biol Chem*, **1988**, 264, 11373–11380.
[7]. Von A, Horn S, Kahl J, Feldheim W, *Eur J Appl Physiol*, **1993,**67,1, 87-91.
[8]. Von AHN, Horn S, Feldheim W, Champaign, AOCS Press, **1995**, 319-325.
[9]. Conlay LA, Sabounjian LA, Wurtman RJ , *Int J Sports Med*, **1992,**13, 141-142.
[10]. Buchman AL, Jenden D, Roch M , *J Am Coll Nutr*, **2000,**18, 6, 598-601.
[11]. Best Ch, Huntsman M E, *J physiol*, **1932,**75, 405-412.
[12]. Daily James W Hi & Sachan S Dileep, **1995,** *J. Mutr*. 125.
[13]. Nobuko H, Sachan S, *J, Nutr*, **2003,** 133, 84– 89.
[14]. Nobuko H, Sachan S, *J, Nutr*, **2000,** 130, 152–157.
[15]. Wolfe RR, klein S, Carraro FW, *J Am J Physical* **1990,** 258- 382.
[16]. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz J, Endert E, *AJP-Endocrinology and Metabolism*, **1993,** 265, 380-391.
[17]. Achten J, Gleeson M, Jeukendrup AE, *Med Sci Sports Exerc*, **2002,** 34- 92.
[18]. Mourtzakis M, Saltin B, Graham T, Pilegaard H, *J Appl Physiol*, **2006,**100, 1822-1830.
[19]. Coyle F Edward, *Am J Clin Nutr*, **2000,** 72, 512–520.
[20]. Bordin D, Bottecchia D, Bettini V, Aragno R, Sartorelli L, *J Sports Med Phys Fitness. Dec*, **1992,** 32, 4, 394-399.
[21]. Carlson A, Ekelund L, Froberg SO, *Europ J Clin Invent*, **1971,** 1:248-255.