



## **The effect of six weeks of high-intensity interval training with and without zinc supplementation on aerobic power and anaerobic power in female futsal players**

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### **ABSTRACT:**

**Purpose:** Different training methods and various supplementations are usually used by the athletes to improve their exercise performance. The aim of this study was to investigate the effect of six weeks of HIT with and without zinc supplementation on aerobic and anaerobic power in female futsal players. **Methods:** Thirty-two female futsal players were randomly divided into 4 groups as placebo, Zinc, HIT and Zinc+HIT. All subjects had to attend futsal-specific training 3 sessions per week. Zinc groups and Placebo group took 30 mg day<sup>-1</sup> of zinc gluconate or dextrose, respectively, and HIT groups performed 6-10 repetitions of a 30-second running at 100% of VO<sub>2peak</sub> with 4 minutes rest between repeats during six weeks. **Results:** decreased VO<sub>2max</sub> in placebo group was not significant (P=0.54), however VO<sub>2max</sub> increased 4.43%, 3.69 % and 9.33% in Zinc, HIT and Zinc+HIT groups, respectively. MPO also augmented 7.84%, 8.78%, 30.85% as well as 11.92% in Placebo, Zinc, HIT and Zinc+HIT groups, respectively. **Conclusions:** The findings suggest that increased number of futsal training sessions per week, solely ameliorated MPO value; while, Zinc, HIT and HIT along with Zinc supplementation increased both aerobic and anaerobic power in female futsal players after six weeks. It seems that zinc improved the exercise performance because of its utile effect on protein synthesis, muscle tissue development and enzymes activities. Consequently, both of HIT and Zinc supplementation were confirmed as the effective ways to improve exercise performance.

**Keywords: HIT, Zinc supplementation, VO<sub>2max</sub>, MPO, Female Futsal Players**



## INTRODUCTION

Futsal is a kind of intermittent sport which contains high tactical, technical and physical requirements. In futsal, the sprint and explosive actions beside maintaining such abilities for a longer time could determine the winner in a futsal competition; therefore, in addition to athletic skills, physical fitness plays a crucial role in sports events, especially futsal ones. Overall, an increase in the aerobic power and anaerobic power sounds a must to futsal players. Such abilities delay the onset of the fatigue during the game and enable the player to perform not only a high intensity activity but also with more frequencies [1]. These days, a kind of training called high-intensity interval training (HIT) has been regarded by the athletes and coaches in order to improve exercise performance. HIT is usually attributed to repeated sessions of intermittent exercise which frequently done with an all-out effort or at an intensity close to  $VO_{2peak}$ . A single effort maybe last from a few seconds to several minutes and multiple efforts are detached by a few minutes of rest or low-intensity exercise from each other [2]. The researchers suggest that factors like an increase in resting muscle glycogen content [3], neural adaptations [4] and improved activities of oxidative and glycolytic enzymes [5] and so on after HIT, ameliorate the exercise capacity; however, results of various investigations on the impact of such a training on exercise performance seems inconsistent to some extent [6,7].

Beside different training methods to improve the performance, the use of supplements is also common [8, 9]; so, checking the effects of supplements as their benefits and possible side effects sounds very important. One of these supplements is zinc; this mineral micronutrient is involved in the growth and development of the tissues, especially the muscle tissue. Zinc also is an essential part of enzymes engaged in

metabolic pathways. It has been identified more than 300 enzymes and 1,000 transcription factors requiring zinc for their activity [10-12]. Zinc reserves are limited in body. It is found more in the bones (29 %) and skeletal muscles (approximately 57%). The recommended daily allowance of zinc is 15 mg for men and 12 mg for women [11, 13]. The beneficial effects of zinc supplementation on a variety of diseases such as diarrhea of children, chronic hepatitis C, diabetes, and so on have been reported in different studies [14-16], the effects of zinc deficiency have also been probed by researchers [17, 18]; but, our data on the effect of zinc supplementation on exercise performance are truly limited. It is represented that 8 weeks of resistance training with zinc supplementation significantly elevated the strength of back muscles as well as hand flexor muscles in healthy and active women [19]. Lukaski et al. (2005) discovered that people with low zinc intake had lower  $VO_{2peak}$  and higher ventilatory equivalent than their peers with supplemental zinc intake during prolonged and submaximal exercise.

Next, it was found no research on the impact of zinc supplementation along with HIT on aerobic and anaerobic power; thus, the aim of this study was to investigate the effect of six weeks of HIT with and without zinc supplementation on aerobic power and anaerobic power in female futsal players.

## METHODS

This semi-experimental study is one of applied researches containing pre-test and post-test phases, with 4 groups. It was organized as single-blind trial. After declaration of the study subject throughout female futsal clubs in Mashhad city, 50 players intended to taking part in the study. Then, 32 female futsal players (mean age  $23.31 \pm 3.89$  years, height  $162.25 \pm 5.85$  cm, weight  $55.21 \pm 6.29$  kg, BMI  $20.93 \pm 1.74$  kg/m<sup>2</sup>) whom were chosen on the study criterion (having at

least 4 years of sports backgrounds, the similar readiness,  $18 < \text{age} < 28$ ,  $18.5 < \text{BMI} < 23$ , being healthy without supplementation use) attended this study, voluntarily. Also, during two months before intervention, all subjects had participated in futsal team trainings on a regular basis of 2 sessions, per week. Our study was approved by the ethics committee of Ferdowsi University of Mashhad by 61681 code. First, the nature of the study was announced in all female futsal clubs around Mashhad city by researcher, then personal and contact information of futsal players who were interested in the cooperation was gathered; a total of Thirty-two individuals were purposefully selected from 50 volunteers. In a meeting, the objectives, the research process and researcher's expectations from the subjects were thoroughly described; next, a consent form (approved by sports physiology department), a demographic questionnaire, medical questionnaire and Kaiser Physical Activity questionnaire were completed by participants. The subjects' height and weight were measured by Seca 220 Stadiometer (Germany) with a sensitivity of 5 mm and 100 g, respectively and their BMI was evaluated by body composition analyzer, Inbody 720 (South Korea). In the next step, the subjects performed 2 trials over consecutive days. 30-second Wingate test was exerted using Monark cycle ergometer, Model 894-E (Sweden) for estimating MPO (anaerobic power); and also Bruce test was carried out using Technogym treadmill (Italy) for assessing  $\text{VO}_{2\text{max}}$  (aerobic power). In Wingate test, bike seat was adjusted to the right height for everyone and the required load of the test was considered according to the body weight (7.5%) for each subject. Totally, the subjects start pedaling after warming-up, at the moment they reach the maximum speed, the required load was immediately applied by researcher; and then, subjects maintain this all-out effort for 30 seconds. Afterwards, they

encountered the Bruce test consisting of seven stages in which the speed and the slope is gradually increasing every 3 minutes. It begins with speed 1.7 mph and inclines at 10%. Finally, the exercise will be stopped when our participants feel extremely exhausted and are not able to continue their efforts. The subjects were randomly divided into 4 groups as placebo (n=8), Zinc (n=8), HIT (n=8) and Zinc+HIT (n=8). Ultimately, after 6 weeks of intervention, Wingate and Bruce tests were conducted again. Our survey began in January and terminated in February 2015 for 6 weeks in Mashhad (Iran). The subjects were supposed to attend futsal league competitions two months later; in this way, the start of our project and their preparation phase, were at the same time. Moreover, the subjects were eliminated from our survey, if they were absent from training more than 2 sessions.

#### Training protocol

Beginning the study, all participants had to attend futsal-specific training 3 sessions per week; HIT and HIT+Zinc groups had to perform HIT protocol as below (Fig 1), in addition to futsal training in every session. HIT was done after a warm-up and before futsal-specific training. HIT contained 6-10 repetitions of a 30-second running at 100% of  $\text{VO}_{2\text{peak}}$  with a 4 minutes rest between repeats [20].

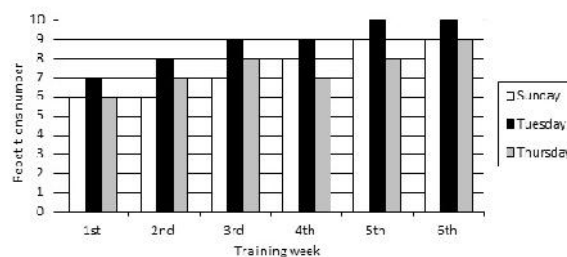


Fig 1. High-intensity Interval Training Protocol

#### Supplement protocol

The subjects in Zinc and Zinc+HIT groups took Zinc (30 mg zinc gluconate [21] distributed by Nature Made

Nutritional Products made in U.S.A as well as the subjects in placebo group took dextrose [22] 2 hours after dinner every day. It should be noted that the subjects had no history of using supplements at least two months prior to the project; they were also asked to avoid taking any other supplement during the study period.

### STATISTICAL RESULTS

Data were lastly analyzed using SPSS version 16. The mean and SD of data were calculated using Descriptive Statistics. Data distribution normality and homogeneity of variance were respectively examined with Shapiro-Wilk and Levene's test. The paired-sample t-test and Tukey's post hoc analysis were applied to compare the differences within groups and between ones, respectively. More, Wilcoxon and Mann-Whitney U tests were respectively used for comparing the differences within groups and between ones in variable with distribution other than normal. Statistical significance was considered at P values 0.05. Whole values were declared as mean±SD. After 6 weeks, MPO augmented from 4.08 to 4.40 (P=0.049) in

placebo group, whilst  $VO_{2max}$  decreased insignificantly. In other group, we witness significant increase in  $VO_{2max}$  from  $39.5\pm3.33$  to  $41.25\pm3.61$  (P=0.009) and MPO from 4.44 to 4.83 (P=0.012) during 6 weeks of zinc supplementation. More, 6 weeks of HIT significantly elevated  $VO_{2max}$ ,  $37.37\pm3.11$  vs  $38.75\pm3.88$  (P=0.028) and MPO, 3.76 vs 4.92 (P=0.012). Eventually, 6 weeks of HIT along with zinc supplementation made a significant increase in  $VO_{2max}$  and MPO from  $38.87\pm4.54$  to  $42.5\pm3.62$  (P=0.002) and from 4.36 to 4.88 (P=0.012), respectively, in female futsal players (Fig 2(a) & 2(b)). We considered significant differences within groups in  $VO_{2max}$  and MPO variables, too (Table 1 & 3). Post-hoc Tukey' test determined that the differences of  $VO_{2max}$  were only statistically significant between Zinc+HIT and placebo groups. It was inferred from the results of Mann-Whitney U test that there was a significant difference between Zinc and HIT groups, and also placebo and HIT groups in MPO variable (Table 2 & 4).

**Table 1**

Differences within groups of  $VO_{2max}$  before and after the interventions. \*P≤0.05 vs before

Variable	Groups	Phases		Differences			
		Pre-test	Post-test	Within Groups		Between Groups	
		Mean ± SD	Mean ± SD	t	Significance Level	F	Significance Level
$VO_{2max}$ ( $ml.kg^{-1}.min^{-1}$ )	Placebo	39.5±3.71	39.12±4.88	0.629	0.549	7.817	*0.001
	Zinc	39.5±3.33	41.25±3.61	-3.564	*0.009		
	HIT	37.37±3.11	38.75±3.88	-2.762	*0.028		
	HIT+Zinc	38.87±4.54	42.5±3.62	-4.963	*0.002		

**Table 2**

Results of Post-hoc Tukey' test on differences between groups of  $VO_{2max}$  before and after the interventions, \*\* $P \leq 0.05$  vs before

	Groups	Mean Difference	Significance Level
<b>HIT+Zinc</b>	Placebo	4	**0.000
	Zinc	1.875	0.191
	HIT	2.25	0.068
<b>Zinc</b>	HIT	0.375	1
<b>Placebo</b>	HIT	-1.750	0.264
<b>Placebo</b>	Zinc	-2.125	0.097

**Table 3**

Differences within groups of MPO before and after the interventions. \* $P \leq 0.05$  vs before

Variable	Groups	Mean Rank	Sum of Ranks	Difference Within Groups		Difference Between Groups	
				Z	Significance Level	K <sup>2</sup>	Significance Level
<b>MPO (w)</b>	Placebo	2	4	-1.960	*0.049	9.058	*0.029
		5.33	32				
	Zinc	0	0	-2.521	*0.012		
		4.5	36				
	HIT	0	0	-2.521	*0.012		
		4.5	36				
	HIT+Zinc	0	0	-2.524	*0.012		
		4.5	36				

**Table 4**

Results of Mann-Whitney U test on differences between groups of MPO before and after the interventions, \*\* $P \leq 0.05$  vs before

	Groups	U	Significance level
<b>HIT+Zinc</b>	Placebo	21	0.247
	Zinc	23	0.344
	HIT	15	0.074
<b>Zinc</b>	HIT	8.5	**0.014
<b>Placebo</b>	HIT	8.5	**0.014
<b>Placebo</b>	Zinc	30	0.833

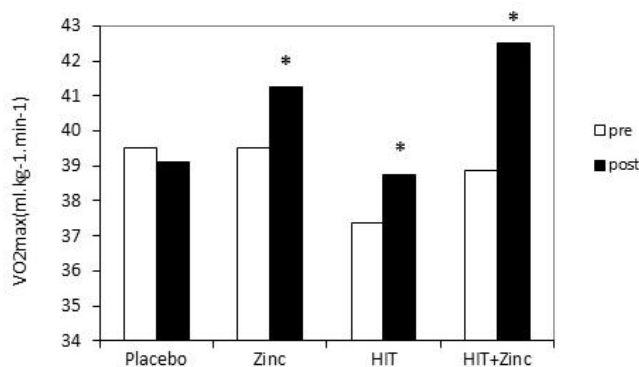


Fig 2(a). VO<sub>2</sub>max levels before and after the interventions

## DISCUSSION

This study revealed that 6 weeks of futsal-specific training solely ameliorated MPO in female futsal players taking dextrose as placebo. Furthermore, Zinc supplementation, HIT and HIT along with Zinc supplementation increased VO<sub>2</sub>max and MPO after 6 weeks, significantly; thus, these interventions resulted in a betterment of exercise performance in female futsal players.

First of all, we point to increased VO<sub>2</sub>max and MPO in HIT group, intending to elaborate possible mechanisms of this phenomenon. We also mark that HIT brought about the most change in MPO variable (30.85%) than Placebo (7.84%), Zinc (8.78%), and Zinc+HIT (11.92%).

Researchers studied the effects of 4 weeks of HIT with and without L-Arginine supplementation on exercise performance. They found out that 4 weeks of HIT resulted in a significant increase in VO<sub>2</sub>max and anaerobic power in female futsal players, more; higher VO<sub>2</sub>max and anaerobic power have been seen after HIT with L-Arginine supplementation [23, 24]. In another study, VO<sub>2</sub>max, absolute mean power and relative mean power values were significantly promoted in college-aged students after 4 weeks of HIT [25]. These

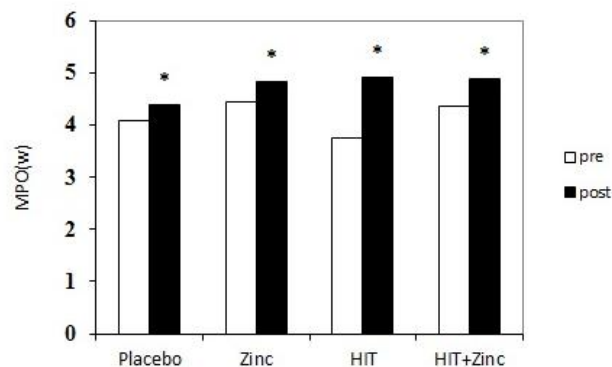


Fig 2(b). MPO levels before and after the interventions

results are consistent with the findings in our study.

Increased VO<sub>2</sub>max is likely to originate from an improvement in the oxygen transport throughout the vascular system, oxygen

delivery to the skeletal muscle and also oxygen consumption by active muscles because of increase in factors as the stroke volume, capillary density and mitochondrial content [26-28]. According to prior studies, changes in oxidative enzymes activity [5,29], skeletal muscle buffering capacity [30], mitochondrial biogenesis markers [31,32] and muscle glycogen content [29,30] associated with HIT implementation, are apparently discussed as the mechanisms responsible for an improvement in VO<sub>2</sub>max.

Beside, HIT-induced adaptations like an increase in resting muscle glycogen content [3], glycolytic enzymes activity [5, 33], FTA percent and the proportion of type II muscle fibers [33], 34 as well as neural adaptations (betterment of motor unit activation [4] and nerve conduction velocity [35]) can clearly justify elevated anaerobic power in our study.

In contrast, it was regarded that 2 weeks of HIT did not improve VO<sub>2</sub>max in active and young men. This different result probably comes from the short-term period of the training and few training sessions, because in most studies, HIT protocol lasting more

than 2 weeks, could make remarkable changes at last [20, 24].

Gharahdaghi et al. (2013) illustrated that 4 weeks of aerobic high-intensity interval training slightly altered  $VO_{2max}$  and time to exhaustion in professional soccer players that was not significant [36]. Allemeier et al. (1994) noted that high-intensity sprint cycle training for 6 weeks changed neither anaerobic nor aerobic performance [7]. These contradictions possibly rooted in the different training programs (various intensity, duration, volume and rest periods of the training, the number of training sessions per week and the kind of engaged muscles) and also diverse individual characteristics (age, gender and fitness level).

As previously mentioned, we have seen a significant increase in  $VO_{2max}$  and MPO variables after 6 weeks in Zinc group. Unfortunately, it was not found any investigation on the effect of zinc supplementation on aerobic and anaerobic power, though researchers cited that 8 weeks of resistance training with zinc supplementation could increase the strength of back muscles and hand flexor muscles [37]. Lukaski et al. (2005) mentioned that people suffering from very mild zinc deficiency had a lower  $VO_{2max}$  and higher ventilatory equivalent than those receiving enough zinc in their diet. It can be concluded that even mild deficiency of zinc has a negative effect on the exercise performance.

As before said, zinc is involved in the growth and development of tissues, especially muscles; it is also expressed that skeletal muscles are the largest reservoir of zinc, containing approximately 57% of all zinc throughout the body [11,13]. To justify the zinc impact on the growth and development of muscles, we can refer to the zinc-requiring metalloenzymes involved in the transcription and synthesis of protein

[38]. In addition, zinc plays an important role in maintaining the structure and stability of protein [18]. Also, it is an essential part of enzymes engaged with metabolic pathways like lactate dehydrogenase (glycolytic enzymes) and carbonic anhydrase [12]. It was perceived that after a short period of zinc supplementation, LDH activity increased in rats with zinc-deficiency [39]. Adequate amounts of zinc in active skeletal muscles and its manifest effect on LDH's function (the conversion of lactic acid to pyruvate) may lead to a reduction of muscle fatigue [22]. Plus, the carbonic anhydrases are other enzymes heavily dependent on zinc and play vital physiological role as efficient catalysts for the reversible hydration of carbon dioxide to bicarbonate<sup>40</sup> and a crucial role in acid/base equilibrium in the tissues, too [41]. They extensively affect the respiration and transport of  $CO_2$ /bicarbonate between the lungs and metabolizing tissues as well as pH and  $CO_2$  homeostasis [40]. According to the key role of zinc in the structure of the enzymes involved in the metabolism and also its effective role in protein synthesis and muscle tissue development, we can expect an increase in aerobic and anaerobic capacity after a period of zinc supplementation. It sounds that zinc supplementation may provide the active muscles with more access to zinc during exercise and boost zinc-requiring enzymes activity.

Our findings in HIT+Zinc group indicate a significant increase in  $VO_{2max}$  and MPO values; it was also determined that HIT along with Zinc supplementation made the most change in  $VO_{2max}$  (9.33%) than HIT solely (3.69%) and Zinc solely (4.43%). In this respect, no study was found probing the effect of HIT and zinc, simultaneously, on aerobic power and anaerobic power; whereas, we are able to justify and expect such changes after 6 weeks of HIT along with Zinc supplementation, regarding

HIT-induced adaptations and the benefits of zinc being reported before.

Results in Placebo group denoted a significant change in MPO and a slightly decrease in  $VO_{2max}$  which was insignificant. On the one hand, the subjects had to participate in futsal-specific training 3 sessions per week with the start of the study; while they used to have futsal training only 2 sessions a week. On the other hand, the start of our study was to coincide with the beginning of preparation phase before their matches which made the subjects take their trainings more seriously.

It should be noted that in futsal, anaerobic and oxidative energy provisions were reported 60% and 40%, respectively [1].

Moreover, its common work-to-rest ratio is 1:1 (moderate and high intensity activities to walking or jogging) ; also numerous player substitutions, extremely maintain the rhythm of the game in a high intensity. According to the above statements, the nature of futsal is regarded as an intense and sprint sport and therefore, elevated MPO due to increasing the number of training sessions is ultimately justified.

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