

Effect of High Intensity Interval Training on Muay Thai Athletes' Mineral Levels

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ABSTRACT There is an evident lack of research focusing on the levels of trace minerals experienced following combat sports and high intensity interval training (HIIT). As a result, the researchers investigated Muay Thai Athletes' (MTA) mineral levels by completing HIIT, and following the International Muay Thai Championship (IMTC). The study was carried out with 21 elite male and female MTAs, which were subjected to the HIIT program before the IMTC. There were some significant changes in the body weight values ($p < 0.01$), and the levels of trace elements of both male and female athletes ($p < 0.05$). For female subjects, erythrocyte (E) Zn levels significantly decreased post training compared to baseline ($p < 0.05$). After the IMTC, E-Cu and plasma (Pl) Zn levels increased ($p < 0.05$), and Pl-Mn levels significantly decreased ($p < 0.05$). For male subjects, E-Zn levels significantly decreased and Pl-Zn levels significantly increased post training compared to baseline ($p < 0.05$). After the IMTC, E-Zn, E-Fe, and Pl-Mn levels significantly decreased ($p < 0.05$), and E-Cu and Pl-Zn levels significantly increased ($p < 0.05$). However there was some decrease or increase in the values of E-Zn, E-Cu, Pl-Zn, Pl-Cu, E-Fe, and Pl-Mn minerals for female subjects, whereas there were no significant changes to the values of E-Cu, Pl-Cu, E-Fe, and Pl-Mn for male subjects post training or IMTC. These results suggest that HIIT and competition could impact the mineral levels of MTA.

INTRODUCTION

There are different forms of martial arts in the world, and each one is practiced with various intensities of physical activity (Hamasaki 2016). Muay Thai (MT) is also a martial art of Thai origin (Rossi 2011), and characterized as anaerobic, high intensity and intermittent in nature, which is similar to that of karate, taekwondo, boxing and wrestling. MT's primary energy systems rely mainly on the immediate and short-term glycolysis (McArdle et al. 1994). From a physical performances viewpoint, MT's sparring consists of very high intensive activity when the athlete is attacking or blocking. It also consists of low intensive activity when the athlete is just moving around, which allows the athlete some level of short-term recovery (Nunan 2006).

During training or in the competition, athletes are continuously exposed to various kinds of stress (Djordjevic et al. 2011) such as increases in heart rate, sweating, breathing, and other autonomic physiological responses as well as feelings of anxiety (Hodges 2015).

Compared to training, competition offers a more challenging environment that imposes a high exercise load on athletes while causing a

high level of psycho-biological stress (Chiodo et al. 2009), which may induce mineral loss (Fogelholm 1999; Jeukendrup and Gleeson 2004; Lukaski 2004; Williams 2005; Wang 2012), and increase the requirements of micronutrients (Fogelholm 1999; Maughan 1999; Cinar et al. 2009).

There are a number of studies that have reported contradictory data when examining the performance outcomes during training and competition to evaluate the effects of minerals, which are important for the physical performance of athletes (Fogelholm 1999; ACSM 2000; Jeukendrup and Gleeson 2004; Williams 2005).

In general, minerals like Cu, Zn, Mn, and Fe, which act as cofactors of antioxidant enzymes, are very important in an athlete's diet (Michalczyk et al. 2016), and essential for a wide variety of metabolic and physiologic processes in the human body (Kara 2012; Chaudhary and Sukhwat 2016) because of their important role in involving in muscle contraction, normal heart rhythm, nerve impulse conduction, oxygen transport, in energy production, synthesis of hemoglobin for the production of red blood cells, building and repair of muscle tissue, oxidative phosphorylation, enzyme activation, adequate immune functions, antioxidant activity, bone health,

and acid base balance of the blood, and the protection of body tissues from oxidative damage (Laxmeshwar and Amarnatha 2015; Chaudhary and Sukhwal 2016), and maintaining health (Chaudhary and Sukhwal 2016; Woolf et al. 2006).

Reasons for the aforementioned differences on the metabolism of trace elements is affected by many factors, including differences in mineral and exercise status of the athlete, type, intensity, duration and volume of exercise, stress, and extent of exercise-induced tissue trauma (Speich et al. 2001; Meludu et al. 2002). Moreover, MT has also weight categories, and most athletes take drastic measures (for example, restriction of fluid intake, sweating and starvation) in order to achieve target body weight (Hamasaki 2016).

However, interactions between high intensity exercise and trace minerals have been largely overlooked. Nevertheless, very little data exists in the literature on the effects of MT training and competition on body trace minerals, namely Zn, Cu, Fe, and Mn levels.

Zinc is an essential co-factor for numerous enzymes (Singh et al. 1989; Lukaski 2004; Wilborn et al. 2004; Margaritis and Rousseau 2008; Nahar et al. 2011; Zamboni et al. 2016), which are involved in functions that play an important role in metabolic processes that occur during physical performance (Gonzalez-Haro et al. 2011), such as protein synthesis and muscle energy metabolism while exercising (Singh et al. 1989; Maughan 1999; Eberle 2000; Manore and Thompson 2000; Peake et al. 2003; Wilborn et al. 2004; Williams 2005; Savas et al. 2006; Margaritis and Rousseau 2008; Marques et al. 2011).

Beside the important role of Zn in the antioxidant defense system, copper and iron are also the key elements for regulating both the hematologic and antioxidant systems of the body (Wang et al. 2012).

Copper is a critical nutrient involved in many aspects of energy metabolism and in other physiological processes that are vital during exercise (Maughan 1999; Manore and Thompson 2000). Copper can alter physical performance in athletes (Marques et al. 2011), and be used to protect ligaments and tendons (Singh et al. 1989; Speich et al. 2001).

Iron is essential for the maintenance of health, and is required for the formation of oxygen-carrying proteins, hemoglobin, myoglobin, and for enzymes involved in energy production (ACSM 2000).

Iron levels in blood contribute to the regulation of the cardiovascular system, immune defenses, and brain function (Zamboni et al. 2016).

Athletic performance may be adversely affected by low Fe levels, which impairs muscle function and limits work capacities (Grout et al. 2016).

Manganese is an essential micronutrient required for normal health, which acts on skeletal growth, synthesis of nucleic acids, hemoglobin, immune functions, and protein metabolism (Speich et al. 2001; Shan et al. 2016).

It plays an important role in metabolic processes of lipids and carbohydrates that occur during exercise (Speich et al. 2001; Gonzalez-Haro et al. 2011), and it is a key component of manganese superoxide dismutase (MnSOD), which plays a critical role in protecting mitochondria from elevated reactive oxygen species (ROS). Insufficient levels of dietary manganese could result in suboptimal levels of MnSOD activity, and glucose uptake and metabolism (Shan et al. 2016).

Moreover, physiological stress, including exercise may increase the need for MnSOD to protect against oxidative stress, which suggests that exercise may increase manganese loss. Thus, it is important for protection against oxidant damage induced by high-intensity training as well as to promote recovery from training (Nielsen 2006).

The purpose of the present study was to examine the trace mineral levels of MTA after completing (HIIT), and following (IMTC).

METHODOLOGY

Participants

In this study, 15 male and 6 female national MTAs were selected as athletic volunteers. The trace minerals (Zn, Cu, Fe, and Mn) and parameters of athletes were measured before their camp period, and used as control values for comparison.

Volunteers had regular exercising and training habits 3 times a week for 120 minutes at an elite level. Each athlete had at least 6 years of training experience. The exclusion criteria for the study were drug and medicine intake in case of illness and cigarette consumption. As a result none of the athletes had to be excluded from the study.

During camp, athletes underwent some of the hematological tests as per the normal camp procedure. Blood samples were taken as a rou-

tine control test of athletes. In addition to routine tests, blood samples were also used for the study parameter. The investigated period included the duration of 10 days of camp before the IMTC. Written informed consent was taken from the athletes.

Before the tests, the participants were given adequate information about the scope and aim of the study. All training sessions took place at the same time of day to control the circadian variation in performance. During the study, the volunteers stayed in the same hotel and they were all provided with the same diet without any other nutritional supplements. The volunteers showed a hundred percent compliance with the exercise-training program. The participants were instructed to refrain from eating or drinking immediately before the tests. They were also instructed not to participate in exercise 24 hours before each testing session. The protocol started one day before the beginning of the training period.

Experimental Protocol

The body mass was measured using calibrated digital scales and height was measured using a portable stadiometer. The age of athletes were recorded in years. Training intensity was determined by using the maximal heart rate method (Fox et al. 1988).

The present HIIT program was designed according to elite MT athletes' needs after taking into consideration "the prerequisites in performance". It is a form of interval training that prioritizes short and intense periods interspersed with short recovery periods (Motta et al. 2016). The method is applied to all athletes as previously described by Ugras (2013).

Sample Collection and Analysis

Blood samples were taken from the participant three times, the first one prior to training, the second one after the training camp and the third one after the IMTC.

The researchers also analyzed the trace mineral (Zn, Cu, Fe, and Mn) levels at three different times, that is, pre-camp, after camp and at the end of the IMTC. Blood samples were centrifuged at 4000 rpm for 10 minutes and plasma frac-

tion of blood was stored at -25°C until analysis. Plasma and erythrocytes samples were diluted with 0.2 percent nitric acid. The standard solutions of trace elements were prepared in 0.2 percent nitric acid at various concentrations. Determinations were performed on a Perkin Elmer AAnalyst 800 Atomic Absorption Spectrometer, a graphite tube atomizer with Zeeman Background Correction and a WinLab32-AA Furnace and Flame program (Shelton, CT 06484-4794 USA). Pyrolytically coated graphite tubes were used.

Statistical Analysis

All statistical results were calculated by the SPSS statistical package.

A paired t-test was used to determine the differences in minerals and fitness parameters between pre and post values. Data was expressed as mean values $\pm$ standard deviation (SD) and standard error (SE). Differences between before and after exercises were reported as mean difference ninety-five percent confidence intervals. Trace minerals' levels were also evaluated by a "repeated measures" t-test.

RESULTS

Physiological characteristics of the Muay Thai male and female athletes are presented in Tables 1 and 2. In male and female recipients, the MTA initial body weight values decreased ($p<0.01$) after the camp. Some of the mineral values of athletes have changed ($p<0.05$) after the camp and championship, and are presented in Tables 3 and 4.

For the female subjects, E-Zn levels significantly decreased post training compared to baseline ($p<0.05$). After the IMTC, E-Cu, and Pl-Zn level increased ($p<0.05$), and Pl-Mn levels significantly decreased ($p<0.05$).

For the male subjects, E-Zn levels significantly decreased and Pl-Zn levels significantly increased post training compared to baseline ($p<0.05$). After the IMTC, E-Zn, E-Fe, and Pl-Mn levels significantly decreased ($p<0.05$), E-Cu and Pl-Zn levels significantly increased ($p<0.05$).

However, there were some decreases and increases in the levels of E-Zn, E-Cu, Pl-Zn, Pl-Cu, E-Fe, and Pl-Mn minerals for females, yet no sig-

Table 1: Changes in selected physiological parameters of male athletes

<i>Selected physiological parameters N= 15</i>	<i>Before X1</i>	<i>After X2</i>	<i>Mean difference ± 95% CI</i>
Age (year)	20.9± 2.4		
Height (cm)	178.6± 6.9 (1.8)		
Body Weight (kg)	68.7±13.0 (3.3)	68.0±12.7 (3.2)****	0.67±0.5 (0.1)

Values are the mean ± SD (S.E), before the camp training, after the camp training (****p<0.01).

Table 2: Changes in selected physiological parameters of female athletes

<i>Selected physiological parameters N= 15</i>	<i>Before X1</i>	<i>After X2</i>	<i>Mean difference ± 95% CI</i>
Age (year)	18.8 ± 1.4		
Height (cm)	170± 1.7 (0.7)		
Body Weight (kg)	57.2± 7.5	56.8±7.5(3.0)****	0.41±0.0(0.0)

Values are the mean ± SD (S.E), before the camp training, after the camp training (****p<0.01)

Table 3: Mineral status of female athletes

	<i>E-Zn</i>	<i>E-Cu</i>	<i>Pl-Zn</i>	<i>Pl-Cu</i>	<i>E-Fe</i>	<i>Pl-Mn</i>
Pre-training	9.1± 1.3	0.50±0.0	0.5±0.0	0.96±0.2	728.0±55.2	12.1±0.7
Post-training	7.9 ± 1.4*	0.47±0.0	0.4±0.3	0.95±0.3	742.3±82.6	12.4±1.5
After the championship	8.3 ± 1.0	0.56±0.1 **	0.8±0.1*	0.95±0.2	699.3±37.7	7.2±1.0***

* p<0.05 when compared with pre-training, **p<0.05 when compared with post-training,

***p<0.05 when compared with after championship. The unit of results is µg/ml. n=15

Table 4: Mineral status of male athletes

	<i>E-Zn</i>	<i>E-Cu</i>	<i>Pl-Zn</i>	<i>Pl-Cu</i>	<i>E-Fe</i>	<i>Pl-Mn</i>
Pre-training	10.0±1.5	0.4±0.1	0.5±0.0	0.7±0.0	805.3± 72.3	12.4±0.5
Post-training	9.1±1.5 *	0.5±0.1	0.7±0.3*	0.7±0.1	779.2±107.4	12.9±1.4
After the championship	8.9±1.3 *	0.5±0.0 *	0.8±0.2*	0.9±0.3	732.6± 62.4*	8.0±1.8***

* p<0.05 when compared with pre-training, **p<0.05 when compared with post-training,

***p<0.05 when compared with after championship. The unit of results is µg/ml. n=15

nificant changes in the levels of E-Cu, Pl-Cu, E-Fe, and Pl-Mn observed in males post training or IMTC.

DISCUSSION

For MT, a comprehensive understanding of changes in physiological and metabolic properties in response to HIIT is critical for coaches to develop practical interventions, such as specific training programs, dietary plans, for preventing the rapid loss of training adaptations.

The research on physiological characteristics and mineral levels of MTA are also limited. In the present study, the researchers tried to investigate trace mineral levels of MTA after completing HIIT, and following IMTC.

There is relatively little data in the literature related to the effects of exercise on plasma or serum trace elements levels during and after exercise (Gonzalez-Haro et al. 2011).

Physical exercise causes some stress on the human systems (Yamaner et al. 2015). The effects of physical exercise on trace elements (Zn, Cu, Fe, and Mn) are often contradictory and incomplete.

Many of the trace elements play a part in the physiological events in the organism (Kara 2012), and play an important role in maintaining the health of the active individual. There are a number of ways that exercise is hypothesized to alter the need for minerals (Laxmeshwar and Amarnatha 2015). Therefore, it is very important to have a balanced nutrition to reduce fatigue and

prevent the occurrence of injuries, improving performance and recovery post-exercise (Zamboni et al. 2016). Thus, an essential trace element status is fundamental to athletics, particularly as physical exercise often creates additional needs, (Speich et al. 2001; Woolf and Manore 2006; Laxmeshwar 2015) depending on the nature, intensity and duration of exercise, training periods and pre-competition as well as nutritional habits, and current mineral levels (Speich et al. 2001). Moreover, exercise stresses many of the metabolic pathways in which these micronutrients are required, while exercise training may cause muscle biochemical adaptations that increase micronutrient needs (Laxmeshwar and Amarnatha 2015). Exercise may also increase the turnover and loss of micronutrients from the body, and the need for these micronutrients to repair and maintain the higher lean tissue mass of the active individual (Woolf and Manore 2006; Laxmeshwar 2015).

Previous studies reported that post-exercise decreases the levels of trace elements in the blood and tissues. This decrease may depend on many factors including the type and intensity of exercise, and health status of subjects. Physical exercise causes a redistribution of various trace elements between body reserves, blood and tissues, and the increased metabolism results in trace element deficiencies (Kara 2012).

A well-balanced diet full of natural antioxidants can minimize the level of oxidative stress produced during high volume and high intensity training (Michalczyk et al. 2016).

It is becoming increasingly clear that an adequate level of minerals is necessary for optimal health (ACSM 2000), and trace elements such as Cu, Mn, Fe, and Zn act as cofactors of antioxidant enzymes, are very important in an athlete's diet (Michalczyk et al. 2016), and are important in the regulation of many physiological processes, including those affecting physical performance (Baker 2011).

Despite abundant data on the influence of training on mineral status of athletes, there have been contradictory reports concerning the effects of different type of exercises. Some studies have demonstrated that brief, high intensity and prolonged endurance exercise increases in PI and serum Zn concentrations immediately after exercise (Marrella et al. 1993; Cordova and Navas 1998; Lukaski 2000; Peake et al. 2003; Savas 2007; Cinar et al. 2009; Baydil 2013; Gonzalez-Haro et

al. 2011), and others have found a decrease in PI-Zn (Couzy et al. 1990; Lukaski 1990; VanLoan et al. 1999; Wang et al. 2012).

Zinc is an essential trace element involved in a range of vital biochemical processes in human organism (Wilborn et al. 2004; Marques et al. 2011), and Zn status may influence exercise capacity (Singh et al. 1989). Zinc is an important to maintain energy metabolism and can alter physical performance in athletes (Marques et al. 2011).

As shown in Tables 3 and 4, researchers found that the athletes' PI-Zn levels increased ($p < 0.05$), after the IMTC for females, and for males both post training and after the IMTC ($p < 0.05$).

Possible explanation for the increased PI-Zn immediately after high-intensity exercise is release of Zn from the muscle due to catabolism (Berning and Steen 2006), hemo-concentration, and Zn mobilization as part of the acute stress response (Anderson et al. 1995).

However, researchers found that female athletes' PI-Zn levels decreased post training. A possible explanation for this includes the expansion of PI volume, increased Zn excretion, redistribution of Zn within the body stores, blood and tissues (Berning and Steen 2006), soft tissue trauma (Lukaski 2000, Gonzalez-Haro et al. 2011) and muscular fatigue (Manore and Thomson 2000), urinary, sweat losses and the development of hematuria. (Jeukendrup and Gleeson 2004; Gonzalez-Haro et al. 2011).

A number of studies have found that E-Zn levels decreased after exercise (Hoshi et al. 2002; Singh et al. 1989; Deuster et al. 1991; Kara 2012). Accordingly, the researchers found the athletes' E-Zn levels decreased post training and after the IMTC for both females and males ($p < 0.05$) (Tables 3 and 4).

Zn protected against the formation of free ROS by Cu-Zn superoxide dismutase. Therefore, in order to resist the increasing amount of free ROS in the period of HIIT of athletes, more Zn would be transported into cells to make up Cu-Zn superoxide dismutase (SOD) (Wang et al. 2012).

Copper is involved in many aspects of energy metabolism (Metin et al. 2003), and is a component of a great number of proteins and enzymes, which contribute to the maintenance of its homeostasis. Due to the changes in the ceruloplasmin and copper-binding enzymes, blood Cu concentrations in athletes can be different

(Resina et al. 1990). The effects of training on PI-Cu concentration are equivocal. Some studies have indicated that PI-Cu levels decreased after exercise (Ohno et al. 1990; Marella et al. 1993; Savas 2007; Bicer et al. 2011; Baydil 2013). However, others reported that these levels increased after exercise (Anderson et al. 1995; Savas 2007; Cinar et al. 2009; Pourvaghari and Shahsavari 2009; Wang et al. 2012).

Copper is important to maintain energy metabolism and can alter physical performance in athletes (Marques et al. 2011). Kara (2012) observed that Cu values of athletes after a 3-month training were significantly lower than those at the beginning of the football training program (Kara 2012). While Lukaski (1995) reported low level of Cu in football players appeared due to increased sweat and urine concentration, Margaritis and Rousseau (2008) reported that losses occurred independently from the training level.

In this study it was also found that the athletes' PI-Cu levels slightly decreased post training and after the IMTC for females, and increased post training and after the IMTC for males (Tables 3 and 4). This appeared to be the result of increased urine and sweat concentration as previously reported by Lukaski (1990). Moreover, the muscle cells need more Cu-Zn SOD, which was caused by the increased ROS so much Cu would be transported into cell with Cu-Zn SOD, which could reduce the increase of PI-Cu (Wang et al. 2012).

The study revealed that the athletes' E-Cu levels increased after the IMTC for females ($p < 0.05$), and in post training and after the IMTC for males ($p < 0.05$). The result may be related to the ceruloplasmin concentrations in plasma. Elevated ceruloplasmin may be related to its ferroxidase action and to endogenous response to an acute phase reaction (Wang et al. 2012).

Some studies have indicated that E-Cu levels increased after exercise (Deusaster 1991), while others reported no change in E-Cu contents after exercise (Deusaster 1991; Pourvaghari and Shahsavari 2009).

However, as shown in Tables 3 and 4, E-Cu levels were found to be lower post training for females. As the studies indicate, the decrease in E-Cu concentrations suggests a mobilization of copper in response to exercise (Wang et al. 2012).

Kara (2012) observed that Fe values of athletes after a 3-month training were not different

from those at the beginning of the football training program (Kara 2012).

Iron deficiency is commonly reported in athletes, undergoing especially in strength and weight class sports, which caloric restrictions are involved (Petrie et al. 2004), and can impair muscle function and limit work capacity (Williams 2005). Correspondingly, this study showed that the athletes' E-Fe levels decreased after IMTC for females ($p < 0.05$), and in the post training and after the IMTC for males ($p < 0.05$). Possible explanations for the decrement of E-Fe level with training, as reported (Nieman 1999; Williams 2005; Meyer et al. 2006), include excessive sweating, mechanical trauma, and impaired Fe absorption, and insufficient dietary intake of Fe. However, erythrocyte levels were found to be higher after training for females in Table 3 and Table 4. It appeared to be the result of hemo-concentration during exercise-induced increased rate of circulation as formerly reported by (Viro and Viro 2001).

Manganese plays key roles in metabolic reactions and is important in many physiological enzymatic processes (Ergen et al. 2013). Gonzalez-Hero (2011) observed that there were no significant changes in PI-Mn levels associated with the test from baseline to the end of the recovery period (Gonzalez-Haro et al. 2011). Kara (2012) also found that Mn levels did not differ from those before the study (Kara 2012).

However, very little has been reported about physiological manganese concentrations and changes in Mn levels during sports and physical activities (Ergen et al. 2013). Physiological stress may increase Mn loss (Nielsen 2006). The researchers found the female and male athletes' PI-Mn levels increased in post training while, their PI-Mn levels decreased after the IMTC ($p < 0.05$) (Tables 3 and 4). The losses of PI-Mn with training appeared to be the result of increased urine and sweat (Jeukendrup and Gleeson 2004). Possible explanations for this include redistribution of Mn within the body stores, blood and tissues. However, further studies are needed to define the mechanism underlying exercise-induced changes in PI-Mn concentration.

CONCLUSION

The results of the present study revealed that the HIIT program appeared to cause some changes ($p < 0.05$) on E and PI values of Zn, Cu, Fe and Mn minerals of male and female athletes follow-

ing HIIT training and competition. Reasons for the above mentioned changes in the reported effects of exercise on the metabolism of the trace elements are affected by many factors, including differences in mineral and exercise status of the athlete, type, intensity, duration and volume of exercise, and extent of exercise-induced trauma.

During the study, the researchers also found some changes ($p < 0.01$) on the physiological parameters because of some biological reasons linked with for high performance training.

RECOMMENDATIONS

Future research pursuits are required to define the mechanisms underlying the effects of exercise on mineral concentration.

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