Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2019

53

29-35

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Original Scientific Article
DOI: https://doi.org/10.35666/ghtbh.2019.53.05
UDC: 612.126:796.015

Changes in mineral content in trainees' blood and urine due to high-intensity training

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Article info
Received: 28/05/2019
Accepted: 07/10/2019

Keywords:
High-intensity training
Minerals
Blood
Urine
Inductively coupled plasma

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Abstract: High-intensity training is becoming more popular nowadays when people have less time to engage in prolonged physical activity. Expertly led high intensity training is a safe way to achieve desired fitness goals. The aim of the study was to check if there were significant changes in the concentrations of sodium, potassium, calcium, magnesium, zinc, iron and copper in the blood and urine of twelve trainees after a short but intense training. Blood and urine sampling was performed before and after high intensity training where bodyweight exercises and exercises with external load were used. Statistical analysis was performed using paired t-test (2-tailed) with $\alpha=0.05$ as statistical significance.

The results obtained showed that the measured mineral concentrations varied as a result of intense physical activity, but these variations were small and did not have a general trend of increase or decrease of analyzed mineral content. Based on these results, it can be concluded that, from the standpoint of the mineral concentrations loss, short high-intensity training is safe for the trainee's health.

INTRODUCTION

Inorganic compounds that are essential to the human body in terms of the metabolic support and function of various physiological processes required for life, growth and/or cellular functions are classified as minerals (Frausto da Silva and Williams, 2001; Lukaski, 2004). The insight into the relationship between health and mineral content, as a result of different types of training, has been intensively studied within the field of mineralomics (Yasuda, Yonashiro, Yoshida, et al., 2006; Uzun, 2013). Understanding of the mentioned interaction can be helpful in diagnosis and treatment of several illnesses caused by elevated or lowered mineral contents, by combining the mineralomic studies with traditional metabolomic studies (Coffey, Durkie, Hague, et al., 2013; Mainous, Wright, Hulihan, et al., 2014). The body's mineral demand is increased as a result of heavy exercise, regarding the minerals loss by kidneys or urine excretion (Keen, Gershwin, Lowney, et al., 1987; Chinevere, Kenefick, Cheuvront *et al.*, 2008). A significant reduction in tissue's fat from the abdomen area, as well as a significant increase in the aerobic capacity of obese young men, is gained by high intensive interval training (HIT), 3 times a week, during 20 minutes within 12 weeks, showing the effectiveness of short, intense training (Heydari, Freund and Boutcher, 2012). Additionally, in comparison to the control group of women who performed long-term training of constant moderate intensity, a significant reduction in subcutaneous fat tissue and insulin resistance of women who performed HIT, 3 times a week within 15 weeks (both tested groups), was obtained (Trapp, Chisholm, Freund, et al., 2008). This implied that a higher intensity of short-term aerobic training was a more effective alternative to the moderate-intensity training used so far to regulate pressure. Therefore, recently HIT has gained popularity as an effective method of improving anaerobic as well as aerobic fitness (Burgomaster, Hughes, Heigenhauser, *et al.*, 2005; Burgomaster, Heigenhauser and Gibala, 2006) in only a few sessions, being widely used not only by healthy trained individuals, but also populations of patients with different metabolic disorders (Gibala, Little, Macdonald, et al., 2012). A combination of high anaerobic demand, mainly in the first bouts, and an increasingly high aerobic contribution, as the high intensity bouts are repeated, resulted in the effectiveness of this type of training stems (Bogdanis, Nevill, Boobis, et al., 1996; Parolin, Chesley, Matsos, et al., 1999). HIT is becoming popular for athletes and other populations with only a few conflicting facts about oxidative stress after an acute session (Bloomer, Falvo, Fry, et al., 2006; Deminice, Trindade, Degiovanni, et al., 2010; Farney, McCarthy, Canale, et al., 2012) or short-term training (Hellsten, Apple and Sjödin, 1996; Fisher, Schwartz, Quindry, et al., 2011). In general, the implementation of high intensity short term interval training improves the antioxidant status of healthy individuals, which supports positive effects not only on physical conditioning, but on overall health (Bogdanis, Stavrinou, Fatouros, et al., 2013). It has been proven that, unlike long running, most respondents perceive moderate intensity interval running as a training that gives greater comfort (Bartlett, Close, MacLaren, et al., 2011). The assumption is that high-intensity training is a safe way to achieve fitness goals in the case of advanced trainees. Cardio training involves exercises that are performed by repeated repetition over a long time (10-40 minutes) involving large muscle groups that require oxygen supply. The aims of this study were to get to know the extent to which changes in mineral content in the blood and urine occur after a short, but intense physical activity and to investigate the above assumption that professionally managed high-intensity training is a safe way of achieving fitness goals. The chosen minerals as the focus of this study were sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) and zinc (Zn), while inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was used for their quantification. Concentrations of seven minerals in urine and blood of twelve trainees were measured before and after high-intensity training. Statistical analysis was performed using paired t-test (2-tailed, paired).

EXPERIMENTAL

Instrumentation

Quantification of minerals in blood and urine was performed in the Laboratory of Quality Control of Rijeka Refinery, INA Oil and Gas Company by Teledyne Leeman Labs Prodigy ICP-AES instrument (Plasma source: RF generator with >78% power consumption; Generator: 27.12 MHz, 1.4 kW; Sampler: glass concentric nebulizer and glass spray chamber; Rinsing time: 20 s; Plasma gas: Argon (20 L/min); Detection system: Charge injection device (CID) cooled to -41°C; Softver: Salsa). Wavelenghts (nm) of tested minerals were: 589.592 (Na); 766.491 (K); 422.673 (Ca); 279.078 (Mg); 213.856 (Zn); 259.940 (Fe) and 324.754 (Cu). Radial plasma observing mode was applied for Na and K analysis. Digestion was performed by microwave oven (High performance Microwave digestion unit, mls 1200 mega, Gmbh). Additionally, analytical balance (Sartorius, d=0.001 g), Vacuette 2 mL (HKO medical systems) and Vacuette Quickshield Complete Plus (HKO medical systems) were used for experimental work.

Chemicals

High purity standard solutions (1000 μg/mL in 4% HNO_3) of Na, K, Ca, Mg, Fe, Cu and Zn were supplied by SCP Science, EU. Nitric acid (65%) was purchased from Zorka Pharma-Kemija, Serbia, while hydrogen peroxide (30%) was purchased from Kemika, Croatia.

Participants

Trainees who had participated in this study were healthy and fit individuals (nine women and three men) performing HIT 45 minutes once a week for last few years. Two trainees were over 48 years old and others were between 25 and 35 years old.

After a thorough explanation of the testing, training protocol, possible risks and the right to terminate participation at will; written informed consent was obtained from each participant for the use of their blood and urine samples.

Testing and training

The entire duration of the HIT had lasted 45 minutes (10 min for warming up + 30 min for main part and 5 min of recovery). A total of three training sessions were conducted with a one-week break. The high-intensity training consisted of changing the intervals of high loads and intervals of rest.

The intensity in the training process involved a load that the trainee was assigned in the form of a force to overcome or in the form of the speed at which the movement is performed. High load intervals consisted of applying great force in the case of an exercise with an external load (weights and kettle bells) or in the pursuit of achieving the highest speed in the case of bodyweight exercises (various jumps, running and as many repetitions of each exercise). Circuit type of training was performed. Work time was 30 seconds and rest time was 1 minute, regardless whether the exercises were used with external load or bodyweight. Breaks included an active rest such as light running and performing less demanding exercises. Prior to the experimental part, the optimal load (weight) which will be used for performing a number of repetitions of complex exercises was defined for each of the trainees. In the case of bodyweight exercises, trainees maintained a high level of perceived effort during the duration of the work interval with the use of heart rate monitoring, because heart rate and intensity of work are closely related.

At high intensity intervals, the heart rate was up to 80% of the maximum heart rate ($HR_{80\%}$). The maximum heart rate (HR_{max}) is the number of heart beats per minute

equal to the number obtained when the age is taken from the number 220, according to the following equation:
$HR_{80\%}=HR_{max}$ x 0.8=(220-age) x 0.8

Blood and urine sampling

Sampling of blood and urine was performed before and after HIT to find out whether acute electrolyte losses are caused by physical effort. The sampling procedure followed the principles of the Helsinki Declaration. Volume of single blood sample was 15 to 20 cm^3. Blood samples (n=24) and urine samples (n=24) of twelve fitness-active trainees were analyzed within three days and total of 72 blood samples and 72 urine specimens were included in the study.

Microwave digestion of blood samples

Blood samples (mass between 2 and 3 g with ± 1 mg of precision) were weighted into a Teflon vessel and 4 mL of HNO_3 (65%) and 0.25 mL H_2O_2 (30%) were added to each vessel. The containers were placed in a carousel and firmly sealed in a microwave oven at the digestion program: 5 minutes at 300 W and 2 minutes at 600 W.
Blood samples of twelve trainees were numbered from 1 to 12, while A and B marks a blood sample before training (BT) or after training (AT).

Urine samples treatment

A 5 mL volume of each urine sample was taken into a 10 mL bottle supplemented with 5 mL of HNO_3 (10%).

RESULTS AND DISCUSSION

Results of mineral concentrations in the urine of trainees before and after HIT

The concentration of analyzed minerals in the urine of individual trainees before and after HIT significantly varied, which is most likely affected by the degree of hydration, fluid consumption during and after training, the amount of excreted sweat (which depends on numerous factors with large individual differences due to physiology, diet, etc.) and similar. The obtained mineral concentrations in the tested urine samples before and after HIT in this study are shown in Table 1 and ranged as follows: sodium from 350 ppm to 2692 ppm; potassium from 157 ppm to 2785 ppm; calcium from 46 ppm to 292 ppm; magnesium from 12 ppm to 71 ppm; while the concentrations of zinc, iron and copper for all trainees were below 1 ppm. Table 1 shows the increase of sodium concentration for trainees who had less than 1000 ppm of Na before the exercises (No. 1, 2, 7, 8 and 12 trainees), except for trainee No. 9, where an insignificant decrease of sodium concentration has been obtained. For other trainees (with Na_{BT} concentration>1000 ppm), a significant decrease was obtained in the urine.
The potassium concentration was ≤1000 ppm for nine of twelve trainees before training (Table 1). The lowest concentration (<200 ppm) was recorded for trainee No. 1. In the case of trainee No. 4, K_{BT} was significantly higher than in other trainees (above 2000 ppm). In general, after training, an increase of potassium concentration in the urine of all trainees has been obtained. Decrease of potassium concentration was observed only for trainees Nos. 5 and 6, while for No. 9 it remained at the same level.
The calcium concentration in all urine samples ranged from 50 to 300 ppm. Concentration of Ca_{AT} increased for five trainees, while for seven it decreased. From the above, in general it can be concluded that HIT does not affect calcium concentrations in the urine.
Magnesium and zinc concentrations increased in the case of eleven of a total of twelve trainees, as a result of HIT. The opposite change only happened with trainee No. 10, for both minerals, which cannot be explained by any significant reason.

Table 1. Concentration (ppm) of seven minerals in 12 urine samples of trainees before (BT) and after training (AT).

Mineral	**Na** (ppm)		**K** (ppm)		**Ca** (ppm)		**Mg** (ppm)		**Zn** (ppm)		**Fe** (ppm)		**Cu** (ppm)	
	BT	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**
1	491	579	157	263	55	67	12	24	0.21	0.25	0.09	0.15	0.07	0.08
2	550	974	1222	1800	81	90	15	25	0.20	0.29	0.09	0.11	0.02	0.03
3	1997	1694	683	884	125	113	25	35	0.16	0.24	0.07	0.10	0.02	0.04
4	2054	1699	2162	2785	292	273	58	61	0.48	0.63	0.06	0.12	0.03	0.06
5	2692	2158	1557	1455	232	191	52	55	0.24	0.30	0.06	0.15	0.06	0.04
6	1124	840	633	437	82	71	14	15	0.12	0.14	0.13	0.09	0.02	0.07
7	350	615	700	1500	49	53	13	23	0.15	0.53	0.10	0.13	0.01	0.03
8	621	743	673	1001	46	64	10	30	0.11	0.15	0.07	0.07	0.02	0.02
9	741	715	673	667	175	170	62	71	0.15	0.26	0.09	0.28	0.05	0.02
10	2296	1317	1000	1398	240	140	40	36	0.24	0.13	0.09	0.08	0.02	0.02
11	1555	1282	636	660	132	104	23	23	0.34	0.36	0.08	0.08	0.01	0.04
12	915	1439	1015	1800	104	130	35	50	0.16	0.35	0.07	0.17	0.01	0.02
$\bar{x}$	1282	1171	925	1220	134	122	29	37	0.21	0.30	0.08	0.13	0.03	0.04

Calcium concentrations decreased for seven trainees, while magnesium and zinc concentrations increased in the case of eleven trainees. The difference is especially evident for trainee No. 10, so it can be noted that HIT had a much more significant effect on trainee No. 10, compared to other trainees.

The concentrations of iron in urine were less than 0.15 ppm and very uniform for all trainees, which was not the case for other analyzed minerals. After HIT, there was an increase in for seven trainees. However, for two trainees the iron concentration remained the same, while it decreased for two trainees. Characteristically, after training, a decrease of Fe, Zn, Mg, Ca and Na concentrations in the urine sample of No. 10 trainee, was obtained. It is noticeable that Cu concentrations were expectedly low and had not reached 0.1 ppm. Only trainees Nos. 5 and 9 had a decrease, while others had an increase or the copper concentration remained the same as before training. In general, the highest measured concentrations in the urine were for sodium and potassium, with almost negligible concentrations of zinc, iron and copper. Therefore, it can be concluded that after training an increase of mineral concentrations in the urine was more often observed. Increased copper concentrations were observed for ten trainees. Furthermore, nine of twelve trainees had increased potassium and iron concentrations after training, while higher concentrations of sodium and calcium were observed for five trainees.

There was also an increase of all tested mineral concentrations in the urine of trainees Nos. 1, 2, 7 and 12, after training. For the trainee No. 8, the concentration of five minerals has also increased and the two other minerals remained at the same concentration level. It is interesting that all of these were females.

The training had a different effect on the change in mineral concentrations for trainee No. 10 compared to the others. There was a decrease in the concentration of up to five minerals after training, the concentration of copper remained at same level and the only obtained increase was for potassium concentration. This trainee was much older than others, except trainee No. 2.

Analyzing the urine sample results after training (Table 1), it can be concluded that there was a change in mineral concentrations, but without any established trend (increase or decrease in general); which indicates that training had no decisive influence to the excretion of minerals.

Results of mineral concentrations in the blood of trainees before and after HIT

Analyzing the results in Table 2, it is clear that training did not significantly affect the change of sodium concentration in the blood of the trainees. For nine of twelve trainees there was an increase of sodium concentration in blood, while for other three trainees a decrease was obtained. Therefore, it cannot be said that HIT in general decreased or increased the sodium concentration in the blood. The effect of HIT on the trend of a change in potassium concentration in the blood of the trainees was even less relevant because six trainees had an increase and other six had a decrease of potassium concentration (Table 2).

For eight trainees, there was an increase in calcium levels in the blood, while the remaining four trainees had Ca_{AT} lower than Ca_{BT}. Concentration decreases were insignificant (only a few ppm) while increases for trainees Nos. 6 and 10 were significant (55 and 75 ppm, respectively).

Unlike the increase of sodium, potassium and calcium concentrations, the concentration of magnesium, zinc and iron decreased for most of the trainees. Magnesium concentration decreased in case of eight of twelve trainees, while nine of twelve trainees had zinc and iron decreased.

Table 2. Concentration (ppm) of seven minerals in 12 blood samples of trainees before (BT) and after training (AT).

Mineral	**Na** (ppm)		**K** (ppm)		**Ca** (ppm)		**Mg** (ppm)		**Zn** (ppm)		**Fe** (ppm)		**Cu** (ppm)	
	BT	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**	**BT**	**AT**
1	1379	1451	1264	1444	249	231	10.50	10.60	2.04	2.33	275	336	0.82	0.80
2	1463	1467	1409	1414	248	245	10.55	9.42	2.56	2.48	297	302	0.76	0.81
3	1350	1435	1362	1403	207	231	10.45	9.67	2.56	2.14	313	261	0.78	0.79
4	1412	1286	1355	1285	234	235	10.85	13.20	2.53	2.80	281	335	0.79	0.83
5	1342	1292	1368	1309	206	213	11.30	10.70	2.76	2.68	353	339	0.71	0.78
6	1294	1419	1348	1361	212	265	11.75	8.94	2.20	1.64	378	320	0.89	0.97
7	1332	1373	1160	1314	248	269	8.85	8.96	1.58	1.55	184	176	0.95	0.99
8	1415	1296	1387	1155	252	244	9.60	9.21	1.84	1.79	232	202	0.88	0.92
9	1332	1399	1282	1269	235	255	9.35	9.59	1.82	2.02	204	200	0.87	0.95
10	1299	1393	1275	1208	215	289	10.15	7.99	1.90	1.52	292	240	0.61	0.63
11	1166	1258	1231	1129	235	228	8.00	7.01	1.88	1.66	186	183	0.60	0.62
12	1205	1248	1108	1121	264	289	5.84	6.11	0.86	0.84	84	53	0.84	0.85
x̄	1332	1359	1295	1284	233	249	9.77	9.28	2.04	1.95	256	245	0.79	0.83

Analyzing the data in Table 2, unlike for the previous six minerals, there was a major increase of Cu_{AT} concentration in the blood (in the case of eleven of twelve trainees).

Observing the individual HIT influence, some trends may be noticed for specific trainees. Therefore when certain mineral concentration AT decreased or increased in the case of trainee No. 7, the same happened with trainee No. 12. The same relations were observed in the case of eleven of twelve tested minerals for trainees Nos. 2 and 11, then for trainees Nos. 5 and 10, trainees Nos. 6 and 7 as well as for the trainees Nos. 6 and 12.

Another characteristic was noticed for trainee No. 8. After training, all mineral concentrations (except Cu) in the blood of this trainee have decreased. The highest measured concentrations in the blood were for sodium and potassium, while the copper concentration was the lowest one. Analyzing the results (Table 2), a similar conclusion as for the urine samples was obtained, meaning that there is no general trend of concentrations increasing or decreasing, which indicates that HIT had no key influence on contents of minerals in blood.

Statistic results for minerals in urine and blood

The influence of HIT on a significant change of the mineral concentration in urine and blood was estimated based on the paired t-test (2-tailed). The results of the t-test for urine and blood are given in Table 3.

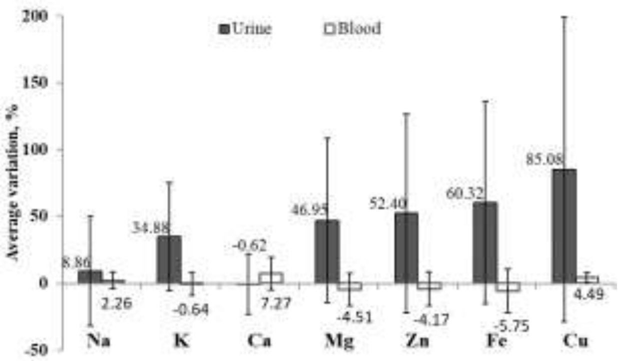

Figure 1. Average of variation percent in urine and blood.

As significance level, value α=0.05 was chosen. Therefore, only values with $p<0.05$ were considered statistically significant. Statistical data analysis obtained by determining contents of sodium, potassium, calcium, magnesium, zinc, iron and copper in the urine and blood of twelve trainees using the ICP-AES method showed that the HIT had a statistically significant effect on the change of potassium, magnesium, zinc and iron concentration in urine, as well as on the change of copper in the blood. However, due to the extremely low concentrations (<1 ppm) of zinc, iron and copper in urine, as well as for copper in blood, the statistical test cannot be considered relevant for changes in the concentration of these minerals in the given samples.

Figure 1 shows the average of variation percent of seven analyzed minerals in urine and blood after intensive training with a standard deviation.

CONCLUSIONS

The statistical data analysis obtained by determining the concentration of sodium, potassium, calcium, magnesium, zinc, iron and copper in the urine and blood of twelve trainees using the ICP-AES method showed that HIT had a statistically significant effect on the change of potassium, magnesium, zinc and iron concentration in urine, as well as on the change of copper in the blood. However, due to the extremely low concentrations (<1 ppm) of zinc, iron and copper in urine, as well as for copper in the blood, the statistical test cannot be considered relevant for changes in the concentration of these minerals in the named samples.

After the intense training, there was a change in the concentration of minerals in the urine and the blood, but the change did not have a general trend of increase or decrease of the analyzed contents of minerals. Therefore, it can be concluded that HIT does not have a key impact on the tested parameters.

In general, expertly led high-intensity training is a safe way to achieve fitness goals in the case of advanced athletes.

Table 3. Statistical values of analyzed minerals in urine and blood samples.

Mineral	Na	K	Ca	Mg	Zn	Fe	Cu
t test (urine)	0.389	0.013	0.237	0.003	0.024	0.031	0.115
t test (blood)	0.283	0.727	0.068	0.227	0.282	0.337	0.002

* the statistical analysis was performed using paired t-test (2-tailed) with α=0.05 as statistical significance

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Summary/Sažetak

Visoko intenzivni trening postaje sve popularniji u današnje vrijeme kada ljudi imaju sve manje vremena za bavljenje dugotrajnim fizičkim aktivnostima. Stručno vođen trening visokog intenziteta može biti siguran način postizanja fitnes ciljeva. Cilj istraživanja bio je da se provjeri dešavaju li se značajne promjene u koncentracijama natrija, kalija, kalcija, magnezija, cinka, željeza i bakra u krvi i urinu 12 vježbača poslije kratke, ali intenzivne fizičke aktivnosti. Uzorkovanja krvi i urina provođena su prije i poslije treninga visokog intenziteta u kojem su se koristile vježbe s vanjskim opterećenjem i vlastitom težinom tijela. Statistička analiza rezultata je napravljena korištenjem parnog t-testa (2-tailed) te je kao nivo značajnosti uzeta vrijednost $\alpha=0.05$. Rezultati su pokazali da se koncentracija mjerenih minerala mijenja usljed naporne fizičke aktivnosti, ali promjene su male i nemaju generalni trend povećanja ili smanjenja koncentracije analiziranih minerala. Na osnovu ovih rezultata može se zaključiti da je, sa stanovišta gubitka minerala, kratak trening visokog intenziteta siguran za zdravlje vježbača.