Annals of Agricultural and Environmental Medicine 2018, Vol 25, No 3, 500–503
www.aaem.pl

ORIGINAL ARTICLE

Sweat iron concentration during 4-week exercise training

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

Saran T, Zawadka M, Chmiel S, Mazur A. Sweat iron concentration during 4-week exercise training. Ann Agric Environ Med. 2018; 25(3): 500–503. doi: 10.26444/aaem/78787

Abstract

Introduction. One possible way of iron loss is sweating. It is unclear how physical activity performed by untrained individuals affects the iron status in sweat.

Objective. The purpose of this study was to analyse iron concentration in sweat during 4-week exercise training to determine the changes in iron excretion during follow-up exercises.

Materials and method. 43 untrained volunteers participated in the study, 29 of whom completed the full exercise programme. The training programme consisted of exercises on a cycle ergometer and cross-trainer. In the first week, participants exercised for 8 minutes on each device, in the second for 10 minutes, and in the third and fourth weeks they exercised for 15 min on each device. Intensity was submaximal and defined as 85% of maximal heart rate. A sterile sweat patch was placed on the skin between shoulder blades.

Results. Concentration of iron on the first and the fifteenth day of exercises was comparable and statistically insignificant. Iron concentration was highly increased on the last day of training in comparison with first ($p<0.001$) and fourteenth day ($p<0.006$). The median of iron concentration in 29 samples on the first day of sampling was 21.2 ppb, in the fifteenth – 52.5 ppb, and on the twenty-eighth day – 286.2 ppb. In relation with the sodium concentration, the iron content was also increased on the twenty-eighth day of the training programme ($p<0.005$).

Conclusions. Iron sweat loss significantly increased during the 4-week exercise programme. A possible explanation may be improvement in the thermoregulation mechanism and secretory activity of sweat glands. Iron sweat loss may be an indicator of iron deficiency observed in active individuals.

Key words

exercise, iron, sweat

INTRODUCTION

The prevalence of iron deficiency and anaemia is generally higher in athletes than in healthy, sedentary individuals, whereas athletes performing endurance sports were the group most affected. Losses of iron from mechanisms such as haemolysis, haematuria, sweating and gastrointestinal bleeding during training may negatively affect the iron status [1]. The well-known consequences of iron deficiency include, *inter alia*, a decline in haemoglobin concentration, decreased size and volume of new red cells, and reduced myoglobin [2].

Athletes trained for endurance may have low levels of blood haemoglobin and haematocrit, which supports the concept of sports anaemia and iron deficiency. However, Convertino et al. suggest that highly trained endurance athletes can have haematocrits of 40 – 42% without reduction in their circulating haemoglobin [3]. They often experience iron deficiency, which implies that there is no decrease in haemoglobin. It is still debatable whether athletes are at higher risk of low iron stores due to an imbalance between absorption and exercise-induced iron excretion. Iron loss is balanced by intestinal absorption [4]. However, supplements should only be used if tests of iron status indicate deficiency [5]. There is no evidence that iron supplementation increases athletic performance [6].

Sweating, which is mainly a mechanism of thermoregulation and thus essential during exercise, is also a mechanism by which the body may lose iron [7]. Iron levels in sweat are higher at the beginning of sweat production than at later phases of a long distance run. Sweat loss may result in daily iron losses of 1 – 2mg [8]. It is still not clear how the amount of sweat iron loss is important in total body iron loss [9]. Several studies [10–13] examined iron loss in sweat as a possible factor which may contribute to iron deficiency, but there are few data on iron loss among untrained subjects during a long-term exercise programme. Thus, the purpose of this study was to analyse iron concentration in sweat during 4-week exercise training to determine the changes in iron excretion during follow-up exercises.

MATERIALS AND METHOD

43 untrained (non-professional in sport) volunteer participant-patients of the Clinic of Rehabilitation took part in this study, of whom 29 completed the full exercise

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Received: 29.08.2017; accepted: 20.10.2017; first published: 15.11.2017

programme. Exclusion criteria were: inflammation or pain disability (acute or chronic), time after surgery, pregnancy, fever, infections, diabetes, endocrine diseases, cardiovascular disorders and autoimmune disorders, such as rheumatoid arthritis.

All subjects gave written informed consent prior to participation in the study. The study was approved by the Ethics Committee at the Institute of Rural Health (3/2015).

Data was collected from April – December 2016.

Training programme. The training programme consisted of exercises on a cycle ergometer and cross-trainer (Orbitrek), and continued daily for 4 weeks (excluding weekends). In the first week, participants exercised for 8 minutes on each device, in the second – 10 minutes, and in the third and fourth weeks they exercised for 15 min on each device. There were no time intervals between exercises on the ergometer and Orbitrek. Participants did not eat or drink liquids during the exercises. Exercises intensity was submaximal. Firstly, maximal HR (heart rate) was calculated for participants based on the formula 208–0.7x age in years [14]. Submaximal intensity was defined as 85% of maximal heart rate (HR), measured by handle sensors on the ergometer and Orbitrek. Exercises took place in an air-conditioned gym of the Institute of Rural Health in Lublin (constant temperature 24 °C). Training courses are presented in Figure 1.

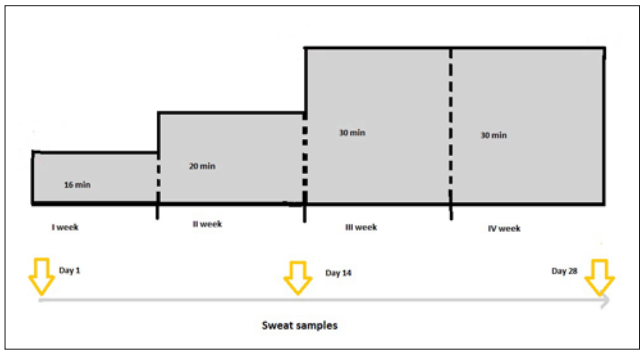

Figure 1. Training programme.

A sterile sweat patch (PharmChek®) was placed on the skin between the shoulder blades and kept in place throughout the training. Samples were collected 3 times: on the f1st day, the 14th day and the last day (28th day) of the training programme. At the end of the exercise session, the sweat patches were removed and placed in individual sealed plastic bags. Sweat samples were stored at -10 °C until laboratory analysis.

Laboratory analysis. The concentration of Fe (and Na) in the collected sweat was determined in samples diluted 50 times with deionized water (0.1 ml sweat sample + 4.9 ml of deionized water).

Determinations were performed using Thermo Scientific XSERIES2 inductively coupled plasma mass spectrometry (*ICP-MS)* with collision-reaction cell.

Parameters of the measurement system: Nebulizer – glass concentric; Spraychamber -glassconical impact bead; Interface option – Xt; Sample uptake rate – 0.4 mL/min, approx. pumped; Quadrupole resolution – standard resolution peak width 0.70 amu at 5% height; Plasma gas – Argon (5 grade) 15 L/min; Plasma gas CCT – 8% Hydrogen in Helium (5 grade) 5 ml/min; Sample uptake time – 15 seconds; Wash delay – 35 seconds; Total time per sample – 3 minutes, 5 seconds; Number of replicates per sample – 3.

The reagents used during the course were designed for trace analysis and deionized water purified with the Millipore Simplicity 185 UV Water Purification System. A spectrophotometer was calibrated using Inorganic Ventures analytical standard 122. The EnviroMat ES-L-2 and EnviroMat ES-H-2 certified reference materials were applied to evaluate the correctness of calibration curves and control the quality of the analyses performed. The recovery of iron from the reference materials in the course of analysis remained within the range 95 – 125%.

Statistical analysis. Statistical analyses were conducted using software IBM SPSS 21. The distribution of data was not normal. Comparisons of the measurements were made using the non-parametric Wilcoxon rank test. The obtained results were supplemented with measurements of effect size using Cohen's correlation coefficient. Values of individual parameters were interpreted on the basis of the median. The values of the quadrant, complementary mean and standard deviation were also given. Significant differences were assumed for $p<0.05$.

RESULTS

29 subjects participated in the study – 21 females and 8 males. Average age – 47.62 (SD=15.91) years. Mean body mass – 70.14 (11.07) kg, body length – 169.62 (8.42) cm.

The median value of Na in the samples collected on the 1st day was 183.8ppm (parts per million); median values of Na in the samples collected on the 14th day and on 28th day of training were 214.7ppm and 204.9 ppm, respectively. None of these values differed significantly statistically from the value obtained on the first day ($p>0.05$).

Concentration of iron on the 1st and the 14th day of exercises was comparable and not statistically significant. Iron concentration was highly increased on the last day of training in comparison with the 1st ($p<0.001$) and 14th day ($p<0.006$). The median of iron concentration in the 29 samples on the first day of sampling was 21.2ppb, on the 14th – 52.5ppb, and on the 28th day – 286.2 ppb (Tab. 1). In relation with the sodium concentration, the level of which remained constant in all 3 samples ($p>0.05$), iron content was also increased on the last day of the training programme (Tab. 2).

DISCUSSION

Iron concentration in sweat during exercises has been investigated previously [7,10–13]. Paulev et al. investigated iron losses in distance runners during cycling. They obtained 3.6 ± 1.1, 2.3 ± 0.2 and 2.4 ± 0.3 µmol/l of iron in 3 samples in group in which the subjects had eliminated initial sweat from the site of collection [13].Lamanca et al. collected sweat using polyethylene bags placed on male and female runners. Differences in iron sweat concentration were found between females (0.417 +/- 0.024 mg/l) and males (0.179 +/- 0.011 mg/l), although males lost more total sweat than females; therefore the sweat rate was similar [12]. The loss of elements is determined by their concentration in sweat and

Table 1. Iron sweat concentration on the 1st, 14th and 28th day of training (ppb)

Sample												intra-class coefficient correlation		
1st day(1)				14th day (2)				28th day (3)						
Me	*Q*	*M*	*SD*	***Me***	*Q*	*M*	*SD*	***Me***	*Q*	*M*	*SD*	X^2	*p*	*W*
21.2	71.9	67.5	115.4	**52.5**	88.1	124.6	228.2	**286.2**	144.9	307.3	122.9	14.51	0.001	0.52
Comparison of samples														
1–2				1–3								2–3		
Z		*P*		*Z*		*p*		r_c		*Z*		*p*		r_c
1.07		0.287		3.53		0.001		0.71		2.77		0.006		0.60

Me – median; Q – quartile deviation; M – mean; SD – standard deviation; X^2 – Friedman test;
W – Wilcoxon test; *Z – Z test*; r_c – Cohen's correlation coefficient

Table 2. Comparison of iron sweat concentration in relation with sodium on the 1st, 14th and 28th day of training (ppb)

Sample												intra-class coefficient correlation		
1st day (1)				14th day (2)				28th day (3)						
Me	*Q*	*M*	*SD*	***Me***	*Q*	*M*	*SD*	***Me***	*Q*	*M*	*SD*	X^2	*p*	*W*
1.6-E-4	2.47-E-4	4.35-E-4	10.4E-4	**1.7-E-4**	2.29-E-4	12.2E-4	38.3-E-4	**15.6-E-4**	20.05-E-4	345-E-4	1240E-4	13.00	0.002	0.46
Comparison of samples														
1–2				1–3						2–3				
Z		*p*		*Z*		*p*		r_c		*Z*		*p*		r_c
0.24		0.809		2.91		0.004		0.58		2.50		0.012		0.55

Me – median; Q – quartile deviation; M – mean; SD – standard deviation; X^2 – Friedman test;
W – Wilcoxon test; Z – Z test; r_c – Cohen's correlation coefficient

the amount of sweat [15]. In the presented study, the presence of iron was expressed in ppb units (parts per billion). Total sweat loss was not measured, which may be a limitation of the study. However, to the best knowledge of the authors of the current study, this is the first investigation of iron content changes in sweat during an exercise training programme. De Rusisseau et al. investigated sweat iron loss during a 2-hour exercise and found that iron concentration was significantly lower during the 2nd hour (0.042 mg/m2/h) than the 1st hour of exercise (0.060 mg/m2/h) [11]. Increased iron loss has also been observed by the authors of the current study, although not during one training session, but during a full 4-week exercise programme.

Sweat loss can be also caused by higher temperature of the environment, and the ion concentration in sweat depends on the degree of acclimatisation [9]. Heat exposure – for example in summer, in combination with exercise – can increase the loss of sweat [7, 16]. In the presented study, the temperature remained constant at 24° C. Thus, the influence of seasonal temperature changes did not affect the results.

The thermoregulatory response during physical training may be helpful in explaining the results of the presented study, in which the participating subjects were not adapted to physical activity. Thus, the process of habituation to physical effort was observed. Lee et al. suggested that habitual long-distance running results in upregulation of the peripheral sweating mechanisms. Routine long-distance runners exhibited higher sweat responses after evoked sweating due to shorter sweat onset time, and higher sweat output per sweat gland [17]. Similar findings were reported by Buono et al. Peripheral sweat rate was significantly higher in trained men and women, compared with sedentary men and women. This suggests that physical training improves the secretory activity of the human sweat gland [18]. The runners thus behaved as if the "set point" of their thermoregulatory system had been reset to a lower level [19]. The increase in iron concentration may therefore be due to the thermoregulation mechanism and secretory activity of sweat glands. A significant and strong correlation was found between VO2max and sweating in the trained subjects, but not in the controls [17, 18]. In the presented study, higher VO2max may occurred during the last 2 weeks of the programme when the time of the exercises was increased to 30 min, which may have implications for the sweating value. However, in this study, the constant level of sodium may also indicate a constant amount of sweat loss. Thus, iron content was compiled with the sodium concentration.

However, knowledge about the iron status in blood during long-term exercise training has not yet been established. Magazanik et al. found that the iron levels in males and females decreased by 65% after 2 weeks of training carried out over a 7 week period, and included 8 h of varying physical activities each day. Ferritin levels decreased by 50% in both genders after 4 weeks of exercise, and remained at this level until the end of the training [20]. Iron and ferritin level were significantly more decreased in athletes than those of control group, and runner's serum iron and ferritin level were lower than cyclists [21]. Opposite results were presented by Bourque et al. who showed that analysis of serum ferritin and values for serum iron concentration did not change significantly during the 12 weeks in a walking/running group or the cycling group, compared with the control group [22]. Thus, the influence of exercises on iron status and sweat loss remains unclear and needs further investigation.

In recent papers, it has been strongly postulated that mechanism of iron deficiency may be indicated by the hepcidin- peptide hormone, which is expressed in response to hypoxia, elevated iron levels, inflammation and exercise [23, 24]. An elevated hepcidin level was observed in response to different forms of physical activity such as long-distance running [25], NordicWalking [26, 27], basketball [28] or rowing [29]. Thus, hepcidine activity is considered as a most important factor of iron homeostasis during exercise. However, connection between serum iron level and amount of exrection needs more reaserch. It may be argued that some part of iron from the skin is a result of contamination from external sources [30]. Thus, the contributory role of sweating to iron deficiency may not be as important as thought. However, exercising for prolonged time periods, over many training sessions may induce a cumulative effect on iron status [31].

CONCLUSION

Iron sweat loss significantly increased during the 4-week exercise programme. A possible explanation may be an improvement in the thermoregulation mechanism and secretory activity of sweat glands.

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