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Zinc supplementation modifies trace element status in exercised rats

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ABSTRACT

Purpose: Investigation of the effect of exercise and zinc (Zn) supplementation on trace element status in rats.
Methods: 24 male Wistar were divided into four groups: control, exercised, Zn-supplemented (15 mg/kg weight Zn asparaginate), exercised Zn-supplemented. Zn was supplemented as Zn asparaginate. Serum lactate and creatinine levels, and creatine kinase activity were assessed. Tissue trace elements were estimated using inductively coupled plasma mass spectrometry.
Results: Exercise significantly increased lactate, and creatinine levels. Exercise significantly decreased muscle, kidney, and hair Zn; liver, muscle and serum Co; serum Fe; myocardial and hair Cu; liver, heart, skeletal muscle and kidney Se levels. Oppositely, exercise results in elevation of liver Zn; heart and skeletal muscle, kidney and hair Fe; kidney Cu; liver and hair Mn; serum and hair Se content. Zn supplementation reduced exercise-induced increase in lactate and creatinine levels, and elevated liver, kidney, heart, and hair Zn content in exercised rats. Supplementation with Zn reversed exercise-induced decrease in Co levels and increased Fe and Se stores in animals with high physical activity.
Conclusions: Beneficial effect of zinc supplementation in exercised organism may be associated not only with modulation of zinc status but regulation of other essential trace elements status and their biological effects.

Introduction

Zinc (Zn) is essential for a wide variety of metabolic and physiologic processes in the human body, especially in athletes (Prasad, 2009). Therefore, maintenance of Zn homeostasis is required for normal organism functioning (Maret and Sandstead, 2006). Zn compounds are one of the most frequently consumed supplements in athletes (Vincent and Neggers, 2013). However, the existing data on changes in tissue Zn concentrations in response to exercise are contradictory (Chu and Samman, 2014). In particular, a number of studies have shown the development of Zn deficiency in athletes (Couzy et al., 1990), whereas the other ones have not revealed significant changes in metal levels (Lukaski et al., 1989). Moreover, some studies showed the increased tissues Zn content after high physical activity (Baydil, 2013; Chu et al., 2016).

The existing data also demonstrate the influence of physical activity on other essential trace elements. In particular, it was shown that physical exercise impacts significantly on iron (Fe) metabolism in athletes (Weaver and Rajaram, 1992). Fe deficiency in athletes was observed in the majority of studies due to the different reasons such as insufficient dietary Fe intake, increased Fe losses, and increased requirements (McClung et al., 2014;

Abbreviations: ANOVA, analysis of variance; C, control rats; CK, creatine kinase; E, exercised rats; ZnS, Zn supplemented rats; E+ZnS, exercised Zn-supplemented rats.

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Latunde-Dada, 2013). At the same time, certain investigations have not revealed any considerable changes in body Fe status after exercise (Govus et al., 2014). Data on copper (Cu) balance in athletes are also contradictory. For example, serum Cu level has been shown to either remain stable (Anderson et al., 1984; Duvan, 2013), decreased (Bordin et al., 1993) or increased (Anderson et al., 1995) after exercise. The existing data on exercise-induced changes in manganese (Mn) and cobalt (Co) levels are also incomplete (Soria et al., 2016).

Earlier data also show a tight interplay between Zn metabolism and other trace elements. Both clinical (Fesyun et al., 2011) and experimental (Sivrikaya et al., 2012) studies demonstrate the possibility of modulation of trace element status in physically active subjects by Zn supplementation. However, the existing data are contradictory and insufficient.

Therefore, the primary objective of the study is investigation of the effect of exercise and Zn supplementation on trace element status, serum lactate, creatinine levels, and creatinine kinase activity in rats.

Materials and methods

Experimental

A total of 24 male three-months-old Wistar rats were used in the current experiment. The protocol of the study was approved by the institutional Local Ethics Committee and carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The animals were acclimatized to laboratory conditions for 14 days prior the experiment. The temperature in the animal room was 23 ± 1 °C with 12-h light cycle (lights on at 8.00). All rats were fed a standard laboratory chow PK-120 (Laboratorkorm Ltd., Moscow, Russia) containing 307 kcal/100 g (20% protein, 70% carbohydrate, 10% fat). The animals had free access to food and drinking water with a total mineralization of less than 250 mg/l. Data on trace elements content in both chow and drinking water are presented in Table 1.

The animals with equal body weight were divided into 4 groups ($n = 6$):

1) Control (C) animals were consumed standard chow and pure drinking water;
2) Zn-supplemented group of animals (ZnS) daily obtained 15 mg/kg weight Zn asparaginate $Zn(C_4NO_4H_6)_2{\cdot}Zn(OH)_2$. Zn asparaginate in a mixture with starch was given by intragastric gavage (at 10.00 a.m.) using silicone flexible catheters;
3) Exercised (E) rats were exposed to treadmill running (Columbus instruments, Columbus, OH, USA) for 10 min. Treadmill speed was set at 23 m/min at a 10° grade;
4) Exercise Zn-supplemented (E + ZnS) rats daily obtained 15 mg/kg weight Zn asparaginate $Zn(C_4NO_4H_6)_2{\cdot}Zn(OH)_2$ in 40 min before exercise.

All procedures were performed daily during 2 weeks.

Table 1
Trace elements content in chow and drinking water.

Element	Chow (μg/g)	Drinking water (μg/ml)
Zn	1.18 ± 0.20	0.235 ± 0.064
Co	0.4335 ± 0.0018	0.0002 ± 0.0001
Cu	17.66 ± 4.79	0.02 ± 0.01
Fe	210.5 ± 10.3	0.52 ± 0.07
Mn	82.50 ± 4.50	0.009 ± 0.001
Se	0.169 ± 0.035	0.009 ± 0.001

Data expressed as mean values and the respective standard deviations.

Sample collection

After the experiment, blood was collected via venesection of the jugular vein with subsequent separation of serum. Rats' liver, kidneys, heart, and muscles (*m. gastrocnemius*) were also collected and separated from connective tissue and rinsed with ice-cold physiological saline. Hair was collected from the cranial part of the spine. The obtained samples were used for subsequent chemical analysis.

Lactate, creatinine, and creatine kinase assessment

Serum samples were used for analysis of lactate and creatinine levels as well as creatine kinase (CK) activity using the respective Randox kits (Randox Laboratories Ltd., Crumlin, UK) on an automated biochemical analyzer Tokyo Boeki (Tokyo Boeki Machinery Ltd., Tokyo, Japan).

Trace element analysis

The collected samples were used for trace element analysis. Hair samples were washed with acetone and rinsed three times with distilled deionized water with subsequent drying at 60 °C on air. Serum was diluted with an acidified diluent (1:15 v/v) containing 1% 1-butanol (Merck KGaA, 64271 Darmstadt, Germany), 0.1% Triton X-100 (Sigma-Aldrich, Co., St. Louis, MO 63103 USA), and 0.07% HNO3 (Sigma-Aldrich, Co., St. Louis, MO 63103 USA) in distilled deionized water.

50 mg of organs, hair and diluted serum were added with concentrated HNO_3 in Teflon tubes with subsequent microwave digestion in Berghof speedwave four (Berghof, Eningen, Germany) system for 20 min at 180 °C. After cooling the obtained samples were used for analysis. Trace elements content in the studied substrates was assessed using NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA) equipped with ESI SC-2 DX4 (Elemental Scientific Inc., Omaha, NE 68122, USA) autosampler. System's calibration was performed using 0.5, 5, 10, and 50 μg/l solutions of the studied trace elements prepared from Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT 06484, USA) by addition of distilled deionized water acidified with 1% HNO_3. Internal on-line standardization was performed using yttrium (^{89}Y) isotope Yttrium Pure Single-Element Standard (PerkinElmer Inc., Shelton, CT 06484, USA). Laboratory quality control was performed using certified Reference Materials of hair (GBW09101; Shanghai Institute of Nuclear Research, Shanghai, China) and serum (ClinCheck Plasma Control, lot 129, levels 1 and 2; RECIPE Chemicals + Instruments GmbH, Germany). The recovery rates for all studied elements exceeded 80%.

Statistical analysis

Statistical treatment of the obtained data was performed using Statistika 10.0 (Statsoft, Tulsa, OK, USA). Data normality was assessed using Shapiro-Wilk test. As the distribution was not normal, data was expressed as Median and the respective values of 25 and 75 percentile values. Log-transformation of the primary data was performed for normalization. Group comparisons were performed using two-way analysis of variance (ANOVA) with Fisher's Least Significant Difference post hoc test. All differences were significant at the significance level 2alpha = 0.05.

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