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Nutrition

The effect of iron and zinc supplementation and discontinuation of this practice on iron and zinc level in tissues in rats fed deficient diets

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ABSTRACT

The effect of iron and iron/zinc supplementation on their levels in tissues of rats fed initially one of the three following regimen: C – control AIN-93 diet, D – iron deficient diet and R – diet with 50% reduction of all vitamins and minerals was investigated. The study was conducted on 6-week male Wistar rats, in 3 stages: (1) 4-week adaptation to the diets (C, D or R); (2) 4-week supplementation with the same regimen enriched with 10-times more iron (CSFe, DSFe, RSFe) or iron/zinc (CSFeZn, DSFeZn, RSFeZn); (3) 2-week post-supplementation period (the same diets as the stage I). Iron and zinc content in serum, the initial segment of intestine, liver and kidney were measured using FAAS method. After supplementation period (stage II) the content of iron in the intestine, liver and kidney in groups of rats fed DSFe and DSFeZn-diet were significantly higher (all p-values ≤ 0.05) than in rats fed D-diet (intestine: DSFe = 50.1 ± 9.0 μg/g wet weight, DSFeZn = 43.0 ± 9.9 μg/g vs. D = 16.5 ± 2.1 μg/g; liver: DSFe = 149 ± 30 μg/g, DSFeZn = 152 ± 25 μg/g vs. D = 56 ± 13 μg/g; kidney: DSFe = 74.0 ± 8.1 μg/g, DSFeZn = 72.7 ± 6.6 μg/g vs. D = 59.3 ± 9.5 μg/g). The same significant associations (all p-values ≤ 0.05) were observed in R rats in the intestine and liver (intestine: RSFe = 60.8 ± 6.6 μg/g, RSFeZn = 54.8 ± 6.6 μg/g vs. R = 31.5 ± 8.2 μg/g; liver: RSFe = 161 ± 10 μg/g, RSFeZn = 166 ± 21 μg/g vs. R = 136 ± 24 μg/g). After post-supplementation period the statistically significant differences between supplemented and non-supplemented rats fed D- and R-diets were still observed. There was not found the effect of applied treatments on zinc status. In conclusion, iron or iron/zinc supplementation increased similarly iron level in tissues of rats fed D-diet or R-diet with prolonged effect after supplementation discontinuation.

Introduction

A lot of studies indicate that iron supplementation is commonly used by some groups of people such as pregnant women or sportsmen, and may have beneficial effects, protect against the consequences of iron deficiency and anemia. Iron supplementation improves attention, concentration and intelligence quotient among anemic children and pre-menopausal women [1], improves exercise capacity [2,3] and quality of life among patients with heart diseases [3]. In contrast, some studies show that high intake of iron can be harmful, may lead to the development of many diseases, such as cardiovascular diseases [4,5], cancers [6,7], type 2 diabetes [8,9], atherosclerosis [10,11] and others. These diseases develop as a result of increased production of hydroxyl radicals ($OH^{\bullet}$) in the body, what initiates oxidative stress and causes peroxidation of lipids [12–20], proteins and DNA damage [16,18,20–22]. Some studies highlight that both iron deficiency and iron overload can lead to lipid peroxidation [13] and DNA damage [21].

In this situation it is necessary to look for solutions, i.e. iron supplementation with minimizing the risk of the adverse effects of high doses and excesses of this element in the organism. There are indications that simultaneous iron and zinc supplementation could be effective in correction of iron depletion [23–28] and reduction of oxidative damages induced by iron [23–25].

Therefore, we conducted animal studies in which were tested the effect of iron or combined iron and zinc supplementation and cessation of this treatment on the iron and zinc status, lipids parameters and oxidative status.

The aim of these paper was to investigate the effect of combination of iron and zinc supplementation on iron and zinc levels in serum and tissues of rats fed iron deficient diet or diet with reduction of vitamins and minerals. Furthermore, the influence of discontinuation of applied treatments on the content of these minerals in serum and tissues were determined.

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Materials and methods

Animals and diets

Two studies were performed among male Wistar rats with a mean initial weight 294 ± 20 g in study I and 296 ± 23 g in study II. Rats were purchased from the Medical Research Center of Polish Academy of Sciences (NIH Certified, No. A5438-01). The animals were housed individually in plexi-glass cages, in controlled temperature (21–22 °C) and humidity (55–60%) with a 12-h light-cycle. The studies were approved by the Third Local Ethics Commission in Warsaw.

Each study was divided into 3 stages: adaptation to the diet (4 weeks), supplementation period (4 weeks) and post-supplementation period (2 weeks) (Table 1). Rats were fed three types of diets: control (C), iron deficient (D) or diet with reduced amount of all vitamins and minerals (R). All diets were based on AIN-93M recommendations [29] with some modifications in added mineral and vitamin mixtures. The content of iron and zinc in experimental diets is shown in Table 2. In adaptation and post-supplementation periods the amount of iron in D diet was 7.4 mg/kg. In R diet the amount of vitamin and mineral mixtures added was reduced by 50% compared to C diet. In supplementation period the amounts of iron (diet CSFe, DSFe and RSFe) or iron and zinc (diet CSFeZn, DSFeZn and RSFeZn) in all diets were 10-times higher compared to C diet. Rats had access to water *ad libitum* and were pair-fed with the group consuming the least amount of diet.

Tissue collection

During tissues collection and analysis the precautions have been taken to avoid iron and zinc samples contamination. Needles and scissors used were made of high quality stainless steel and all tubes and tips were decontaminated in 10% HCl and 3-times rinsed in ultrapure water.

At the end of each period, after overnight starvation, rats were anesthetized with an intraperitoneal injection of thiopental and blood was collected by heart puncture. Serum was separated after 45 min blood samples incubation in room temperature by centrifugation at 3000× *g* for 10 min at 4 °C. The small intestine samples (the first 5 cm initial segment – duodenum) and the liver and the kidney were removed then washed with an ice-cold solution of 0.9% NaCl, dried on filter paper, weighted and then stored at −80 °C before analysis.

Iron and zinc determination

Approximately 1 cm^3 serum, 0.15–0.20 g wet weight of intestine and 0.5–1.0 g wet weight of kidney and liver samples were taken to determine the content of iron and zinc. The samples were digested with 65% HNO_3 (Merck 1.00456.1000; Germany) for 10 min at 210 °C at a pressure of 160 PSI using microwave digestion system (MARS5, CEM, USA).

The iron and zinc content in serum and aliquots of intestine, liver and kidney tissues samples was determined by flame atomic absorption spectrometry (FAAS) (UNICAM 989, SOLAAR, UK). Iron and zinc standard curves were prepared by diluting iron and zinc standard reference materials (Merck 1.19781 and 1.19806, respectively) in a range from 0 to 5.0 μg/cm^3. Certified reference materials i.e. serum (Seronorm™ Trace Elements Serum L-1, SERO AS, JL4409; Norway) and bovine liver (National Institute of Standards and Technology INIST, 1577B, Gaithersburg, MD) were used to test the accuracy of the methods. Recovery in serum was 102.0% for iron and 99.9% for zinc, while in bovine liver was 105.4% and 105.3%, respectively. The coefficients of variation in serum were 2.6% for iron and 6.3% for zinc, while in bovine liver −1.5% and 1.3%, respectively.

Statistical analysis

Data were analyzed using Statistica software version 9. The results were presented as the mean ± SD. Group comparisons were conducted using a two-way analysis of variance and LSD post hoc test. The results with p-values ≤ 0.05 were considered as statistically significant.

Results

In each phase of study I and study II the diet intake and body weight of animals did not differ among experimental and control groups. Moreover, the weights of the liver and the kidney were similar in rats fed a various type of C and D diets, while after supplementation period (stage II) the mean liver weight of rats fed RSFeZn and R diet were statistically significantly different (12.3 ± 1.1 vs. 10.4 ± 1.1 g, respectively).

Table 1
Experimental designs.

Diet/group	Stage I adaptation to diets (4 weeks)	Stage II supplementation period (4 weeks)	Stage III post-supplementation period (2 weeks)
Study I (n = 132 rats)			
C	Control diet	Control diet	Control diet
CSFe		Supplemented with Fe	
CSFeZn		Supplemented with Fe and Zn	
D	Fe-deficient diet	Fe-deficient diet	Fe-deficient diet
DSFe		Supplemented with Fe	
DSFeZn		Supplemented with Fe and Zn	
Study II (n = 77 rats)			
R	Diet with reduced quantity of vitamins and minerals	With reduced quantity of vitamins and minerals	Diet with reduced quantity of vitamins and minerals
RSFe		With reduced quantity of vitamins and minerals supplemented with Fe	
$RSFeZ_n$		With reduced quantity of vitamins and minerals supplemented with Fe and Zn	

Experimental diets based on AIN-93M recommendation [29]: C – control; D – iron-deficient, mineral mixture without iron; CSFe and DSFe – supplemented with iron, 10× more than in C diet; CSFeZn and DSFeZn – supplemented with iron and zinc, 10× more than in C diet; R – with reduced quantity of vitamins and minerals, 50% of vitamin and mineral mixtures were added compare to C diet; RSFe – with reduced quantity of vitamins and minerals supplemented with iron, 10× more iron than in C diet; RSFeZn – with reduced quantity of vitamins and minerals supplemented with iron and zinc, 10× more iron and zinc than in C diet. Number of animals is 6–7 in each group.