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Original article

Adverse effect after cessation of rats' unjustified iron or iron and zinc supplementation on hematological parameters but not ferritin concentration

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SUMMARY

Background & aims: Studies on the impact of supplementation cessation are lacking. We investigated the effect of iron and iron/zinc supplementation and cessation of this intervention on iron status parameters.
Methods: The study was conducted on 6-week male Wistar rats, in 3 stages: 4-week adaptation to the diets: C – control (AIN-93M) and D – iron deficient (mineral mix without iron); 4-week supplementation: 10-times more iron (CSFe, DSFe) or iron/zinc (CSFeZn, DSFeZn) compared to C; 2-week post-supplementation period (the same diets as in the first stage). Red blood cell count, hemoglobin, hematocrit, transferrin saturation (TSAT) and ferritin concentration were determined.
Results: After stage II D rats had statistically significantly (p-value ≤ 0.05) lower hemoglobin and TSAT in comparison to DSFe rats, but not DSFeZn, and significantly lower ferritin concentration in comparison to DSFe and DSFeZn rats. After stage III, CSFe and CSFeZn rats had a significantly lower level of all analyzed hematology parameters compared to C, in contrast rats fed DSFe and DSFeZn diets had higher hemoglobin concentration and hematocrit in comparison to D group. Moreover, in comparison to D rats those fed DSFe diet had higher TSAT and those fed DSFe and DSFeZn diets had significantly higher ferritin concentration.
Conclusions: After cessation of unjustified both iron and iron/zinc supplementation resulted in an adverse effect on hematological but not other iron status parameters. In the situation of iron deficiency in the diet, iron supplementation alone had a prolonged beneficial effect and was more effective than simultaneous iron/zinc supplementation in the improvement of the iron status.

1. Introduction

According to the World Health Organization (WHO) about 1.62 billion people worldwide suffer from anemia and insufficient iron status,[1] and justified iron supplementation improves attention, intelligence quotient and concentration among anemic children and pre-menopausal women,[2] improves physical work capacity[3,4] and quality of life among patients with heart diseases.[4] In contrast, the results of prospective studies indicated that a high iron intake is associated with increased risk of stroke,[5] coronary heart disease,[6] cardiovascular mortality[7] and some types of cancers.[8,9] It is known that iron has pro-oxidative properties and its supplementation can lead to lipid peroxidation, protein modification and DNA damage,[10] also among anemic people.[11]

In this situation it is necessary to look for solutions, i.e. replenishment of iron deficiency with minimizing the risk of the adverse effects of high doses and overload of this element in the organism. There are suggestions that zinc administration simultaneous with iron can be effective in the complement of iron deficiency[12–14] and can protect body against harmful effects of high iron doses, for example by reduction of oxidative damage.[12,15]

Both population[16–18] and animal[12,19] studies have analyzed the impact of iron and zinc supplementation on hematological parameters, but neither have studied the effect after the cessation of this intervention.

Therefore, the aim of this study was to investigate the effect of combined iron and zinc supplementation and the cessation of this treatment on iron status in rats fed control (C) and iron deficient (D) diets.

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2. Materials and methods

2.1. Animals and diets

The study was approved by the Third Local Ethics Commission in Warsaw. A hundred thirty-two male Wistar rats with initial weight 294 ± 20 g were purchased in the Polish Academy of Sciences Medical Research Center (no. A5438-0, NIH Certified, Warsaw, Poland). The animals were housed individually in glass–propylene cages in a temperature (21–22 °C) and humidity (55–60%) controlled laboratory with a 12-h light/dark cycle. Rats had access to ultrapure water *ad libitum* and were pair-fed with the group consuming the least amount of diet.

The study was divided into 3 stages (Fig. 1): 4-week adaptation to the diets (C – control or D – iron deficient), 4-week supplementation period where appropriate groups of animals received iron (CSFe, DSFe) or iron and zinc (CSFeZn, DSFeZn) supplement diet and 2-week post-supplementation period (the same diets as in the adaptation stage). All diets were based on AIN-93M recommendations[20] with some modifications in iron or iron and zinc contents in mineral mixtures. The content of iron and zinc in experimental diets is shown in Table 1. The mineral mixture without iron was added to the D diet, while in supplemented diets the amounts of iron (CSFe, DSFe) and iron and zinc (CSFeZn, DSFeZn) in mineral mixtures were 10-fold compared to the C diet.

Table 1
Content of iron and zinc in experimental diets.

Element[a] (mg/kg diet)	Diet[b]					
	C	CSFe	CSFeZn	D	DSFe	DSFeZn
Fe	48.4	470	490	7.4	470	490
Zn	42.6	44.7	412	43.4	44.7	412

C – control diet; D – iron deficient diet; CSFe, DSFe – diets supplemented with iron; CSFeZn, DSFeZn – diets supplemented with iron and zinc.
[a] The contents of Fe and Zn in diets determined by FAAS.
[b] Moreover, diets included (per kg diet): wheat starch 621 g, casein 140 g, sucrose 100 g, soybean oil 40 g, cellulose 50 g, modified mineral mix AIN-93M 35 g, vitamin mix AIN-93-VX (MP Biomedicals, LLC, no. 960402) 10 g, L-cystine 1.8 g, choline bitartrate 2.5 g, and t-buthlyhydroquinone 0.008 g.

2.2. Blood collection

At the end of each stage, after overnight starvation, the rats were anesthetized with an intraperitoneal injection of thiopental. Blood was collected by heart puncture and immediately transferred into tubes containing serum separator or potassium-EDTA. After 45 min blood samples incubation in room temperature, serum was separated by centrifugation at 3000× *g* for 10 min at 4 °C.

2.3. Iron and zinc determination

Approximately 1 ml of serum was taken to determine the content of iron and zinc. The samples were digested in 65% HNO_3 (no. 1.00456, Merck, Darmstadt, Germany) for 10 min at 210 °C and pressure 160 PSI using a microwave digestion system (MARS5, CEM, USA). The iron and zinc content in serum was determined by flame atomic absorption spectrometry (FAAS) (Unicam 989, Solaar, United Kingdom) as described previously.[21]

2.4. Iron status parameters

Hematological parameters such as red blood cell count, hemoglobin and hematocrit concentration were determined in whole blood samples (tubes with potassium-EDTA) in the Laboratory of the Veterinary Center of WULS – SGGW (Warsaw, Poland) with an Abacus Junior Vet Analyzer (Diatron MI PLC, USA). Transferrin saturation (TSAT) was calculated as the ratio of serum iron and total iron-binding capacity (TIBC) multiplied by 100. TIBC was determined in serum samples in the Diagnostic Medical Laboratory (Warsaw, Poland) with an Olympus AU680 Analyzer (Beckman Coulter Inc., USA). To assess ferritin serum concentration a quantitative ELISA-based test kit was used for detecting rat ferritin in biological samples (Cat. No. E-25F, Immunology Consultants Laboratory INC, USA).

2.5. Statistical analysis

The data were presented as mean values ± standard deviation (SD) and were analyzed using Statistica software version 10.0. Homogeneity of variance was analyzed using Levene's test. Comparisons between groups were conducted using a two-way analysis of variance and Fisher's Least Significant Difference (LSD) *post-hoc* test. The results with p-values ≤ 0.05 were considered as statistically significant.

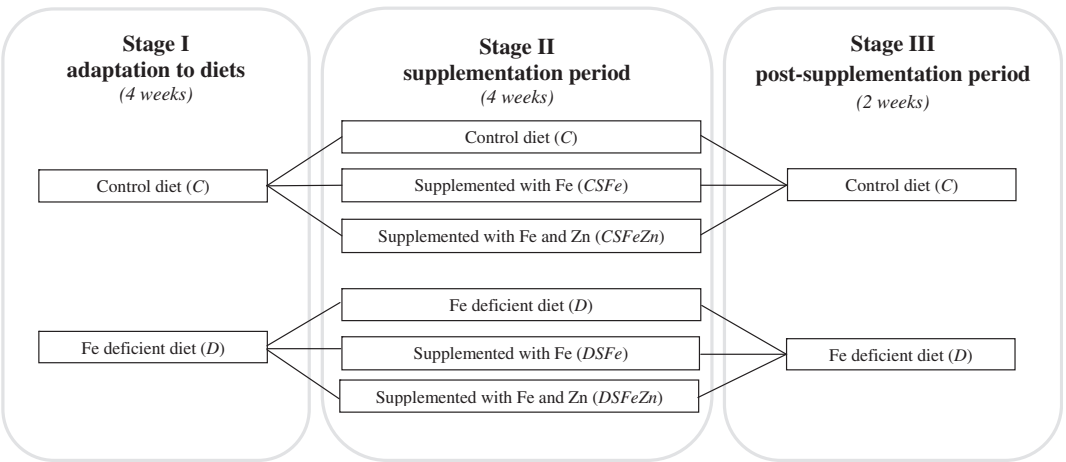

Fig. 1. Study design.

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