Journal of Trace Elements in Medicine and Biology 35 (2016) 77–82

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Iron absorption after introducing and discontinuation of iron and zinc supplementation in rats

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ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form
31 December 2015
Accepted 26 January 2016

Keywords:
Apparent absorption
Iron
Rats
Supplementation
Zinc
Post-supplementation

ABSTRACT

The aim of this study was to investigate the changes in iron apparent absorption (IAA%) during and after iron and zinc supplementation in rats.

The study was conducted on 6-week old male Wistar rats in 3 stages: 4-week period of adaptation to the control (C) and iron deficient (D) diets (stage I); 4-week period of supplementation with 10-time more iron (CSFe, DSFe), zinc (CSZn, DSZn) or both iron and zinc (CSFeZn, DSFeZn) compared to C diet (stage II); 2-week of post-supplementation period (rats were fed the same diets as in the adaptation period, stage III).

IAA% was measured in five consecutive days directly after introducing and discontinuation of iron and zinc supplementation as well as in the end of stage II (days: 22–24th) and stage III (days: 8–10th).

Overall in the second day after introducing and in the fifth day after discontinuation of iron or iron and zinc supplementation, the IAA% had undergone to the level compatible with the values in the end of each stage. At the end of stage II, IAA% in CSFeZn (54.1 ± 2.7%) rats was not different from the IAA% in CSFe rats (53.9 ± 1.9%), but in DSFeZn group IAA% (49.4 ± 2.1%) was significantly lower than in DSFe (57.4 ± 2.3%) group. Moreover, IAA% after stage II and stage III in DSZn group was significantly lower (39.2 ± 2.8% and 38.6 ± 2.6%, respectively) than in group D (60.7 ± 1.9% and 54.3 ± 3.0%, respectively).

In conclusion, zinc administered simultaneously with iron (Zn:Fe weight ratio = 1:1) decreased IAA% in adult rats fed on iron deficient diet, but not in rats fed on control diet. IAA% reduction by zinc supplementation has been extended to 10 days after discontinuation of the treatment. Adaptation of the rats to high doses of iron or iron and zinc and also to the cessation of these treatments was relatively fast. However, IAA% was stabilized faster after introducing the supplementation than it's discontinuation.

1. Introduction

Iron as an important microelement plays various fundamental role in the body. Its deficiency in the diet can lead to anemia and health consequences associated with this disease [1]. On the other hand, it is known that excessive iron intake may be harmful. It increases production of reactive hydroxyl radicals ($OH^{\bullet}$), which impair antioxidant and pro-oxidant balance in the body [2]. In consequences, lipid peroxidation [3–5], protein modification and DNA damage take place [5,6]. In contrast, zinc as indirect antioxidant can protect the body cells against the damage caused by reactive oxygen species. Among others as an inhibitor of NADPH oxidase and an integral metal of Cu,Zn-SOD zinc reduces formation of free radicals [2,7,8].

Although, combined supplementation of iron and zinc seems to be an effective strategy to improve the iron status along with minimizing the adverse effect of high doses of iron [9–12], animal [13–15] and human [16–19] studies on combined impact of iron and zinc supplementation on iron absorption are limited only to show the overall effect. To the best of our knowledge none of them has investigated the changes in iron absorption in the consecutive days directly after introducing as well as discontinuation of the supplementation.

Therefore, the aim of this study was to analyze the impact of iron and zinc supplementation not only on iron apparent absorption (IAA), but also on the changes in IAA in the consecutive days directly after introducing and discontinuation of the treatment.

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http://dx.doi.org/10.1016/j.jtemb.2016.01.013
0946-672X/

2. Materials and methods

2.1. Animals and diets

The study was conducted on 132 certified male Wistar rats with a mean initial weight of 294 ± 20 g. The animals were obtained from the Medical Research Centre of Polish Academy of Sciences (NIH Certified, No. A5438-01). The study was approved by the Third Local Ethics Commission in Warsaw. During the 10-week period of experiment, rats were housed individually in glass-propylene cages in the animal room at a temperature of 21 °C with a 12:12-h light:dark schedule and 50–60% humidity. The rats were weighed once in every two weeks.

The study included three stages: 4-week period of adaptation to the diets (C—control and D—iron deficient) (stage I); 4-week period of supplementation where specific groups of animals received diet supplemented with iron (CSFe, DSFe), zinc (CSZn, DSZn) or both iron and zinc (CSFeZn, DSFeZn) (stage II); and 2-week of post-supplementation period (rats were fed the same diets as in the adaptation period) (stage III). The study design has been presented in Fig. 1. All diets were prepared in accordance with the recommendations of the American Institute of Nutrition (AIN-93M) [20], with some modifications in the mineral contents. The mineral mixture added to the C diet contained 6.06 g ferric citrate and 1.65 g zinc carbonate per kg. The mineral mixture without iron was added to the D diet, while those used to prepared the supplemented diets contained 10-fold higher amount of iron (CSFe and DSFe), zinc (CSZn and DSZn) or simultaneous of iron and zinc (CSFeZn and DSFeZn) compared to the diet C. The levels of iron and zinc in diets were determined by flame atomic absorption spectrometry (FAAS) and they are presented in Table 1. Throughout the study, rats had free access to deionized water and they were pair fed, i.e. amounts of diet were given according to the amounts took by the animals with the lowest intake. The amount of diet consumed by the rats and feed spills were recorded daily separately for each animal.

2.2. Feces collection

In order to determine the apparent absorption of iron after adaptation to the treatment, 24-h feces were collected for 3 consecutive days at the end of each stage i.e. for stage I and stage II during 22nd to 24th day and for stage III during 8th to 10th day. To determine changes in IAA% feces were collected for the first five consecutive days after introducing (stage II) and discontinuation (stage III) the supplementation. In total 600 24-h feces were collected. Feces were purified from residuals of feed and fur. The collected material was dried and stored in decontaminated (in 10% HCl) plastic test-tubes until the analysis.

2.3. Iron determination in feces

Approximately 1.0–1.5 g dry weight of feces were digested for 10 min in 65% HNO_3 (no. 1.00456, Merck, Darmstadt, Germany) at a temperature of 210 °C and pressure 160 PSI in a microwave digestion system (Mars5, CEM, Matthews, USA). Then the samples were diluted with deionized water and the iron content in feces was determined by FAAS using a spectrophotometer Solaar (Unicam 989, United Kingdom) as described previously [10]. Iron standard curves were prepared by diluting iron standard reference materials (Merck 1.19781, Darmstadt, Germany) with deionized water in a range from 0 to 5.0 μg/cm^3. Certified reference materials i.e. serum (SeronormTM Trace Elements Serum L-1, No. JL4409; Norway) and bovine liver (National Institute of Standards and Technology INIST, No. 1577B, Gaithersburg) 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.

2.4. IAA% estimation

The apparent absorption of iron (IAA%) was calculated using the following equation:

$$\text{IAA\%} = \frac{I - E}{I} \times 100$$

where:IAA%—iron apparent absorption [%],I—daily intake of iron [μg],E—daily excretion of iron with feces [μg].

To calculate the IAA% after introducing and discontinuation of the supplementation this parameter was calculated separately for each of five consecutive 24-h periods. Diet iron intake was taken a day before. To calculate the IAA% after adaptation to the treatment, data on diet intake for three days (i.e. 21–23 day for stage I and II and 7–9 day for stage III) and excretion of iron with feces for three days (i.e. 22–24 day for stage I and II and 8–10 day for stage III) were used.

2.5. Statistical analysis

Data were analyzed using Statistica software version 10.0PL. The results were presented as the mean ± SE. Homogeneity of variance was analyzed using Levene's test. The effects of treatments were evaluated by means of three-way analysis of variance, while comparisons between groups were conducted using LSD post-hoc test. The results with p-values ≤ 0.05 were considered as statistically significant.

3. Results

3.1. Body weight, diet consumption, and biological parameters

After each stage of experiment, the average body weight and total diet consumption did not differ between rats fed various type of diets (Table 2). Also during the 21st to 23rd days of the stage II and the 7th to 9th days of the stage III the diet consumption did not differ.

Our previous studies indicated that in iron deficient rats combined supplementation with iron and zinc was effective to a similar extent as the iron supplementation alone in the correction of iron level in serum, intestine, liver and kidney, but not impact in the same way on transferrin saturation. This treatment did not affect the zinc status [10,21].

3.2. Dynamic of the iron excretion with feces and IAA%

The changes in IAA% and iron excretion in the first days after introducing and discontinuing the supplementation in stage II and III has been presented in Fig. 2 and Fig. 3 (in the On-line Data Supplement), respectively.

During the first few days after introducing iron or zinc or iron and zinc supplementation (stage II), iron excretion with the feces of rats became comparable to the values which observed at the end of this stage (Fig. 3. in the On-line Data Supplement). Similarly, the IAA% became stable already on the second day of balance period and it was similar to the IAA% at the end of stage II (Fig. 2).

In the second day after discontinuing the supplementation (stage III) iron excretion was significantly higher in CSFe (718 ± 232 μg/g d.w. of feces), DSFe (209 ± 46 μg/g d.w.), CSFeZn (718 ± 245 μg/g d.w.) and DSFeZn (147 ± 41 μg/g d.w.) rats in comparison to the end of stage III (Fig. 3. in the On-line Data Supplement). Groups supplemented with zinc showed the same tendency.