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ORIGINAL PAPER

THE EFFECT OF IRON AND/OR ZINC DIET SUPPLEMENTATION AND TERMINATION OF THIS PRACTICE ON THE CONTENT OF THESE ELEMENTS IN MALE RATS' REPRODUCTIVE TISSUES*

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Abstract

Iron and zinc concentrations in testes, epididymis and prostate are important for the proper functioning of male reproductive tract. Therefore, the aim of this study was to investigate the effect of diet supplementation with high doses of iron and/or zinc and the termination of this practice on the content of these minerals in the testes, epididymis and prostate. The experiment was conducted on 132 male Wistar rats, in three stages: I) 4-week adaptation to diets (C – control or D – iron deficient), II) 4-week supplementation (10-times more vs. C diet of iron: CSFe, DSFe; zinc: CSZn, DSZn; or iron and zinc: CSFeZn, DSFeZn), and III) a 2-week post-supplementation period (the same diets as during stage I). The content of iron and zinc in tissues was determined by flame atomic absorption spectrometry. Directly after stage II (after 4-week supplementation), the content of iron in the epididymis in the DSFe rats was significantly higher than in the DSZn rats; similar differences in the iron content were not observed in the testes and prostate. The effect of the supplementation was observed mainly after stage III (after 2-week post-supplementation period); the content of iron in testes, epididymis and prostate in DSFe and DSFeZn rats was statistically significantly higher than in D and DSZn rats (all *P*-values ≤0.05). There was no effect of the applied treatments on the zinc status. Simultaneous iron and zinc supplementation was effective to a similar extent as in the case of iron supplementation alone in the correction of the iron level in reproductive tissues in rats fed an iron-deficient diet. The observed effect was delayed and it was mainly visible 2 weeks after the termination of the supplementation.

Keywords: iron, zinc, supplementation, rats, male, reproductive tissues.

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INTRODUCTION

The problem of iron deficiency is very rare in men who use iron supplementation. Long-term iron supplementation, especially with high doses, can lead to exceeding safe doses (the tolerable upper intake level for iron is 45 mg day^{-1} (Trumbo et al. 2001)). Moreover, iron and zinc can interact and a high dose of iron reduces zinc absorption (Zago, Oteiza 2001); on the other hand, being an indirectly antioxidant, zinc can protect from the pro-oxidative damage caused by high doses of iron (Zago, Oteiza 2001, Kamp, Donangelo 2008, Kaluza et al. 2013, 2014, Januszko et al. 2016). It is known that both an excess as well as a deficiency of iron and zinc may cause harmful effects in the male reproductive tract. Epidemiological studies indicated that it can lead to defects of spermatogenesis, reduced libido, oxidative damage of the testicular tissue and spermatozoa, and ultimately may cause impairment of fertility and problems in male sexuality (Tvrda et al. 2015, Liu et al. 2016, Arafa et al. 2017, Kovac 2017).

Numerous studies indicate that zinc can protect male reproductive tissues against the damage caused by reactive oxygen species (Oteiza et al. 1995, Powell 2000, Prasad 2009, Walczak-Jedrzejowska et al. 2013), and its supplementation has a positive effect on the male reproductive tract, playing an important role in creating and maintaining sperm viability (Taravati, Tohidi 2016, Anjum et al. 2017).

Combined supplementation of iron and zinc seems to be a good strategy for improving the iron status along with minimising the adverse effect of high doses of iron. To the best of our knowledge, there are no studies which have examined the effect of high doses of iron and zinc supplementation on the content of these minerals in the male reproductive tissues, and no-one has investigated the effect after termination of this intervention.

Therefore, we have conducted an animal study on the effect of iron, zinc or combined iron and zinc supplementation and then the termination of this treatment on the content of these minerals in the testes, epididymis and prostate of rats.

The study was a part of one of two animal experiments on advantages and disadvantages of high doses of iron and zinc supplementation on chosen health status parameters.

MATERIAL AND METHODS

Animals and diet

The study was conducted on 132 male Wistar rats with a mean initial body weight of 294 ± 20 g. The animals were purchased from the Medical Research Centre of the Polish Academy of Sciences (NIH Certified,

No. A5438-01). The rats were kept individually in plexiglass cages in a room under controlled conditions of temp. (21-22°C), humidity (55-60%) and a 12-h light-cycle. The animals had an ad libitum access to ultrapure water and were pair-fed with the group consuming the least amount of the diet.

The study was divided into 3 stages (Figure 1): I) 4-week adaptation to the diets (C – control or D – iron deficient), II) 4-week iron and/or zinc sup-

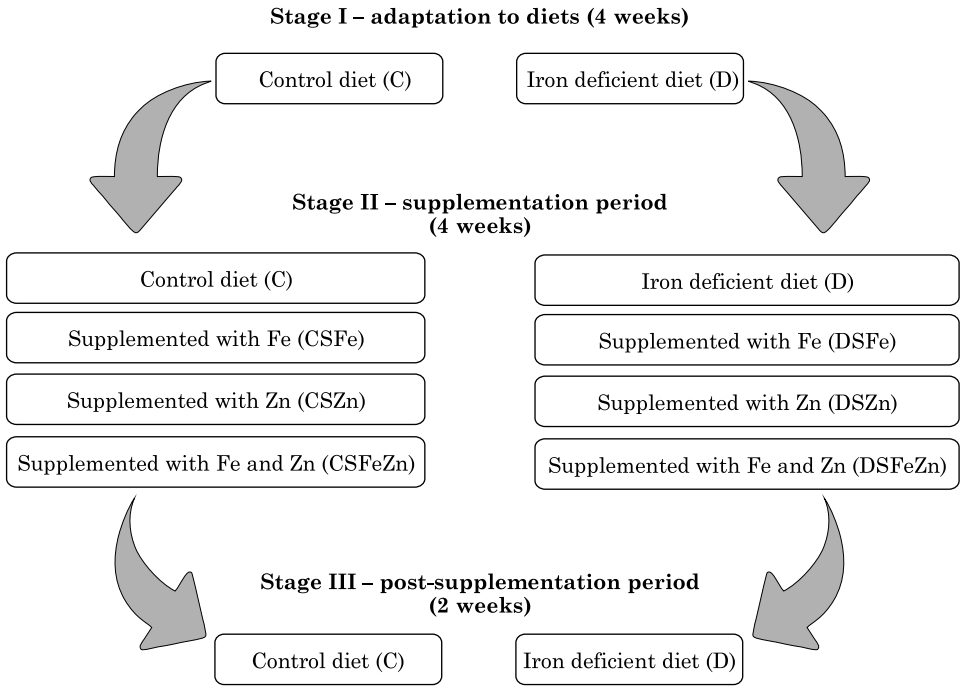

Fig. 1. Experimental design

plementation (diets containing 10-times more than the control diet of iron: CSFe, DSFe; zinc: CSZn, DSZn; or iron and zinc: CSFeZn, DSFeZn), and III) a 2-week post-supplementation period (the same diets as in stage I). Diets based on the C diet were named as C-type diets (C, CSFe, CSZn, CSFeZN), and those based on the D diet – D-type diets (D, DSFe, DSZn, DSFeZn). All diets were based on AIN-93M recommendation (Reeves et al. 1993). A ready-to-use vitamin mix was added to the diets (Cat. No. 960402 MP Biomedicals, LLC). Mineral mixes were prepared in the laboratory of the Human Nutrition Department WULS, Poland, and depending on what modification of the dietary iron and zinc content was made. Iron and zinc were added to the mineral mixes as ferric citrate and zinc carbonate, respectively. The mineral mix used to prepare D and DSZn diets did not contain iron. The content of iron and zinc in the experimental diets was determined by flame atomic absorption spectrometry (FAAS, UNICAM 989, SOLAAR, UK) and was shown in our previous articles (Kaluza et al. 2013, Kaluza,

Madej 2014, Kaluza et al. 2014, Kaluza, Madej 2015, Januszko et al. 2016). The intake of iron and zinc in each group and stage of the experiment was calculated based on the amount of diets consumed by the rats and on the content of iron and zinc in the diets.-

The study was approved by the Third Local Ethics Commission in Warsaw, Poland (Resolution No. 16/2008).

Tissue collection

At the end of each stage, after overnight starvation, the rats were anesthetised with an intraperitoneal injection of thiopental. The blood was taken by heart puncture, and then the testes, epididymis and ventral prostate were removed, rinsed with an ice cold 0.9% NaCl solution (Merck, Darmstadt, Germany), dried on filter paper, weighed and stored at -80°C.

Iron and zinc determination

Samples of reproductive tract tissues (0.3 to 1.0 g) were taken to determine the content of iron and zinc. For this purpose, the samples were digested with 65% HNO_3 (Cat. No. 1.00456.1000 Merck, Germany) for 10 min at 210°C, at a pressure of 160 PSI, using a microwave digestion system (MARS5, CEM, USA). The content of elements in the testes, epididymis and prostate 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 in deionised water (Marck 1.19781 and 1.19806, respectively) in a range from 0.0 to 5.0 $\mu g\ ml^{-1}$.

In order to control the accuracy of the method, certified reference materials were used, i.e. serum (Seronorm™ Trace Elements Serum L-1, SERO AS, JL4409, Norway) and bovine liver (National Institute of Standards and Technology INSITE, 1577B, Gaithersburg, MD). The coefficients of variation in serum were: 2.6% for iron, 6.3% for zinc, and in bovine liver: 1.5% for iron, and 1.3% for zinc.

Statistical analysis

The data were analysed using Statistica software version 10 and presented as mean values ± standard deviation (SD). The homogeneity of variance was analysed using the Levene's test. The normality of the distribution was checked by the Shapiro-Wilk test, data with non-normal distribution were logarithmically transformed. Comparisons between groups were conducted using a two-way analysis of variance and the Least Significant Difference (LSD) *post-hoc* test. Results with p-values ≤ 0.05 were considered to be statistically significant.

RESULTS

Iron and zinc intake

The intake of iron and zinc in each group and stage of the experiment is presented in Table 1. In stage I and III, C-type groups consumed about 6.7-time more iron than the rats fed D-type diets. According to the assumptions of the study, in stage II the iron intake was about 10-time higher in the CSFe, CSFeZn, DSFe and DSFeZn groups than in the C, CSZn, D and DSZn rats. Similarly, in the case of zinc, the CSZn, CSFeZn, DSZn and DSFeZn groups consumed about 10-time more this mineral than other groups of rats.

Reproductive tissues weight

The wet weights (w.w.) of the testes, epididymis and prostate were similar in animals fed C-type and D-type diets, and statistically significant differences between groups were not observed (Table 2).

Content of iron in rats fed C or D diet through all stages in reproductive tissues

Taking into account the differences between the study stages, a regular increase of the iron content from stage I to stage III in the testes and epididymis was found in rats fed the C diet (Table 3), but the differences were only statistically significant in the testes (from 21.78 ± 4.290 in I stage to 28.37 ± 2.133 μg g^{-1} w.w. in III stage). In animals fed the D diet, a regular statistically significant decrease from stage I to stage III was found in the prostate (from 8.834 ± 1.813 to 5.930 ± 0.861 μg g^{-1} w.w.). Moreover, a difference between stage II and stage III in the epididymis was observed in rats fed the D diet (14.44 ± 2.841 *vs.* 9.903 ± 1.296 μg g^{-1} w.w.).

Content of iron after the supplementation period (stage II) in reproductive tissues

No statistically significant differences were observed in the content of iron in the reproductive tissues of rats fed C-type diets after supplementation with high doses of iron and/or zinc (Table 3). In rats fed D-type diets, the content of iron in the epididymis in the DSFe group was significantly higher than in the DSZn group (15.29 ± 2.047 *vs.* 12.68 ± 3.277 μg g^{-1} w.w.). We did not observe any similar statistically significant associations in the testes and prostate.

Content of iron after the post-supplementation period (stage III) in reproductive tissues

Taking into account the C-type diets, a statistically significant difference in the iron content was only found in the epididymis (Table 3); higher

Table 1

Iron and zinc intake (mg per day); mean ± SD (n = 6-7)

Diet/grup		Iron		Zinc	
stage I		stage I		stage I	
stage II	stage III	stage II	stage III	stage II	stage III
C		1.029 ± 0.067$^{\#}$		0.906 ± 0.059	
C	C	0.971 ±0.077$^{a\,\#}$	0.913 ± 0.079 $^{\#}$	0.855 ± 0.068^{a}	0.804 ± 0.070
CSFe	C	9.356 ± 0.529^{b} *	0.938 ± 0.033 $^{\#}$	0.890 ± 0.050^{a}	0.825 ± 0.029
CSZn	C	0.961 ± 0.073$^{a\,\#}$	0.939 ± 0.044 $^{\#}$	8.082 ± 0.611^{b} *	0.826 ±0.038
CSFeZn	C	9.666 ± 0.274^{b} *	0.948 ± 0.089 $^{\#}$	8.127 ± 0.230^{b} *	0.835 ± 0.078
Variance analysis, p-value					
Supl (non-suppl x Fe x Zn x FeZn)		<0.001		<0.001	
Stage (II x III)		0.002		<0.001	
Supl x stage		<0.001		0.001	
D		0.155 ± 0.011		0.914 ± 0.062	
D	D	0.145 ± 0.008^{a}	0.141 ± 0.012	0.856 ± 0.050^{a}	0.830 ± 0.069
DSFe	D	8.946 ± 0.834^{b} *	0.140 ± 0.013	0.851 ± 0.079^{a}	0.822 ± 0.076
DSZn	D	0.142 ± 0.010^{a}	0.139 ± 0.008	7.860 ± 0.633^{b} *	0.818 ± 0.050
DSFeZn	D	9.623 ± 0.739^{b} *	0.137 ± 0.007	8.091 ± 0.622^{b} *	0.809 ± 0.044
Variance analysis, p-value					
Supl (non-suppl x Fe x Zn x FeZn)		<0.001		<0.001	
Stage (II x III)		0.003		<0.001	
Supl x stage		0.002		0.001	

C – control diet, D – iron deficient diet, CSFe, DSFe – diets supplemented with iron, CSZn, DSZn – diets supplemented with zinc, CSFeZn, DSFeZn – diets supplemented with iron and zinc;
a,b – different letters indicate statistically significant differences within a stage, p-value ≤0.05 (test LSD); A, B – different letters indicate statistically significant differences between stage I, stage II and stage III for C- and D- diet only, p-value ≤0.05 (test LSD); ns – not significant, p-value >0.05;
$^{\#}$ – statistically significant differences between a group of rats fed C-type of diets compared to a corresponding group of rats fed D-type of diets, p-value ≤0.05 (LSD test);
* – statistically significant differences compared to stage III.

Table 2

Effect of iron and zinc supplementation on weight of testes, epididymis and prostate (g wet weight); mean ± SD (n = 6-7)

Diet/grup		Testes		Epididymis		Prostate	
stage I		stage I		stage I		stage I	
stage II	stage III	stage II	stage III	stage II	stage III	stage II	stage III
C		1.890 ± 0.181		0.630 ± 0.121		1.258 ± 0.203	
C	C	1.935 ± 0.136	2.062 ± 0.148	0.807 ± 0.076	0.809 ± 0.082	1.348 ± 0.097	1.285 ± 0.082
CSFe	C	1.947 ± 0.077	2.016 ± 0.146	0.750 ± 0.146	0.841 ± 0.137	1.211 ± 0.123	1.268 ± 0.178
CSZn	C	1.921 ± 0.217	1.926 ±0.144	0.783 ± 0.094	0.797 ± 0.148	1.244 ± 0.110	1.358 ± 0.144
CSFeZn	C	1.987 ± 0.078	2.040 ± 0.137	0.810 ± 0.057	0.805 ± 0.154	1.334 ± 0.088	1.460 ± 0.114
Variance analysis, p-value							
Supl (non-suppl x Fe x Zn x FeZn)		ns		ns		ns	
Stage (II x III)		ns		ns		ns	
Supl x stage		ns		ns		ns	
D		1.794 ± 0. 204		0.690 ± 0.072		1.335 ± 0.172	
D	D	1.928 ± 0.188	1.997 ± 0.062	0.832 ± 0.080	0.762 ± 0.148	1.400 ± 0.095	1.386 ± 0.139
DSFe	D	1.839 ± 0.127	1.809 ± 0.228	0.770 ± 0.057	0.777 ± 0.085	1.420 ± 0.143	1.283 ± 0.084
DSZn	D	1.896 ± 0.114	1.802 ± 0.158	0.764 ± 0.078	0.742 ± 0.066	1.400 ± 0.126	1.359 ± 0.140
DSFeZn	D	1.941 ± 0.217	1.788 ± 0.179	0.797 ± 0.070	0.776 ± 0.050	1.411 ± 0.111	1.281 ± 0.131
Variance analysis, p-value							
Supl (non-suppl x Fe x Zn x FeZn)		ns		ns		ns	
Stage (II x III)		ns		ns		ns	
Supl x stage		ns		ns		ns	

Explanations see Table 1.

Table 3

Effect of iron and zinc supplementation on iron level in rats' reproductive tissues (μg g^{-1} wet weight); mean ± SD (n = 6-7)

Diet/grup		Testes		Epididymis		Prostate	
stage I		stage I		stage I		stage I	
stage II	stage III	stage II	stage III	stage II	stage III	stage II	stage III
C		21.78 ± 4.290^{A}		16.86 ± 4.417 $^{\#}$		10.24 ± 2.376	
C	C	27.18 ± 3.875$^{B\#}$	28.37 ± 2.133$^{B\#}$	16.97 ± 2.776	17.53 ± 1.145$^{b\#}$	9.503 ± 1.013$^{\#}$	8.832 ± 0.982$^{\#}$
CSFe	C	26.64 ± 3.694	30.33 ± 2.595	15.14 ± 0.652	15.44 ± 2.555^{ab}	9.411 ± 0.931$^{\#}$	8.917 ± 0.957
CSZn	C	26.83 ± 4.044	30.10 ± 3.834$^{\#}$	15.36 ± 1.280	14.67 ± 3.241^{a}	9.127 ± 0.889$^{\#}$	8.323 ± 1.149$^{\#}$
CSFeZn	C	26.43 ± 5.409	28.75 ± 4.044	15.40 ± 1.715	17.77 ± 1.486^{b}	10.31 ± 1.066$^{\#}$	9.404 ± 1.324$^{\#}$
Variance analysis, p-value							
Supl (non-suppl x Fe x Zn x FeZn)		ns		0.048		ns	
Stage (II x III)		0.016		ns		0.014	
Supl x stage		ns		ns		ns	
D		17.36 ± 3.922		11.81 ± 1.630^{AB}		8.834 ± 1.813^{A}	
D	D	20.10 ± 3.557	16.28 ± 3.091^{a}	14.44 ± 2.841$^{ab}_{A}$	9.903 ± 1.296$^{a\,B}$	8.032 ± 1.144^{A}	5.930 ± 0.861$^{a\,B}$
DSFe	D	25.16 ± 6.124	29.25 ± 5.153^{b}	15.29 ± 2.047^{b}	15.54 ± 1.775^{b}	7.866 ± 0.566	9.046 ± 1.126^{b}
DSZn	D	22.79 ± 6.793	20.23 ± 2.774^{a}	12.68 ± 3.277^{a}	10.87 ± 1.804^{a}	7.652 ± 1.313	6.858 ± 0.748^{a}
DSFeZn	D	24.87 ± 6.144	28.78 ± 6.503^{b}	15.03 ± 3,990^{ab} *	18.23 ± 1.721^{b}	8.914 ± 0.977	7.948 ± 0.700^{b}
Variance analysis, p-value							
Supl (non-suppl x Fe x Zn x FeZn)		<0.001		<0.001		<0.001	
Stage (II x III)		ns		ns		0.045	
Supl x stage		ns		0.006		<0.001	

Explanations see Table 1.

amounts of iron were found in groups the fed C and CSFeZn diets *vs.* those fed the CSZn diet (17.53 ± 1.145 and 17.77 ± 1.486 *vs.* 14.67 ± 3.241 μg g^{-1} w.w., respectively).

In rats fed D-type diets, the iron content in the testes, epididymis and prostate was significantly higher in rats fed the DSFe diet (29.25 ± 5.153, 15.54 ± 1.775, and 9.046 ± 1.126 μg g^{-1} w.w., respectively) and the DSFeZn diet (28.78 ± 6.503, 18.23 ± 1.721, and 7.948 ± 0.700 μg g^{-1} w.w., respectively) than in rats fed D (16.28 ± 3.091, 9.903 ± 1.296, and 5.930 ± 0.861 μg g^{-1} w.w., respectively) and DSZn diets (20.23 ± 2.774, 10.87 ± 1.804, and 6.858 ± 0.748 μg g^{-1} w.w., respectively). Moreover, after cessation of the supplementation of iron and zinc (DSFeZn) and a return to the D diet, we observed a statistically significant higher iron content in the epididymis after stage III compared to stage II (18.23 ± 1.721 μg g^{-1} w.w. *vs.* 15.03 ± 3.990 μg g^{-1} w.w.). Similar associations between stages (II and III) were not observed in rats fed other types of diets as well as in the other reproductive tissues.

Comparison of the content of iron in rats fed C-type vs. D-type diets in reproductive tissues

After stage II and stage III in the testes and prostate, and after stage III in the epididymis, the iron content in rats fed the D diet was lower than in rats fed the C diet (Table 3).

In rats fed D-type diets, iron depletion was especially found in the prostate. After stage II, the depletion of iron in the prostate was found in all group of rats regardless of the type of the supplementation used; similarly, after stage III, the depletion was observed in the prostate of all supplemented rats except the DSFe group (Table 3). In the testes and epididymis, there were no differences after stage II and stage III (except in the testes in the CSZn group) in the iron content in rats fed D-type supplemented diets compared to rats fed the corresponding C-type diet.

Content of zinc through all stages in reproductive tissues

Only in the D group, the highest amount of zinc in the testes was achieved after II stage (29.85 ± 2.404 μg g^{-1} w.w.) in comparison to the content of this mineral after stage I (25.56 ± 2.186 μg g^{-1} w.w.) and stage III (25.71 ± 1.413 μg g^{-1} w.w.) – Table 4. The effect of the applied treatments on the zinc status was not observed.

DISCUSSION

Our study reflects a situation often encountered among humans (Pietruszka, Brzozowska 1999, Jeruszka-Bielak et al. 2011), when dietary supplements are used unnecessarily (i.e. diet contains proper amounts

Table 4

Effect of iron and zinc supplementation on zinc level in rats' reproductive tissues (μg g^{-1} wet weight); mean ± SD (n = 6-7)

Diet/grup		Testes		Epididymis		Prostate	
stage I		stage I		stage I		stage I	
stage II	stage III	stage II	stage III	stage II	stage III	stage II	stage III
C		24.95 ± 1.562		29.71 ± 6.331		11.82 ± 2.526	
C	C	28.76 ± 4.126	26.60 ± 1.909	33.44 ± 4,966	30.29 ± 1,325	13.20 ± 2.059	13.75 ± 1.691
CSFe	C	27.25 ± 1,997	28.17 ± 2.501	31.59 ± 5.011	35.62 ± 1.224	12.68 ± 1.335	13.37 ± 1.111
CSZn	C	26.74 ± 1.769	26.79 ± 1.456	29.84 ± 2.738	33.44 ± 1.442	13.37 ± 0.777	13.16 ± 0.815
CSFeZn	C	27.06 ± 1.809	25.18 ± 3.522	31.22 ± 2.666	35.64 ± 1.002	13.23 ± 1.085	13.92 ± 0.358
Variance analysis, p-value							
Supl (non-suppl x Fe x Zn x FeZn)		ns		ns		ns	
Stage (II x III)		ns		ns		ns	
Supl x stage		ns		ns		ns	
D		25.56 ± 2.186^{B}		27.93 ± 9.429		11.65 ± 2.315	
D	D	29.85 ± 2.404^{A}	25.71 ± 1.413^{B}	32.13 ± 5.500	30.13 ± 4.506	12.58 ± 0.312	12.97 ± 1.853
DSFe	D	27.89 ± 2.656	25.54 ± 1.930	34.55 ± 1.788	30.70 ± 5.624	11.86 ± 1.102	13.81 ± 2.089
DSZn	D	27.41 ± 2.264	26.71 ± 2.608	32.33 ± 6.813	30.76 ± 3.444	12.70 ± 1.648	12.83 ± 1.006
DSFeZn	D	28.67 ± 2.668	27.40 ± 2.740	30.72 ± 7.309	28.86 ± 1.722	12.35 ± 0.660	13.35 ± 1.088
Variance analysis, p-value							
Supl (non-suppl x Fe x Zn x FeZn)		ns		ns		ns	
Stage (II x III)		0.019		ns		ns	
Supl x stage		ns		ns		ns	

Explanations see Table 1.

of nutrients – C-type diets) and in excessive doses (supplemented groups: CSFe, CSZn, CSFeZn, DSFe, DSZn, and DSFeZn). Dietary supplements are often used for a certain time only, and importantly, without an improvement of the habitual diet. This situation has been reflected by groups of rats fed D diets supplemented with high doses of iron and/or zinc in stage II, and especially in stage III where the animals were again fed iron-deficient diets.

According to our knowledge, this is the first study to introduce the post-supplementation period (stage III) after an intervention consisting of high doses of iron and/or zinc supplementation on the content of these minerals in the male reproductive tissues.

The effect of the supplementation was delayed and it was mainly visible 2 weeks after the termination of this treatment in rats fed various types of iron-deficient diets (D-type of diets), i.e. rats fed DSFe and DSFeZn diets had a statistically significant higher iron concentration in testes, epididymis and prostate than animals fed the D and DSZn diets. Directly after the 4-week supplementation period (stage II), a higher content of iron in DSFe *vs.* DSZn was found in the epididymis only. In contrast, similar results of the used intervention were not observed in rats fed well-balanced diets (C-type diets). In the same experiment, after both stages (stage II and III), the effect of iron or iron and zinc supplementation was observed in the intestine, liver and kidney of rats fed iron deficient diet, the higher content of iron in DSFe and DSFeZn rats compared to D rats was found (Kaluza et al. 2013). We can hypothesise that observed changes in the liver, intestine and kidney in iron concentrations directly after introducing the supplementation relative to delayed changes in reproductive tissues may be the result of higher protection of the reproductive system than other organs against negative external factors (such as high doses of iron supplements) and probable oxidative damage.

In our study, the simultaneous supplementation with iron and zinc increased the content of iron in the testes, epididymis and prostate to a similar level as did iron supplementation alone. Similar effects after simultaneous iron and zinc supplementation and after iron supplementation on iron status were also found in the same experiment in the liver, intestine and kidney, but not in serum (Kaluza et al. 2013), and in human studies (Kamp, Donangelo 2008, Chang et al. 2010, Olivares et al. 2012, Mujica-Coopman et al. 2015). Adult women aged 22-31 who received iron supplements for 8 weeks (50 mg/d of iron gluconate) and then for the next 8 weeks iron and zinc (25 mg/d zinc gluconate) supplements had a statistically significantly higher iron serum concentration in both supplemented stages versus a non-supplemented group (Kamp, Donangelo 2008). A positive effect of iron and zinc supplementation on the iron status has been shown in a study with childbearing age women, who received iron (30 mg/d of ferrous sulfate) and zinc supplements (30 mg/d of zinc sulfate) for 3 months (Mujica-Coopman et al. 2015). In contrast, results of an animal study (Bodiga, Krishnapillai 2007) and some hu-

man studies (Wasantwisut et al. 2006, Wieringa et al. 2007) demonstrated that combined iron and zinc supplementation improves iron status but less effectively than iron supplementation alone.

In this study, in rats fed the D diet, a significant decrease of the amounts of iron from stage I to stage III in the prostate and between stage II and stage III in the epididymis was found. Also, in the same experiment, animals fed the D diet compared to those fed the C diet had a depleted iron level in the liver, intestine and kidney (Kaluza et al. 2013) and had statistically significantly lower haematological parameters and serum ferritin concertations (Kaluza, Madej 2015). Lower haematological parameters and iron content in the liver were also found in other studies where animals were fed an iron-deficient diet (Arce, Keen 1993, Alferez et al. 2011). These results are in line with findings based on human studies, i.e. lower haemoglobin concentration (Parikh et al. 2011), serum ferritin (Ahmed et al. 2018), serum iron level, total iron binding capacity and transferrin saturation (Patterson et al. 2001) were observed in people with an iron-deficient diet. It is known that consumption of habitual diet insufficient in iron can lead to iron depletion and anaemia (de Benoist et. al. 2008), and the effect of iron supplementation depends on one's nutritional status. In the case of iron deficiency, the regulation at the level of mucosa enables completion of body stores as well as protection, to a certain extent, from excessively high absorption (Anderson et al. 2009).

It is known that iron and zinc absorption is determined by the quantity of these minerals in diet and by the competition of both cations for a limited number of shared transporters at enterocytes (Olivares et al. 2012). An interaction between iron and zinc could be explained by their competitive binding to the divalent metal transporter 1 (DMT1) and a proton-coupled transporter of a variety of divalent metals (Gunshin et al. 1997). In the study, zinc addition to a diet supplemented with iron does not significantly interfere with the deposition of iron in the reproductive tissues and other tissues such as the intestine, liver and kidney (Kaluza et al. 2013). The same effect of combined iron and zinc supplementation on the iron status was also observed in the blood of adult people (Olivares et al. 2012) and children (Chang et al. 2010). The authors emphasise that the interaction depends on the Fe:Zn ratio in the diet, and indicate that the inhibition weight threshold of iron bioavailability is 5.9:1. In our experiment, zinc administered simultaneously with iron (at the weight ratio 1:1) did not affect the apparent iron absorption in rats fed C-type diets, while the inhibition of iron absorption was found in rats fed D-type diets (Januszko et al. 2016). After stage II, significantly lower iron apparent absorption in the DSZn and DSFeZn compared to the D and DSFe groups was observed, and the lower level of iron absorption stayed in the DSZn group after 2-week cessation of the supplementation. However, in a relatively short time after the beginning of iron or iron and zinc supplementation (on the second day) and after discontinuation of these treatments (on the fifth day), the iron apparent absorption has been

stabilized to a similar level as in the end of the each stage (Januszko et al. 2016). Absorption of iron and zinc depends on the chemical form and food matrices (Sandstrom 2001). In our experiment, rats were fed semi-synthetic diets and iron and zinc were administered as ferric citrate and zinc carbonate, respectively. In a study conducted among children, iron absorption was inhibited significantly when zinc was administered in dumplings as zinc sulfate but not in the zinc oxide form (Herman et al. 2002). In another study carried out among adult women, iron absorption was inhibited when women consumed bread fortified with iron and zinc with black tea (Olivares et al. 2013). Also, iron absorption was inhibited when high doses of zinc (zinc sulfate) were administered to healthy women in aqueous solutions (Olivares et al. 2007). Results of studies suggest that inhibition of iron absorption by zinc is a complex process which depends on many factors and may be controlled by different mechanisms.

Neither in rats fed C-type nor in ones receiving D-type diets did we observe the effect of iron or iron and zinc supplementation on the zinc content in the reproductive tissues and liver, intestine and kidneys (Kaluza et al. 2013). In contrast, in a study conducted among growing rats (initial body weight of 35-45 g), 2-week simultaneous iron (4.0 mg/d of iron citrate) and zinc (3.3 mg/d zinc carbonate) supplementation was associated with a statistically significantly higher zinc concentration in plasma and liver than iron supplementation alone; however, the simultaneous iron and zinc supplementation was less effective than zinc supplementation alone (Bodiga, Krishnapillai 2007). Results obtained by Bodiga and Krishnapillai (2007) can be considered beneficial due to a possible harmful effect of excess of iron as well as zinc on reproductive tissues.

Checking the effect of using supplementation after the termination of the intervention can be considered as the most important strength of the study. The controlled conditions in which the experiment was conducted enabled us to eliminate factors that might have distorted the results. However, differences in the absorption of various chemical forms of iron between rats and humans should be considered. Unlike humans, iron-deficient rats absorb heme iron less effectively than ferrous iron (Wallace 2016). Moreover, feeding animals with semisynthetic diets and hosting them in controlled conditions can also be recognised as a downside due to the limitations in extrapolating the results to humans.

CONCLUSION

A significantly higher content of iron in the testes, epididymis and prostate after the termination of iron or iron and zinc supplementation was found in rats fed an iron-deficient diet, but not in those fed a well-balanced diet. Simultaneous supplementation with iron and zinc was effective to

a similar extent as in the case of iron supplementation alone in the correction of the iron level in reproductive tissues. There is a need for further analysis to explain if the negative effect of high doses of iron can be reduced by simultaneous supplementation of zinc.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study was approved by the Third Local Ethics Commission in Warsaw, Poland (Resolution No. 16/2008).

REFERENCES

Ahmed F., Khan M.R., Shahenn N., Ahmed K.M.U., Hasan A., Chowdhury I.A., Chowdhury R. 2018. *Anemia and iron deficiency in rural Bangladeshi pregnant women living in areas of high and low iron in groundwater*. Nutrition, 51-52: 46-52. DOI: 10.1016/j.nut.2018.01.014

Alferez M.J.M., Diaz-Castro J., Lopez-Aliaga I., Rodriguez-Ferrer M., Perez-Sanchez L.J., Campos M.S. 2011. *Development of nutritional iron deficiency in growing male rats: haematological parameters, iron bioavailability and oxidative defence*. Brit J Nutr, 105(4): 517-525. DOI: 10.1017/S0007114510004046

Anderson G.J., Frazer D.M., McLaren G.D. 2009. *Iron absorption and metabolism*. Curr Opin Gastroenterol, 25: 129-35.

Anjum M.R., Madhu P., Reddy K.P., Reddy P.S. 2017. *The protective effects of zinc in lead--induced testicular and epididymal toxicity in Wistar rats*. Toxicol Ind Health, 33(3): 265-276. DOI: 10.1177/0748233716637543

Arafa M., Agarwal A., Al Said S., Majzoub A., Sharma R., Bjugstad K.B., AlRumaihi K., Elbardisi H. 2017. *Semen quality and infertility status can be identified through measures of oxidation-reduction potential*. Andrologia. DOI: 10.1111/and.12881

Arce D.S., Keen C.L. 1993. *Effects of marginal and severe iron-deficiency on hepatic proteins in developing rats are reversible with dietary iron repletion*. Reprod Toxicol, 7(1): 61-72. DOI: 10.1016/0890-6238(93)90011-U

Bodiga S., Krishnapillai M. N. 2007: *Concurrent repletion of iron and zinc reduces intestinal oxidative damage in iron- and zinc-deficient rats*. World J Gastroenterol, 13: 5707-5717.

Chang S., El Arifeen S., Bari S., Wahed M.A., Rahman K.M., Rahman M.T., Mahmud A.B., Begum N., Zaman K., Baqui A.H., Black R.E. 2010. *Supplementing iron and zinc: double blind, randomized evaluation of separate or combined delivery*. Eur J Clin Nutr, 64(2): 153-160. DOI: 10.1038/ejcn.2009.127

de Benoist B.M., McLean E., Egli I., Cogswell M. 2008. *Worldwide prevalence of anemia 1993e2005: WHO global database on anemia*. World Health Organization, Geneva.

Gunshin H., Mackenzie B., Berger U.V., Gunshin Y., Romero M.F., Boron W.F., Nussberger S., Gollan J.L., Hediger M.A. 1997. *Cloning and characterization of a mammalian proton--coupled metal-ion transporter*. Nature, 388(6641): 482-488. DOI: 10.1038/41343

Herman S., Griffin I.J., Suwarti S., Ernawati F., Permaesih D., Pambudi D., Abrams S.A. 2002. *Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children*. Am. J. Clin. Nutr., 76: 813-817.

Januszko O., Madej D., Brzozowska A., Kaluza J. 2016. *Iron absorption after introducing and*

discontinuation of iron and zinc supplementation in rats. J Trace Elem Med Bio, 35: 77-82. DOI: 10.1016/j.jtemb.2016.01.013

Jeruszka-Bielak M., Brzozowska A., Kaluza J., Pietruszka B. 2011. *Supplementation and fortified product intake versus iron status among female students of WULS-SGGW*. Ann. Nat. Inst. Hyg., 92(3): 603-609.

Kaluza J., Madej D. 2015. *Adverse effect after cessation of rats' unjustified iron or iron and zinc supplementation on hematological parameters but not ferritin concentration*. Clin Nutr, 34(1): 44-48. DOI: 10.1016/j.clnu.2013.12.016

Kaluza J., Madej D., Rusaczonek A., Siedlecka E., Pietruszka B. 2014. *The effect of iron and zinc supplementation and its discontinuation on liver antioxidant status in rats fed deficient diets*. Eur J Nutr, 53(4): 1083-1092. DOI: 10.1007/s00394-013-0610-y

Kaluza J., Madej D., Brzozowska A. 2013. *The effect of iron and zinc supplementation and discontinuation of this practice on iron and zinc level in tissues in rats fed deficient diets*. J Trace Elem Med Bio, 27(4): 334-338. DOI: 10.1016/j.jtemb.2013.03.004

Kamp F., Donangelo C.M. 2008. *Supplementing young women with both zinc and iron protects zinc-related antioxidant indicators previously impaired by iron supplementation*. J Nutr, 138(11): 2186-2189. DOI: 10.3945/jn.108.093260

Kovac J.R. 2017. *Vitamins and antioxidants in the management of male fertility*. Ind J Urol, 33(3): 215-215. DOI: 10.4103/iju.IJU_188_17

Liu Q., Xu C., Ji G., Liu H., Mo Y., Tollerud D.J., Gu A., Zhang Q. 2016. *Sublethal effects of zinc oxide nanoparticles on male reproductive cells*. Toxicology in vitro: an international journal published in association with BIBRA, 35: 131-138. DOI: 10.1016/j.tiv.2016.05.017

Mujica-Coopman M.F., Borja A., Pizarro F., Olivares M. 2015. *Effect of daily supplementation with iron and zinc on iron status of childbearing age women*. Biol Trace Elem Res, 165(1): 10-17. DOI: 10.1007/s12011-014-0226-y

Olivares M., Pizarro F., Gaitan D., Ruz M. 2007. *Acute inhibition of iron absorption by zinc*. Nutr. Res., 27: 279-282.

Olivares M., Pizarro F., Ruz M., de Romana D.L. 2012. *Acute inhibition of iron bioavailability by zinc: studies in humans*. Biometals, 25(4): 657-664. DOI: 10.1007/s10534-012-9524-z

Olivares M., Castro C., Pizarro F., de Romana D.L. 2013. *Effect of increasing levels of zinc fortificant on the iron absorption of bread co-fortified with iron and zinc consumed with a black tea*. Biol. Trace Elem. Res., 154: 321-325.

Oteiza P.I., Olin K.L., Fraga C.G., Keen C.L. 1995. *Zinc deficiency causes oxidative damage to proteins, lipids and DNA in rat testes*. J Nutr, 125(4): 823-829.

Parikh A., Natarajan S., Lipsitz S.R., Katz S.D. 2011. *Iron deficiency in community-dwelling us adults with self-reported heart failure in the national health and nutrition examination survey III: prevalence and associations with anemia and inflammation*. Circ-Heart Fail, 4(5): 599-606. DOI: 10.1161/Circheartfailure.111.960906

Patterson A.J., Brown W.J., Roberts D.C.K., Seldon M.R. 2001. *Dietary treatment of iron deficiency in women of childbearing age*. Am J Clin Nutr, 74(5): 650-656.

Pietruszka B., Brzozowska A. 1999. *Vitamin and mineral supplement use among adults in Central and Eastern Poland*. Nutr Res, 19(6): 817-826. DOI: 10.1016/S0271-5317(99)00043-3

Powell S.R. 2000. *The antioxidant properties of zinc*. J Nutr, 130(5): 1447-1454.

Prasad A.S. 2009. *Zinc: role in immunity, oxidative stress and chronic inflammation*. Curr Opin Clin Nutr, 12(6): 646-652. DOI: 10.1097/MCO.0b013e3283312956

Reeves P.G., Nielsen F.H., Fahey G.C., Jr. 1993. *AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet*. J Nutr, 123(11): 1939-1951.

Sandstrom B. 2001. *Micronutrient interactions: effects on absorption and bioavailability*, Br. J. Nutr., 85(2) 181-185.

Taravati A., Tohidi F. 2016. *Association between seminal plasma zinc level and asthenozoospermia: a meta-analysis study*. Andrologia, 48(6): 646-653. DOI: 10.1111/and.12494

Trumbo P., Yates A.A., Schlicker S., Poos M. 2001. *Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. J Am Diet Assoc, 101(3): 294-301. DOI: 10.1016/S0002--8223(01)00078-5

Tvrda E., Peer R., Sikka S.C., Agarwal A. 2015. *Iron and copper in male reproduction: a double--edged sword*. J Assist Reprod Gen, 32(1): 3-16. DOI: 10.1007/s10815-014-0344-7

Walczak-Jedrzejowska R., Wolski J.K., Slowikowska-Hilczer J. 2013. *The role of oxidative stress and antioxidants in male fertility*. Cent Europ. J Urol, 66(1): 60-67. DOI: 10.5173/ /ceju.2013.01.art19

Wallace D.F. 2016. *The regulation of iron absorption and homeostasis*. Clin Biochem Rev, 37(2): 51-62.

Wasantwisut E.,Winichagoon P., Chitchumroonchokchai C., Yamborisut U., Boonpraderm A., Pongcharoen T. 2006. *Iron and zinc supplementation improved iron and zinc status, but not physical growth, of apparently healthy, breast-fed infants in rural communities of northeast Thailand*. J Nutr, 136: 2405-2411.

Wieringa F.T., Berger J., Dijkhuizen M.A., Hidayat A., Ninh N.X., Utomo B. 2007. *Combined iron and zinc supplementation in infants improved iron and zinc status, but interactions reduced efficacy in a multicountry trial in southeast Asia*. J Nutr, 137: 466-471.

Zago M.P., Oteiza P.I. 2001. *The antioxidant properties of zinc: interactions with iron and antioxidants*. Free Radical Bio Med, 31(2): 266-274.