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Can zinc supplementation ameliorate cadmium-induced alterations in the bioelement content in rabbits?

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The study was designed to investigate the influence of zinc (Zn) supplementation on cadmium-induced alterations in zinc, copper (Cu), and magnesium (Mg) status in rabbits. For this purpose, the concentrations of cadmium (Cd), Zn, Cu, and Mg were estimated in the blood, liver, kidney, and bone. The rabbits were divided in a control group, a Cd group-animals intoxicated orally with Cd (10 mg kg^{-1} bw, as aqueous solution of Cd-chloride), and a Cd+Zn group-animals intoxicated with the same dose of Cd and co-treated with Zn (20 mg kg^{-1} bw, as aqueous solution of Zn-sulphate). Solutions were administered orally, every day for 28 days. Sample mineralisation was performed with concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) (4:1) and metal concentrations were determined by atomic absorption spectrophotometry (AAS). Zinc supplementation improved some of Cd-induced disturbances in bioelement levels in the investigated tissues. Beneficial effects of Zn on Zn and Cu levels were observed in blood, as well as on the Cu kidney level. The calculated values for Cu/Zn, Mg/Zn, and Mg/Cu ratios in blood suggest that Zn co-treatment reduces Cd-induced changes in bioelement ratios in blood.

KEY WORDS: *biometals; biometal ratio; blood; bones; copper; kidney; liver; magnesium; interactions*

Cadmium (Cd) is a widely dispersed toxic metal of current occupational and environmental concern. It is responsible for numerous adverse effects, especially in the liver, lung, and testes following acute intoxication and in the kidney after chronic exposure. Recent data have confirmed the negative effects of low-level cadmium exposure on bone mineral density and calciotropic hormones (1). There are also examples of a significant association between Cd and myocardial infarction (2) as well as between Cd and prediabetes and diabetes mellitus in humans (3). Furthermore, cadmium acts as an inorganic xenoestrogen in humans: there is evidence that Cd possesses estrogenic activity (4, 5), while its thyroid-disrupting activities have been observed in experimental studies (6-8).

Over the past several decades, numerous experimental and epidemiological studies have demonstrated that Cd toxicity involves various cytotoxic and metabolic effects and multiple mechanisms, such as the induction of oxidative stress and apoptosis, aberrant gene expression, altered DNA structure, inhibition of ATP production in mitochondria, etc. (9-14). Furthermore, Cd toxic effects, especially as a result of chronic low-level exposure, can be connected with altered homeostasis of some bioelements (15, 16).

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In 1970s, the TASK group on metal interactions (17) underlined the fact that toxic metals, Cd being one of them, can change the fate of biometals and *vice versa*, that some biometals can influence the absorption and distribution of Cd. This was followed by many studies on Cd interactions with zinc (Zn), copper (Cu), iron (Fe), calcium (Ca), or magnesium (Mg), which revealed that Cd intoxication influenced the homeostasis of biometals, causing predominantly their secondary deficit (15-19). Our previous investigation also confirmed disbalance of Zn, Cu, and Mg in rabbits exposed to prolonged Cd intoxication (20-22), as well as in mice and rats exposed to acute and subacute Cd intoxication (23-25). Furthermore, our results pointed to a significant decrease in Zn and Mg levels in blood and an increase in Zn content in the urine of nickel-cadmium battery workers (26).

On the other hand, there is growing evidence that supplementation with certain essential elements, especially Zn, could have a protective role against Cd toxicity (27-31). The antagonism between Cd and Zn is well documented and is probably one of the most investigated toxic metal-bioelement interactions (32, 33). Thus, Rogalska *et al*. (29) concluded that Zn supplementation during chronic cadmium exposure may have a protective role against the proatherogenic action of Cd by preventing hyperlipidemia and lipid peroxidation in rats. The hepatoprotective Zn impact, observed in a prolonged Cd treatment of rats, was attributed to the antioxidative, antiapoptotic, and anti-inflammatory properties of Zn, as well as to its ability to stimulate regenerative processes and to reduce non-MT-bound Cd levels in the liver (30, 34). A Zn co-treatment for four weeks succeeded in preventing Cd accumulation in the kidneys of rabbits (27), as well as Cd-MT induced proteinuria and calciuria in rats (35). A beneficial effect on the renal function was also observed in acute cadmium exposure in the investigation performed on the proximal tubules of rats. This was explained by Zn and Cd competition for transport proteins DMT1 and ZnT1 (28, 36). Furthermore, Zn supplementation lowered the risk of bone fractures, and increased bone density and biochemical bone properties in animals intoxicated with Cd for six months (37, 38). A beneficial, i.e. protective role of Zn against Cd toxicity is rather well documented and is prevalently explained by the ability of Zn to ameliorate Cd-induced oxidative stress, apoptosis, and necrosis (28, 29, 39).

Hu et al. (40) have even shown a protective effect of Zn against Cd carcinogenicity in rats, as they observed a decrease in proto-oncogene, c-jun and c-fos, expression and the increase in metallothionein (MT) genes' expression in prostate and p53 genes in testes. Additionally, Zn treatment of HeLa cells completely abolished the inhibition of Cd-induced DNA-protein interactions, which are essential for DNA repair (41). A recent investigation proved that Zn treatment boosted the immune function and the proliferation of lymphocytes in cadmium-treated rats (42).

Since our previous study confirmed the beneficial effect of Zn on Cd content in blood and organs of rabbits exposed to prolonged Cd intoxication (27), the aim of this study, performed under the same experimental conditions, was to find out whether Zn supplementation could counteract Cd-induced disbalance of bioelements.

MATERIALS AND METHODS

Chemicals

All reagents and chemicals used were of analytical grade quality or higher purity. Cadmium chloride ($CdCl_2$ x H_2O) and zinc sulphate ($ZnSO_4$ x $7H_2O$), trace-pure concentrated nitric (HNO_3) and perchloric ($HClO_4$) acids, as well as metals standard solutions for atomic absorption spectrometry (AAS) were purchased from Merck (Darmstadt, Germany). Double-distilled water was used in the metal analysis.

Animals and experimental protocol

The experiment was performed on *Oryctolagus cunniculus*–Belgian hare rabbits, weighing 2.5-3.5 kg. Throughout the experiment, the animals were maintained in accordance with institutional and international guidelines (European Community Guidelines). The experimental protocol was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia.

Animals were kept under controlled conventional conditions (temperature 22±2 ºC, relative humidity of 50±10 %, 12 h light-dark cycle) and were housed individually in standard cages. They had free access to drinking water and standard pellet diet (Complete mixture for young rabbits "Smeša K 16 % proteina" The Veterinary Institute Subotica, Republic of Serbia) which contained min. 16 % protein, max. 12 % cellulose, min. 1.0 % Ca, min. 0.8 % P, min. 50 mg kg^{-1} Zn, and min. 8 mg kg^{-1} Cu (manufacturer's data). The following concentrations of metals were determined in our laboratory: 91 mg kg^{-1} Zn, 21 mg kg^{-1} Cu, 2.4 g kg^{-1} Mg, and 19.2 µg kg^{-1} Cd in diet and 148 µg L^{-1} Zn, 10 µg L^{-1} Cu, 15 mg L^{-1} Mg, while Cd concentration was under 0.1 µg L^{-1} in drinking water.

The rabbits were randomly divided into three groups, eight animals in each:

Control: non-treated animals.

Cd group: rabbits given a dose of 10 mg kg^{-1} body weight (bw) Cd orally, by orogastric tube, every day for four weeks, in the form of an aqueous solution of $CdCl_2$ (the same Cd dose was applied in our previous investigation) (43).

Cd+Zn group: rabbits exposed first to the same dose of Cd and then, one hour later, supplemented orally with Zn, 20 mg kg^{-1} bw, as an aqueous solution of $ZnSO_4$.

Before and during intoxication (0, 10th, 14th, 18th, 22nd, 25th, and 28th day), blood samples were taken from the ear arteries using a cannula and collected in tubes with sodium heparin as anticoagulant.

At the end of the experiment (28th day), all animals were sacrificed by injection of 3 mL of a 50 g L^{-1} sodium pentobarbitone solution in the marginal vein of the ear, followed by air embolism. The liver, kidney, and bone were excised and stored frozen (-20 °C) until analysis.

Sample preparation and analytical method

Samples of whole blood and organs were mineralised with concentrated HNO_3 and $HClO_4$ in 4/1 ratio. After mineralisation and dilution with 0.1 mol L^{-1} HNO_3, metals were determined by flame atomic absorption spectrophotometry (FAAS, instrument GBC 932AA, Dandenong, Australia). The accuracy of the AAS analyses was validated with a reference sample from the National Bureau of Standards (NIST SRM 1577a bovine liver, National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

Statistical analyses

Statistical analyses of results were conducted by one-way analysis of variance (ANOVA) followed by the LSD multiple comparison test for metal concentrations in blood and organs, as well as for Cd-bioelement ratios in blood.

All values are presented as the means±SD. The acceptable level of significance was set at $P<0.05$. All calculations were prepared with EXCEL 2007 and SPSS package PASW Statistics 18.

RESULTS

Zn, Cu, and Mg concentrations in blood and organs of rabbits exposed to Cd and co-treated with Zn

As for Zn, its level was reduced by about 30 % at the end of the experiment in the Cd group, when compared to controls. However, in the Cd+Zn group, Zn levels did not differ significantly from controls (Figure 1A). Although cadmium intoxication significantly elevated hepatic Zn, the Zn liver content did not change in the Cd+Zn group when compared with the Cd group. A similar pattern was observed in bones. In the kidney, no statistically significant changes of the Zn content were observed in either Cd or Cd+Zn group if compared with controls (Table 1).

Cu blood levels in the Cd+Zn group were significantly reduced if compared with Cu in the blood of Cd intoxicated animals, although these levels were still significantly higher than in controls (Figure 1B). Zn supplementation had a beneficial effect on the Cu content in the kidney: Cu levels significantly increased in the Cd group but did not change significantly in the Cd+Zn group if compared with controls. No changes in the Cu content in the liver and bone were observed either between Cd and Cd+Zn groups or between these groups and controls (Table 1).

Zn supplementation had no effect on the Mg level in blood, which reduced after Cd intoxication (Figure 1C). Mg concentration in the liver, kidney, and bone did not change significantly in both Cd and Cd+Zn group if compared with controls.

The results on bioelement concentrations in the blood and organs of animals treated with Cd have already been published (43).

Zn, Cu, and Mg ratios in the blood of rabbits exposed to Cd or Cd+Zn

Bioelement ratios Cu/Zn, Mg/Zn, and Mg/Cu were calculated for blood. Figure 2 presents the ratios of bioelements for the control, Cd (results previously presented in Ref. 43), and Cd+Zn groups in blood. Zinc co-treatment counteracted the Cd-induced increase in Cu/Zn and Mg/Zn ratios and the Cd-induced decrease in Mg/Cu ratio in blood.

DISCUSSION

The obtained results indicate that Zn supplementation improved the Cd-induced changes in the Zn and Cu contents in certain rabbit tissues but had no beneficial effect on the Mg status in rabbits.

In blood, supplementation with Zn had a beneficial effect on the Zn content, which was reduced by about 30 % in Cd-intoxicated animals if compared with controls. Co-treatment with Zn elevated blood Zn to control levels. This could be explained by the fact that Cd and Zn are absorbed from the gastrointestinal tract using the same divalent transport systems, such as divalent metal transporter-1 (DMT-1) and ZIP transporter family (18). Both metals compete for the same sites on these transporters and, consequently, the supplementation with Zn favours its absorption and increases its blood level. This finding is in accordance with the results of Brzóska et al. (37) who confirmed that Zn application in rats exposed to 5 mg L^{-1} Cd (by drinking water containing a concentration of either 30 or 60 mg L^{-1} Zn for 12 months) resulted in Zn serum levels that did not differ from controls. However, the same

Table 1 *The effect of Zn supplementation on Zn, Cu, and Mg concentrations in organs of rabbits after 28 days of Cd intoxication*

	Controls[1)]	Cd group[2)]	Cd+Zn group[3)]
Zn (µmol kg^{-1})			
Liver	640.73±112.27	1037.02±128.61**	1133.85±360.42***
Kidney	677.15±106.09	825.39±178.96	755.49±155.56
Bone[a)]	3.75±0.42	2.61±0.32*	2.72±0.42*
Cu (µmol kg^{-1})			
Liver	52.11±9.64	55.55±9.26	46.32±9.23
Kidney	55.07±8.58	74.88±11.37*	48.53±5.58†††
Bone	199.65±34.67	201.74±40.27	200.46±32.35
Mg (mmol kg^{-1})			
Liver	14.37±1.91	14.94±1.94	12.17±2.70†
Kidney	14.19±2.87	14.99±3.12	12.07±3.05†
Bone	343.46±22.31	340.34±20.41	333.29±31.46

*[1)]Controls – non-treated animals; [2)]Cd group - intoxicated orally every day for four weeks with Cd (10 mg kg^{-1} bw); [3)]Cd+Zn group – given Zn (20 mg kg^{-1} bw) one hour after Cd treatment; [a)]mmol kg^{-1}; Values are presented as the means±SD. Marked values differ significantly (ANOVA + LSD test) from * control group, † Cd group; *, † P<0.05; **, †† P<0.01; ***, ††† P<0.001*

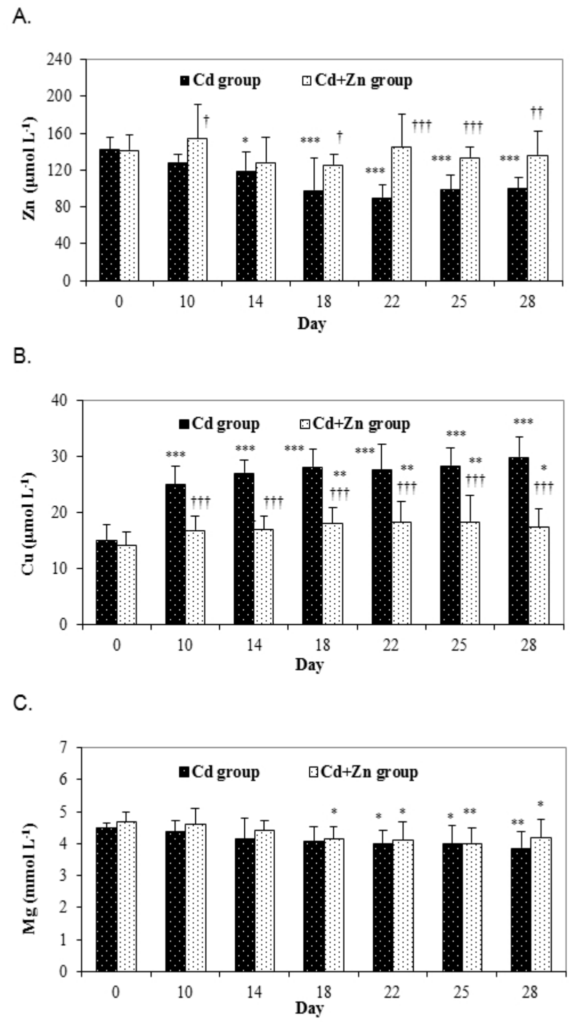

Figure 1 *Zn (A), Cu (B), and Mg (C) concentrations in the blood of rabbits intoxicated with Cd and co-treated with Zn after 10, 14, 18, 22, 25, and 28 days; Cd group – intoxicated orally for four weeks with Cd (10 mg kg^{-1} bw per day); Cd+Zn group – given Zn (20 mg kg^{-1} bw) one hour after Cd treatment. Marked values differ significantly (ANOVA + LSD test) from: * day 0 and † Cd group; *, † P<0.05: **, †† P<0.01; ***, ††† P<0.001*

authors did not prove the protective effect of Zn when a higher dose of Cd was used (50 mg L^{-1}). A positive effect of Zn co-treatment has also been proven in an *in vitro* study (44) performed on Caco-2 TC7l cells – a model system for the investigation of intestinal epithelium. Moreover, literature data indicate that not only does Zn supplementation ameliorate the Cd-induced disturbances of Zn levels, but it also influences and prevents Cd atherogenic effects through its effect on lipid metabolism whereby it prevents hyperlipidemia and hypercholesterolemia (29, 45).

A pronounced and rapid increase in blood Cu observed in Cd-intoxicated animals was not completely counteracted by Zn supplementation although Zn produced a significant decrease in blood Cu if compared with rats treated with Cd only. This phenomenon may be of concern since Cu is a Fenton metal that induces reactive oxidative species production and could be explained by Zn and Cu competition for the same metal transporters in cell membranes, as well as for MT in intestinal mucosa cells (46, 47).

Contrary to the effect on Zn and Cu, co-treatment with Zn had no beneficial effect on the Mg blood content, which was significantly lowered in rabbits intoxicated with Cd only. It could be explained by Mg homeostasis, which is strictly controlled by intestinal absorption, its accumulation in bones, and elimination via urine. Furthermore, up-to-date literature data on metal transporters indicate that Zn and Mg, *in vivo*, probably use different transport systems that are hardly mutually influenced. The protein ZIP family is involved in Zn transport, as well as in the transport of some other metals but unlikely in Mg transfer (18, 48). High affinity of TRPM7 transporters is proposed predominantly for Mg and Ca, and to a lesser extent for Zn (49). Thus, the omitted beneficial effect of Zn could be more likely the consequence of forced Mg urine elimination induced by Cd (our unpublished data). However, in the study with rats simultaneously treated with Cd and Zn, the plasma Mg levels and rate of urinary Mg elimination were in the range of controls indicating a beneficial effect of Zn (28). Similar results were obtained in sheep intoxicated with Cd and supplemented with Zn: Zn succeeded to restore normal Mg levels after their initial increase (50).

Cadmium intoxication had a strong influence on the Zn liver level resulting in two-times elevated Zn levels if compared with controls. Zn treatment was unable to sufficiently ameliorate this effect. Our results are in accordance with the study of Rogalska *et al.* (30) who also demonstrated zero effect of Zn supplementation on Cd-induced enhancement of hepatic Zn level (six months of exposure to 50 mg L^{-1} Cd and 30 or 60 mg L^{-1}Zn in drinking water). These findings could be explained by the fact that Zn supplementation did not induce a decrease in the Cd content in rabbits intoxicated with Cd (27) although a strong influence of Cd on MT synthesis induction in the liver should also be taken into account. Furthermore, the protective effect of Zn against Cd toxicity could be also connected with some specific Zn mechanisms and properties that cannot be simply explained by Zn and Cd direct interactions. Thus, in a study of Jihen *et al*. (34), 500 mg L^{-1} of Zn supplementation in drinking water induced a strong protective Zn effect against Cd toxicity in rats treated with 200 mg L^{-1} Cd for five weeks, although no influence on hepatic Zn level was observed.

It should be emphasised that the effect of excessive Zn intake on Cu kidney levels was similar to the Zn effect obtained in blood but was even more pronounced as the levels of Cu reached the control ones. This finding is of special importance having in mind that the kidney is the target organ of Cd toxicity and is in accordance with our previous investigation that pointed to a significant reduction of Cd levels caused by Zn co-treatment (27). Contrary to our results, administration of Zn concomitant with Cd in the form of Cd-MT did not change Cu concentration neither

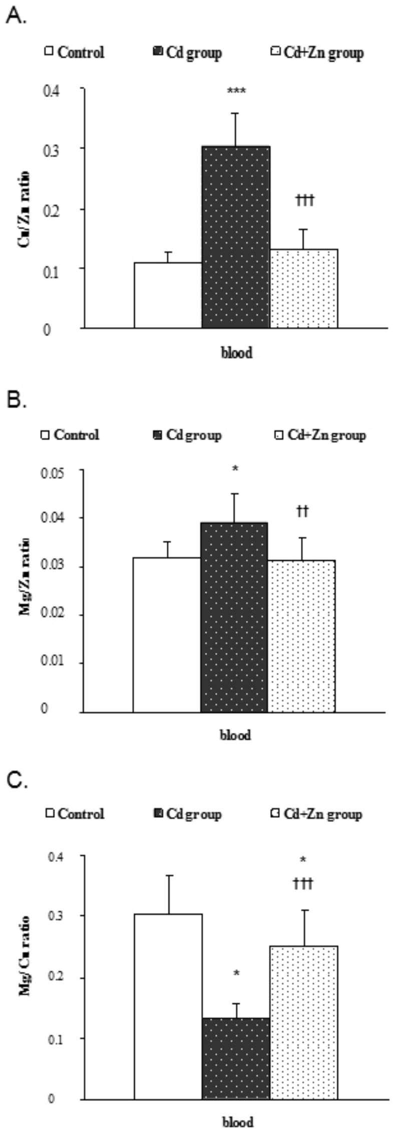

Figure 2 *The effect of Cd exposure and Zn supplementation on the Cu/Zn ratio (A), Mg/Zn ratio (B), and Mg/Cu ratio (C) in the blood of rabbits after 28 days of treatment; Controls – non-treated animals; Cd group intoxicated orally for four weeks with Cd (10 mg kg^{-1} bw per day); Cd+Zn group given Zn (20 mg kg^{-1} bw) one hour after Cd treatment. Marked values differ significantly (ANOVA + LSD test) from * control group and † Cd group; *, † P<0.05: †† P<0.01; ***, ††† P<0.001*

in the liver nor in the kidney of rats (35). The effect of Zn supplementation on Cu levels can be at least partly explained by the reduction of Cd body burden induced by Zn co-treatment. However, the observed Zn effect on Cu levels could be the consequence of interactions between these bioelements in the organism. Up-to-date knowledge on Zn and Cu interactions suggests their physiological antagonism, which is confirmed by the usage of Zn in the treatment of Wilson's disease (46, 47).

Osteotoxicity of Cd is well documented and could be at least partly explained by the interactions between Cd and bioelements in bones (37, 38). However, under our experimental conditions, the applied Cd dose of 10 mg kg^{-1} bw did not induce significant changes in the Cu and Mg content, although a significant decrease was achieved for Zn (43). No beneficial effect of Zn supplementation on the content of bioelements in bone was confirmed. Brzóska *et al*. (37) investigated the effect of Zn supplementation with two different doses (30 and 60 mg L^{-1} in drinking water) on the femur Zn concentration in rats exposed to 5 or 50 mg L^{-1} Cd for 6 and 12 months. Both administered doses of Zn succeeded in preventing Zn deficit caused by low dose of Cd (5 mg L^{-1}). In rats exposed to a high dose of Cd (50 mg L^{-1}), the protection was observed only at a higher dose of Zn (60 mg L^{-1}) after six months. Our results and the results of Brzoska *et al*. (37) suggest that the interactions between Cd and Zn are dose-dependent. Furthermore, these investigations underline the duration of treatment as an important issue, indicating that the beneficial effect of Zn on its bone content in an organism exposed to Cd can be achieved only after prolonged Zn treatment.

With the goal to obtain more information on the effect of Zn supplementation on Zn, Cu, and Mg distribution pattern in the organism, the ratios of Cu/Zn, Mg/Cu, and Mg/Zn in blood were calculated. Literature data, although rare, indicate that the ratios of bioelements provide a better insight into bioelement fate and their interactions in the organism under specific pathological conditions (51). Our results show that Zn had a beneficial effect on all investigated ratios in blood suggesting its positive effect on biometal distribution in blood. It should be emphasised that literature data underscore the Cu/Zn ratio as a potential blood biomarker of different health disorders. Thus, increased values of the Cu/Zn ratio were determined in developmental and behavioural disorders in children (52) and in some cases of cancer (53).

On the basis of our results, it can be concluded that Zn supplementation under the conditions of Cd exposure prevents the accumulation of Cd in the organism. Furthermore, it exerts beneficial effects, at least partly, on Cd induced disturbances in bioelements Zn and Cu, with the most pronounced beneficial effect observed in blood.

Acknowledgements

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Može li utjecaj suplementacije cinkom poboljšati kadmijem izazvane promjene u razinama bioelemenata u kunića?

Istraživanje je provedeno da bi se ispitao utjecaj suplementacije cinkom (Zn) na promjene u razinama cinka, bakra (Cu) i magnezija (Mg) u kunića tretiranih kadmijem (Cd). U tu su svrhu koncentracije Cd, Zn, Cu i Mg određivane u krvi, jetri, bubregu i kostima. Kunići su bili podijeljeni u tri skupine: kontrolna skupina, Cd skupina – životinje koje su trovane kadmijem (10 mg kg^{-1} tjelesne mase, vodena otopina Cd-klorida) i u Cd+Zn skupinu – životinje koje su primale istu dozu kadmija i suplementaciju cinkom (20 mg kg^{-1} tjelesne mase, vodena otopina Zn-sulfata). Otopine su davane 28 dana oralnim putem. Uzorci su mineralizirani koncentriranom dušičnom kiselinom (HNO_3) i perklornom kiselinom ($HClO_4$) (4:1), a koncentracija metala određena je primjenom atomske apsorpcijske spektrofotometrije (AAS). Suplementacija cinkom uspjela je ublažiti poremećaj u razini bioelemenata, do kojega je dovela izloženost kadmiju u ispitivanim organima. Primjena cinka imala je povoljan učinak na razinu Zn i Cu u krvi i bubregu. Analiza odnosa bioelemenata u krvi, izražena kao Cu/Zn, Mg/Zn i Mg/Cu, upućuje na to da primjena cinka može u značajnoj mjeri smanjiti promjene u odnosima ispitivanih bioelemenata do kojih dovodi izloženost kadmiju.

KLJUČNE RIJEČI: *bakar; biometali; bubreg; interakcije; jetra; kosti; krv; magnezij; omjer biometala*