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EFFECTS OF VANADIUM COMPLEXES SUPPLEMENTATION ON V, Fe, Cu, Zn, Mn, Ca AND K CONCENTRATION IN STZ DIABETIC RAT'S SPLEEN

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Abstract: The objective of the study was to assess the effects of five vanadium organic complexes administered with small insulin injection, on V, Fe, Cu, Zn, Mn, Ca and K concentration in STZ (streptozotocin) diabetic rats tissues during a 5-week treatment with the tested complexes. In all groups of animals, metal concentration in a dry spleen samples was investigated by the proton induced X-ray emission (PIXE) method. Obviously, vanadium tissue concentration was higher in vanadium-treated rats. Concentration of vanadium in the spleen was $\bar{x}$ = 21.3 μg/g of dry sample. Vanadium administration influenced other metals concentration of rats tissues. The most pronounced influence of vanadium was observed on iron concentration in the spleen. All results were calculated for correlation between different groups of animals. Present study showed small interferences between trace element changes in diabetic, or non diabetic rats after vanadium treatment. Measured elements, especially zinc, manganese and copper, are co-factors of enzymes and their content changes can influence on organism homeostasis in diabetes treatment. Understanding and recognizing these relationship may permit better diabetes treatment in the future.

Keywords: rats, PIXE, diabetes, vanadium, trace elements, spleen

Vanadium is one of the trace elements essential for living organisms. Its concentration in typical tissues ranges from 0.1 to a few μg/kg. Several studies have shown its important role in biochemical mechanisms, especially for azotobacter (1, 2), ascidians (3, 4), mushrooms (5), chickens (6) and mammals (7). In the process of experiments on animals, vanadium demonstrated anti-diabetic (8), anti-HIV and anti-cancer (9) activity, the influence on oxidative defense (10), on bone growth (11) and several others. The most of the animal experiments were devoted to vanadium anti-diabetic role in both types – 1 and 2 – of diabetes. Some results have also been presented in the papers concerning vanadium treatment in patients (8). However, despite these positive aspects, vanadium could accumulate in living organisms and have toxic effects. Toxic effects were associated with the type of vanadium compounds and with the administered doses. In most cases, biochemical changes were studied after vanadium application. In the present study, the changes in concentration of iron, copper, zinc, manganese, calcium, and potassium in the spleen are presented. The spleen is a limphopoietic organ which enables the portal circulation and in healthy subjects, it has limited volume. The role of the spleen and its functions change during ontogeny. In adults, the spleen is an organ primarily limphopoietic and acts as a filter, which comes into contact with foreign antigens in immunologically competent cells. In people with diabetes, the spleen may accumulate the elements that protect the body from an excess of free radicals.

MATERIALS AND METHODS

Animals and vanadium administration

Male Wistar rats weighing between 220–250 g were adapted for a 12 h/12 h day/night cycle, (day

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was from 10 a.m. to 10 p.m.) with humidity ranging between 75–85%. The animals were divided into 7 groups of 6 animals in each group. After 3 days from the beginning of the experiment, 55 mg of streptozotocin in citric buffer, pH 4.5 (0.1 mol/L) solution per 1 kg of body mass was injected into the caudal vein in the volume of 1 mL/kg of body mass in all groups of animals. Three days subsequent to the injections, the content of glucose was measured by Exac Tech (Medisense) strip glucometer. The glucose content, measured in animal blood, was higher than at least 17 mmol/L. After the measurement, the rats were separated into the following tested groups: diabetic control rats (D), diabetic rats with insulin (Di), 5 groups of diabetic rats with insulin and tested complexes (Di 1–5). The tested complexes of vanadium in water solution were administered once a day before 10 a.m. by gavage in dose of 50 μmol/kg and 1 U/kg of insulin was injected subcutaneously. Five weeks after the beginning of the complexes administration, the rats were anesthetized (thiopental 50 mg/kg) and then the spleens were collected. The organs were kept frozen at –20°C until the time of analysis. Experiments got the agreement from Local Ethic Commission in Kraków.

Synthesis of vanadium complexes

Bisperoxo complexes of vanadium(V) of $Na[VO(O_2)_2 L] \times n\ H_2O$ type (complexes 1-3)

Ten millimoles of $NaVO_3$ was dissolved in molar excess of 10% H_2O_2 (molar ratio of H_2O_2 to vanadium 1:3). To the obtained clear, yellow solution cooled in the ice bath, 20 mL of ethanolic solution containing 10 mmol of proper polypyridine (L = 2,2'-bipyridine, 1,10'-phenanthroline or 4,4'-dimethyl-2,2'-bipyridine) was added dropwise with constant stirring. Temperature of the reaction mixture did not exceed 10°C during the synthesis. Afterwards, 50 mL of cooled ethanol was added to precipitate yellow crystals. The solid phase was filtered off *via* a glass frit and washed with 10 mL of cold ethanol. The obtained vanadium complex was dried in the air, in a dark place, for 24 h, then was collected and stored in refrigerator.

Vanadyl complexes of $[VO(SO_4) L] \times n\ H_2O$ type (complexes 4 and 5)

To a hot solution of $VOSO_4 \times 5\ H_2O$ (5 mmol) in ethanol (30 mL), containing the amount of water required to dissolve $VOSO_4$, an equimolar hot solution of proper polypyridine (L= 2,2'-bipyridine, 1,10'-phenanthroline) in ethanol (30 mL) was added dropwise with stirring. The solution was cooled to room temperature and concentrated if required. The green precipitate which occurred was filtered off *via* a glass frit, washed with ethanol and ethyl ether and dried *in vacuo*. The synthesis was carried out under argon atmosphere in order to prevent the oxidation of vanadium(IV). The purity of the complexes was confirmed by microanalysis and IR spectroscopy. Five different complexes of vanadium were used in the experiments:

1. $Na[VO(O_2)_2(2,2'\text{-bpy})] \times 8\ H_2O$ – sodium (2,2'-bipyridine)oxooxidobisperoxovanadate(V) octahydrate;
2. $Na[VO(O_2)_2(1,10'\text{-phen})] \times 5\ H_2O$ – sodium (1,10'-phenanthroline)oxooxidobisperoxovanadate(V) tetrahydrate;
3. $Na[VO(O_2)_2(4,4'\text{-}Me_2\text{-}2,2'\text{-bpy})] \times 8\ H_2O$ – sodium (4,4'-dimethyl-2,2'-bipyridine)oxooxidobisperoxovanadate(V) octahydrate;
4. $[VO(SO_4)(1,10'\text{-phen})] \times 2\ H_2O$ – oxooxido(1,10-phenanthroline)[tetraoxosulfato(2-)-O,O']vanadium(IV) dihydrate;
5. $[VO(SO_4)(2,2'\text{-bpy})] \times H_2O$ -(2,2'-bipyridine) oxooxido[tetraoxosulfato(2-)-O,O']vanadium (IV) monohydrate.

Spleen tissue preparation

Frozen spleen tissue was transferred directly to lyophilizing cabinet and lyophilized using Labconco Freezone 4.5 L at the temperature from –40 to –53°C, and under pressure of 1–14 Pa. Then, the lyophilized spleen tissue was homogenized and pressed into pellets, about 1 mm thick and 10 mm in diameter, using pressure of 15 MPa. Such pellets were placed on Scotch tape and attached to an aluminum frame.

PIXE analysis

The PIXE (proton induced X-ray emisson) analysis was performed at the Institute of Nuclear Physics of PAN (IFJ PAN) in Kraków. A 2 MeV proton beam from the Van de Graaff accelerator was directed at the sample for a multi-trace element analysis. In order to get a good statistics of the X-ray characteristic spectra, each sample was measured for 20 min. The series of 14 samples with 2 standards (IAEA H-8 Horse Kidney and National Standards & Technology Standard Reference Material 1577b Bovine Liver) were installed simultaneously in the PIXE chamber. All the spectra were detected with Si-Li detector using the energy resolution of 190 eV for the 5.9 keV line of the emitted X-ray spectra. The normalization was calculated on the basis of detected back-scattered protons. Both the X-ray and back-scattered protons were registered

with CAMAC system. All the collected spectra were analyzed by GupixWin ver. 2.0 software.

RESULTS

All results, showing the content of metal concentration in the rat spleen, are presented below as tables containing the information on statistical differences between the groups of rats ($p < 0.05$). The statistical calculations were performed using Statistica 7.1 program. Differences between the studied rats groups were estimated using a non-parametric Kruskal-Wallis test that enables to compare three or more unpaired groups. To quantify the metal concentration in the rat spleen, median values have been calculated.

Vanadium

The content of vanadium in diabetic not-treated rats (D) and insulin injected diabetic ones (Di) was in the range of 0.27–0.36 mg/kg of dry tissue. Similar data have been reported by Frank et al. (12). In all rat groups treated with vanadium compounds, the metal was administered in the dose of 50 μmol/kg of body mass. The results show that independently of the chemical structure, the concentration of vanadium in the rat's spleen was more than 20 times higher as compared to the uptake content of healthy rats (Tab. 1). This observation was similar to data presented by Cremer et al. (13) where rats were treated with isotope ^{48}V. The total absorption of vanadium after oral administration of the tested complexes was not calculated, but since it is widely known that the content of vanadium measured in intestinal system equals roughly ten percent of administered dose (14), we assumed such content of vanadium uptake. Concentration of vanadium in spleens observed in the group of vanadium-treated rats ranged from 20.82 to 21.73 mg/kg of dry tissue. Our results have shown no differences between 5 rat groups tested with different vanadium complexes, which was confirmed by the statistical non-parametric Kruskal-Wallis test. It is known that the presence of metal administration can influence the content of other metals such as iron, copper, zinc, manganese, calcium or potassium (7). Therefore, in our analysis the concentrations of these metals have been determined.

Iron

Natural iron concentration in the spleen was remarkably high (216.3–474.4 mg/kg dry mass, Tab. 1) in comparison to other tissues with good blood supply (liver, heart). Our data were similar to those reported by Olusola et al. (15) for the spleen of Wistar albino and Wild Black rats. Also, Hamdaoui et al. (16) observed that the spleen shows the highest iron concentration compared to other tested organs. Iron concentration is strongly associated with the spleen function in mammals. Indeed, the spleen eliminates damaged erythrocytes and stores iron needed for hemoglobin synthesis, which takes place in bone marrow. More than 60% of the total amount of iron in the organism is bound to hemoglobin in erythrocytes and myoglobin in muscles. In our measurements, the concentration of iron in the spleen was much diversified depending on the tested rat group. In the diabetic not-treated rats (D), and also in the insulin injected diabetic group (Di), iron concentration was the lowest. The values for these groups were statically insignificant and, furthermore, they were lower than the value obtained for Di5 vanadium rats (330 mg/kg *vs*. 652 mg/kg). A similar effect was also observed by Guenno et al. (17). The treatment of rats with different vanadium compounds shows the sta-

Table 1. Concentration of V, Fe, Cu and Zn in the spleen of the investigated groups of rats.

Group	V [mg/kg dry mass]	Fe [mg/kg dry mass]	Cu [mg/kg dry mass]	Zn [mg/kg dry mass]
D	0.27 ± 0.04	330.56 ± 94.58	13.21 ± 1.59	22.43 ± 2.36
Di	0.36 ± 0.04	347.99 ± 97.24	23.96 ± 10.00	24.07 ± 28.81
Di1	21.38 ± 0.85*	460.03 ± 136.51	36.07 ± 12.48*	102.90 ± 15.65*
Di2	21.73 ± 0.82*	597.28 ± 98.52*	36.90 ± 9.18*	109.71 ± 25.38*
Di3	21.05 ± 1.18*	647.78 ± 169.51*	36.79 ± 6.10*	65.51 ± 8.08*
Di4	20.82 ± 0.74*	652.48 ± 68.86*	28.95 ± 8.87*	78.21 ± 20.10*
Di5	21.19 ± 0.93*	652.06 ± 106.56*	26.52 ± 5.50*	56.91 ± 14.68*

Diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1 – $Na[VO(O_2)_2(2,2'\text{-bpy})] \times 8\ H_2O$, Di2 – $Na[VO(O_2)_2(1,10'\text{-phen})] \times 5\ H_2O$, Di3 – $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \times 8\ H_2O$, Di4 – $[VO(SO_4)(1,10'\text{-phen})] \times 2\ H_2O$, Di5 – $[VO(SO_4)(2,2'\text{-bpy})] \times H_2O$). * Statistical differences ($p < 0.05$) measured between concentrations of trace elements.

tistical differences between diabetic vanadium not-treated groups (D and Di) and diabetic vanadium treated groups (Di2–5). The smallest difference was observed for the Di1 group while the other vanadium treated groups (Di2–5) exhibited higher iron contents. All vanadium complexes (2–5) – excluding complex 1 – established the Fe concentration in the spleen the same as in the content found in the control group (D).

Copper

The copper content in the rat spleen shows similar behavior as that observed for iron (Tab. 1). Analogously, the copper concentration was the lowest in the group of diabetic not-treated rats (D) and also in the insulin injected diabetic group (Di). However, for the latter group, a large value of distribution was obtained, indicating probable distinct uptake of copper by the rats. All vanadium-treated animals showed higher Cu concentration in the organ in comparison to those of the D group. Uriu-Adams et al. (18) reported that copper concentration is usually higher in diabetic animals, in some organs such as the thymus and the uterus. Since there are no papers reporting the influence of vanadium and diabetes on copper concentration in the spleen, it is difficult to discuss the copper concentration in the spleen in diabetic rats. However, our results show a general trend of higher copper contents in the diabetic rats.

Zinc

As shown in Table 1, zinc concentration was the lowest in the case of diabetic not-treated rats (D – 22 mg/kg), similarly to the contents of iron or copper. In the diabetic group with insulin administration (Di), Zn concentration was very low (24 mg/kg) but still higher than in the diabetic group (D). In the vanadium-treated animal groups, the Zn concentration increased but the content was dependent on the type of vanadium complexes used. In the Di1 and Di2 animal groups Zn reached 102 and 109 mg/kg, respectively (Tab. 1). The other vanadium compounds demonstrated lower uptake content, comparable with that observed in the control group of rats. In the vanadium complex-treated animals (Di3, Di5), zinc concentration content was similar in both groups.

Manganese

The manganese content in the spleen, shown in Table 2, remained almost stable in all tested groups of animals except for the Di3 group. The significant differences between vanadium complex-treated rats (groups Di3 and Di5) and control rats (D) were noticed (Tab. 2). Both tested complexes (3 and 5) contained the same ligand (bipyridine) but they differed in vanadium oxidation state. Apparently, either vanadium oxidation state or ligand type may affect manganese concentration in the spleen. In the other tested cases, vanadium showed no influence on the Mn content in the spleen. These results demonstrated that vanadium supplementation affects manganese concentration in the same pattern as reported by Thompson et al. (19).

Calcium

The calcium level in the spleen of rats treated with vanadium(V) was significantly higher compared to the amount of this element in the animals in

Table 2. Manganese, calcium and potassium content in animal in the spleen.

Group	Mn [mg/kg dry weight]	Ca [mg/kg dry weight]	K [mg/kg dry weight]
D	5.84 ± 1.96	114.50 ± 19.29	8.16 ± 0.69
Di	7.07 ± 1.77	110.28 ± 30.92	13.92 ± 3.46*
Di1	8.53 ± 3.35	130.62 ± 68.86*	16.70 ± 4.04*
Di2	8.67 ± 2.73	157.36 ± 28.74	16.09 ± 2.92*
Di3	9.74 ± 0.82*	109.59 ± 16.99*	15.44 ± 0.62*
Di4	8.52 ± 2.16	162.99 ± 60.62*	13.40 ± 1.77*
Di5	7.47 ± 1.90*	145.30 ± 33.02*	10.72 ± 1.95*

Diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1 – $Na[VO(O_2)_2(2,2'\text{-bpy})] \times 8\ H_2O$, Di2 – $Na[VO(O_2)_2(1,10'\text{-phen})] \times 5\ H_2O$, Di3 – $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \times 8\ H_2O$, Di4 – $[VO(SO_4)(1,10'\text{-phen})] \times 2\ H_2O$, Di5 – $[VO(SO_4)(2,2'\text{-bpy})] \times H_2O$). * Statistical differences ($p < 0.05$) measured between concentrations of trace elements

Table 3. Correlation between concentrations [mg/kg] of trace elements measured in spleen for all investigated rats groups.

	V	Fe	Cu	Zn	Mn	Ca	K
V	1.00	0.39*	0.60*	0.61*	0.30*	0.09	0.19
Fe	0.39*	1.00	0.28*	0.18	0.24	0.09	0.21
Cu	0.60*	0.28*	1.00	0.71*	0.24	0.11	0.59*
Zn	0.61*	0.18	0.71*	1.00	0.15	0.25	0.64*
Mn	0.30*	0.24	0.24	0.15	1.00	-0.08	0.01
Ca	0.09	0.09	0.11	0.25	-0.08	1.00	0.23
K	0.19	0.21	0.59*	0.64*	0.01	0.23	1.00

* Statistical differences ($p < 0.05$) measured between concentrations of trace elements.

the control group (D) and diabetic groups of insulin (Di). In our study, the calcium concentration was the lowest in the diabetic animals with insulin injection and the highest in the vanadium complex-treated diabetic rats (Di5) (Tab. 2). Pittas et al. (20) reported that Ca inhibits the diabetes in the Nurses' Health Study. Our study results are opposite to that observation – in diabetic animals, a decreased calcium concentration in spleen samples was found in comparison to the control group. Furthermore, in the vanadium treated rats, differences were observed only between the groups Di2 and Di3. The animals in those groups were treated with vanadium complexes 2 and 3, respectively. Ligands in the vanadium complexes at the +IV oxidation state differ in their influence on the calcium concentration.

Potassium

Results presented in Table 2 show the potassium contents. The lowest concentration was observed for the diabetic, non-treated animals (D). An injection of insulin increases potassium concentration in diabetic rats. The group of animals treated with vanadium complexes (Di4, Di5) had lower potassium concentration than the other animals, excluding the diabetic group (D). Supplementation of complexes containing vanadium with the oxidation state of +IV caused the decrease in potassium concentration compared to the effect of complexes with oxidation state +V.

Correlations

To study the correlation between the tested rat groups, the correlations between all measured elements (V, Fe, Cu, Zn, Mn, Ca, and K) were calculated with $p < 0.05$. The results are included in Table 3. Calcium did not affect the contents of other measured elements. Vanadium had small but statistically significant influence on manganese ($p = 0.30$) and iron $p = 0.39$) concentration in the spleen. Influence of vanadium on the copper ($p = 0.60$) and zinc ($p = 0.61$) content was more significant. Correlations between copper and vanadium, and zinc are statistically significant ($p = 0.60$, $p = 0.71$ respectively). In case of zinc, statistically significant correlations were observed with vanadium, copper and potassium ($p = 0.61$, $p = 0.71$, $p = 0.64$, respectively).

DISCUSSION

The content of metal concentration in different organs has influence on metabolism (enzyme activity) of living organisms. It has been shown that vanadium possesses anti-diabetic activity, and it can be used as a potential therapeutic agent in diabetes treatment in several diabetic models (8). Simultaneously, supplementation of this metal can influence the content of other metals in tissues and, indirectly, many metabolic parameters such as enzymatic activity. The spleen is an organ where old or damaged erythrocytes are eliminated from the circulation. This organ also participates in immunoglobulin formation but it is not necessary for living mammals. Considerable percentage of circulating blood passes through the spleen and, therefore, all changes taking place in the organism are exhibited in this organ.

From our results, one can clearly observe the high vanadium uptake in the spleen of animals treated with vanadium compounds in comparison with the healthy (D) and diabetic rats (Di). The injection of insulin did not influence the natural content of vanadium. The vanadium uptake was not dependent on the chemical composition of the vanadium com-

plexes. The interaction between the vanadium content and other materials was dependent on the studied elements and on the chemical composition of vanadium compounds. The iron concentration in the insulin-injected (Di) and non-insulin injected (D) diabetic rats was lower than in the control group of healthy rats, that agrees with the observation by Olusola et al. (15) and Hamdaoui et al. (16) indicating a natural, low content of iron in the diabetes mellitus disease, in the heart and spleen of Wistar rats. However, the iron concentration depends on the degree of vanadium uptake defined by the chemical composition of vanadium complexes. The lower iron concentration was observed for the Di1 and Di2 groups. The increase of an iron content to the value obtained for healthy rats indicates a normalizing/stabilizing influence of vanadium. Vanadium administration decreases iron concentration in the spleen. Perhaps it is correlated with the function of this organ (elimination and degradation of red cells).

The character of copper uptake in the presence of high vanadium concentration was similar to the iron uptake. However, in this case the Cu content significantly above the higher content was observed in the diabetic and insulin-injected diabetic rats. The presence of vanadium probably enhances the Cu uptake in this organ. In opposition, like in case of iron, the vanadium complexes (Di4, Di5) decreased the copper spleen content. The character of copper uptake in the presence of high vanadium concentration was similar to the iron-uptake. In the paper of Cempel and Janicka (21), the influence of nickel on copper content of spleen was not observed. In the present investigation, the influence of vanadium complexes on copper content in spleen was noticed. It suggests possibility of increased activity of those enzymes for which copper is cofactor.

The zinc content increased from 20 mg/kg dry tissue (D, Di) to 120 mg/kg dry tissue (Di1) in the diabetic rats treated with vanadium. For this element, almost a statistically significant decrease of zinc concentration as a function of a type of vanadium complexes was observed. Also, the content of zinc is higher in all insulin treated rats. It is possibly associated with zinc supplementation during insulin injection where insulin is stabilized by zinc (22).

For manganese, a weak influence of vanadium on the concentration of Mn was observed. It seems that only the vanadium-complex no. 3 enhanced the uptake of this metal. Similarly, the differences in the calcium content in the studied rats groups did not show after vanadium administration. The concentration was similar to the content observed for diabetic, and insulin-injected diabetic rats.

The potassium content showed the same behavior as copper, as the two first vanadium compounds (Di1, Di2) increased the K concentrations. The potassium content showed the same behavior as copper for two first vanadium compounds (Di1, Di2) – an increase in the K concentrations. The content of healthy rats was reached when the diabetic insulin-injected rats were treated with the vanadium-complex no. 3. In order to properly interpret the obtained information, an extended analysis should be made along with further gathering of additional data.

The observed changes can influence especially the enzyme activity where metals are cofactors and finally the organism homeostasis. Probably during treatment of some diseases, it would be desirable supplementation with chosen elements, which can help to regulate activity of enzymes with metal cofactors. In the similar investigation it would be important to measure not only elements content, but also enzymes activity.

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REFERENCES

1. Fisher K, Dilworth M.J., Newton W.E.: Biochemistry 45, 4190 (2006).
2. Rüttimann-Johnson C., Staples C.R., Rangaraj P., Shah V.K., Ludden P.W.: J. Biol. Chem. 274, 18087 (1999).
3. Michibata H., Uyama T., Ueki T., Kanamori K.: Microsc. Res. Techn. 5, 421 (2002).
4. Ueki T., Yamaguchi N., Michibata H.: Comp. Biochem. Physiol. B Biochem. Mol. Biol. 136, 91 (2003).
5. Garner C.D., Armstrong E.M., Berry R.E., Beddoes R.L., Collison D., Cooney J.J., Ertok S.N., Helliwell M.: J. Inorg. Biochem. 80, 17 (2000).
6. Qureshi M.A., Hill C.H., Heggen C.L.: Vet. Immunol. Immunopathol. 68, 61 (1999).
7. Jelikić-Stankov M., Uskoković-Marković S., Holclajtner-Antunović I., Todorović M., Djurdjević P.: J. Trace Elem. Med. Biol. 21, 8 (2007).
8. Thompson K.H., Orvig C.: J. Inorg. Biochem. 100, 1925 (2006).

9. Sun R.W., Ma D.L., Wong E.L., Che C.M.: Dalton Trans. 43, 4884 (2007).
10. Tas S., Sarandol E., Ayvalik S.Z., Serdar Z., Dirican M.: Arch. Med. Res. 38, 276 (2007).
11. Barrio D.A., Etcheverry S.B.: Can. J. Physiol. Pharmacol. 84, 677 (2006).
12. Frank A., Madej A., Galgan V., Petersson L.R.: Sci. Total Environ. 181, 73 (1996).
13. De Cremer K., Van Hulle M., Chéry C., Cornelis R., Strijckmans K., Dams R., Lameire N., Vanholder R.: J. Biol. Inorg. Chem. 7, 884 (2002).
14. Azay J., Brès J., Krosniak M., Teissedre P.L., Cabanis J.C., Serrano J.J., Cros G.: Fundam. Clin. Pharmacol. 15, 313 (2001).
15. Olusola A.O., Obodozie O.O., Nssien M., Adaramoye A., Adesanoye O., Odama L.E., Emerole G.O.: Biol. Trace Elem. Res. 98, 265 (2004).
16. Hamdaoui M.H., Chahed A., Ellouze-Chabchoub S., Marouani N., Ben Abid Z., Hédhili A.: Ann. Nutr. Metab. 49, 118 (2005).
17. Le Guenno G., Chanséaume E., Ruivard M., Morio B., Mazur A.: Diabetes Res. Clin. Pract. 77, 363 (2007).
18. Uriu-Adams J.Y., Rucker R.B., Commisso J.F., Keen C.L.: J. Nutr. Biochem. 16, 312 (2005).
19. Thompson K., Molina R., Donaghey T., Brain J.D., Wessling-Resnick M.: Toxicol. Appl. Pharmacol. 210, 17 (2006).
20. Pittas A.G., Dawson-Hughes B., Li T., Van Dam R.M., Willett W.C., Manson J.E., Hu F.B.: Diabetes Care 29, 650 (2006).
21. Cempel M., Janicka K.: Biol. Trace Elem. Res. 90, 215 (2002).
22. Dunn M.F.: Biometals 18, 295 (2005).

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