ORIGINAL PAPERS

Adv Clin Exp Med 2007, **16**, 4, 479–491
ISSN 1230-025X

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Influence of Physical Training on Level of Oxidative Damages in Kidneys of Rats Intoxicated with Cadmium as Well as Simultaneously Cadmium and Copper

Wpływ treningu fizycznego na poziom uszkodzeń oksydacyjnych w nerkach szczurów intoksykowanych kadmem oraz jednocześnie kadmem i miedzią

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Abstract

Background. The toxic effect of exposure to cadmium and copper is a result of their concentrations and interactions in the body. Free radical mechanisms and disorders of zinc metabolism have been implicated in the damage of kidneys caused by Cd and Cu. Physical exercise causes the oxidative stress and also changes the metal distribution and disturbs its metabolism. On the other hand, moderate physical training influences favourably on the antioxidative protection and can reduce the toxic effect caused by cadmium and copper.
Objectives. The aim of this study was to investigate the influence of both physical training and intoxication with cadmium, copper, and cadmium and copper simultaneously on the concentrations of metals (Cd, Zn, Cu) in the blood and kidneys; the concentration of indicators of oxidative stress (malonyldialdehyd (MDA), sulfhydryl groups) in the kidneys of rats; and the changes in some antioxidant components such as: superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and reduced glutathione (GSH).
Material and Methods. Fifty-four rats (female, Buffalo strain) were split into 9 groups of 6 rats each. One group represented the control and eight groups were intoxicated with cadmium (50 ppm Cd in drinking water) and/or copper (0.5 or 2.5 Cu mg/kg m.b., intraperitioneally, three times a week). Half of the intoxicated groups were subjected to physical training. The experiment was carried out over 15 weeks.
Results. The training decreased the concentration of Cd in the blood and increased the concentration of zinc and copper in kidneys. The administration of metals, both alone or in combination with physical training, caused a significant decline of renal content of the sulfhydryl groups (SH groups) and a rise in the content of MDA. The highest increase of Cd concentration was observed in untrained rats intoxicated with cadmium only. Injecting copper reduced the concentration of cadmium, but increased the oxidative processes. In trained rats intoxicated only with cadmium, the concentration of Cd in the kidney was lower, and the concentration of GSH, SH groups and the activity of CAT were higher, in comparison with untrained rats.
Conclusions. Physical training partially alleviates the oxidative stress induced by metals and increases the concentration of zinc (a nutrient which protects against toxicity of Cd) in kidneys, especially in rats treated only with Cd. The combination of physical training, cadmium and a high dosage of copper, considerably intensifies the oxidative stress in the kidneys of rats (**Adv Clin Exp Med 2007, 16, 4, 479–491**).

Key words: cadmium, copper, zinc, physical training, oxidative stress.

Streszczenie

Wprowadzenie. Działanie toksyczne związane z ekspozycją na kadm i miedź wynika z ich stężeń w organizmie i wzajemnego oddziaływania. Mechanizmy wolnorodnikowe oraz zaburzanie metabolizmu cynku biorą udział w uszkodzeniach nerek powodowanych przez Cd i Cu. Wysiłek fizyczny powoduje stres oksydacyjny, a także za-

burza metabolizm i rozmieszczenie metali. Jednocześnie jednak umiarkowany trening fizyczny korzystnie wpływa na obronę antyoksydacyjną i może łagodzić skutki toksyczne wywoływane przez kadm i miedź.

Cel pracy. Zbadanie wpływu treningu fizycznego i zatrucia organizmu kadmem, miedzą oraz kadmem i miedzią jednocześnie na stężenie metali (Cd, Zn, Cu) we krwi i nerkach, stężenie wskaźników stresu oksydacyjnego w nerkach szczurów (malonylodialdehyd (MDA), grupy SH) i aktywności/stężenia niektórych antyoksydantów, takich jak: dysmutaza ponadtlenkowa (SOD), peroksydaza glutationowa (GPx), katalaza (CAT) i zredukowany glutation (GSH).

Materiał i metody. Pięćdziesiąt cztery szczury (samiczki, szczepu Buffalo) podzielono na 9 grup (po 6 sztuk w każdej grupie): jedna stanowiła grupę kontrolną, a pozostałym 8 grupom podano kadm (50 Cd ppm w wodzie do picia) i/lub miedź (0,5 lub 2,5 Cu mg/kg m.c., dootrzewnowo, 3 razy w tygodniu). Połowę grup intoksykowanych metalami poddano treningowi fizycznemu. Eksperyment trwał 15 tygodni.

Wyniki. Trening zmniejszał stężenie Cd we krwi i zwiększał stężenie Zn i Cu w nerkach. Podawanie samych metali oraz w połączeniu z treningiem znacząco zmniejszało zawartość grup SH i zwiększało stężenie MDA w nerkach. Największe stężenie Cd w nerkach zaobserwowano u zwierząt nietrenowanych, którym podano tylko kadm. Podawanie miedzi zmniejszało stężenie kadmu w nerkach, ale nasilało procesy oksydacyjne. U szczurów trenowanych, którym podano tylko kadm stężenie Cd w nerkach było istotnie mniejsze, a stężenie GSH, grup SH i aktywność CAT były większe w porównaniu z grupą nietrenowaną.

Wnioski. Trening częściowo łagodzi powodowany przez metale stres oksydacyjny oraz zwiększa stężenie cynku (chroniącego przed toksycznością kadmu) w nerkach, zwłaszcza u szczurów intoksykowanych tylko kadmem. Trening, kadm i miedź (większa dawka) działając jednocześnie, znacznie nasilają stres oksydacyjny w nerkach (**Adv Clin Exp Med 2007, 16, 4, 479–491**).

Słowa kluczowe: kadm, miedź, cynk, trening fizyczny, stres oksydacyjny.

In industrial areas people are exposed to several heavy metals simultaneously. The interaction between metals in the human body can occur at absorption level, when binding with specific proteins, during transport, distribution in the body, detoxification and excretion. These interactions can lead to an increase in the toxic effects or alleviation the toxic action of metals.

Cadmium is a widespread, environmental pollutant and a very toxic heavy metal. Cadmium is a poor electron acceptor and donor under physiological conditions, however oxidative stress has been implicated in cadmium induced organ damage and dysfunction. Cd is known to deplete glutathione and other endogenous antioxidants (vitamin C, Se) and to decrease antioxidative enzymes activity, which results in enhanced production of reactive oxygen species (ROS) [1, 2].

Cadmium also induces enzyme dysfunction by metal enzyme substitution or cadmium/apoenzyme interaction. Disorders of copper, zinc and other biogenic metal metabolisms also play a very important role in the oxidative processes caused by cadmium [3–5].

Copper, like cadmium, is a component of the pollutants acting on people in industrialized societies. Copper is capable of catalyzing Haber-Weiss and Fenton reactions, which create a very reactive hydroxyl radical. Many of the toxic effects of copper, such as lipid peroxidation, enzyme inactivation and nucleic acids damage, are related to its role in the generation of oxygen free radicals [6, 7].

Cytotoxicity of cadmium and cooper is also the result of the binding of thiol protein groups by these metals. It increases the proteins sensitive to oxidative attack and in consequence causes its inhibitions [2, 7].

On the other hand, copper is an essential trace element involved in a variety of important metabolic processes, such as cellular respiration, neurotransmitter functions, melanin and connective tissue biosynthesis and iron metabolism. Copper is also an element, which takes part in the antioxidative defence of the human body. Cu serves as a cofactor of superoxide dismutase, which is a very important antioxidative enzyme [8]

Copper can reduce cadmium intake. The supplementation of copper alleviates the toxic effects caused by cadmium. The mechanism for this protection has also been postulated to be due to the induction of metallothionein (MT) synthesis by copper. The cysteine-rich protein sequesters cadmium and results in a lesser distribution of cadmium to the critical particulate fraction and induces a tolerance to the toxicity of cadmium. Cadmium–copper interactions are dependent on the doses of those elements. Disorders of copper metabolism play an important role in the cadmium induction oxidative processes [9, 10].

Depending on the concentration, copper can be a kind of protection and reduce the cadmium toxicity or cooperates with cadmium to cause an increase of damages in the tissues.

Zinc is a microelement, which also protects against cadmium toxicity. Liu et al [10] show that the administration of zinc or copper 24 hours before cadmium-thionein (Cd-MT) intoxication reduces damage to the kidney caused by Cd. Treatment with zinc or copper causes an induction of metalothionein synthesis and increase its con-

centration in the kidney. Therefore, there is an increased probability that toxic cadmium ions remain bound with metalothionein.

Increased Zn supplies may reduce Cd absorption and accumulation, and prevent or reduce the adverse actions of Cd, whereas Zn deficiency can intensify Cd accumulation and toxicity. The Zn status of the body is important in relation to the development of Cd toxicity [11].

Physical exercise causes the oxidative stress and also changes the metals distribution and disturbs their metabolism [12]. So, physical activity can be a problem in cadmium- and copper-contaminated environment conditions. On the other hand, systematical training influences favourably on the antoxidative protection in the different tissues. Exercises influence the increase of reduced glutathione concentration and on the activities of antioxidative enzymes [13, 14], factors which play an important role in the protection against copper toxicity and which can mitigate the toxic effects indicated by cadmium.

Moreover, Rodriges et al. [15] showed that physically-active people have lower blood levels of cadmium and higher levels of Zn and Cu than people, that do not do physical exercise.

The aim of this study was to investigate the influence of intoxication with cadmium, copper and cadmium and copper simultaneously and physical training on the concentrations of metals (Cd, Zn, Cu) in blood and kidneys of rats, concentration of indicators of oxidative stress (malonyldialdehyd (MDA), SH groups) in kidneys and the changes in some antioxidant components such as: superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and reduced glutathione (GSH).

The most important purpose of these investigations was the evaluation of possible influences of moderate training on the reinforcement of antioxidative defence mechanisms in the kidney and the allieviation of oxidative damages caused by cadmium and copper.

Material and Methods

Female Buffalo rats weighing 120–150g were used in this study. The animals were housed in a ventilated room, at 22 ± 2°C, under natural lighting conditions and had free access to standard rat feed and water. Rats were randomly divided into 9 groups of 6 rats each: One represented the control and the remaining eight groups were exposed to cadmium and/or copper for 15 weeks. The animals in the cadmium treatment groups received drinking water containing 50 ppm Cd (as $CdCl_2$). Copper (as solution $CuSO_4$) was injected to rats intraperitonealy (i.p.), in two doses: 0.5 or 2.5 mg Cu/kg mass body, three times a week. The control animals received clear, tap water and were injected intraperitioneally with physiological saline (0.9% NaCl). Additionaly, animals which were intoxicated only with cadmium received physiological saline intraperitionally.

Table 1. The protocol of the rats treatment

Tabela 1. Procedury, którym poddano poszczególne grupy szczurów

Group of rats (Grupy szczurów) (n = 6)	Treatment (Procedury)
C (control)	tap water to drink + NaCl (*i.p*)
Cd	50 ppm Cd in drinking water + NaCl (*i.p*)
Cu2.5	tap water to drink + 2.5 mg Cu/ kg m.b (*i.p*)
CdCu0.5	50 ppm Cd in drinking water + 0.5 mg Cu/kg m.b (*i.p*)
CdCu2.5	50 ppm Cd in drinking water + 2.5 mg Cu/kg m.b (*i.p*)
CdT	50 ppm Cd in drinking water + NaCl (*i.p*) + training
Cu2.5T	tap water to drink + 2.5 mg Cu/kg m.b (*i.p*) + training
CdCu0.5T	50 ppm Cd in drinking water + 0.5 mg Cu/kg m.b (*i.p*) + training
CdCu2.5T	50 ppm Cd in drinking water + 2.5 mg Cu/kg m.b (*i.p*) + training

Half of the intoxicated groups were subjected to physical training. Exercise training was given by subjecting the rats to treadmill running, through 15–20 min (speed 15–20 m/min), 3 days a week. Rats were adapted to the exercise for one week before starting intoxication with metals. The protocol of the rats' treatment is given in Table 1.

Approximately 72 h after the last training session all animals were anaesthetized by a intramuscular ketaminum injection. Blood was collected via tubes (both with and without EDTA as an anticoagulant) after a cardiac puncture, and then the animals were sacrificed through the dislocation of cervival circles. Plasma samples were prepared by centrifugation (2500 × g, 10 min) from the blood. Kidneys were removed, cut into pieces and stored at –80°C until used.

The experimental design was approved by the Local Bioethics Committee for Animal Experiments in Wrocław, Poland.

Biochemical Assays

The concentrations of reduced glutathione (GSH) and malondialdehyde (MDA), and the activities of superoxide dismutase (SOD) and glu-

tathione peroxidase (Gpx) in the kidney's homogenate were measured spectrophotometrically, according to instructions from OxisResearch kits (GSH-400 Assay No 21011, MDA-586Assay No 21044, SOD-525 Assay No 21010 and GPx-340 Assay No 21017, respectively). Catalase activity was measured in a homogenate of tissues in a phosphate buffer, pH 7.0. Catalase activity was assayed following the decrease of H_2O_2 at 240 nm, by the method of Aebi [16]. One unit of CAT is defined as 1 μmol of H_2O_2 degraded/min/mg protein, at 25°C.

The concentration of total sulphydryl groups in kidney homogenates was assayed spectrophotometrically as described by Ellman [17]. 5.5-dithiobis 2-nitrobenzoic acid (DTNB) reacts with SH groups to yield disulfide and a yellow product – 2-nitro-5-thiobenzoic acid, which has a maximal absorbance at 412 nm. A 10% homogenate of the tissues was made in 5mM sodium phosphate buffer, pH 8.0. After centrifugation, 0.3 ml aliquots of supernatant (with known concentrations of protein) were mixed with 0.3 ml 10% sodium dodecyl sulfate (SDS) and with 0.3 ml of DTNB (0.0198g diluted to 50 ml phosphate buffer). Samples were diluted to 3.3 ml with phosphate buffer, and incubated for 60 min at 37°C. Absorbance was measured at 412 nm against sample-free and DTNB-free blanks. GSH was used as a standard.

Protein was assayed using a commercial kit (Sigma Diagnostics, procedure No. P5656) according to the method of Lowry. Bovine serum albumin was used as standard.

Cd, Cu and Zn Determination

Cadmium was measured in the whole blood and the kidney. Copper and zinc was measured in the kidney and serum. Kidney samples of known weight were subjected to mineralization in concentrated nitric acid (65% HNO_3, spectral pure, BAKER, No 9598), under pressure 45–42 Atm, using the mineralizer Uni Clever (PLASMATRONICA). Reference materials (Beef Liver CRM NCS ZC 85005 (T) were mineralized with each run. The concentrations of metals (Cd, Zn) were measured by the Atomic Absorption Spectrophotometer (AAS) (Spectrophotometer SOLAAR M6, Thermo Elemental). Cd concentration in the whole blood (after dilutions in 5% HNO_3) and the kidney was determined by AAS method with electro thermal atomization in a graphite cuvette, at length of wave $\lambda = 228.8$ nm. Zn and Cu concentration in the serum and kidney was determined by AAS with flame atomization in an air-acetylene burner, at length of waves: $\lambda = 213.9$ nm (Zn) and $\lambda = 324.8.9$ nm (Cu).

Statistical Analysis

Statistical analysis for differences among rats in the experimental groups was performed by Student *t*-test and by Cochran-Coxa test (at heterogeneous variances in analyzed groups of data) using STATISTICA 5.1 (for Windows) computer software. When the data did not follow the Gaussian distribution, the Mann-Whitney non parametric test was used. Data are expressed as means ± SD. Pearson's or Spearman's correlation analysis (for non-parametric data) was conducted for the relationship between blood and renal levels of metals (Cd, Zn, Cu) and between renal concentrations of biochemical parameters and levels of metals in the blood and kidney. Values of $p < 0.05$ were considered statistically significant.

Results

The activities of antioxidant enzymes in kidneys of control and experimental rats are shown in Table 2 (mean ± SD).

In the kidneys of rats exposed to cadmium (Cd group) and cadmium and copper simultaneously (both doses of copper: CdCu0.5 and CdCu2.5 groups) for 15 weeks a significant decrease of catalase activities compared to the control group could be observed.

Physical training caused a significant increase of catalase activity in rats intoxicated with cadmium alone (CdT) and co-exposed to cadmium and a lower dose of copper (CdCu0.5T) compared to analogical, untrained groups (Cd, CdCu0.5), by 24% and 55%, respectively. In contrast, in rats co-exposed to cadmium and a higher dose of copper (CdCu2.5T) CAT activity decreased with training and was lower by about 55% compared to untrained rats (CdCu2.5) CAT activity in the CdT-group was lower than in CdCu0.5T and higher compared to the CdCu2.5T group. CAT activity in trained rats co-exposed to cadmium and a lower dose of copper (CdCu0.5T) was higher (about 54%) compared to the rats injected with a bigger dose of copper (CdCu2.5T group).

A significant decline of glutathione peroxidase activities in comparison with the control group (C) were observed only in the kidneys of trained rats exposed to cadmium and copper simultaneously (both doses of copper: CdCu0.5T, CdCu2.5T), by 24% and 26%, respectively. In rats subjected to

Table 2. Effect of physical training on activities of antioxidant enzymes (CAT, GPx, SOD) in kidney of rats intoxicated with cadmium, copper and copper and cadmium simultaneously (different doses of Cu), mean ± SD

Tabela 2. Wpływ treningu fizycznego na aktywność enzymów antyoksydacyjnych w nerkach szczurów, którym podano kadm, miedź oraz kadm i miedź jednocześnie (różne dawki Cu), średnia ± SD

No (Lp.)	Groups of rats (Grupy szczurów) n = 6	CAT U/mg protein (U/mg białka)	GPx mU/mg protein (mU/mg białka)	SOD U/mg protein (U/mg białka)
1.	C	8.943 ± 0.404	650.33 ± 143.00	316.73 ± 80.99
2.	Cd	6.933[a] ± 0.684	587.11 ± 54.13	508.44[a] ± 141.48
3.	Cu2.5	8.074 ± 0.897	594.97 ± 75.5	645.82[a] ± 138.46
4.	CdCu0.5	6.852[a] ± 0.417	516.64 ± 50.61	472.64[a] ± 158.33
5.	CdCu2.5	7.599[a] ± 0.736	622.65 ± 83.35	597.12[a] ± 129.69
6.	CdT	8.625* ± 0.950	533.31 ± 69.12	443.07[a] ± 111.70
7.	Cu2.5T	8.336 ± 0.673	517.56 ± 66.14	597.40[a] ± 125.94
8.	CdCu0.5T	10.636[a, b, d, *] ± 1.419	495.69[a] ± 66.41	444.65 ± 132.61
9.	CdCu2.5T	6.906[a, c, d, e] ± 0.433	482.35[a, *] ± 58.51	540.10[a] ± 128.61

Groups of rats: C – control, Cd – intoxicated with cadmium, Cu2.5 – intoxicated with copper (2.5 mg Cu/kg b.m.), CdCu0.5 – intoxicated with copper (0.5 mg Cu/kg b.m.), CdCu2.5 – intoxicated with cadmium and copper (2.5 mg Cu/kg b.m.), CdT – intoxicated with cadmium, subjected to physical trained, Cu2.5T – intoxicated with copper (2.5 mg Cu/kg b.m.), trained, CdCu0.5T – intoxicated with copper (0.5 mg Cu/kg b.m.), trained, CdCu2.5T – intoxicated with cadmium and copper (2.5 mg Cu/kg b.m.), trained.

[a, b, c, d, e, *] – significant differences between groups of rats, $p < 0,05$; [a] – control group C significantly different from intoxicated with metals groups: Cd, CdCu0.5, CdCu2.5, CdT, CdCu0.5T, CdCu2.5T, [b] – intoxicated with cadmium group: Cd significantly different from intoxicated with cadmium and copper groups: CdCu0.5, CdCu2.5, CdCu0.5T, CdCu2.5T, [c] – CdT significantly different from CdCu0.5T, CdCu2.5T, [d] – CdCu0.5 significantly different from CdCu2.5, $p < 0.05$ and CdCu0.5T, significantly different from CdCu2.5T, $p < 0.05$, [e] – Cu2.5 significantly different from CdCu2.5 and Cu2.5 T significantly different from CdCu2.5T, $p < 0.05$, * – untrained groups: Cd, CdCu0.5, CdCu2.5 significantly different from analogical groups, subjected to physical training: CdT, CdCu0.5T, CdCu2.5T.

Grupy szczurów: C – kontrolna, Cd – intoksykowana kadmem, Cu2.5 – intoksykowana miedzią (2.5 mg Cu/kg m.c), CdCu0.5 – intoksykowana kadmem i miedzią (0.5 mg Cu/kg m.c), CdCu2.5 – intoksykowana kadmem i miedzią (2.5 mg Cu/kg m.c), CdT – intoksykowana kadmem, trenowana, Cu2.5T – intoksykowana miedzią (2,5 mg Cu/kg m.c), trenowana, CdCu0.5T – intoksykowana kadmem i miedzią (0,5 mg Cu/kg m.c), trenowana CdCu2.5T – intoksykowana kadmem i miedzią (2,5 mg Cu/kg m.c), trenowana.

[a, b, c, d, e, *] – różnice statystycznie istotne między grupami danych, $p < 0,05$; [a] – między grupą kontrolną C a grupami intoksykowanymi metalami: Cd, Cu2.5, CdCu0.5, CdCu2.5, CdT,Cu2.5T,CdCu0.5T, CdCu2.5T, $p < 0,05$, [b] – między grupą intoksykowaną kadmem Cd a grupami intoksykowanymi kadmem i miedzią: CdCu0.5, CdCu2.5, CdCu0.5T, CdCu2.5T, $p < 0,05$, [c] – między grupą CdT a grupami: CdCu0.5T, CdCu2.5T, $p < 0,05$, [d] – między grupą CdCu0.5 a grupą CdCu2.5, $p < 0,05$ oraz między grupą CdCu0.5T a grupą CdCu2.5T, $p < 0.05$, [e] – między grupą Cu2.5 a grupą CdCu2.5, $p < 0,05$ oraz między grupą Cu2.5T a grupą CdCu2.5T, $p < 0,05$, * – między grupami nietrenowanymi a analogicznymi grupami trenowanymi, np. Cd wzg. CdT; Cu2.5 wzg. Cu2.5T itd., $p < 0.05$.

physical training and intoxicated with cadmium and a higher dose of copper (CdCu2.5T) glutathione peroxidase activity had fallen compared to the analogical, untrained rats(CdCu2.5) and the untrained animals injected with a bigger dose of copper alone (Cu2.5), by 22 and 19%, respectively, $p < 0.05$.

The kidney activities of SOD increased as a result of Cd and/or Cu administration and physical training in all experimental groups: Cd, Cu2.5, CdCu0.5, CdCu2.5, CdT, Cu2.5T and CdCu2.5T, compared to the control (C) by 60, 104, 49, 88, 40, 89 and 70%, respectively, $p < 0.05$. There were no significant differences in SOD activities in kidneys among the other experimental groups of rats.

Concentration of Malondialdehyde, Reduced Glutathione and Thiol Groups

Malonylodialdehyde (MDA), reduced glutathione (GSH) and sulphydryl groups (SH) concentrations in the kidneys of the control and experimental rats are shown in Table 3 (mean ± SD).

A significant decline in the concentrations of renal reduced glutathione in comparison with the control group (C) were observed in the trained and untrained rats intoxicated with metals (except trained animals, co-exposed to cadmium and a higher dose of copper – CdCu2.5): Cd, Cu2.5, CdCu0.5, CdCu2.5, CdT, Cu2.5T, CdCu0.5T, by

Table 3. Effect of physical training on concentration of MDA, GSH and SH groups in kidney of rats intoxicated with cadmium, copper and copper and cadmium simultaneously (different doses of Cu), mean (± SD)

Tabela 3. Wpływ treningu fizycznego na stężenie MDA, GSH i grup SH w nerkach szczurów szczurów intoksykowanych kadmem, miedzią oraz kadmem i miedzią jednocześnie (różne dawki Cu), średnia (± SD)

No (Lp.)	Groups of rats (Grupy szczurów) n = 6	GSH µmol/g tissue (µmol/g tkanki)	SH-groups (Grupy SH) µmol/g protein (µmol/g białka)	MDA µmol/g tissue (µmol/g tkanki)
1.	C	3.268 ± 0.174	186.38 ± 27.49	36.63 ± 6.63
2.	Cd	2.557[a] ± 0.087	151.40[a] ± 10.56	43.54 ± 6.66
3.	Cu2.5	2.911[a] ± 0.201	154.67[a] ± 8.27	51.07[a] ± 4.97
4.	CdCu0.5	2.841[a, b] ± 0.238	160.28[a] ± 10.06	51.92[a, b] ± 4.15
5.	CdCu2.5	2.493[a, d, e] ± 0.117	158.41[a] ± 14.26	47.96[a, b] ± 5.80
6.	CdT	2.792[a, *] ± 0.181	165.01[a, *] ± 8.41	51.45[a] ± 6.41
7.	Cu2.5T	2.856[a] ± 0.215	152.87[a] ± 9.12	44.10 ± 10.11
8.	CdCu0.5T	2.787[a, b] ± 0.218	152.32[a, c] ± 10.48	48.15[a] ± 10.87
9.	CdCu2.5T	3.266[b, c, d, e,*] ± 0.230	145.28[a, c] ± 11.87	54.51[a, b, *] ± 2.82

Explanation – see Table 2.

Objaśnienia pod tabelą 2.

22, 11, 13, 24, 15, 13 and 15%, respectively, $p < 0.05$. The administration of cadmium and a lower dose of copper (CdCu0.5) simultaneously caused an increased concentration of renal GSH in comparison with the group treated with Cd alone. However, no significant differences in GSH concentration was observed between the group exposed to cadmium alone (Cd) and the group co-exposed to cadmium and a higher dose of copper (CdCu2.5). Treatment with both metals simultaneously (groups: CdCu0.5 and CdCu2.5) caused a decrease in the concentrations of GSH in comparison with the group intoxicated only with copper (larger dose) (Cu2.5).

In rats co-exposed to cadmium and a lower dose of Cu (CdCu0.5) the concentration of GSH was significantly higher in comparison with the group treated with cadmium and a higher dose of Cu (CdCu2.5). By contrast, in rats subjected to physical training the renal concentration of GSH in the group intoxicated with cadmium and a lower dose of Cu (CdCu0.5T) was lower than in the group intoxicated with cadmium and a higher dose of Cu (CdCu25T). In both the group of rats intoxicated with cadmium-only, and the group co-exposed to cadmium and a higher dose of copper, physical training significantly enhanced the renal content of GSH compared to untrained rats, by 9 and 31% respectively, $p < 0.05$.

The administration of metals alone or in combination with physical training (groups: Cd, Cu2.5, CdCu0.5, CdCu2.5, CdT, Cu2.5T, CdCu0.5T, CdCu2.5T) caused a significant decline in the renal content of sulphydryl groups (-SH), by 19, 17, 14, 15, 11,5, 18, 18 and 22%, respectively ($p < 0.05$) in comparison with the control group.

The results show that physical training slightly, but statistically-significantly, elevated the renal concentration of SH content in rats exposed to cadmium-only (CdT), by 7% ($p < 0.05$) compared to their untrained co-partners (Cd). There were no significant differences in the concentrations of the SH groups between the other, trained groups of rats and analogical untrained groups. In rats subjected to physical training, the concentration of sulphydryl groups was significantly higher in the group treated only with cadmium (CdT) compared to the groups intoxicated with both metals (groups: CdCu0.5T, CdCu2.5T), by 8 and 12%, respectively ($p < 0.05$).

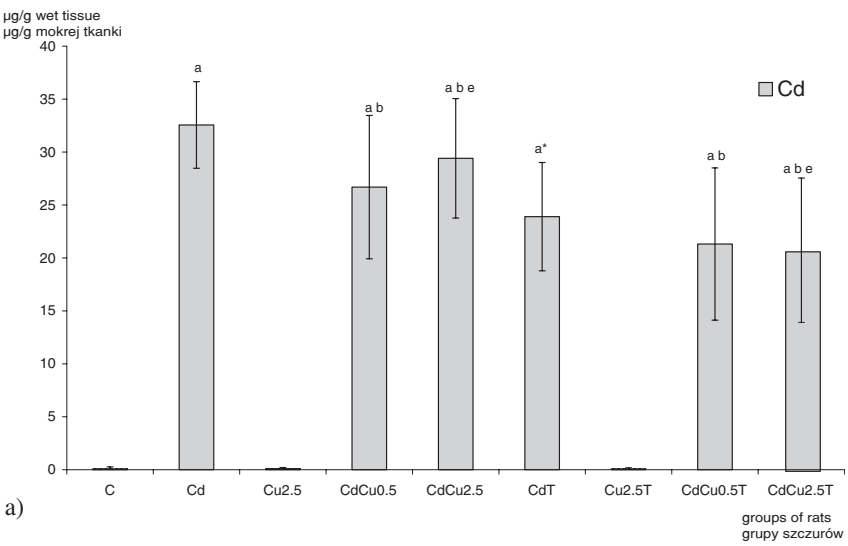
The groups exposed to metals-only, or in combination with physical training (groups: Cd, Cu2.5, CdCu0.5, CdCu2.5, CdT, Cu2.5T, CdCu0.5T, CdCu2.5T) raised the concentration of malonylodialdehyde (MDA) by 40%, 42%, 31%, 40%, 40%, 31% and 49% respectively, ($p < 0.05$) compared to the control group (C).

The rats treated with cadmium-only (Cd) exhibited a significantly lower level of MDA in kidneys than rats intoxicated with both metals simultaneously (CdCu0.5, CdCu2.5), ($p < 0.05$).

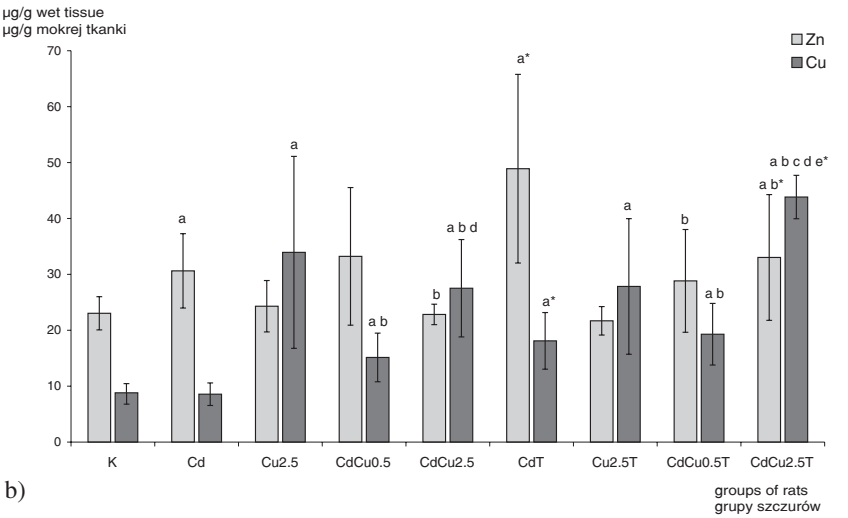
The renal concentration of MDA in the CdCu2.5T group was higher than in the analogical untrained group (CdCu2.5) ($p < 0.05$).

Cd, Cu and Zn Concentration

The results of measuring the concentrations of cadmium, zinc and copper in the kidneys are shown in Figure 1.



a)



b)

Fig. 1. Effect of physical training on concentration of metals (Cd, Zn, Cu) in kidney of rats intoxicated with cadmium, copper and cadmium and copper simultaneously (different doses of Cu), mean ± SD: a) concentration of cadmium in kidney, b) concentration of zinc and copper in kidney

Ryc. 1. Wpływ treningu fizycznego na stężenie metali w nerkach szczurów intoksykowanych kadmem, miedzią oraz kadmem i miedzią jednocześnie (różne dawki Cu), średnia ± SD: a) stężenie kadmu w nerkach, b) stężenie cynku i miedzi w nerkach

In all the groups of rats intoxicated with cadmium (groups: Cd, CdCu0.5, CdCu2.5, CdT, CdCu0.5T, CdCu2.5T) the concentrations of Cd in the kidney were significantly higher compared to the control group.

In both trained and untrained rats co-exposed to cadmium and copper (CdCu0.5, CdCu2.5, CdCu0.5T, CdCu2.5T) the cadmium level was significantly lower in comparison to the group treated only with cadmium (Cd), ($p < 0.05$).

In the groups treated only with copper, the concentration of cadmium in the kidney was similar to the control group.

In rats intoxicated with cadmium alone, training significantly increased the renal level of Cd (by 26%) in comparison with their untrained copartners. In rats intoxicated with copper alone and co-exposed to cadmium and copper simultaneously, exercise did not significantly alter the concentration of Cd in the kidneys.

The concentration of zinc in the kidneys was higher in the following groups: Cd, CdT and CdCu2.5T than the control group. The highest zinc concentration was observed in the group exposed to cadmium and subjected to physical effort (CdT).

Training significantly elevated the renal level of Zn only in rats intoxicated with cadmium-only and those co-exposed to cadmium and a higher dose of copper. The concentration of zinc in the CdT group was higher by 60% than the Cd group. The zinc level in the CdCu2.5T group was higher by 45% than the CdCu2.5 group. In rats subjected to physical training and co-exposed to Cd and Cu (2.5 mg Cu/kg m.b.) the zinc concentration was higher in comparison with trained rats intoxicated with copper-only (the Cu2.5T group).

In the kidneys of rats exposed to metals (apart from the group intoxicated with Cd-only) the concentration of copper was significantly increased compared to the control group, ($p < 0.05$).

The copper content in rats co-exposed to cadmium and copper (the CdCu0.5, CdCu0.5T, CdCu2.5, and CdCu2.5T groups) was higher than in untrained rats intoxicated only with cadmium (Cd), ($p < 0.05$).

The resuts show that exercise training significantly elevated the level of copper in the kidney.

The highest concentration of copper (five times higher than in the control group) – was observed in trained rats co-exposed to cadmium and a higher dose of copper (CdCu2.5T), $p < 0.05$. In groups subjected to physical effort, groups CdT and CdCu2.5T, the concentration of copper was significantly higher in comparison to analogical, untrained groups: Cd and CdCu2.5.

Moreover, in both trained and untrained rats co-exposed to cadmium and a lower dose of copper (CdCu0.5T and CdCu0.5) it could be observed that the concentration of copper was twice as high compared to the control group ($p < 0.05$).

In trained rats intoxicated only with cadmium (CdT) a higher concentration of Cu compared to the control group ($p < 0.05$) could be found.

In both untrained rats intoxicated with a high dose of copper-only (Cu2.5), and those co-exposed to cadmium and higher dose of copper (CdCu2.5), the content of copper in the kidney was four- and three-times higher respectively in comparison to the control group (C), $p < 0.05$.

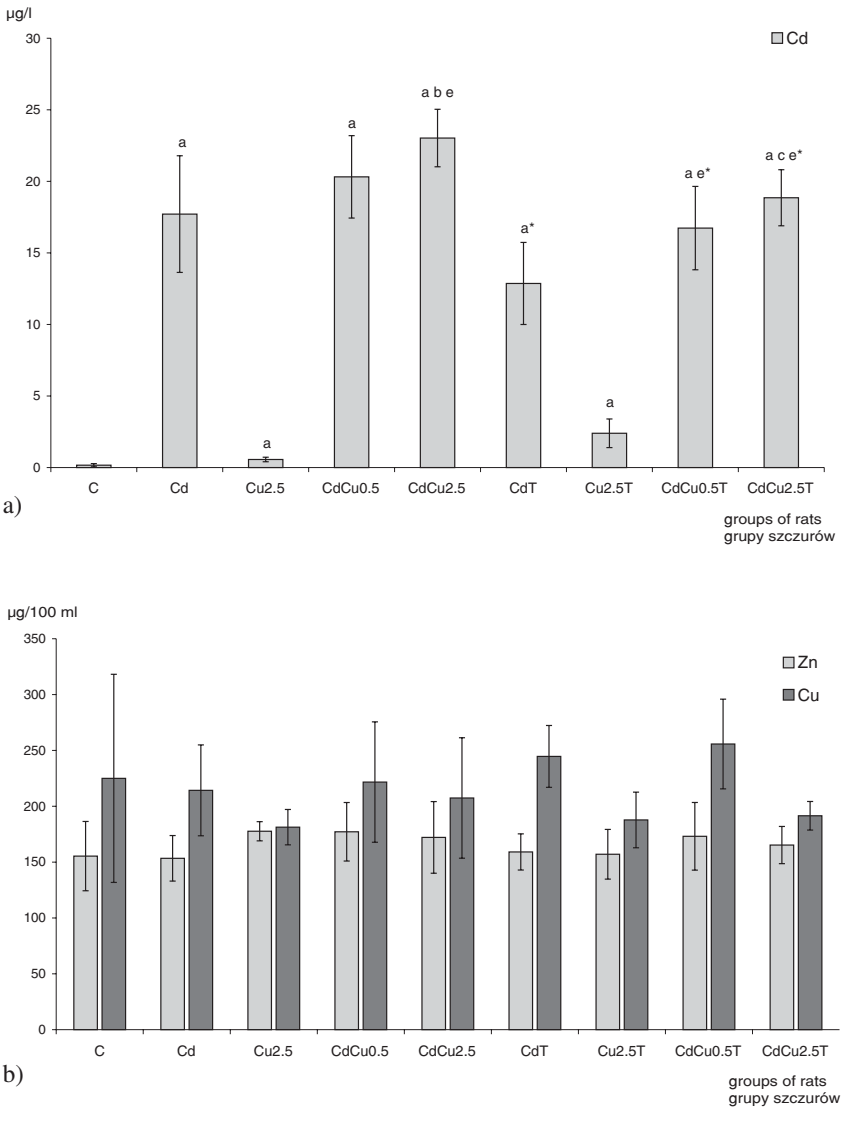
The concentration of copper in the CdCu2.5T group was higher (by 30%) compared to the Cu2.5 group, ($p < 0.05$).

The levels of copper in rats co-exposed to cadmium and a higher dose of Cu (CdCu2.5, CdCu2.5T) were higher compared to the analogical rats co-exposed to cadmium and a lower dose of Cu (CdCu0.5, CdCu0.5T), ($p < 0.05$).

However, no significant differences in Cu concentration was observed between trained rats intoxicated only with cadmium (CdT) and trained rats co-exposed to cadmium and a lower dose of copper (CdCu0.5T).

The results of measuring the concentrations of cadmium in the whole blood and zinc and copper in serum are shown in Figure 2.

Serum cadmium levels were significantly higher in trained and untrained rats, both intoxicated with cadmium or co-exposed to cadmium and copper, than in the control group.



a)

b)

Fig. 2. Effect of physical training on concentration of metals (Cd, Zn, Cu) in blood of rats intoxicated with cadmium, copper and copper and cadmium simultaneously (different doses of Cu), mean ± SD: a) concentration of cadmium in blood of rats, b) concentration of zinc and copper in serum of rats

Ryc. 2. Wpływ treningu fizycznego na stężenie metali we krwi szczurów intoksykowanych kadmem, miedzią oraz kadmem i miedzią jednocześnie (różne dawki Cu). Średnia (± SD): a) stężenie kadmu we krwi szczurów, b) stężenie cynku i miedzi w surowicy szczurów

The highest blood cadmium concentration was found in untrained rats intoxicated with cadmium and a higher dose of copper (CdCu2.5).

The results show that physical training significantly alters the blood levels of cadmium. Training significantly decreased the blood levels of Cd in rats both intoxicated with cadmium-only and co-exposed to Cd and Cu, in comparison with their untrained co-partners.

The serum concentration of Cu and Zn in experimental groups was similar to the control group.

Correlation Coefficients (*r*-values) Between Blood and Renal Levels of Metals (Cd, Zn, Cu)

The *r*-values and levels of significant (p) were extracted in Table 4. Positive, significant correlations were found between the renal and blood content of cadmium (r = 0.59, p = 0.000004) and also between the renal concentration of cadmium and the renal concentration of zinc (r = 0.56, p = 0.000017).

Correlation Coefficients (*r*-values) Between Renal Biochemical Parameters and Levels of Metals (Cd, Zn, Cu) in Blood and Kidney

The *r*-values and levels of significant (p) were extracted in Table 5. Negative correlations were found between the renal activity of catalase and the concentrations of cadmium in the kidney (r = –0.30, p = 0.0277) and in the blood (r = –0.40, p = 0.0037) and also between the renal activity of catalase and renal concentrations of MDA (r = –0.35, p = 0.0118).

Positive correlations were observed between the blood content of Cu and the renal activity of catalase (r = 0.43, p = 0.0014), however negative correlations were found between the blood content of Cu and the renal activity of GPx (r = –0.30, p = 0,0288).

Negative correlations were found between the renal activity of SOD and the renal concentrations of cadmium (r = –0.28, p = 0.040) and also between the renal activity of SOD and the content of SH groups (r = –0.29, p = 0.039).

Table 4. Correlations between levels of metals (Cd, Zn, Cu) in blood and kidney (rats from all groups)

Tabela 4. Zależności między stężeniami metali (Cd, Zn, Cu) we krwi i nerkach (szczury ze wszystkich grup łącznie)

	Cd Kidney (Nerka)	Zn Kidney (Nerka)	Cu Kidney (Nerka)	Cd Kidney (Nerka)	Zn Kidney (Nerka)
Cd Kidney (Nerka)					
Zn Kidney (Nerka)	r = 0.56 p = 0.000017				
Cu Kidney (Nerka)	ns.	ns.			
Cd Blood (Krew)	r = 0.59 p = 0.000004	ns.	ns.		
Zn Blood (Krew)	ns.	ns.	ns.	ns.	
Cu Blood (Krew)	ns.	ns.	ns.	ns.	ns.

r – coefficient of correlation.
p – level of significant.

r – współczynnik korelacji.
p – poziom istotności.

Table 5. Correlations between renal biochemical parameters and levels of metals (Cd, Zn, Cu) in blood and kidney

Tabela 5. Zależności między stężeniami metali (Cd, Zn, Cu) i badanymi parametrami biochemicznymi we krwi i nerkach szczurów (szczury ze wszystkich grup łącznie)

	CAT	GPx	SOD	GSH	SH groups (Grupy SH)	MDA
CAT						
GPx	ns.					
SOD	ns.	ns.				
GSH	ns.	ns.	ns.			
SH Groups (Grupy SH)	ns.	r = 0.2695 p = 0.0504	r = –0.29 p = 0.039	ns.		
MDA	r = –0.35 p = 0.0118	ns.	ns.	ns.	r = –0.29 p = 0.035	
Cd Kidney (Nerka)	r = –0.30 p = 0.0277	ns.	r = –0.28 p = 0.040	r = –0.39 p = 0.0047	ns.	ns.
Zn Kidney (Nerka)	ns.	ns.	ns.	ns.	ns.	ns.
Cu Kidney (Nerka)	ns.	ns.	r = 0.34 p = 0.0146	ns.	r = –0.31 p = 0.0258	r = 0.37 p = 0.0075
Cd Blood (Krew)	r = –0.40 p = 0.0037	ns.	ns.	ns.	r = –0.33 p = 0.0167	ns.
Zn Blood (Krew)	ns.	ns.	r = 0.27 p = 0.0503	ns.	ns.	ns.
Cu Blood (Krew)	r = 0.43 p = 0.0014	r = –0.30 p = 0.0288	ns.	ns.	ns.	r = –0.28 p = 0.0454

r – coefficient of correlation.
p – level of significant.

r – współczynnik korelacji.
p – poziom istotności.

Renal SOD activity and the copper content in kidney were found to show a significant, positive correlation (r = 0.34, p = 0.0146).

Renal GSH content and the renal concentrations of cadmium were found to show a significant, negative correlation (r = –0.39, p = 0.0047).

Moreover, negative correlations were observed between the renal content of SH groups and the renal concentration of MDA (r = –0.29, p = 0.035); the renal concentration of copper (r = –0,31, p=0,0258) and also the cadmium concentration in the blood (r.= –0.33, p = 0.0167)

Positive correlations were found between the renal content of copper and the renal concentration of MDA (r = 0.37, p = 0.0075), however negative correlations were found between the blood content of Cu and the renal concentration of MDA (r = –0.28, p = 0.0454).

Discussion

Cadmium is a widespread and very toxic metal. It promotes oxidative stress and afterwards contributes to the development of serious pathological changes in the kidney because of its long retention in this organ [1, 2]. In industrial areas, people are exposed to several heavy metals simultaneously. Interactions between metals can lead to increasing or alleviating toxic effects caused by metals.

According to expectations, the results of our investigations show that the ingestion of cadmium chloride (50 ppm Cd in drinking water) for a period of 15 weeks caused an increase in Cd concentration in the blood, the accumulation of cadmium in the kidney and the elevation of renal lipid peroxidation.

In rats co-exposed to cadmium and copper (both higher and lower doses) we observed

a decrease in the cadmium concentration in kidneys and an increase of Cd concentration in the blood. It means that copper prevents the accumulation of cadmium in kidneys. Other researchers had already observed this phenomenon [18].

In our study, we did not find any differences in renal and blood cadmium concentration depending on the dose of copper.

The training program used in our investigations caused a decrease in cadmium concentration in rats' kidneys and blood. These results are consistent with other authors' conclusions [15, 19]. It seems that systematic physical effort interferes with cadmium accumulation in kidneys and /or accelerates its elimination from the organism.

The influence of physical training on the decrease of cadmium concentration in kidneys was significant in the case of rats which were intoxicated only with cadmium. No changes were observed after physical training in the case of rats intoxicated with cadmium and copper (both higher and lower doses) simultaneously. However, at this stage of the study, it is difficult to conclude the role of copper in these processes.

The administration of cadmium alone, as well as the administration of cadmium and a higher dose of copper simultaneously, caused the modification of zinc metabolism and an increase of its concentration in kidneys. There was a significant, positive correlation between Cd and Zn concentrations in kidneys ($r = 0.56$, $p = 0.000017$). Oishi et al [20] obtained similar results. This phenomenon can be explained by various mechanisms. The increase of zinc concentration in the kidney mobilizes the defence system against cadmium toxicity. Metalothionein and zinc act as antioxidants and influence the stabilisation of tissue membranes. Zinc is also a component of Zn, Cu SOD (Zn, Cu dismutase superoxide), which neutralises reactive superoxide radicals [11].

A higher increase in the renal concentration of zinc was found in kidneys of animals subjected to the physical training. Mishima et al [21], suggest that protective-acting mechanisms of zinc against cadmium toxicity depend on zinc concentration. In the case of higher concentrations, zinc inductes the synthesis of MT and at lower concentrations, in spite of a significant protective effect, the rise of this protein concentration was not observed.

According to these authors, the protective effect of low Zn doses results from the similarities between physical and chemical specificities of zinc and cadmium.

Zinc imitating cadmium ions, accelerate the stabilization of equality between internal and external concentration of cadmium and it is also possible that zinc disturbs the process of cadmium to passing through biological membranes.

The aforementioned research [21] suggest that protective mechanisms, independent from MT, play a more important role in natural physiological conditions.

Milnerowicz [19] has observed that lower Cd concentration in kidneys, during physical training, was accompanied by the lowering of MT content.

It is possible that the mobilization of zinc in kidneys, during physical effort, lowers the toxicity of cadmium resulting from mechanisms independent from MT.

Systematic physical effort also influences copper metabolism. In the case of rats intoxicated only with cadmium, physical training caused the increase of copper concentration in kidneys. It can be considered also as a positive effect because copper, similar to zinc, is an important component of the antioxidant defence system and induction MT synthesis, which bind reactive cadmium ions.

In animals intoxicated with cadmium and a lower dose of copper, the renal concentration of copper did not change significantly during training.

However, in the case of rats co-exposed to cadmium and a higher dose of copper, physical effort caused a further increase in the renal content of copper.

It seems that physical effort escalates pathological changes induced by cadmium and a high dose of copper, and additionally it disrupts copper metabolism and enlarges the retention of Cu in the kidney.

In rats exposed to cadmium and cadmium and copper simultaneously (both higher and lower doses) we observed a significant decrease of catalase.

The depletion of catalase activity in the kidney correlated with the rise of blood and renal concentration of cadmium and also with the rise in renal concentration of products of lipid peroxidation (MDA).

These dependences confirm the influence of cadmium on the decrease of catalase activity and induce oxidative processes. The inhibitory effect of cadmium on catalase activity was observed in other investigations [22].

The data show that exercise training on treadmill increased the renal activity of catalase in rats treated with cadmium and co-exposed to cadmium and a lower dose of copper. A higher growth in the activity of catalase was observed in the group co-exposed to cadmium and a lower dose of copper. By contrast, in rats co-exposed to cadmium and a higher dose of copper, CAT activity decreased with training.

These results indicate that physical training, exposure to Cd and the injection of lower dose of

Cu synergistically affects an increase of catalase activity. In the presence of a high renal concentration of copper, training additionally intensified the inhibition of CAT activity.

The activity of GPx decreased in the kidneys of trained rats exposed to cadmium and copper. In the case of rats intoxicated with a higher dose of copper, it might also be a consequence of an interaction between Cd, Cu and physical effort.

However, in the case of rats intoxicated with a smaller dose of copper, a fall in activity of glutathione peroxidase under the influence of training can be explained by the compensatory rise of catalase.

In trained rats exposed to cadmium alone the activity of SOD was lower than in untrained co-partners.

The activity of SOD increased in groups exposed to a higher dose of copper alone, and also in group co-exposed to cadmium and a higher dose of copper, and did not exhibit any further changes during training.

The rise of SOD activity and the significant negative correlation between the activity of this enzyme and the concentrations of SH-groups ($r = –0.29$, $p = 0.039$) indicate a considerable generation of superoxide radical, which causes oxidative damages of thiol group.

The activation of the antioxidative defence system connected with the rise of SOD activity was insufficient therefore in rats intoxicated with a higher dose of copper, where a significant elevation in the concentration of lipid peroxidation products could be observed. The level of oxidative stress (measured as MDA) concentration was greater in rats subjected to physical training than in their untrained co-partners.

The highest activity of SOD (twice higher than in the control group) was found in rats intoxicated only with a high dose of copper. An increase of renal SOD activity positively correlated with renal copper ($r = 0.34$, $p = 0.0146$) and negatively with cadmium content in the kidney($r = –0.28$, $p = 0.040$).

These associations indicate an important role of renal copper in both the antioxidative defence system and oxidative processes.

During the intoxication with metals, we observed a decrease of GSH content in kidney Glutathione status shown to have an impact on the ability of the body to handle heavy metals such as cadmium and copper. Glutathione is an important antioxidant. When glutathione status is elevated or increased by supplementation, the tissues were able to stop the damage caused by the lipid peroxides induced to the metals [23–25].

The negative correlation between the content of GSH and the concentration of cadmium in the kidney confirms the escalation of oxidative processes by Cd and the participation of reduced glutathione in the neutralization of toxic Cd ions. The data of the present study shows that exercise training significantly increased the concentration of GSH but only in rats intoxicated with Cd alone and co-exposed to cadmium and a high dose of copper

The increase of GSH content in the kidneys of trained rats co-exposed to cadmium and a high dose of copper, can be explained by the inhibition of GPx activity, which uses reduced glutathione as a substrate.

According to expectations, the level of oxidative stress (measured as the elevation of MDA concentration and the decline of SH-groups) increased in rats treated with metals.

The depletion of SH-groups' concentration correlated with the increase in the renal concentration of copper and MDA and with blood cadmium concentration.

A positive correlation was found between the renal concentration of MDA and copper content in kidneys. However, negative correlations were found between the renal concentration of MDA and blood content of Cu.

The highest increase of products of lipid peroxidation and the highest decline of SH-groups were observed in the kidneys of rats subjected to physical training and co-exposed to cadmium and a high dose of copper.

The results of this study show that physical training significantly alters blood and renal levels of cadmium. Training significantly decreased plasma Cd levels in rats intoxicated with cadmium or co-exposed to cadmium and copper in comparison with their untrained equivalens. Physical training reduces the concentration of cadmium in kidneys and also increases the renal concentration of zinc (a nutrient which protects against cadmium toxicity), especially in animals exposed to cadmium alone.

Systematical physical exercise intensifies the antioxidant defence system. We observed a higher concentration of reduced glutathione, SH-groups and activity of CAT in trained rats compared to their untrained equivalents.

In rats which were intoxicated by cadmium and a higher dose of copper, training only enlarged the oxidative stress induced by these metals and lowered the activity of antioxidative enzymes such as GPX and CAT.

Copper intoxication (higher dose) lowered the concentration of cadmium in rats, but increased oxidative processes. Training, in conjunction with intoxication with cadmium and copper (high dose), considerably intensifies the oxidative stress in the kidneys of rats.

However, physical training intensifies the antioxidant defence system, but does not reduce lipid peroxidation.

The results of this study show that the influence of physical training on oxidative processes and antioxidant defences depend on doses of metals and their concentrations in tissues.

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Conflict of interest: None declared

Received: 11.04.2007
Revised: 14.06.2007
Accepted: 22.08.2007