Individual approach to treatment and cobalt - a new look at an old problem.

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Abstract

An individual approach in the cobalt-induced stress dependently hypoxic states properties was analyzed. Effects of cobalt (Co) chloride treatment (30 mg kg bw, 3 h) on metals content (Na, K, Ca, Fe, Zn, Mn, Mg, Co, Cd and Pb) in femur, plasma, and liver Wistar rats with individual resistance to hypoxia (low resistance - LR and high resistance - HR) were studied. Effects of cobalt chloride were mediated by individual response to hypoxia factor and reduction of iron, lead and cobalt in the long bones with subsequent accumulation in liver tissue. Regression analysis showed a high dependence of the individual organism's reactivity from a low content of free Co. Possibly, a parameter of low content of Co in the blood can be used as a test system for assessing individual resistance to hypoxia and subsequent pharmacological correction. Effects of Co in our investigation cause the cadmium accumulation in bones and liver. Effects Co show substantial reduction of erythrocyte membranes resistance to the action of hemolytic agents (osmotic, peroxide and acid) with retaining a high level of total antioxidant capacity in erythrocytes and plasma, but not in liver. Individual constitutional resistance to low oxygen tension may be an important criterion for an individual approach in pharmacotherapy.

Keywords Cobalt, bones, heavy metals, antioxidant defense, different resistance to hypoxia, oxidative stress.

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Introduction

Oxygen is needed for the implementation of oxygen-dependent processes in the mitochondria by production of cellular energy through ATP synthesis. From the other hand, reactive oxygen species (ROS) generated as byproducts of energy generation and have been implicated in the genesis of many diseases [1-3] and heavy metals impact. Therefore, eukaryotic cells have evolved elaborated oxygen-sensing mechanisms decreases in oxygen levels defined as hypoxia [4]. A decrease in ROS production, such as during hypoxia or anoxia, may protect cells from death or injury caused by different free radicals. Conditions that cause hypoxia or different levels of tissue hypoxia are very common in the human and animal population. High level of ROS and the disruption of the antioxidant system in the tissues are among the leading causes of morbidity, disability, and mortality [5]. The molecular pathophysiology of hypoxia states and its potential effects on organism's health are important issues at the forefront of biomedical research [6].

The states of moderate hypoxia or hypoxia/reoxygenation alternation have a great importance in the formation of body adaptation mechanisms. Cellular mechanisms of adaptation to hypoxia are regulating the expression of more than one hundred genes involved in various biological processes [7]. It has been shown that the adaptation to hypoxia leads to a set of hematological shifts and an increase in blood oxygen capacity, as well as activates a multifactorial cascade of the antioxidant defense systems, mitochondria functioning, improves muscle performance, glucose transport, utilization of lipids etc. [8-10].

Adaptation to periodic hypoxia has effectively been used in different fields of clinical and preventive medicine and sports physiology [11]. There has been a renewed interest in the role of cellular responses to hypoxia specific metabolic stimulus which undoubtedly belongs to, as shown by recent studies, cobalt chloride. Mitochondria and oxygen-dependent processes are a key target of cobalt toxicity [12]. Cobalt interferes with cellular respiration leading to oxygen deprivation [13]. Mitochondrial dysfunction can lead to functional and metabolic pathology due to dose-dependent ATP depletion and may involve of a number of mechanisms. However, using cobalt chloride as a well-established hypoxia mimetic factor and inducer of hypoxia-like responses [14,15] was connected with doping tests in sports. Cobalt chloride administration is an alternative and dangerous blood doping technique, which is virtually undetectable by anti-doping testing [16,17].

Main effectors of oxygen homeostasis are Hypoxia-Inducible Factors (HIF) that enhancement of oxygen delivery (angiogenesis) and cellular energy metabolism [18-20]. HIF-α is constitutively expressed in cells but is post inactivated under normoxic conditions. Exposing cells to hypoxic conditions are due to HIF-stabilization with next gene transcription and association with hypoxia response elements [21]. Co causes gene modulation at the HIF pathway to stimulate the

erythropoietin (EPO) transcription or its recombinant form, and increase its levels in blood [17,22]. Erythropoietin using for controlling erythropoiesis [23] in bone marrow, therefore similar stimulating agents often was used in athletes [16,24]. Although these doping may possibly have some physiological effects on tissues, there are significant risks associated with the illicit use of these substances in athletes [25]. Often in literature provides data on the high cobalt content in racehorses [15]. In the some countries there have been reports of unexplained deaths in horses that were found to have elevated blood levels of cobalt chloride [15].

Recently cobalt salts have medical applications for the treatment of anaemia as well-known haematopoietic system agent; it is an essential micronutrient that is important for the formation of the vitamin B12 complex [26]. Co acts as a coenzyme to catalyse various metabolic reactions [27]. Co realizes effects into three classes of enzymes: isomerases, methyl transferases and reductive dehalogenases. These enzymes participate in reactions essential to DNA synthesis, fatty acid synthesis and energy production [28].

But cobalt in high doses can be highly toxic [29]. It exerts neurotoxic effects, cardiomyopathy and ischaemic heart disease, a variety of toxic effects on the nervous system as well as the respiratory system, skin, heart and thyroid [30]. High doses of Co in patients exposed to abnormal levels from damaged hip prostheses induce some negative effects because promote secretion of cytokines from osteoblasts, which leads to inflammation [31-33]. High serum Co has been associated with optic atrophy and retinal dysfunction as possible complications of cobaltism [34].

It has been showed that the bone is a dynamic organ with well-regulated turnover mediated by bone resorption by osteoclasts and bone formation by osteoblasts [35]. For this reason, the study of the pattern of systemic responses of the bones and tissues has an important prognostic value for assessing the functional reserves of the human and animal body, monitoring the process of adaptation of athletes and horses during intense trainings, prediction of the working capacity under extreme living conditions, and prediction of the incidence of risk [36]. However, the pattern of heavy-metal distribution and levels of heavy metals in various tissues of rodents are similar to those found in humans. Therefore, in research rodents frequently serve as mammalian surrogates for humans [37].

ROS generated in the bones by extra- or intra-osteoclast have been shown to act as signaling molecules that expedite the differentiation of osteoclasts, and enhance bone resorption [38,39]. Therefore, deregulation of the redox balance by excessive ROS levels under different toxic agents can accelerate bone resorption and lead to bone fragility [40,41]. On the other hand, ROS play a critical role in the pathogenesis of ageing and osteoporosis. In the old mouse, loss of bone mass and decreases in bone formation and osteoblast function are associated with an increase in ROS levels simultaneously with decreasing of antioxidant defense system in bone. Investigation by [41] study shows that progressive, age-related bone loss correlates with increased oxidative stress in osteoblasts or osteoclasts. Key role in these processes is associated with dihydrogene peroxide in bones [42-44].

It has been investigated that the effects of adaptation to hypoxia depend in many respects on individual sensitivity to it, which, provides an individual reaction of mitochondria functioning, biotransformation of xenobiotics, the sensitivity of target cells to nitric oxide (NO), and formation of individual cell resistance to oxygen deficiency [6,7]. Some known mechanisms of question of sensitivity/resistance to hypoxia is determined by the phenotypic and adaptive-acquired features of the body manifesting themselves at the level of systemic, local, tissue, and molecular-cellular properties [11]. Individual constitutional resistance to low oxygen tension may be an important criterion for an individual approach in pharmacotherapy diseases, sport and early prevention diseases complications [6].

Different toxic and pharmacological agent influences may impact opposite changes in metabolism pathways (oxidative stress first) in tissues of animals with genetically determined differences in the sensitivity to hypoxia. Genetic and phenotypic mechanisms providing the resistance of organs and tissues to hypoxia was shown [9,10]. Individual resistance to hypoxia provides an individual reaction of differences in the parameters of hepatocytes functioning [8], mitochondrial transport, biotransformation of xenobiotics, drug-metabolizing system and hepatic cytochrome P450 activities, properties of mitochondrial enzymes and energy metabolism [8], state of mitochondrial respiration and calcium capacity in liver [9].

The rats highly resistant (HR) and low resistant (LR) to hypoxia differ in the intensity of lipid peroxidation (LPO) and activity of the antioxidant system [9]. In our earlier investigation, we observed that rats with low and high resistance to hypoxia had a different level of capacity. Animals with a high resistance, endured the physical training better than rats with a low resistance, which represented the explored index of dynamic endurance. L-arginine as precursor NO and modulator at hypoxic states [10] increased dynamic endurance to the physical training of rats with LR to the level of HR in the control. This data suggest individual resistance to hypoxia/ stress may be a serious criterion for individual approach in pharmacotherapy of diseases and metabolic ways corrections.

The aim of this study was to evaluate the effect of cobalt impact on elucidate physiological and heavy-metal contents in long bones, plasma and liver, the resistance of erythrocytes to haemolytic agents and oxidative stress tissues' ability in animals with different predisposition to hypoxia factor.

Materials and Methods

Animals and experimental design

The experiments were conducted with the Guidelines of the European Union Council and the current laws in Poland, according to the Ethical Commission. Male white Wistar rats (180-220 g) were used in the study. The rats were housed at a constant temperature of 20 ± 2oC. The animals had free access to feed and water throughout the experiments. Previously, the

animals were divided into two groups: rats with low resistance and high resistance to hypoxia. Resistance of rats to hypoxia was evaluated as survival time (min) in the altitude chamber 11,000 m above sea level. Survival time was measured after achieving the altitude. Cessation of breathing served as the criterion for resistance to hypoxia. Animas were used in experiments after two weeks from this dividing. Animals with middle resistance to hypoxia weren't used in this study. To eliminate diurnal rhythm changes, all examinations started at 10 and ended at 12 am.

Experimental groups

The rats were randomly assigned into four groups (divided into two subgroups) : untreated control group (I) consisting eight rats with low resistance and rats with high resistance to hypoxia; Co group (II) - rats with low resistance (n = 8) and high resistance to hypoxia (n = 8) receiving 30 mg of cobalt chloride/kg b.w. for 3 hours. Before the experiment a group of control animals was injected 1 ml of saline.

Tissue isolation

The liver, brain, kidney, lung, heart were removed from rats after their decapitation. One rat was used for each homogenate preparation. Briefly, the excised liver were weighed, washed in ice-cold buffer, and minced. The minced tissues were rinsed with cold isolation buffer to remove blood and homogenised in a glass Potter-Elvehjem homogenising vessel with a motor-driven Teflon pestle on ice. The isolation buffer contained 120 mM KCl, 2 mM K2CO3, 10 mM HEPES, and 1 mM EDTA; pH of 7.2 was adjusted with KOH.

Sampling

We collected 2,5 mL blood samples in tubes with EDTA. After centrifugation, plasma samples were frozen at -20°C and stored until analysis. For isolation of erythrocytes, blood samples were centrifuged at 3,000g for 10 min. The plasma was removed; the erythrocytes were washed three times with five volumes of saline solution and centrifuged at 3,000g for 10 min. Plasma was used for the determination of total antioxidant capacity.

Resistance of erythrocytes to haemolytic reagents

Acid resistance of erythrocytes. The acid resistance of erythrocytes was measured spectrophotometrically following the method with 0.1 N HCl [45]. The method is based on the measuring of the dynamics of erythrocytes disintegration under haemolytic reagent action. Disintegration of erythrocytes (%) for every period of time was expressed in curve. The time of haemolytic reagent action serves as the measure of erythrocytes resistance. The absorbance was read at 540 nm in every 30 second after addition of HCl till the end of haemolysis. Difference of absorbance at the beginning and at the end of haemolysis was determined as 100%.

Osmotic resistance of erythrocytes. The osmotic resistance of erythrocytes was measured spectrophotometrically at the wave length of 540 nm as described Kamyshnikov [46]. The method is based on the determination of differences between osmotic resistance of erythrocytes to a mixture with a different concentration of saline solution and urea.

Peroxide resistance of erythrocytes. The peroxide resistance of erythrocytes was determined spectrophotometrically as described by Gzhegotskyi et al. [47] at 540 nm by monitoring the rate of erythrocytes disintegration with hydrogen peroxide. The peroxide resistance of erythrocytes was expressed in %. Absorbance of mixture contained erythrocytes and phosphate buffer without hydrogen peroxide was determined as 100%.

Analyses of chemical element concentration

Samples of left femora and liver tissue were taken for analyses of chemical element concentration (Na, K, Ca, Fe, Zn, Mn, Mg, Co, Cd and Pb). These were preceded by mineralization of samples, which was done using the Berghof Speedwave MWS-2 system (microwave pressure digestion unit with built-in in situ temperature measurement) and using 1.5ml of nitric acid 65% pure for analysis by Sigma-Aldrich to receive a clear solution. The contents of elements (ppm dry weight) were then determined according to Weltz [48] using a ‘‘Perkin-Elmer AAnalyst 800’’ atomic absorption spectrophotometer. Standard curves were prepared using standardized Merck samples for each of the element.

Biochemical assays

Total antioxidant capacity (TAC) assay

The TAC level in the plasma and tissues was estimated spectrophotometrically at 532 nm following the method with Tween 80 oxidation by measuring TBARS level [49]. Plasma inhibits Fe2+/ascorbate-induced oxidation of Tween 80, resulting in a decrease of TBARS level. The absorbance of the obtained solution was measured at 532 nm. Absorbance of blank was determined as 100%. The level of TAC in sample (%) was calculated in respect to the absorbance of blank.

Statistical analysis

Results are expressed as mean ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors tests ($p>0.05$). Homogeneity of variance was checked using the Levene's test. Significance of differences in the chemical element concentration between control and cobalt group depending individual resistance of hypoxia was examined using Mann-Whitney U test according to Zar [50]. Differences were considered significant at $p<0.05$. In addition, the relationships between data of all individuals were evaluated using Pearson's correlation analysis. Multivariate linear regression adjusted for high and low resistance to hypoxia of bones, blood and liver of rats was performed to evaluate the effect of metals content on resistance parameters function. All statistical calculation was performed on separate data from each individual with Statistica 8.0 software (StatSoft Inc., Poland).

Results

Concentration of metals in bone, plasma and liver

Concentration of selected metals (Na, K, Ca, Cu) in the femora, plasma and liver during cobalt treatment dependent resistance to hypoxia are listed in Table 1. Lowering concentration of Na and higher for Ca (for HR animals) was detected in the liver of rats at cobalt treatment.

Table 1. Concentration of selected metals (Na, K, Ca and Cu) in the femora, plasma and liver of rats with different resistance to hypoxia at cobalt treatment.

		Control	Co
Parameters		**Bone**	
	LR	26.01 ± 0.29	24.92 ± 0.03*
	HR	26.01 ± 0.29	24.93 ± 0.025*
	plasma		
	LR	29.15 ± 1.76	24.99 ± 0.10
	HR	25.35 ± 0.06#	24.94 ± 0.09
	liver		
	LR	25.54 ± 0.28	25.54 ± 0.49
Na, mg/g	HR	25.26 ± 0.84	25.51 ± 0.80
	bone		
	LR	4.99 ± 0.001	4.99 ± 0.00
	HR	4.99 ± 0.000	4.99 ± 0.00
	plasma		
	LR	7.49 ± 0.06	4.99 ± 0.01
	HR	4.99 ± 0.004#	4.99 ± 0.004
	liver		
	LR	5.00 ± 0.02	5.01 ± 0.21
K, mg/g	HR	5.00 ± 0.12	5.00 ± 0.12
	bone		
	LR	11.11 ± 0.37	11.15 ± 0.04
	HR	10.20 ± 0.01	10.66 ± 0.06*
	plasma		
	LR	11.93 ± 0.12	10.58 ± 0.11*
	HR	10.98 ± 1.74	9.93 ± 0.67
	liver		
	LR	47.35 ± 3.77	54.62 ± 3.79
Ca, mg/g	HR	88.34 ± 9.14#	145.03 ± 24.64*
	bone		
	LR	27.08 ± 0.07	27.21 ± 0.04
	HR	26.98 ± 0.13	27.07 ± 0.02
	plasma		
Cu, µg/g	LR	29.42 ± 1.87	25.43 ± 2.97*

HR	24.91 ± 3.60#	25.28 ± 3.07
liver		
LR	33.91 ± 0.01	33.93 ± 0.01
HR	33.94 ± 0.01	33.95 ± 0.01

Values are expressed as the mean ± SEM, n = 6-8.

LR = rats with low resistance to hypoxia and HR = rats with high resistance to hypoxia.

- the significant change was shown as $p<0.05$ when compared rats with low and high resistance to hypoxia.

* - the significant change was shown as $p<0.05$ when compared rats Co treated group and two groups with different resistance to hypoxia.

Co effects in bone, plasma and liver for selected elements (Mg, Fe, Zn and Mn) was presented in Table 2. We observed statistically differences in Fe content between control and Co treated HR animals in bones. For these gropes of rats, we observed higher concentration of Mn in femur vs. control too.

Table 2. Concentration of selected metals (Mg, Fe, Zn and Mn) in the femora, plasma and liver of rats with different resistance to hypoxia at cobalt treatment.

		Control	**Co**
Parameters		**Bone**	
	LR	1160 ± 0.001	1157 ± 0.89*
	HR	1148 ± 1.34	1150 ± 0.89
	Plasma		
	LR	1165 ± 2.97	1143 ± 2.69
	HR	1009 ± 8.84	1149 ± 5.33
	liver		
	LR	852.84 ± 33.99	950.68 ± 20.45
Mg, µg/g	HR	950.65 ± 2.84	879.46 ± 34.72
	bone		
	LR	699.15 ± 28.64	460.4 ± 104.8
	HR	682.9 ± 80.72	246.25 ± 13.21*
	plasma		
	LR	637.11 ± 33.03	415 ± 20.02*
	HR	561.56 ± 12.21	283.34 ± 14.23*
	liver		
	LR	692.41 ± 37.16	809.74 ± 24.21*
Fe, µg/g	HR	657.74 ± 43.14	682.05 ± 16.66
	bone		
	LR	239.85 ± 11.11	250.9 ± 2.73
	HR	245.35 ± 1.99	236.7 ± 5.37
	plasma		
	LR	220.85 ± 10.01	230.1 ± 20.03
	HR	220.35 ± 15.09	221.4 ± 15.04
	liver		
Zn, µg/g	LR	455.56 ± 8.03	450.95 ± 3.58

	HR	456.46 ± 8.98	452.52 ± 12.80
	bone		
	LR	13.1 ± 0.24	13.27 ± 0.17
	HR	13.15 ± 0.16	13.87 ± 0.03*
	plasma		
	LR	15.06 ± 3.22	12.18 ± 3.38
	HR	11.62 ± 4.01	12.48 ± 3.48
	liver		
	LR	17.37 ± 0.01	17.59 ± 0.06
Mn, µg/g	HR	17.40 ± 0.03	17.37 ± 0.01

Values are expressed as the mean ± SEM, n = 6-8.

LR = rats with low resistance to hypoxia and HR = rats with high resistance to hypoxia.

- the significant change was shown as p<0.05 when compared rats with low and high resistance to hypoxia.

* - the significant change was shown as p<0.05 when compared rats Co treated group and two groups with different resistance to hypoxia.

Co, Cd and Pb in the femora, plasma and liver of animals with different resistance to hypoxia at Co treatment was also investigated (Table 3). Co administration was changed levels these three selected elements and dependent on individual sensitivity to hypoxic factor. So, we observed statistically decreased level of Co in bone and increased ones in liver. Toxic effects of Co at Co treatment were accompanied by statistically increased level of Cd in bone and liver for HR animals. Similar tendencies were observed for LR animals at Co treatment too; however, these differences were not statistically significant. Co effects reflected also on Pb level. Lower concentration of Pb level in bone at Co impact for HR animals was detected. In these conditions we observed the tendencies to accumulation of Pb in liver HR rats.

Table 3. Concentration of selected heavy metals (Co, Cd and Pb) in the femora, plasma and liver of rats with different resistance to hypoxia at cobalt treatment.

Parameters		**Control**	**Co**
		bone	
	LR	1.55 ± 0.53	0.96 ± 0.03
	HR	0.82 ± 0.10	0.37 ± 0.079*
	plasma		
	LR	5.23 ± 0.06	1.03 ± 0.35*
	HR	2.72 ± 0.24#	2.81 ± 0.71
	liver		
	LR	0.10 ± 0.001	0.13 ± 0.02*
Co, mg/g	HR	0.10 ± 0.02	0.20 ± 0.001*
	bone		
	LR	3.54 ± 0.27	4.85 ± 0.56
	HR	3.26 ± 0.07	4.64 ± 0.42*
	plasma		
	LR	6.65 ± 0.21	3.02 ± 1.65*
	HR	2.80 ± 0.99#	3.17 ± 0.93
	liver		
Cd, µg/g	LR	0.32 ± 0.07	0.43 ± 0.05

	HR	0.32 ± 0.06	0.43 ± 0.04*
	bone		
	LR	3.12 ± 0.001	3.80 ± 0.45
	HR	5.45 ± 0.26	4.17 ± 0.06*
	plasma		
	LR	5.62 ± 0.11	3.20 ± 1.87*
	HR	3.47 ± 1.81	3.16 ± 0.98
	liver		
	LR	0.18 ± 0.05	0.09 ± 0.001
Pb, μg/g	HR	0.19 ± 0.004	0.40 ± 0.09

Values are expressed as the mean ± SEM, n = 6-8.

LR = rats with low resistance to hypoxia and HR = rats with high resistance to hypoxia.

- the significant change was shown as $p<0.05$ when compared rats with low and high resistance to hypoxia.

* - the significant change was shown as $p<0.05$ when compared rats Co treated group and two groups with different resistance to hypoxia.

Thus, the effects of cobalt in different tissues (bone, plasma, liver) were dependent on the physiological levels of elements redistribution, especially heavy metals. The cobalt effects were dependent of the level of physiological reactivity to hypoxia. Several correlations between elements in the bones of rats with different resistance to hypoxia were found (Tables 4 and 5). We observed that increased Co level modifies physiological and heavy metals content and participated in significant element-element relations depending on congenital resistance to hypoxic states.

Parameters	**Correlative coefficient, p**	**Parameters**	**Correlative coefficient, p**
Low Resistance			
Control		**Co**	
Na-Zn	-0.993 (0.000)	Na-Mg	-0.944 (0.005)
Mg-Ca	0.992 (0.000)	Mg-Co	-889 (0.018)
Ca-Cd	-0.841 (0.037)	Zn-Cu	0.857 (0.029)
Fe-Co	0.890 (0.019)	Zn-Mn	0.851 (0.023)
Fe-Pb	0.991 (0.000)	Cu-Mn	0.969 (0.001)
Co-Pb	0.920 (0.011)	Co-Mg	-0.889 (0.018)
High Resistance			
Control		**Co**	
Ca-Mg	-0.884 (0.020)	K-Cu	0.844 (0.033)
Zn-Cu	0.926 (0.008)	K-Mn	0.895 (0.016)
Zn-Mn	0.948 (0.005)	Ca-Pb	0.954 (0.003)
Cu-Mn	0.983 (0.012)	Zn-Cu	0.849 (0.033)
Cd-Cu	0.909 (0.012)	Zn-Mn	0.834 (0.039)
Cd-Mn	0.886 (0.019)	Cu-Mn	0.988 (0.000)
Pb-Cu	0.815 (0.048)		

Table 4. Analysis of correlations between metals level in the plasma of rats with different resistance to hypoxia at cobalt treatment.

Table 5. Analysis of correlations between metals level in the liver of rats with different resistance to hypoxia at cobalt treatment.

Parameters	Correlative coefficient, p	Parameters	Correlative coefficient, p
Low Resistance			
Control		**Co**	
Na-K	0.783 (0.021)	Ca-Cu	-0.840 (0.009)
Cu-Mn	-0.945 (0.001)	Ca-Mn	-0.876 (0.005)
Cu-Co	0.981 (0.001)	Ca-Cd	0.865 (0.004)
Cu-Cd	0.980 (0.000)	Ca-Pb	0.839 (0.009)
Cu-Pb	0.956 (0.000)	Cu-Pb	0.835 (0.008)
Co-Cd	0.978 (0.000)		
High Resistance			
Control		**Co**	
Fe-Cu	0.731 (0.032)	K-Zn	0.858 (0.006)
Fe-Mn	0.729 (0.040)	Cu-Pb	-0.987 (0.000)
Fe-Co	- 0.739 (0.033)	Co-Pb	-0.978 (0.001)
Fe-Cd	-0.726 (0.023)		
Co-Cu	- 0.745 (0.024)		
Co-Cd	0.987 (0.000)		
Co-Pb	0.976 (0.001)		

Multiple linear regression analysis is a statistical tool for determining the relationship between single depended variable and a set of independent variables to best represent a relationship in a population. We used regression analysis as a way of predicting an outcome variable from a predictor variable individual organism's reactivity and metals content in tissues (bones, blood, liver). Stepwise linear multiple regression method was applied to find the dominant factors influencing resistance to hypoxia forming. Our data represented in Table 6 shows dependencies between resistance to hypoxia and metals content in the plasma. Table content the prediction equation, where are dependent from metals variable, the different set of independent variables, the regression coefficient (R2), the corrected regression coefficient (R2 corr), the random error, and probability (p). Multiple linear regression analysis fit a model to our data metal content in the plasma (Na, K, Cu, Co, Cd) in individual resistance to hypoxia in rats. Statistical model used to describe to resistance and predict a value of the dependent variable from set of independent variables. In this study, a research model is based on the selected metals in different tissues to explain the individual resistance factor.

Table 6. Multiple linear regression equation for metal concentrations in plasma rats with different resistance to hypoxia.

Metals	Regression equation (Resistance)	R^2	R^2 corr	Significance of regression, p
Plasma				
Cu	-1.127Cu+143.213 ± 2.868	0.426	0.368	0.021
K	1.247K - 120.97 ± 1.323	0.516	0.468	0.009
Cd	-0.963Cd+103.883 ± 1.498	0.665	0.631	0.001
Na	1.899Na - 166.46 ± 1.402	0.688	0.656	0.001
Co	-1.126Co+118.982 ± 1.334	0.774	0.751	0.000
Liver				
Mg	24.45Mg - 1616.83 ± 81.458	0.292	0.241	0.031
Ca	2.482Ca - 987.678 ± 19.799	0.551	0.519	0.001

Coefficient of determinations (R2) as so as corrected coefficient of determinations (R2corr) for selected (only statistical important) data shows dependencies of metals in model resistance to hypoxia. R2 values (Table 6) revealed that Cu, K, Cd, Na, and Co contents explained the variability of the metal in plasma individual organism's reactivity to hypoxia to the extent of 42.6% (36.8%), 51.6 % (46.8%), 66.5% (63.1%), 68.8% (65.6%) and 77.4% (75.1%) respectively, and indicated a significant relationship ($p<0.05$). Regression model in this Table indicated inverse higher relationships for Co content in plasma dependent other metals. This suggests that decrease in blood Co content may lead to increase in resistance factor (Tables 7,8).

Table 7. Analysis of correlations between metals level in the bones of rats with different resistance to hypoxia at cobalt treatment.

Parameters	**Correlative coefficient, p**	**Parameters**	**Correlative coefficient, p**
Low Resistance			
Control		**Co**	
Na-Co	0.937 (0.006)	Ca-Mg	-0.884 (0.020)
Fe-Zn	0.821 (0.045)	Zn-Cu	0.926 (0.008)
Fe-Cu	0.834 (0.039)	Zn-Mn	0.943 (0.005)
Fe-Cd	0.841 (0.036)	Cu-Cd	0.909 (0.012)
Fe-Pb	0.856 (0.030)	Cu-Pb	0.815 (0.048)
Zn-Mn	0.859 (0.030)	Cd- Mn	0.886 (0.019)
Cu-Mn	0.978 (0.001)		
Cd-Mn	0.848 (0.033)		
Cd-Pb	0.880 (0.021)		
High Resistance			
Control		**Co**	
Ca-Mg	-0.884 (0.020)	K-Cu	0.844 (0.034)
Zn-Cu	0.926 (0.008)	K-Mn	0.895 (0.016)
Zn-Mn	0.943 (0.005)	Zn-Cu	0.849 (0.033)
Cu-Cd	0.909 (0.012)	Zn-Mn	0.834 (0.039)
Cu-Pb	0.815 (0.048)	Pb-Ca	0.954 (0.003)
Mn-Cd	0.886 (0.019)		

Table 8. Total antioxidant status (%) in the blood and tissues of rats with different resistance to hypoxia at cobalt treatment.

Parameters		**Control**	**Co**
Plasma	LR	23.64 ± 4.52	32.11 ± 6.37
	HR	16.59 ± 1.99	41.39 ± 3.33**
Erythrocytes	LR	14.12 ± 1.23	43.30 ± 4.44**
	HR	18.48 ± 2.02*	46.13 ± 5.98**
Brain	LR	10.91 ± 1.34	12.82 ± 3.17
	HR	12.48 ± 2.14	12.02 ± 2.45
Kidney	LR	7.71 ± 1.12	7.94 ± 1.45
	HR	4.42 ± 0.89*	5.18 ± 0.97
Lung	LR	10.21 ± 3.11	11.58 ± 3.45
	HR	12.48 ± 3.23	8.93 ± 3.12

	LR	23.05 ± 2.36	16.89 ± 2.58**
Liver	HR	16.04 ± 0.25*	8.77 ± 0.25**
	LR	6.83 ± 0.94	4.63 ± 0.70
Heart	HR	8.99 ± 1.28	7.25 ± 0.79

Values are expressed as the mean ± SEM, n = 8.

LR = rats with low resistance to hypoxia and HR = rats with high resistance to hypoxia.

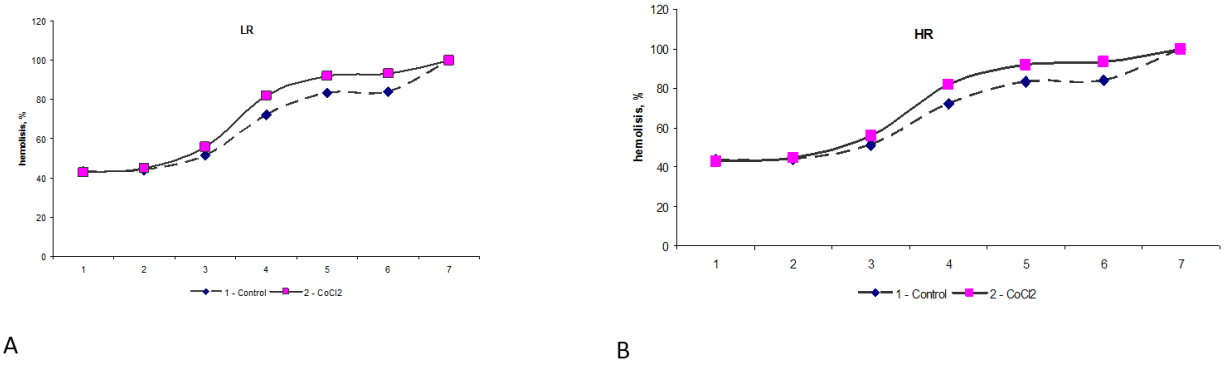
* - the significant change was shown as $p<0.05$ when compared rats two groups with different resistance to hypoxia.

** - significant difference ($p<0.05$) between Co-treated group rats with low and high resistance to hypoxia.

Resistance of erythrocytes to haemolytic reagents

Erythrocytes are the one of the most important indicators of the internal exposure of an individual to increased generation of reactive oxygen species and intensification of lipid peroxidation. Therefore, the next goal of our investigation was the measurement of erythrocytes' resistance to haemolytic reagents in the blood rats at cobalt treatment (Figures 1-3). Using different methods for determining the resistance of red blood cells to hemolytic agents has allowed us to identify the main differences of the cobalt effects dependent of the individual hypoxia reaction.

Osmotic resistance of erythrocytes is shown in Figure 1. Percent of hemolysated erythrocytes under incubation with different concentration of urea was lower in the blood of LR (part A) and was higher for HR (part B) at cobalt treatment.



A B

Figure 1: Osmotic resistance of erythrocytes (% of hemolysated erythrocytes in different concentration of urea) of rats with different resistance to hypoxia at cobalt treatment (n = 8). Horizontal axis: different urea concentration (1 - 0.12, 2 - 0.135, 3 - 0.15, 4 - 0.165, 5 - 0.18, 6 - 0.195, 7 - 0.3 mol/L).

Resistance of erythrocytes to hydrogen peroxide is shown in Figure 2. Resistance of erythrocytes to hydrogen peroxide was found to be lower in LR rats at Co impact and has not been changed at simple condition in HR animals compared to the control group.

Our work clearly establishes that erythrocytes of LR rats at Co impact subjected to the haemolytic agents undergo high level of haemolysis compared to control group due to the oxidizing effect of the acid, urea and hydrogen peroxide. Erythrocyte haemolysis is associated with peroxidation of erythrocytes membranes by Co-induced oxidative stress only for HR animals. This may confirm the participation of cobalt metabolism in the formation in individual hypoxia resistance at blood level.

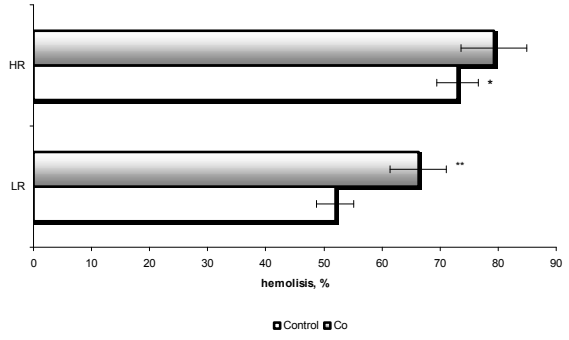


Figure 2: Resistance of erythrocytes to peroxide of rats with different resistance to hypoxia at cobalt treatment (n = 8). * - the significant change was shown as $p<0.05$ when compared two groups rats with different resistance to hypoxia; ** - significant difference ($p<0.05$) between Co-treated group rats with low and high resistance to hypoxia.

Total antioxidant status in the blood and tissues

For the purpose of concept proof for activating oxidative stress under the influence of cobalt in various rat tissues, we determined in the blood and tissues total antioxidant properties.

These data are presented in Table 6. Total antioxidant status (TAS) of tested tissues (plasma, erythrocytes, brain, kidney, lung, liver and heart) was significantly affected by Co and changed dependently on tissue specify and basic resistance to hypoxia. It was shown that Co treatment led to the TAS increasing in plasma and erythrocytes and decreasing in liver. Acid resistance of erythrocytes (Figure 3) was significantly lower at Co impact independently resistance to hypoxia. Percent of hemolysated erythrocytes per first min of initiation of haemolysis was higher in LR animals at Co treatment.

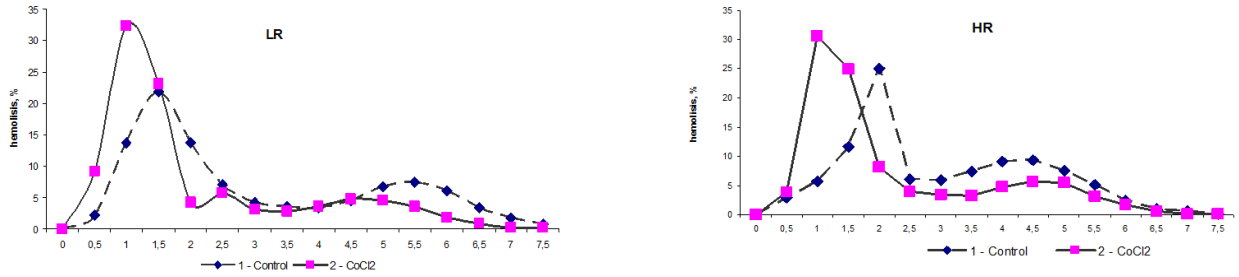


Figure 3: Acid resistance of erythrocytes (% of hemolysated erythrocytes per min) of rats with different resistance to hypoxia during cobalt treatment (n = 8).

Discussion

In the present work we focused on the effects of Co-associated changers of element content and biomarkers of oxygen metabolism damage pathways in different tissues dependent to physiological predisposition to hypoxia.

Oxidative stress, such as lipid peroxidation, is induced by an imbalance between reactive oxygen species (ROS) production and antioxidant defense capacity [51-52]. Various model organisms diseases hypothesized that oxidative stress can play a role in the regulation of important processes heavy metals and biotransformation of xenobiotics through influencing synthesis of antioxidant enzymes, modulation of signal pathways, inflammation, apoptosis, and cell proliferation, and thus oncogenic transformation [23].

In our study, Co impact was changed levels Na, Ca, Fe, Mn, Co, Cd and Pb elements and dependent on individual sensitivity to hypoxic factor. So, we observed statistically decreased level of Co in bone and increased ones in liver. Selected metals belong to the group of the most important physiological and heavy metals. They are predominantly bivalent metals, which are also included as a coenzyme in the active centers of antioxidant enzymes. Toxic effects of Co at Co treatment were accompanied by statistically increased level of Cd in bone and liver for HR animals. Co effects in bone and liver was focused at Fe and Mn level changers. However, some authors [53] reported that iron ions are themselves free radicals, and ferrous ions can take part with molecular oxygen in electron transfer reaction. It was important that generation of superoxide in the presence of high Fe ions induced to the formation of more reactive hydroxyl radicals by Fenton chemistry. Hydroxyl radicals can initiate lipid peroxidation by hydrogen abstraction. Using different scavengers system was studied superoxide-dependent Fenton reaction. Recently shows that superoxide providing the hydrogen peroxide and also reducing Fe3+ to Fe2+. Another iron complex can stimulate peroxidation by lipid decomposition reactions too [54].

Our results suggest higher sensitivity of erythrocytes membranes to hemolytic agent (peroxide, acid and osmotic) treatment at Co impact. The hemolytic activity at cobalt chloride administration was predominantly associated with ROS production by iron ions [55] realized by heme modification and hemoglobin. Our data was demonstrated higher Co level vs. control group animals. Similar to these observations cobalt ions can penetrate into the liver cells and modify intracellular heme and heme proteins. Increased contents of free heme or its analog in liver cells induced HO-1 [53].

High doses of cobalt exert a variety of dangerous effects including cardiotoxicity in man and in animals [13]. The cobalt (II) ion has recently been reported to react with hydrogen peroxide and to produce reactive oxygen intermediates such as superoxide radicals and hydroxyl radicals [44,56]. The liver and the heart have a higher uptake of cobalt compared to other organs [29]. Our data demonstration that bone's physiological and heavy metals level was important affected by Co too. This cobalt effects mediated by lowering erythrocytes membranes functioning as suggest our data with different hemolytic agents using. The mechanisms of this toxicity are not entirely clear. The cobalt (II) ion has recently been reported to react with hydrogen peroxide under physiological conditions to form reactive oxygen intermediates [57-59]. Such of them, hydroxylate aromatic compounds and degrade deoxyribose can involve formation of oxygen free radicals [60].

However, some data revealed in vitro and in vivo that cobalt influences bone resorption and bone formation by modulating bone cell metabolism [61]. Dermience et al. [62] showed that cobalt ions affect osteoblast proliferation, size, and shape. Co influences on proliferation and function of human osteoblast-like cells, osteoblastic activities decreases by alkaline phosphatase levels and calcium accretion, which inhibits release of osteocalcin and collagen type 1 proteins [57,63-65]. Co ions effects may be mediate by oxidative stress state in osteoblast-like cells by an unknown mechanism [59,66]. Co leads to inflammation and osteoclast differentiation, maturation, and stimulation by cytokines (TGF-β1, TNF-α, IL-β1, IL-6) secretion [65,67]. In contrast, some studies [68,69] on cobalt-chromium wear particles indicate antagonistic actions, such as induction of apoptosis in osteoclasts and decreased secretion of some inflammatory factors such as

prostaglandin E2 and interleukin-6. Effects cobalt-chromium interaction are associated with oxidative stress, oxidation and nitration of proteins, and misregulation of antioxidant enzyme expression [57,59,65].

Cobalt toxicity [33] has been an emerging problem of recent years with the introduction of metal-on-metal hip replacements [70-73]. Direct toxicity by Co and Cr ions represents some data of metal-on-metal hip prostheses, made of cobalt-chromium (Co-Cr) alloys [74-76]. CoCr alloys have widespread and extensively use in the orthopaedics industry as an alternative to titanium alloys due to their superior stiffness and corrosion resistance. Recent studies have shown that Co and Cr particles can enter the bloodstream and accumulate both in tissues and organs of patients. Toxic effects of these ions may cause hypersensitivity to these metals, led to chromosome aberrations and changers of lymphocytes proportions [66,76]. Cobalt nanoparticles could induce primary DNA damage in a concentration-dependent manner. The International Agency for Research on Cancer (IARC) has classified soluble Co2+ as possible carcinogenic and implanted orthopedic alloys as unclassifiable carcinogenic [61].

Our results shows higher level TAC in blood and plasma animals at Co treatment may be mediated by as non-classical antioxidant enzyme heme oxygenase-1 (HO-1). In bones similar mechanism was described by Nishishita and Tsukuba [77] in osteoclasts. ROS was identified as an additional essential factor for the osteoclasts differentiation by cobalt protoporphyrin functioning. Cobalt protoporphyrin was known as metalloprotoporphyrin and inducer of heme oxigenase-1 (HO-1) in cells. Recent studies these authors have demonstrated that induction of HO-1 inhibited differentiation and activation of osteoclasts cells. Induction of HO-1 connected with cobalt protoporphyrins has been shown as effective mechanism to protect apoptosis in the liver from ischemia/reperfusion or in human cardiac cells.

Effects of cobalt nanoparticles on human T cells in vitro was connected with significant decreasing in the SOD, GPX, and CAT activities, inducing oxidative stress and next cytotoxicity and genotoxicity [61]. These authors suggested that nanometer cobalt particles, generated from the articular surfaces metal-on-metal hip prostheses could gradually enter the immune cells, disturb the antioxidant balance, and next induce tissue damage. Similar data of oxidant-antioxidant balance and induced a depletion of cerebral and cerebellar antioxidant activities at Co effects was represented Garoui et al [78] too.

Some studies indicated important role of plasma proteins in toxic cobalt effects and individual's particular albumin binding capacity. It's connected with developing next influences in patients with high level cobalt blood concentration such as liver pathology, kidney disease and cancer, trauma, scleroderma and diabetes as well as poor protein diet [71,79]. However, cobalt can result in chemically induced hypoxia-mimicking conditions by stimulating hypoxia-inducible factors and modify oxidative phosphorylation [80].

The published data [11] and early results of our studies [10] partially answer the question about the causes of individual variability in the hypoxic resistance and show the contribution of individual functional systems to the oxygen supply of tissues. These two types of animal's models (low and high resistance to hypoxia) are described by two different functional and metabolic patterns not only hypoxia factor but stress and diseases predominance. They are associated with typical differences in activity of central nervous system and neurohormonal regulation, connected stress-activating and stress-limiting systems, oxygen-transporting function of the blood, mitochondrial functioning, Crebs cycle metabolites functioning [4]. Moreover, these parameters are coupled with energy metabolites exchange and functional activity of the mitochondrial respiratory change in animal tissues [9].

Low individual resistance to hypoxia was due with initially predominant activity sympathetic nervous system, with an even greater increase in the sympathetic activity during the development of the stress response. High individual resistance to hypoxia connected with domination of the parasympathetic activity in the autonomic regulation. Its reserve of the heart rate variability range and was used for a more adequate compensation as showed study [11].

Concluding, the results presented here demonstrate the importance of division animals at two different groups with hypoxia predominance in cobalt impact in conjunction with metal analysis and oxidative stress responses. Effects of cobalt chloride were associated with the formation of individual response to hypoxia and reduction of iron, lead and cobalt in the long bones with subsequent accumulation in liver tissue. Effects of cobalt in our investigation cause the cadmium accumulation in bones and liver. Effects cobalt show substantial reduction of erythrocyte membranes resistance to the action of hemolytic agents with a high level of total antioxidant capacity in erythrocytes and plasma, but not in liver.

References

1. Halliwell B. Free radicals and antioxidants-quo vadis? Trends Pharmacol Sci. 2011; 32(3):125–30.
2. Mitsiev AK. Role of activation of lipid peroxidation in the mechanisms of cardiovascular disease system under the action of heavy metals in the experiment. Patol Fiziol Eksp Ter 2015; 59(1):60-4
3. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. J Clin Biochem 2015; 30(1):011-026
4. Kurhaliuk N. Effect of tricarboxylic acid cycle intermediates on nitric oxide system during acute hypoxia. Ukr Biochim Zh 2002; 74(4):85-90
5. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological function and human disease. Int J Biochem Cell Biol 2007; 39(1):44–84
6. Glazachev OS, Dudnik EN. Microcirculatory Reactivity Features in Apparently Healthy Individuals during Acute Moderate Hypoxia and Hyperoxia Modeling. Human Physiology 2013; 39(4):400–06

7. Garcia-Heredia JM, Felipe-Abrio B, Cano DA, et al. Genetic modification of hypoxia signaling in animal models and its effect on cancer. Clin Transl Oncol 2015; 17(2):90–102.
8. Lukyanova LD. Problems of Hypoxia: Molecular, Physiological, and Clinical Aspects, Ed. L D Lukyanova and I B 2004
9. Tkachenko H, Kurhalyuk N. Protective role of pinacidil against adrenaline myocardium dystrophy in guinea pig liver mitochondria. Cen Eur J Biol 2007; 2(4):547-62
10. Tkachenko HM, Kurhalyuk NM, Vovkanych LS. Effect of pinacidil, KATP channel opener, on the liver mitochondria function in rats with different resistance to hypoxia during stress. Ukr Biokhim Zh 2013; 76(1):56–64.
11. Krivoshchekov SG, Balioz NV, Nekipelov NV, et al. Age, Gender, and Individually-Typological Features of Reaction to Sharp Hypoxic Influence. Human Physiology 2014; 40(6):613–22.
12. Karovic O, Tonazzini I, Rebola N, et al. Toxic effects of cobalt in primary cultures of mouse astrocytes: similarities with hypoxia and role of HIF-1. Biochem Pharmacol 2007; 73(5):694–708
13. Seghizzi P, D'Adda F, Borleri D, et al. Cobalt myocardiopathy: a critical review of literature. Sci Total Environ 1994; 150(1-3):105–9.
14. Piret JP, Mottet D, Raes M, et al. Cobalt chloride, a chemical inducer of hypoxia-inducible-factor-1, and hypoxia reduce apoptotic cell death in hepatoma cell line HepG2. Ann N Y Acad Sci 2002; 973:443–7.
15. Shrivastava K, Sairam M, Bansal A, et al. Cobalt supplementation promotes hypoxic tolerance and facilitates acclimatization to hypobaric hypoxia in rat brain. High Alt Med Biol 2008; 9(1):63–75.
16. Lippi G, Franchini M, Guidi GC. Cobalt chloride administration in athletes: a new perspective in blood doping. Br J Sports Med 2005; 39(11):872–3
17. Debeljak N, Sytkowski AJ. Erythropoietin and erythropoiesis stimulating agents. Drug Testing and Analysis 2012; 4(11):805–12.
18. Zhang N, Fu Z, Linke S, et al. The asparaginyl hydroxylase factor inhibiting HIF-1a is an essential regulator of metabolism. Cell Metab 2010; 11(5);364–78.
19. Myllyharju J. Prolyl-4-hydroxylases, master regulators of the hypoxia response. Acta Physiol 2013; 208(2):148–65.
20. Chen Z, Liu X, Mei Z, et al. EAF2 suppresses hypoxia-induced factor 1a transcriptional activity by disrupting its interaction with coactivator CBP/p300. Mol Cell Biol 2014; 34(6):1085–99.
21. Wang Y, Tang Z, Xue R, et al. Differential response to CoCl2-stimulated hypoxia on HIF-1α, VEGF, and MMP-2 expression in ligament cells. Mol Cell Biochem 2012; 360(1-2):235–42
22. Ho EN, Chan GH, Wan TS, et al. Controlling the misuse of cobalt in horses. Drug Testing and Analysis 2015; 7(1):21–30
23. Ebert B, Jelkmann W. Erythropoiesis-stimulating agents. Pharmacoeconomics 2014(2); 26:99-120
24. Lippi G, Franchini M, Guidi GC. Albumin cobalt binding and ischemia modified albumin generation: An endogenous response to ischemia? International Journal of Cardiology 2006;108(3):410 –1.
25. Mobasheri A, Proudman CJ. Cobalt chloride doping in racehorses: Concerns over a potentially lethal practice The Veterinary Journal 2015; 205(3):335-8.
26. Banerjee R, Ragsdale SW. The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes. Annu Rev Biochem 2003; 72:209–47.
27. Varela-Moreiras G, Murphy MM, Scott JM. Cobalamin, folic acid, and homocysteine. Nutr Rev 2009; 67(1):S67–S72.
28. Bleackley MR, Ross TA, MacGillivray RT. Transition metal homeostasis: from yeast to human disease Biometals 2011; 24(5):785–809.
29. Paustenbach DJ, Tvermoes BE, Unice KM, et al. A review of the health hazards posed by cobalt. Crit Rev Toxicol 2013; 43(4):316-62.
30. Catalani S, Rizzetti MC, Padovani A, Apostoli P. Neurotoxicity of cobalt. Hum Exp Toxicol 2012; p:31.
31. Simonsen LO, Harbak H, Bebbekou P. Cobalt metabolism–A brief update. Sci Tot Env 2012; 432:210-5.
32. Apel W, Stark D, Stark A, et al. Cobalt–chromium toxic retinopathy case study. Doc Ophthalmol 2013; 126(1):69–78.
33. Apostoli P, Catalani S, Zaghini A, et al. High doses of cobalt induce optic and auditory neuropathy. Exp Toxicol Pathol 2013; 65(6):719-27
34. Jantzen C, Jorgensen HL, Duus BR, et al. Chromium and cobalt ion concentrations in blood and serum following various types of metal-on-metal hip arthroplasties: a literature overview. Acta Orthop 2013; 84(3):229–36.
35. Wauquier F, Leotoing L, Coxam V, et al. Oxidative stress in bone remodeling and diseases. Trends Mol Med 2009; 15(10):468–77.
36. Kousteni S. Fox OS: Unifying Links Between Oxidative Stress and Skeletal Homeostasis. Curr Osteoporos Rep 2011; 9(2):60–6.
37. Shore RF, Rattner BA. Ecotoxicology of wild mammals. Wiley, London 2011.
38. Fraser JHE, Helfrich MH, Wallace HM, et al. Hydrogen peroxide, but not superoxide, stimulates bone resorption in mouse calvaria. Bone 1996; 19(3):223–6.
39. Lee NK, Choi YG, Baik JY, et al. A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. Blood 2005; 106(3):852–9.
40. Almeida M, Han L, Martin-Milla M, et al. Skeletal involution by age-related oxidative stress and its acceleration by loss of sex steroids. J Biol Chem 2007; 282(37):27285–27297.
41. Banifi G, Iorio EL, Corsi MM. Oxidative stress, free radical and bone remodeling. Clin Chem Lab Med 2008; 46(11): 1550–5.
42. Bax BE, Alam AS, Banerji B, et al. Stimulation of osteoclastic bone resorption by hydrogen peroxide. Biochem Biophys Res Commun 1992; 183(3):1153–8.

43. Basu S, Michaelsson K, Olofsson H, et al. Association between oxidative stress and bone mineral density. Biochem Biophys Res Commun 2001; 288(1):275–9.
44. Baek KH, Oh KW, Lee WY, et al. Association of oxidative stress with postmenopausal osteoporosis and the effects of hydrogen peroxide on osteoclast formation in human bone marrow cell cultures. Calcif Tissue Int 2010; 87(3):226–235.
45. Terskov IA, Hitelzon II. To the question about the dynamics of changes of red blood. Biophysics 1957; 3:523-535.
46. Kamyshnikov VS. Reference book on clinic and biochemical researches and laboratory diagnostics. Moscow, MED press-uniform 2004 .
47. Gzhegotskyi M, Kovalchuk S, Panina L, et al. Method for determination of erythrocyte membranes peroxide resistance and its informativeness under physiological conditions and at intoxication of organism. Exp Clin Physiol Biochem 2004; 3:58-64.
48. Weltz B Atomic absorption spectrometry. VCH Veincheim, Berlin. Atomic absorption spectrometry. VCH Veincheim, Berlin 1985.
49. Galaktionova LP, Molchanov AV, Elchaninova SA, et al. Lipid peroxidation in patients with gastric and duodenal ulcers. Klinicheskaia Labaratornaia Diagnostika 1998; 6:10-4
50. Zar JH. Biostatistical Analysis. Prentice-Hall Inc 1999.
51. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology 2011; 283(2-3): 65-87.
52. Slimen IB, Najar T, Ghram A, et al. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. Int J Hyperthermia 2014; 30(7):513-23.
53. Kaliman PA, Nikitchenko IV, Sokol OA. Regulation of Heme Oxygenase Activity in Rat Liver during Oxidative Stress Induced by Cobalt Chloride and Mercury Chloride. Biochemistry 2001; 66(1):77-82.
54. Gutteridge JMC. Lipid reoxidation and antioxidants as biomarkers of tissue damage Clin Chem. Clin Chem 1995; 41(12 pt 2):1819-28.
55. Bresgen N, Eckl PM. Oxidative stress and the homeodynamics of iron metabolism. Biomolecules 2015; 5(2):808-47.
56. Battaglia V, Compagnone A, Bandino A, et al. Cobalt induces oxidative stress in isolated liver mitochondria responsible for permeability transition and intrinsic apoptosis in hepatocyte primary cultures. Int J Biochem Cell Biol 2009; 41(3):586-94.
57. Fleury C, Petit A, Mwale F, et al. Effect of cobalt and chromium ions on human MG-63 osteoblasts in vitro: morphology, cytotoxicity, and oxidative stress. Biomaterials 2006; 27(5):3351–60.
58. Tkaczyk C, Huk OL, Mwale F, et al. Effect of chromium and cobalt ions on the expression of antioxidant enzymes in human U937 macrophage-like cells. J Biomed Mater Res 2010; 94(2):419–25.
59. Zijlstra WP, Bulstra SK, van Raay JJAM, et al. Cobalt and chromium ions reduce human osteoblast-like cell activity in vitro, reduce the OPG to RANKL ratio, and induce oxidative stress. J Orthop Res 2012 30(5):740–7.
60. Hatory N, Pehrsson SK, Clyne N, et al. Acute cobalt exposure and oxygen radical scavengers in the rat myocardium. Biochim Biophys Acta 1993; 1181(3):257-60.
61. Jiang H, Liu F, Yang H, et al. Effects of Cobalt Nanoparticles on Human T Cells In Vitro Biol Trace Elem Res 2012; 146(1):23–29.
62. Dermience M, Lognay G, Mathieu F. Effects of thirty elements on bone metabolism. J Trace Elem Med Biol 2015; 32:86–106.
63. Anissian L, Stark A, Dahlstrand H, et al. Cobalt ions influence proliferation and function of human osteoblast-like cells. Acta Orthop Scand 2002; 73(3):369–374.
64. Queally JM, Devitt JS, Butler AP, et al. Cobalt ions induce chemokine secretion in primary human osteoblasts. J Orthop Res 2009; 27(7):855–864.
65. Sansone V, Pagani D, Melato M. The effects on bone cells of metal ions released from orthopaedic implants. Clin Cases Mineral Bone Metab 2013; 10(1):34–40.
66. Tkaczyk C, Petit A, Antoniou J, et al. Significance of elevated blood metal ion levels in patients with metal-on-metal prostheses: An evaluation of oxidative stress markers. OpenOrthop 2010; 4:221-227.
67. Devitt BM, Queally JM, Vioreanu M, Butler JS, et al. Cobalt ions induce chemokine secretion in a variety of systemic celllines. Acta Orthop 2010; 81(10):756–764.
68. Haynes DR, Rogers SD, Hay S, et al. The differences intoxicity and release of bone-resorbing mediators induced by titanium and cobalt-chromium-alloy wear particles. J Bone Joint Surg 1993; 75(6):825–834.
69. MacQuarrie RA, Fang Chen Y, Coles C. Year-particle–induced osteoclast osteolysis: the role of particulates and mechanical strain. J Biomed Mater Res B Appl Biomater 2004; 69B:104–112.
70. Andrews RE, Shah KM, Wilkinson JM. Effects of cobalt and chromium ions at clinically equivalent concentrations after metal-on-metal hip replacement on human osteoblasts and osteoclasts: Implications for skeletal Heath Bone 2011; 49(4):717-723.
71. Tower S. Arthroprosthetic cobaltism: Identification of the at-risk patient. Alaska Med 2010; 52:28-32.
72. Tower SS. Arthroprosthetic cobaltism: Neurological and cardiac manifestations in two patients with metal-on-metal arthroplasty: a case report. J Bone Joint Surg Am 2010; 92(17):2847–2851.
73. Tower SS. Arthroprosthetic cobaltism associated with metal on metal hip implants. BMJ 2012; pp:344-430.
74. Grandfield K, Palmquist, Goncalves S, et al. Free form fabricated features on CoCr implants with and without hydroxyapatite coating in vivo: A comparative study of bone contact and bone growth induction. J Mater Sci Mater Med 2011 22(4):899–906.
75. Lim CA, Khan J, Chelva E, et al. The effect of cobalt on the human eye. Doc Ophthalmol 2015; 130(1):43-8.

76. Fritzsche J, Borisch C, Schaefer C. High Chromium and Cobalt Levels in a Pregnant Patient with Bilateral Metal-on-Metal Hip Arthroplasties. Clin Orthop Relat Res 2012; 470(8):2325–31.
77. Nishishita K, Tsukuba T. Cobalt. Protoporphyrin represses osteoclastogenesis through blocking multiple signaling pathways Biometals 2015(2); 28:725–32.
78. Garoui E, Amara IB, Driss D, et al. Effects of Cobalt on Membrane ATPases, Oxidant, and Antioxidant Values in the Cerebrum and Cerebellum of Suckling Rats. Biol Trace Elem Res 2013;154(3):387–95.
79. Catalani S, Leone R, Rizzetti MC, et al. The role of albumin in human toxicology of cobalt: Contribution from a clinical case. Hematology 2011.
80. Hara A, Niwa M, Aoki H, et al. A new model of retinal photoreceptor cell degeneration induced by a chemical hypoxia-mimicking agent, cobalt chloride. Brain Res 2006; 1109(1):192–200.

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