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EFFECTS OF MODERATE PHYSICAL EXERCISE ON BLOOD AND URINE CONCENTRATIONS OF CADMIUM AND METALLOTHIONEIN IN RUNNERS

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Abstract. The aim of the study was to investigate the effect of combined exposure to physical exercise and tobacco smoke on the human body. The selected groups of runners similar in age, weight, height and body mass index were subjected to physical exercise, 2000 m and 4000 m run. The study covered two groups composed of smokers and non-smokers, each of the 12 subjects. Protein, creatinine, cadmium and metallothionein concentrations were measured three times: before the run (A); 30 min after the run (B); and 24 h after the exercise (C). In the group of smokers, higher urine and blood concentrations of cadmium were found. In the group of smokers who ran 2000 m, a post-exercise decrease in blood cadmium concentration that maintained also 24 h after the exercise was observed. Urine cadmium concentration increased with its post-exercise decrease in blood. The changes in urine differed significantly between smokers and non-smokers, and they significantly depended on the distance covered by the runners. Metallothionein proved to be a very effective marker of changes occurring in the runners' body. Its statistically significant increase in urine of non-smokers was observed 30 min after the run, and the longer the distance the higher the increase. In urine of smokers, the decrease in metalliothionein concentration was observed after the run, regardless of the distance covered. The results of the study provide evidence that metallothionein, due to its specificity, can be regarded as an essential antioxidant. They also proved that this protein is a useful marker to monitor physical effort and disorders of the pro- and anti-oxidative balance. A moderate physical exercise may be a good avenue to remove cadmium deposits resulting from the environmental exposure to this metal.

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Introduction

The progress of civilization entails a growing environmental threat to the human and animal health, resulting from its contamination with chemical, physical and biological agents. Among numerous harmful substances put on the list of the International Register of Potentially Toxic Chemical Substances, published by the UN Environment Program, there are heavy metals, including cadmium (Cd), whose toxicity even at very low concentrations has already been well documented [11,15,32]. Cd is accumulated in the body and deposited in parenchymatous organs and its half-life equals even 20 years. Cadmium ions may be responsible for the disruption of hydrogen bonds and despiralisation of nucleic acids; the decreased synthesis of fatty acids owing to changed acetyl-CoA activity; and the inhibition of the succinate oxidase activity with sequential disorders of the mitochondria respiratory chain [8,17].

Cadmium also induces chromosome aberrations, inhibits the activity of RNA polimerase and protein synthesis to polyribosomes, accelerates cellular translation processes, and affects processes of free radicals by intensified lipid peroxidation in hepatocytes [15,20]. In addition, this metal exerts an oncogenic effect, leading to the development of neoplasms of many organs [11,12,15,17,18], which is closely related with cigarette smoking. Twenty cigarettes smoked a day contributes to an additional daily inhalation of 3.6-6.0 μg Cd, providing that in the main stream of smoke there are 12% of tobacco-contained Cd, and the lung retention accounts for 48-82% [5,11]. Smoking is responsible for the development of many diseases. As depicted by statistical data, nine persons die every minute throughout the world due to this habit.

It is believed that a systematic physical exercise extends a life span by two years on average, which may play a role in the prevention and treatment of arterial hypertension, disorders of fat and carbohydrate metabolism, obesity, diseases of the locomotor system and depression [7,28,34]. Physical exercise is one of the most effective intervention methods of preventing various diseases, including ischemic heart disease, the main killer that endangers modern societies. Physical activity may also contribute to the promotion of health-enhancing forms of behavior, e.g. non-smoking, better balanced diet and body mass control [7]. Physical exercise increases oxygen consumption, and thus the production of reactive oxygen species (ROS) is growing [6]. The balance denoted by Sies [30] as pro- and anti-oxidative equilibrium is set between ROS generation and their inactivation by antioxidative systems. Regular and moderate physical exercise releases oxidative stress, stimulating at the same time cellular adaptation and diminishing the amount of lipid peroxidation products developed during exercise performance [2,34]. In such conditions, cells in response to the stress produce varied proteins, however, their role has not as yet been fully elucidated. Metallothionein (MT) is one of them. It is a cytoplasmic protein with molecular mass of 7-10 kDa, in which 30% of aminoacids constitute cysteine residues [12]. This protein sequestrates heavy metals thus diminishing their toxicity [8]. Metallothionein recognized as a protein of acute phase serves as an active scavenger of free radicals, being at the same time a well documented marker of exposure to cadmium, the strongest inducer of MT synthesis [9,16,18]. It proved to be a more effective free radical scavenger than glutathione, which together with sulfuric aminoacids are categorized into the third group of the antioxidative system [26,29]. MT is also significantly involved in the stress-induced adaptive mechanisms of the body [13,14,31]. The value of MT determination in physical exercise monitoring has already been evidenced [1,25,33].

The effect of physical exercise on cadmium concentrations in tissues and body fluids has scarcely been reported in the literature. The aim of this study was to investigate the effect of physical exercise on Cd and MT concentrations in body fluids of runners in respect of health-enhancing influence of exercise on Cd deposits, resulting from smoking or environmental exposure to this xenobiotic.

Material and Methods

The study covered two groups composed of smokers (S) and non-smokers (NS), each of the 12 running-trained air force recruits. They were divided into subgroups of those who completed running test of 2000 m (I NS-2, II S-2) or 4000 m (I NS-4, II S-4). The selected groups of runners (smokers and non-smokers, running 2000 or 4000 m) were similar in age (mean, 21 years), weight (mean, 73 kg), height (mean, 177 cm) and body mass index (BMI). The mean time to run the distance of 2000 m and 4000 m was 425±17 s, and 956±24 s, respectively. The mean speed for 2000 m was 4.62±0.14 $m \cdot s^{-1}$ and for 4000 m 4.1±0.1 $m \cdot s^{-1}$, and this difference was statistically significant ($P<0.001$). Of a large team of runners trained to participate in the Air Force Championship at the central level, the study groups were selected following the running tests.

The subjects eligible for the study underwent periodical medical examinations. According to the information provided, the subjects did take any medication. All the subjects were accommodated in one place and were on the same diet.

The study design was approved by the Bioethic Committee of the Wrocław Medical Academy, and informed consent was obtained in all persons.

Blood and urine samples were collected three times: before the run (A); 30 min after the run (B); and 24 h after the exercise (C). Blood was drawn from the ulnar vein and collected in original SARSTEDT test-tubes with heparin as anticoagulant. Immediately after collection, urine samples were centrifuged at 800 x g, then freezed at - 80^{0}C until determinations. Blood and urine Cd concentrations were measured by electrothermal atomic absorption spectrometry (ET-ASA) with a graphite cuvette [22,24]. The division of subjects into groups of smokers and non-smokers was based on an interview. The obtained information was verified by measuring the level of urine cotinine with the enzyme-linked immunosorbent assay (ELISA) (IDSC, USA) [22]. The tanine method was used to determine protein levels in urine using bovine serum albumin as standard.

Metallothionein concentration was determined with ELISA, using as standards MT-I and MT-II from human liver and polyclonal antiserum against MT isoforms [14,22]. The Stat Fax 2200 incubator and Stat Fax 2100 reader produced by the Analco GB (USA) were used.

Statistical analysis

The results are expressed as arithmetic means ±SD and examined by Student's t-test.

Results

Table 1
Urine protein/creatinine index values (μg/mmol) in the study groups of runners

	Group I NS-2			Group I NS-4		
	A	B	C	A	B	C
Protein / creatinine	4.3±1.0	24.3±6.2	4.5±1.6	4.2±1.4	46.9±14.4	4.8±1.9
	Group II S-2			Group II S-4		
Protein / Creatinine	4.1±1.4	25.1±7.1	4.4±1.7	3.9±1.3	43.2±11.9	6.7±2.2
	I		II	I		II
A : B	0.001		0.001	0.001		0.001
A : C	NS		NS	NS		NS
B : C	0.001		0.001	0.001		0.001
NS : S	NS	NS	NS	NS	NS	NS

S = smokers; **NS** = non-smokers;
Group I NS -2 = non-smoking runners for 2000 m; Group I NS - 4 = non-smoking runners for 4000 m;
Group II S - 2 = smoking runners for 2000 m; Group II S - 4 = smoking runners for 4000 m;
Statistical analysis summarized in the table gives P≤ values;
NS- = not significant;
Measurements were taken at three time points: before the run (A); 30 min after the run (B); and 24 h after the run (C)

Protein index value. Urine creatinine measurements and their statistical analysis are given in Table 1. Physical exercise taken by runners to cover the distance of 2000 and 4000 m induced statistically significant increase (P<0.001) in the value of urine protein index immediately after the run and its statistically significant decrease (P<0.001) 24 h after the exercise. The fact that both groups were well trained for the task was evidenced by the absence of significant differences between the values obtained in two points of time (A) and (C). As depicted by the statistical analysis, there were no differences between the groups of smokers and non-smokers at all the three points of time.

Table 2
Blood cadmium concentrations (μg/l) in the study groups of runners

	Group I NS-2			Group I NS-4		
	A	B	C	A	B	C
Blood Cd	0.97±0.19	0.39±0.09	1.06±0.41	1.15±0.32	0.59±0.27	0.84±0.37
	Group II S-2			Group II S-4		
Blood Cd	2.63±0.32	1.29±0.4	1.67±0.26	2.28±0.31	1.37±0.3	2.27±0.39
	I		II	I		II
A : B	0.001		0.001	0.05		0.001
A : C	NS		0.001	NS		NS
B : C	0.05		NS	NS		0.001
NS : **S**	0.001	0.001	0.05	0.001	0.001	0.001

Data on blood Cd concentrations in groups I and II are summarized in Table 2. Statistically significant decrease in blood Cd concentrations was observed after the exercise; it was maintained in group II S-2 also 24 h after the run for 2000 m. Cd level was significantly higher in smokers at all points of time.

Table 3
Urine cadmium concentrations (ng/mmol creatnine) in the study groups of runners

	Group I NS-2			Group I NS-4		
	A	B	C	A	B	C
Urine Cd	61.9±12.3	82.1±29.3	56.1±16.4	70.2±18.3	88.4±27.9	64.2±28.2
	Group II S-2			Group II S-4		
Urine Cd	189.1±29.3	263.6±41.2	174.2±33.6	174.6±23.4	242.3±26.8	180.1±21.

	I		II	I		II
A : B	NS		0.05	NS		0.05
A : C	NS		NS	NS		NS
B : C	NS		0.05	NS		0.05
NS : S	0.001	0.001	0.001	0.001	0.001	0.001

Table 3 presents measurements of urine Cd concentrations in both groups of runners after physical exercise. In smokers, a significant increase (P<0.05) in Cd excretion with urine was observed after the exercise, followed by a decreased concentration of this metal in the C point of time. Significant statistical differences (P<0.001) between smokers and non-smokers who covered 2000 and 4000 m are worthy of special mention.

Urine MT concentrations in both groups of runners are given in Table 4. In group I - NS, a significant increase in MT concentration was observed at time point B, and it was higher in runners who covered 4000 m. In group II - S, MT concentration significantly decreased (P<0.001) at time point B, regardless of the covered distance. The comparison of both groups revealed higher MT concentrations in the group of smokers at time points A and C and a reverse trend immediately after the run (time point B).

Table 4
Urine metallothionein concentrations (ng MT/mmol creatinine) in the study groups of runners

	Group I NS-2			Group I NS-4		
	A	B	C	A	B	C
MT/creatinine	26.0±6.0	62.3±32.6	154.8±73.5	33.7±10.8	78.5±47.2	102.4±33.9
	Group II S-2			Group II S-4		
MT/creatinine	118.6±33.3	29.2±7.1	40.2±11.8	157±79.2	16.6±8.1	58.3±1.7
	I		II	I		II
A : B	0.05		0.001	0.05		0.001
A : C	0.002		0.001	0.001		0.02
B : C	0.05		NS	NS		0.001
NS : S	0.001	0.05	0.005	0.005	0.01	0.05

Discussion

The results of the studies at a molecular level provide evidence that exhaustive physical exercise induces oxidative stress [10]. An enhanced production of oxygen free radicals followed by the process of lipid peroxidation is a normal sequence of the increased oxygen consumption after an exercise [31]. As a result of lipid peroxidation, membranes become stiff, lose their selective permeability and may even deplete their integrity, leading finally to tissue damage or decay [6,15,33]. These effects are not desired in persons who actively practice sports. Therefore, environmental exposure to xenobiotics present in tobacco smoke among active or passive smokers is an additional body burden. Cadmium is one those xenobiotics. Cd toxicity is associated with its ability to induce free radical processes [15,20]. Experimental studies of rats chronically intoxicated with cadmium acetate showed an increased Cd accumulation in the kidney, pancreas, heart and skeletal muscles [23]. These organs are especially vulnerable to damage induced by free radicals, because of intensive metabolic processes [8,11,17].

The intensity of free radical processes, generated by physical exercise, depends greatly on how well the athletes are trained.

The present study carried out in the groups of running-trained air force recruits showed their satisfactory physical fitness to undertake the programmed exercise - 2000 and 4000 m run. The measured Cd concentrations in blood and urine showed their significantly higher values in smokers, which confirms earlier observations [11,24].

Significantly decreased blood Cd was observed in the subjects immediately after the run, its concentration did not evidently change during 24 h and remained significantly different from Cd initial level, whereas an exhaustive run enhanced the level of this metal in urine, and after 24 h it decreased below the initial values. Our results are in agreement with those obtained by Rodriguez *et al.* [28] who assessed the level of heavy metals in persons living in contaminated areas. Lower Cd and Pb and higher Cu and Zn concentrations in blood were found in active sporting persons as compared to those who lead a sedentary life.

It may be assumed that owing to physical exercise, the larger amounts of cadmium permeate from blood to urine, and thus are eliminated from the body. It is possible that Cd is additionally released to blood and urine from tissues. It may be concluded that extensive physical exercise persistently change Cd reserves in tissues. This could be regarded as a very positive occurrence, especially in view of Cd long half-life and the range of its toxicity.

Cadmium concentration in tissues and body fluids is closely associated with the level of MT, as Cd is the best inductor of its synthesis. The MT measurement in urine is of particular significance owing to strong nephrotoxicity shown by this element [27]. MT excretion with urine is the earliest sign of Cd exposure, preceding the excretion of enzymes and proteins [1,16,18]. MT is a potential marker of physical exercise [19,33]. It may be induced in response to oxidative stress and it is able to protect tissues against damage caused by free radicals [9,10,14]. The mechanism responsible for MT participation in this process involves the neutralization of hydroxyl radical, owing to its stronger affinity than that of glutathione [9.29], and the assurance of relevant concentrations of Zn essential to maintain activity of enzymes, which neutralize the effect of free radical reactions [4,14,26]. Shinogi *et al.* [31] experimented with rats to investigate the effect of different types of stress on MT level in the rat liver. They applied dietary restrictions and subjected the animals to intensive physical exercise. The MT level increased when the animals were deprived of food, and then decreased when the animals were fed again. A long period of starvation decreased MT concentrations in the liver. On the other hand, an intensive physical exercise increased MT concentration in the liver when the rats were at rest. After combining these effects, it appeared that MT synthesized during dietary restrictions, preceding the effort, suppressed the generation of lipid peroxidation products induced by physical exercise [10,30].

In the present study, urine MT concentrations measured after the exercise differed essentially between smokers and non-smokers. The increase in MT concentration in non-smokers 30 min after the run can be undoubtedly associated with the enhanced synthesis of this protein in the liver, owing to oxidative stress induced by intensive physical exercise. A more intensive physical exercise (4000 m run) contributed to a higher increase in concentration of MT excreted with urine. However, 24 h after the run, MT concentration was higher in runners for 2000 m than in those for 4000 m. Thus at a less intensive exercise, the ability of the body to moderate changes induced by physical stress was sufficient to stimulate MT synthesis and to excrete part of protein with urine. Whereas, the 4000 m run appeared to evoke the stress beyond the ability of the body to rapidly neutralize its effects, and thus much lower excretion of MT was observed. These observations are supported by the results obtained in smokers, in whom MT is involved in the neutralization not only of the effects of physical exercise, but mainly of the effects of Cd binding and its adverse reactions. In this group, higher MT concentrations were observed before the run as a result of Cd exposure, and 30 min after the run, MT concentrations decreased in those who ran 2000 m and retained at the decreased level for 24 h. A similar trend was noted in runners for 4000 m, however, the MT decrease after the run was even larger, probably due to more extensive physical exercise.

Metallothionein a strong scavenger of free radicals extensively generated during the run [3,6,7,]. In non-smokers, an increased urine MT concentration as a result of physical stress is observed together with its further increase at rest - 24 h after the run. It may be concluded that the ability of the body to rapidly react to oxidative stress induced by physical exercise is diminished by cigarette smoking, and metallothionein is a useful marker of this process.

Conclusions

1. A moderate physical exercise (2000 m run) may be a good avenue to eliminate cadmium deposits from the body, accumulated due to environmental exposure to this metal.

2. Practice of exhaustive physical exercise concomitant with Cd exposure should be monitored in view of the limited ability of the body to neutralize the effects of oxidative stress induced by these two factors.

3. Evidence that MT proteinuria is increased in non-smoking runners and decreased in smokers may indicate a specific property of metallothionein that makes it an essential antioxidant in the antioxidative defence.

4. Metallothionein is a marker useful in monitoring physical exercise and disorders of pro- and antioxidative equilibrium.

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