Can Metallothionein be Considered a Diagnostic Marker in Physical Exercise?*

Czy metalotioneina może być markerem wysiłku fizycznego?

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Abstract
On the basis of biochemical results the metallothionein (MT) synthesis regulation as well as the mechanism of MT action as a free radicals scavenger and its function during the physical exercise was presented in this paper. From the results of many years’ research, authors of this publication state that metallothionein is a valuable marker of exercise, and can be used in monitoring and management of athletes’ sports form. Results of the study point to the necessity of using MT in diagnostic evaluation of athletes’ organism undergoing strenuous physical training (Adv. Clin. Exp. Med. 2003, 12, 5, 641–645).

Key words: metallothionein, marker, physical exercise.

Streszczenie

Słowa kluczowe: metalotioneina, marker, wysiłek fizyczny.

Modern professional sport requires optimization of training process, with the main goal being the achievement of supremacy in contest. Realization of this aim requires a discerning look at the considered problem, as well as a thorough integration of combined efforts of coaches, doctors, biochemists, dietitians and athletes as subjects of training process. In professional sport, there is a constant need for new indicators of physical exertion, called markers, that would allow, through biochemical monitoring, to perceive the state of a superb sport form, the rate of post-training exaltation, or early episodes of overtraining, and would warn of possible negative results caused by application of too much of a training load. The key role in this search is played by scientists working on the advancement of physical exercise biochemistry; the discipline that would provide us with explanation of mechanisms of changes taking place in organism at the molecular level.

Intensification of physical exercise frequently leads to an acute phase reaction in various areas of

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metabolic transformations, manifesting itself by muscular pain, changes in hematological and hormonal parameters, elevation of body temperature, and changes in the activity of some enzymes [1–4]. The alternation of levels of certain metal ions in serum was also reported; for example in marathon runners zinc and copper levels were elevated [5] (Fig. 1).

Poortmans showed that among post-exercise urine proteins, acute phase reaction proteins are an important group [6]. It is worth noticing that urine level of these proteins increases after physical exercise. It concerns especially low molecular weight proteins (3S–gamma1–globulin, orosomucoid), but then – also high molecular ones, like ceruloplasmin and IgG.

As it was proved by recent years’ research, acute phase reaction proteins concentration changes not only due to factors causing disturbances in homeostasis such as infection, neoplasms, or stress, but also in relation to physical exercise [7]. Among these proteins ceruloplasmin, C-reactive protein, haptoglobin, alpha1–proteinase inhibitor, and others should be mentioned [1, 2, 7].

The fact deserving special attention is that a low molecular weight protein (6–10 kDa), called metallothionein (MT) [8], which was identified several decades ago as a protein responsible for natural kidney cadmium accumulation in mammals, has been recently recognized as an acute phase reaction protein [9]. It has been proven that MT has a high affinity to metals such as Cd, Hg, Cu, Zn, Ag, Bi, and that it plays an important role in metabolism of toxic and essential metals. In mammals, MT molecule is built of a polypeptide chain containing 61 amino acids, 20 of which are cysteines, and seven bound bivalent ions [10]. The N-terminal amino acid of polypeptide chain is N-acetylmethionine, and the C terminal-alanine. Amino acids from 1 to 30 form a β center, and from 31 to 61 an α center. The β center contains nine cysteine residues, and has the ability to bind three atoms of Zn or Cd, or in the absence of them six atoms of Cu. The sulphydryl group/bivalent metal ratio in metallothionein is 4:1 [11]. The characteristic features of MT are [12]:

- low molecular weight,
- high metal content,
- lack of aromatic amino acids or histidine,
- a unique sequence of amino acids (with a characteristic location of cysteine residues: -Cys-X–Cys-).

The existence of four isoforms of metallothionein has been proved: MT-1, MT-2, MT-3, MT-4. MT-1 and MT-2 are present in most mammalian tissues; MT-3 has only been found in the brain [13], and MT-4 in stratified epithelium [14].

**Regulation of metallothionein synthesis**

Heavy metals cause induction of metallothionein synthesis by increasing the frequency of metallothionein gene transcription. Cd was found to be the best metallothionein inductor in the liver, followed successively by Zn, Cu and Hg. In the kidney, however, Hg proved to be the most effective, followed in turn by Cd, Zn and Cu [15]. It has been demonstrated that a wide variety in mutual metal–tissue interactions exists in the regulation of MT synthesis. It is thought that enhanced synthesis rather than reduced degradation rate cause the elevation of metallothionein concentration, resulting from the exposure to metals. Other factors influencing metallothionein synthesis are:

- glucocorticoids, glucagon, adrenaline,
- interferon, interleukin-1, interleukin-6, tumor necrosis factor (TNF),
- physiological stress (food deprivation, temperature), radiation, diet,
- physical exercise.

An increase of metallothionein synthesis causes changes in cellular –SH groups pool. Because of metallothionein high cysteine content (30%), the availability of cysteine and methionine is the limiting factor in the synthesis of this protein [16].
Mechanism of metallothionein action as a free radicals scavenger

Metallothionein is known as a free radicals scavenger; it also takes part in the repairing of oxidative stress caused damages. As it was shown by Bobillier-Chaumont et al., MT is capable of scavenging hydroxyl and superoxide radicals produced in xanthine oxidase reaction in vitro [17]. Thiol groups of MT are considered responsible for binding of both these radicals (Zn-MT binding rate constant for anion superoxide radical is $4 \times 10^5$ mol$^{-1}$s$^{-1}$, and for hydroxyl radical $2.7 \times 10^{12}$ mol$^{-1}$s$^{-1}$).

In a case of a known and important antioxidant, glutathione, those figures are $6.7 \times 10^5$ mol$^{-1}$s$^{-1}$, and $8 \times 10^9$ mol$^{-1}$s$^{-1}$, respectively. Therefore, metallothionein can be considered a multi-effective hydroxyl radical scavenger.

According to Thomas et al., the main protective factor in MT action is Zn liberation and its uptake by membranes, which reduces lipid peroxidation and therefore, leads to membrane stabilization [18]. Liberation of Zn ions may repress lipid peroxidation by changing numerous functions, such as: decreasing Fe ions uptake, increasing glutathione peroxidase activity, suppressing NADPH cytochrome reductase and causing induction of synthesis of new MT molecules [19].

It has been demonstrated that MT – mRNA level in the liver is very low, and the rapid elevation of the liver Zn concentration causes immediate activation of MT synthesis. It is considered to be a compensating mechanism in case of genetic deficit of synthesis of copper and zinc dependent superoxide dismutase [20].

Metallothionein and glutathione function during physical exercise

The research, conducted by Gohil et al., concerning glutathione function in the blood of training athletes’, showed considerable changes in GSH and GSSG levels. During exertion GSH level drops by 60%, whereas total glutathione concentration undergoes only slight changes [21] (Fig. 2).

GSH oxidation during submaximal exercise and its normalization during rest strongly suggest that reactive oxygen species are generated in the blood of moderately trained competitors.

Research conducted on athletes (marathon and long-distance runners following an over 60 minute training scheme in anaerobic zone) showed a statistically significant reduction in urine MT/creatinine ratio, immediately after exercise, which still lasted after 24 hours [22], (Fig. 3 and 4).

Contrary to various other proteins, urine concentration of which increases after physical exercise, MT concentration decreases. This decline resembles changes in concentrations of some amino acids such as glycine, serine, histidine and others [2], as well as 3-methylhistidine [5].

It is supposed that the changes in urine MT concentration following exercise may be caused by increasing demand for this protein in athletes’ organisms. Increased quantities of Zn and Cu appearing in the blood after exercise may be connected to the process of MT synthesis in the liver.

The results of our earlier studies [22–24] demonstrated, that greater needs for this protein occur in marathon runners with higher blood concentrations of Zn and Cu [5]. Copper and zinc are co-factors of superoxide dismutase. Higher levels of Cu are profitable, because both ceruloplasmin, and superoxide dismutase are strong antioxidants protecting cytomembrane lipids from peroxidation caused by free radicals [25]. Reduced level of Zn may lower superoxide dismutase activity, making hepatocytes more prone to the damage by radicals. Cu deficit is connected to superoxide dismutase defect and handicapped scavenging, which in turn promotes development of undesired organic damage.

Taking into account the amount of work done by MT, the difference in energy transformations accomplished by marathon and long-distance runners, and MT concentration changes under physical exercise...
exercise, it seems that as a low molecular weight protein, MT plays a particular physiological role. The role of MT in protecting against free radicals generated during exercise is critical. Our own research [23, 24], concerning exercise in marathon and long-distance runners, allowed us to come to the following conclusions:

1. After exercise, urine MT concentration decreases unlike concentrations of various proteins, which may be related to mineral balance of Zn and Cu, and to MT role in protection against free radicals generated during exercise.

2. MT/creatinine ratio may be considered as an indicator useful in regulating training load and in monitoring the condition of sports form (Fig. 5). Diagnosing numerous biochemical reactions taking place in an organism during physical exercise using sensitive markers may contribute to identification of current athletes’ fitness. Because metallothionein level results from multiple biochemical processes taking place in human organism, it can be considered a valuable marker of exertion, which is essential in monitoring and managing athletes’ training and their sports form, as well as to eliminating the dangers posed by the application of excessive exercise load. Only the early and precise diagnosing of organism during exercise will undoubtedly contribute to supremacy in contest. Results of the research strongly suggest the necessity of using MT in diagnostic evaluation of human and animal organisms under physical exercise [26–28].
References


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