Changes of lysosomal enzymes activity in the skeletal muscle fibers exposed to endurance exercise

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Abstract

Purpose: To evaluate the effect of endurance exercise on the activity changes of selected lysosomal enzymes in particular types of rat muscle fibers, occurring by 0-4 days following the trial.

Material and methods: The experiment was performed on 3 month old male Wistar rats with body mass 250 ± 25 g, exposed to single physical exercise on moving track (speed 17 m x min^{-1}, decline 0°, duration 87.5 ± 27.5 min). Biochemical analyses were performed on homogenized fast-twitch FTa and FTb (*m. gastrocnemius*) and slow-twitch ST (*m. soleus*) muscle fibers of animals sacrificed 2 h (group II), 6 h (III) or 96 h (IV) after exercise and control group. The measurements considered protein concentration and the activities of beta-glucuronidase (β-GRS), N-acetyl-β-D-glucosaminidase (NAG), and arylsulphatase A (ASA).

Results: In FTa fibers, ASA and β-GRS activities were elevated in all the exercised groups, with the most evident changes in animals tested 96 h post trial (group IV), while the peak of NAG activity was demonstrated 2 h after exercise (group II). In contrast, in FTb and ST fibers the levels of all the enzymes studied peaked 96 h after exercise, following the transient decrease in activity.

Conclusions: The present study demonstrated that maximal running exercise, without the eccentric components, affects the activities of lysosomal enzymes in all types of rat muscular fibers. The lack of uniform activity profile for the lysosomal enzymes studied probably reflects the variety of their cellular functions.

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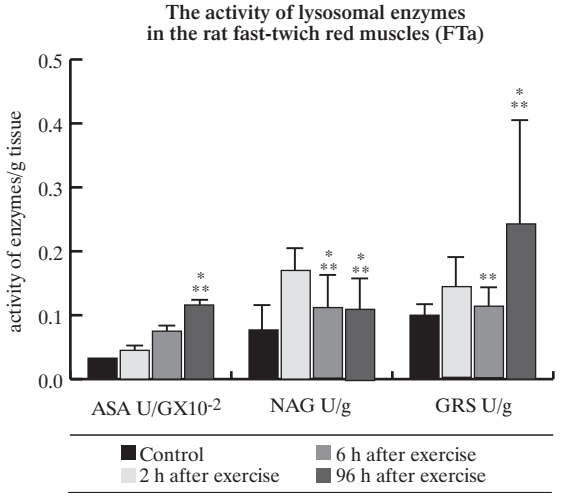
Introduction

It is widely known that physical exercise reflects in the imbalance of homeostasis. The resulting changes depend on the intensity of stimulation and its duration. One of possible exercise-associated consequences is the damage of muscle fibers [1], which might consider either sarcolemma, or myofibrils and cell organelle [2]. Free radicals [3], increased acidification, hypoxia, some metabolites and elevated intracellular Ca^{2+} concentration are mentioned as the factors responsible for those injuries [4,5]. Exercise stress reflects in the changes of protein composition either in cytosol or in plasma membranes and extracellular matrix [6,7].

Lysosomes are the main structures where occurs the degradation of proteins, proteoglycans, mucopolysaccharides, glycoproteins and sulpholipids. They participate in composed, intracellular system decomposing either extra- or intracellular compounds. Lysosomes are present in cells of various tissues, but are particularly frequent in secretive or excretive organs (liver, kidneys, and lungs), enterocytes and leucocytes (mainly granulocytes). In contrast, they are of rare evidence in myocytes and pancreatic glandular vesicles [8]. Lysosomes are intracellular organelles surrounded with single-layered protein-lipid membrane, which, due to specific activity of ATP-dependent proton pump, maintains their internal pH between 5.0 and 5.5. They exhibit the activity of numerous hydrolytic enzymes, including arylsulphatase A (EC 3.1.6.1), catalyzing decomposition of sulphate lipid esters and other sulphconjugates, N-acetyl-beta-D-glucosaminidase (EC 3.2.1.30), splitting off sugar moieties (N-acetylglucosamine) from glycoproteins and glycolipids, and beta-glucuronidase (EC 3.2.1.31) degrading glucosaminoglycanes. The latter one is known as the marker of lysosomes and approved quantitative indicator of muscular damage [9,10].

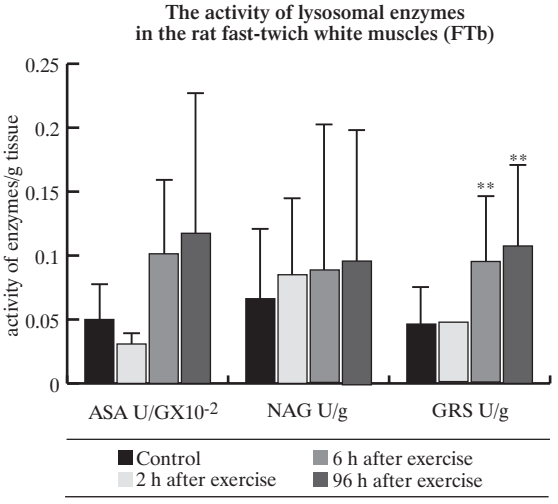
The purpose of present study was to evaluate the effect of

Figure 1. **Lysosomal enzymes activity in FTa fiber muscles. ASA – arylsulfatase A (Uxg^{-1}x10^{-2}), NAG-N-acetyl-β-D-glucosaminidase (Uxg^{-1}), GRS – β-glucuronidase (Uxg^{-1}). Results are means ± SEM from 5 animals**



* p<0.05 (vs control) ** p<0.05 (vs 2 h after exercise)

Figure 2. **Lysosomal enzymes activity in FTb fiber muscles. ASA – arylsulfatase A (Uxg^{-1}x10^{-2}), NAG-N-acetyl-β-D-glucosaminidase (Uxg^{-1}), GRS – β-glucuronidase (Uxg^{-1}). Results are means ± SEM from 5 animals**



* p<0.05 (vs control) ** p<0.05 (vs 2 h after exercise)

endurance exercise on the activity changes of selected lysosomal enzymes in particular types of rat muscle fibers, occurring by 0-4 days following the trial.

Material and methods

The experiment was performed on 3 month old male Wistar rats with body mass 250 ± 25 g. All the animals were kept under the equal microclimatic conditions and given water and standard laboratory fodder ad libidum. The rats were exposed to single physical exercise on moving track (speed 17 m x min^{-1}, decline 0°). The exercise trial was performed "until refuse", which corresponded to average duration 87.5 ± 27.5 min.

The animals were divided into the following groups (n=5):

I – controls – non-exposed to physical exercise,
II – examined 2 h after trial,
III – examined 6 h after trial,
IV – examined 96 h after trial.

The rats were sacrificed by ketamin (Bioketan) injection (100 mg x kg^{-1} b.w.), followed by the dislocation of cervical vertebra. All the procedures were performed according to the guidelines of Local Ethical Commission in Wrocław. The material analyzed considered hind-limb muscles: FTa and FTb fibers dissected from *musculus gastrocnemius*, and ST fibers from *musculus soleus*. The tissues were frozen at -80°C directly post dissection. Prior the biochemical analyses the tissues were homogenized in Potter homogenizer at +4°C, with the solution containing 0.15M NaCl; 0.01M Tris/HCl buffer (pH 7.4), and 0.1% Triton X-100.The homogenates (10% w/v) were subsequently spinned by 8000 x g for 30 min (+4°C) and the supernatant was used for further analyses.

The following analyses were performed on the material studied: 1) protein concentration measured by means of Bradford method [11] with Coomassie Brillant Blue G-250, 2) beta-glucuronidase activity (β-GRS) determined by Maruhn method [12] with p-nitrophenyl-β-D-glucuronide (Sigma), 3) N-acetyl-β--D-glucosaminidase activity (NAG) with p-nitrophenyl-N-acetyl-β-D-glucosaminide (Sigma) [13], 4) arylsulphatase A activity (ASA) by means of Baum method [14] in own modification with 2-hydroxy-5-nitrophenol sulphate (Flucka) as a substrate. The samples were dialyzed to distilled water (+4°C) prior the latter analysis to remove the chloric anions which might alter the enzymatic activity.

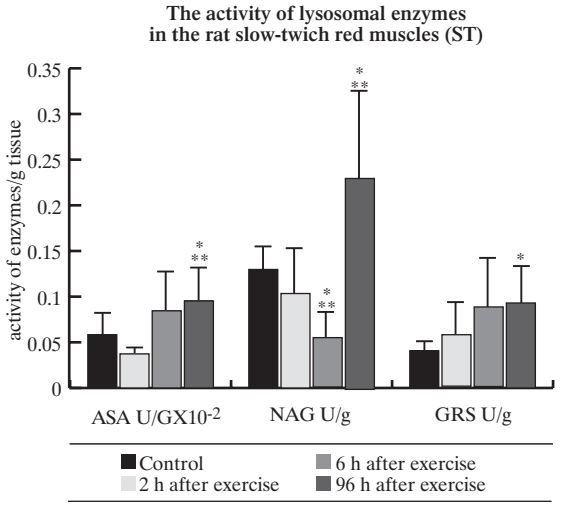
The results of measurements underwent statistical analysis by means Friedman's ANOVA and non-parametric Wilcoxon's test (p<0.05), using Statistica 6.0 PL (StatSoft, Poland) package.

Results

The present study revealed that physical exercise changed lysosomal enzyme activity in all types of muscular fibers. The sequence of that changes was however different in particular experimental groups.

In FTa fibers (*Fig. 1*) ASA activity was elevated in all the exercised groups, with the most evident changes (3.5-fold increase) in animals tested 96 h after trial (group IV). Similar sequence was observed for β-GRS activity, which was also the highest in group IV (2.5-fold increase). However, the nature of NAG changes was different. The peak of activity was demonstrated 2 h after exercise (2-fold increase, p<0.05), while 6 and 96 h post trial the enzyme level remained lower than in group II, but it was still 1.5-fold higher than in the

Figure 3. **Lysosomal enzymes activity in ST fiber muscles. ASA – arylsulfatase A ($Uxg^{-1}x10^{-2}$), NAG-N-acetyl-β-D-glucosaminidase (Uxg^{-1}) GRS – β-glucuronidase (Uxg^{-1}). Results are means ± SEM from 5 animals**



* p<0.05 (vs control) ** p<0.05 (vs 2 h after exercise)

controls. Enzymatic profile of FTb fibers (*Fig. 2*) was different than described for FTa ones. The activity of ASA in group II was decreased by 44% of the basal level. The most prominent, 2.4-fold, increase was demonstrated in group tested 96 h after exercise. The aforementioned changes were insignificant, however, as well as the alterations of NAG activity, which was also the highest in group IV. Two hours after exercise, the activity of β-GRS in FTb fibers was similar as in the controls, while 2- and 2.3-fold increase was observed 6 and 96 h post trial (group III and IV), respectively ($p<0.05$).

In slow-twitch fibers ST (*Fig. 3*) the decrease of ASA activity by 40% was observed 2 h after exercise (group II), while the increase by 40% and 70% of the basal level was demonstrated in groups III and IV, respectively. The activities of NAG decreased by 17% and 54% of the normal values in groups II and III, respectively. The twofold increase was, however, observed in group IV, when compared with the controls.

Discussion

Present study revealed that the single endurance exercise reflects in the changes of lysosomal enzyme activities in all types of rat muscle fibers. The most pronounced increase of enzymatic values was demonstrated in red, fast-twitch FTa fibers. Most of the authors proved significant effects of eccentric exercise on muscular damage. The lesions were indicated by the increase of enzymatic activity 1-3 days after exercise and the subsequent decrease by 7th day post trial [15-17]. Takala et al. revealed 8-fold increase of muscular β-GRS activity after eccentric exercise, while concentric trial reflected in only 2-fold elevation. Forty-eight hours and 4 days after the electrical stimulation of isolated anterior tibial muscle of rat, the rise of β-GRS activity was 4- and 12-fold, respectively [17]. The eccentric exercise-associated increase of β-GRS level was the most pronounced in red muscle fibers, followed by the white and slow-twitch ones [10].

The present study revealed that the endurance exercise without the eccentric components might cause the similar biochemical changes in muscle fibers, manifested by the highest increase of β-GRS activity in FTa fibers 4 days after exercise. The comparable sequences of NAG and β-GRS, i.e. increase 2 days after endurance test and return to the basal level on day 3, were revealed in biopsy specimen of equine muscle (middle gluteal muscle) [18]. In present study the changes of ASA activity in all the types of muscular fibers followed the similar sequence like in case of β-GRS, and were parallel to described by Kihlström et al. in the muscle of exercise-exposed mice [19]. The elevation of serum ASA activity by 40%, occurring 7 days after exercise, was also demonstrated in sportsmen [20]. It is suggested that exercise-associated peak of lysosomal enzyme activities in muscles might reflect the infiltration of macrophages and granulocytes into the injured tissue and/or result from the activation of endogenous lysosomal system of myocytes [6]. In present study, the peak of NAG activity in fast- and slow-twitch fibers was demonstrated 96 and 2 hours after exercise, respectively. In contrast, Raulo et al. measured the maximal activities of NAG and β-GRS on 3rd day after exercise [18].

The lack of uniform activity profile for the lysosomal enzymes studied probably reflects the variety of their cellular functions. β-GRS and NAG participate in the metabolism of glucosaminoglycanes, which is particularly intensive after exercise [21]. The variations described may also result from changes in the permeability of lysosome membranes and the subsequent selective transport of various compounds. Several factors, including hypoxia, free radical generation or the rise of intracellular Ca^{2+} concentration, might enhance the permeability of lysosomal membranes [22]. Fusion of lysosomes with the plasma membrane and their exocytosis is triggered by elevated intracellular concentration of Ca^{+2} and is required for the repair of cellular disruptions [23]. Lysosomal membranes are in turn stabilized by such hormones as cortisol and ACTH [24]. The transient decrease of ASA activity in FTb and ST fibers observed 2 h after exercise in our study might be the effect of those hormones.

Conclusions

Concluding, the present study demonstrated that maximal running exercise, without the eccentric components, affects the activities of lysosomal enzymes in all types of rat muscular fibers. The lack of uniform activity profile for the lysosomal enzymes studied probably reflects the variety of their cellular functions.

Acknowledgements

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References

1. Armstrong RB, Ogilvie R, Schwane JA. Eccentric exercise-indused injury to rat skeletal muscle. J Appl Physiol, 1983; 54: 80-93.

2. Takekura H, Fujinami N, Nishizawa T, Ogasawara H, Kasuga N. Eccentric-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. J Physiol, 2001; 533.2: 571-83.

3. Niess AM, Dickhuth HH, Northoff H, Fehrenbach E. Free radicals and oxidative stress in exercise – immunological aspects. Exerc Immunol Rev, 1999; 5: 22-56.

4. Ebbling CB, Clarkson PM. Exercise – induced muscle damage and adaptation. Sports Med, 1989; 7: 207-34.

5. McArdle A and Jackson MJ. Intracellular mechanisms involved in sceletal muscle damage. [In:] Salmons S edditors. Muscle Damage. Oxford University Press, Oxford, UK; 1997, p. 90-106.

6. Farges Ch, Balcerzak Fisher B, Attaix D, Bechet D, Ferrara M, Baracos V. Increased muscle proteolysis after local trauma mainly reflects macrophage-associated lysosomal proteolysis. Am J Endocrinol Metab, 2002; 282: E326-35.

7. Yu JG, Malm C, Thornell LE. Eccentric contractions leading to DOMS do not cause loss of desmin nor fibre necrosis in human muscle. Histochem Cell Biol, 2002; 118: 29-34.

8. Sheeler P, Bianchi DE. Cell biology: structure biochemistry, and function. New York, John Willey& Sons; 1980, 410-1.

9. Salminen A, Kihlström M. Lysosomal changes in mouse skeletal muscle during repair of exercise injuries. Muscle Nerve, 1985; 8: 269-79.

10. Koskinen SOA, Wang W, Ahtikoski AM, Kjaer M, Han XY, Komulainen J, Kovanen V and Takala TES. Acute exercise induced changes in rat skeletal muscle mRNAs and proteins regulating type IV collagen content. Am J Physiol Regulatory Integrative Comp Physiol, 2001; 280: R1292-300.

11. Bradford MM. A rapid and sensitive method for the quantitation of microgramquantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 72: 248-54.

12. Maruhn D, Fuchs I, Mues G, Bock KD. Normal limits of urinary excretion of eleven enzymes. Clin Chem, 1976; 22: 1567-74.

13. Maruhn D. Rapid colorimetric assay of β-galactosidase and N-acetyl-β-glucosaminidase in human urine, Clin Chim Acta, 1976; 73: 453-61.

14. Baum H, Dodgson KS, Spencer B. The assay of arylsulfatases A and B in human urine. Clin Chim Acta, 1959; 4: 453-5.

15. Frieden J, Lieber RL. Ultrastructural and mechanical basis of exercise induced muscle injury. Med Sci Sport Exerc, 1992; 24: 521-30.

16. Takala TES, Koskinen SOA, Uotila R, Hesselink M, Kuipers H, Vihiko V, Kovanen V, Komulainen J. Effects of forced eccentric and concentric contractions on prolyl 4-hydroxylase activity in skeletal muscle. Sceletal Muscle Research PART III Cellular and Molecular Adaptation, Jyväskylä, 1995; 394-5.

17. Komulainen J., Takala TES., Kuipers H, Hesselink MKC. The disruption of myofibre structures in rat skeletal muscle after forced lengthening contractions. Pflügers Arch Eur J Physiol, 1998; 436: 735-41.

18. Raulo SM, Hyyppa S, Rasanen LA, Poso AR. Exercise-induced changes in the activities of beta-glucuronidase and N-acetyl-beta-D-glucosaminidase in plasma and muscle of standardbred trotters. Zentralbl Veterinarmed A, 1996; 43: 119-26.

19. Kihlström M, Salminen A, Vihko V. Prednisolone decreases exercise-induced acid hydrolase response in mouse skeletal muscle. Eur J Appl Physiol, 1984; 53: 53-6.

20. Drewa G, Maciak R, Woźniak A, Chęsy G, Rakowski A, Woźniak B, Rozwadowska M. Influence of exercise on arylsulfatase and acid phosphatase activities in blood serum of kayakers and rowers. Biol Sport, 2000; 17: 289-97.

21. Stauber WT, Clarckson PM, Fritz VK, Evans WJ. Extracellular matrix disruption and pain after eccentric muscle action. J Appl Physiol, 1990; 69: 868-74.

22. Fisher B, Attaix D, Bechet D, Ferrara M, Baracos V. Increased muscle proteolysis after local trauma mainly reflects macrophage-associated lysosomal proteolysis. Am J Endocrinol Metab, 2002; 282: E326-35.

23. Reddy A, Caler EV, Andrews NW. Plasma membrane repair is mediated by Ca^{2+} – regulated exocytosis of lysosomes. Cell, 200; 106: 157-69.

24. Toncsev H, Frenkl R. Studies on the lysosomal enzyme system of the liver in rats undergoing swimming training. Int J Sports Med, 1984; 5: 152-5.