

**THE EFFECT OF THE PHYSICAL EFFORT ON THE ACTIVITY OF BRUSH BORDER ENZYMES AND LYSOSOMAL ENZYMES OF NEPHRON EXCRETED IN THE URINE**

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**Abstract.** The lysosomal enzymes activities in the athletes urine were designated and presented in this work: N-acetyl- $\beta$ -D-glucosaminidase (NAG),  $\beta$ -glucuronidase (GSR), arylsulfatase A (ASA). The brush border enzymes activities: leucyloaminopeptidase (LAP), alanine aminopeptidase (AAP),  $\gamma$ -glutamyltransferase (GGT), the trypsin inhibitor activity (UTI) and the total protein and creatinine concentrations were determined as well. Values of examined parameters are presented after its conversion to mmol creatinine units. Nine athletes subjected to physical effort in the frame of the training unit with the speed endurance accent were taken under the examination. The urine was taken before, immediately after and 24 h after effort. 9-fold increase of the protein/creatinine index was observed in the postexercise urine. In the urine taken after next 24h this index decreased to over 2-fold higher value than it presented itself before effort. Almost 3-fold increase of the NAG activity and 4-fold decrease of the ASA activity were noticed in the after effort urine. The brush border enzymes values were higher for over 2-3-fold in the postexercise urine but after next 24h they went down below values observed before effort. The correlation between NAG and brush border enzymes was observed at the level of  $r=0.7$ . All changes of examined parameters point at the passing glomerular-tubular troubles of nephrons. It may also be suggested that various forms of changes in the lysosomal enzymes activity are connected with their functions in organism and not with the degree of the renal cells structure damage.

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*Key words:* Lysosomal enzymes – Brush border enzymes – Urine – Physical exercise

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## Introduction

In physiological conditions, with the correct renal filtration and reabsorption function, the majority of proteins and enzymes present in urine come from renal cells and in some part from the plasma filtration and urinary tracts. Haemodynamic changes in the kidney take place during the physical effort – the blood pressure rises and its flow through the kidney falls [21]. The concentration of hydrogen ions and the partial pressure  $\text{CO}_2$  grow simultaneously. Those changes lead to disorders in the glomerular filtration and in mechanisms of the reabsorption which as a consequence influence the after effort urine content. The growth or the fall of the enzyme activity in urine might be the reflection of physiological or pathological changes in the kidney.

Determining the activity of enzymes and protein concentration in urine is a non-invasive and sensitive way for the discovery of early changes in kidneys while the traditional indices of kidneys function are within the standard [14]. Lysosomal enzymes of proximal tubule: arylsulfatase A (ASA),  $\beta$ -glucuronidase (GSR), N-acetyl- $\beta$ -D-glucosaminidase (NAG) as well the brush border enzymes e.g.: leucylaminopeptidase (LAP), alanine aminopeptidase (AAP),  $\gamma$ -glutamyltransferase (GGT) display particular usability in the evaluation of changes taking place in kidney [5]. Owing to the specific localisation of those enzymes (by the surface of cells) even after slight functional changes of tubules their liberation from the cells. Denoting the concentration or some specific activity of protein in an acute phase is also useful. One of the acute phase proteins is an inter- $\alpha$  proteinase inhibitor. The trypsin inhibitor present in urine (UTI) is produced as a consequence of the limited proteolysis of the inter- $\alpha$  proteinase inhibitor present in blood. This glycoprotein has a very low isoelectric point ( $pI=2.1$ ) what causes that the pace of its excretion with urine is highly depended on the blood acidifying [1].

As for avoiding urine time collection it is useful to relate gained results of concentrations and activity to the concentration of creatinine. This method of results presentation is profitable in the case of clinical analysis [15,16,19] as well as in the physical effort diagnostics [3].

The proper planing of a training unit as a part of the whole training cycle is needed in the process of the athlete optimal preparation. Biochemists and physiologists aim is to search for sensitive effort markers which might be useful in managing and monitoring athletes' effort adaptation, determining the readiness for taking the maximal effort or gaining information about athletes condition during effort load. Metabolic effects of the physical effort which initiate anaerobic lactate metabolism [23] cause significant functional changes in kidneys [20]. Such



metabolic changes is observed in the endurance – speed training which is the subject of this paper research.

The aim of the study was the evaluation of after effort changes taking place in the specific renal structures. It was done through measuring the activity of the arylsulfatase A (ASA),  $\beta$ -glucuronidase (GRS), N-acetyl- $\beta$ -D-glucosaminidase (NAG), leucyloaminopeptidase (LAP), alanine aminopeptidase (AAP),  $\gamma$ -glutamyltransferase (GGT), trypsin inhibitor (UTI) and measuring the total protein and creatinine concentration in urine. The trial of the evaluation of energetic changes taking place in athletes' organisms was also the aim.

### Material and Methods

The subjects of the study were nine athletes preparing for a 3000 m race. The examined group characteristics are presented in Table 1. The physical effort during training was to be conducted in anaerobic lactate zone according to Wolkow's and Koriagin's classification of exercise metabolism zones [23]. The training unit was characterised by the speed endurance and was conducted with the interval method. The training lasted for 60 min and it consisted of 8 runs x 400 m in the maximal pace i.e. 70 s/400 m with intervals: 2-2.5 min depending on the pulse. The next distance was beginning when the after effort pulse lowered itself from 180 to 120 beats/min.

**Table 1**

The examined group characteristic

No	Age	Height (cm)	Body mass (kg)	Training practice (years)
1	20	180	72	3
2	20	173	65	4
3	21	179	71	4
4	21	179	71	2
5	21	172	67	4
6	22	179	75	3
7	23	174	70	4
8	23	179	66	4
9	22	175	69	2



The urine taken from athletes before (A), after (B) and 24 h after (C) the training was the examined material. It was immediately cooled and centrifuged by 3000 x g in +4°C. The supernatant was divided into 2 portions. One was dialysed in +4°C to PBS and then its part was dialysed to the distilled water with the aim of eliminating ions disturbing the determination of the arylsulfatase A activity.

The creatinine concentration was measured with the colorimetric method using the picric acid in the not dialysed part of urine. The respective indices were determined in the urine dialysed to PBS: the  $\beta$ -glucuronidase (GRS) EC 3.2.1.31 activity was measured with the colorimetric method according to Maruhn [17] using the p-nitrophenyl- $\beta$ -D-glucuronide as a substrate, the N-acetyl- $\beta$ -D-glucosaminidase (NAG) EC 3.2.1.30 with the colorimetric method using the p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide as the substrate [18]. The activity of the trypsin inhibitor (UTI) was also determined with the Fritz *et al.* method [9] using the benzol arginine-4-nitroanilide as the trypsin substrate. The trypsin was assayed by the Chase and Shaw method [6]. The 50% inhibition of the trypsin activity 2  $\mu$ g was defined as the activity unit. The total protein concentration was determined with the Bradford's method [4] using Coomassie Brilliant Blue G-250 using the bovine albumin as a standard. The activity of  $\gamma$ -glutamyltransferase (GGT) EC 2.3.2.2 was determined by the Wahlefeld's and Bergmeyer's method [24]. The leucyloaminopeptidase activity (LAP) EC 3.4.11.1 was assayed with the method according to Haschen [12], the alanine aminopeptidase (AAP) EC 3.4.11.2 with the method of Jung and Scholz [13]. The activity of arylsulfatase A (ASA) EC 3.1.6.1 was determined in the urine dialysed to the water with the Baum's method [2] using the sulphate 2-hydroxy-5-nitrophenol as the substrate.

Results obtained were analysed statistically with the t-Student test. The Pearson's correlation coefficients were also established.

## Results

The statistically significant (20%) decrease of the concentration of creatinine in relation to urine A and C was observed in the postexercise urine B. Moreover, 9-fold increase of the protein/creatinine index in the after effort urine and 5-fold decrease of this index in urine C was observed. The activity of UTI/creatinine in the postexercise urine grew 4-fold. Results are presented in Table 2. The lysosomal enzymes activity changed in various ways (Table 3). The activity index of NAG/creatinine grew 2.7-fold and next reduced itself below the before effort values. The lack of changes in the GRS activity in urine B and statistically significant (20%) increase of its activity in urine C were observed. The statistically



significant – almost 4-fold drop of the ASA/creatinine activity in urine C in relation to the before effort values (A) and in urine B was also stated.

**Table 2**

The creatinine and protein concentration and the trypsin inhibitor activity in the athletes before and after effort urine

	Creatinine (mmol/l) x±SD	Protein mg/mmol creatinine x±SD	UTI U/μmol creatinine x±SD
Before effort	10.23	2.82	2.42
(A)	±7.67	±1.72	±1.72
After effort	7.93*	25.20**	10.00**
(B)	±2.59	±6.20	±4.98
24 h after effort	9.59	5.80	1.56
(C)	±2.43	±1.42	±1.10

\*-P≤0.05; \*\*-P≤0.01; the statistical significance in relation to urine A

**Table 3**

The lysosomal enzymes - ASA, NAG, GRS – activity in the athletes before and after effort urine

	ASA U/mmol creatinine x±SD	NAG U/mmol creatinine X±SD	GRS U/mmol creatinine x±SD
Before effort	0.54	0.48	0.24
(A)	±0.28	±0.18	±0.12
After effort	0.50	1.30*	0,24
(B)	±0.20	±1.06	±0.08
24 h after effort	0,14 *	0,36	0.30
(C)	±0.18	±0.10	±0.10

\*-P≤0.05; the statistical significance in relation to urine A



Examined brush border enzymes (Table 4) displayed similar profile of the activity changes. As considering LAP 2.5-fold increase was determined in urine B. Similar was the change of AAP activity which reached 3-fold increase in urine B and activity of GGT increased more than twice. The activity of all enzymes was lowering below restful values in urine C (24 h after effort) namely, AAP/creatinine for 2.3-fold, LAP for 1.2-fold and GGT for 6-fold in relation to urine A.

Established correlation coefficients in the after effort urine shows the dependence between the activity of NAG and brush border enzymes: NAG/GGT  $r=0.67$ ; NAG/LAP  $r=0.71$ ; NAG/AAP  $r=0.72$ ; LAP/AAP  $r=0.94$ . The correlation between the protein concentration and the activity of examined enzymes was not stated.

**Table 4**

The brush border enzymes – GGT, LAP, AAP - activity in the athletes before and after effort urine

	GGT U/mmol creatinine x±SD	LAP U/mmol creatinine x±SD	AAP U/mmol creatinine x±SD
Before effort (A)	9.22 ±4.32	0.46 ±0.160	0.72 ±0.36
After effort (B)	22.26** ±8.10	1.12** ±0,54	1.90** ±1,34
24 h after effort (C)	1.52** ±1.32	0.38 ±0.10	0.32** ±0.08

\*\* -  $P \leq 0.01$ ; the statistical significance in relation to urine

## Discussion

Changes of concentration and activity of metabolites, proteins, enzymes and other markers present in blood and urine are counted among the biochemical parameters used in the training control [21,23]. For various reasons invasive methods connected with blood taking or biopsy used in the physical effort control are inconvenient for examined and therefore they undergo some more restricted law. The stress connected with the blood taking may also influence some parameters results. Therefore, the search for indices useful in the evaluation of the effort influence on organism in non-invasively taken materials is continuing.



Chosen lysosomal enzymes and brush border enzymes were determined as the physical effort indices in urine basing on our own previous research [1, 3, 15].

According to Poortmans [20] the significant increase of the protein concentration in the postexercise urine suggests the physical effort with high intensity. This intensity, characteristic for effort in the anaerobic zone, causes the accumulation of lactic acid in blood what leads among others to changes in the permeability of the glomerular membrane and changes in renal tubules. Additionally, changes of the blood flow in kidney occur. Significant increase of the protein level in postexercise urine points to troubles in the permeability of the glomerular membrane as a result of the reduction of blood flow through kidneys enhances protein diffusion into the tubular lumen [20]. Similar changes were observed during efforts conducted strictly in the anaerobic lactate zone where an over 30-fold increase of the protein/creatinine index was observed [1]. However, the transitory character of filtration disorder was confirmed by returning to the before effort value after next 24 h. The decrease of the UTI level after 24 h in relation to after effort values is the evidence for the lack of the acute phase reaction in examined athletes [3]. Slight lowering of the creatinine level in urine B (middling about 20 %) suggests that athletes were correctly watered and that the aerobic conversions presented significant intensity beside anaerobic ones [21].

According to Gilli *et al.* and de Padi *et al.* [7,10] the increase of the GGT activity suggests the hypoxia of the kidneys brush border. Similarly the increase of the AAP and LAP activity placed in the same cells area as GGT confirm the hypoxia and damage of the brush border. However, decrease of the activity of GGT, LAP and AAP in urine C suggests that changes in the brush border were transitory.

Various character of changes in the lysosomal enzymes activity – ASA, NAG, GRS – may prove the lack of damage of lysosomes, characteristic for heavy damages of the nephron cells. It points at existence of links between the observed changes and the various functions of those enzymes [22]. The selective outflow of the lysosomal enzymes after physical effort was observed by Tsuboi in livers of rats subjected to the physical effort. Our results in urine are similar to this observation. Tsuboi connects this variation with the change of pH inside the lysosomes caused by the physical effort.

The activity of NAG in urine is an accepted renal lysosomal marker of troubles with the proximal tubules. Its increase in the postexercise urine and decrease after next 24 h showed at the higher permeability of the lysosome membrane for this enzyme during the effort. Changes in activity of ASA in the after effort urine have a different character than those observed in blood by Drewa *et al.* [8]. Authors



connect the increase of ASA activity in blood after effort with the release of this enzyme from myocytes as a consequence of ischemia and hypoxia or releasing of this enzyme by macrophages and leucocytes. Unchanged ASA activity in after effort urine B and its decrease in urine taken after next 24 h C were observed in our research. It suggests some stabilisation of lysosomal structures and cells membranes. The decrease in the activity of examined enzymes (apart from GRS) in 24 h after effort may prove the enzymatic protein degradation after its usage and insufficient synthesis which allows gaining some restful values. On the other hand, the lack of significant changes in the GSR activity may suggest the absence of heavy renal damages [5,11].

Growing excretion of the brush border enzymes and lowering of the lysosomal enzymes were observed as a result of applied effort.

The lack of correlation between postexercise proteinuria and enzymuria points at different mechanisms of glomerular and tubular malfunctions.

Conducted studies allow to evaluate the influence of physical effort occurring in the training unit on nephron functioning. Changes which were measured confirm that the training was conducted in the anaerobic lactate zone of exercise metabolism with the presence of significant component of aerobic changes. The training, the spite of its high intensity, did not cause long lasting changes within renal structures: the brush border and tubular.

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