Polish Journal of Veterinary Sciences Vol. 14, No. 4 (2011), 635-642

DOI 10.2478/v10181-011-0094-8

Original article

Na^+, K^+-ATPase activity and ATP concentration in horses of the Wielkopolski breed in relation to age

M. Suska, E. Brucka-Jastrzębska, D. Kawczuga

University of Szczecin, Faculty of Biology, Department of Physiology,
Felczaka 3c, 71-412 Szczecin, Poland

Abstract

This study aimed at determining relationships between the age of the Wielkopolski horses, ATP in whole blood and in the erythrocytes, and between erythrocyte Na^+, K^+-ATPase activity, and serum concentrations of mineral components. ATP was measured in whole blood and in erythrocytes by HPLC method. Serum concentrations of Ca^{2+} and Mg^{2+} were measured spectrophotometrically, while Na^+ and K^+ by flame photometry. In horses aged from 4 to 48 months, a dynamic decrease in ATP activity was found. Erythrocyte Na^+, K^+-ATPase activity decreased proportionally with the decrease in ATP activity. The results of this study may enable planning physical effort of horses at optimum use of energetic efficiency of their erythrocytes and mineral components in relation to their age.

Key words: RBC, horses, age, ATP, Na^+/ K^+-ATPase

Introduction

Red blood cells are classically considered to be the major supplier of oxygen to tissues. Ellsworth et al. (1995) proposed that they are not only a carrier of oxygen, red blood cells ourselves are involved in the regulation of oxygen supply. When the red blood cell is transiently exposed to low pO_2, low pH, or mechanical deformation, ATP release increases (Ellsworth et al. 1995, Sprague et al. 2001). Following release, the ATP binds to purinergic receptors initiating a conducted vasomotor response that increases blood flow to the tissue (McCullough et al. 1997, Collins et al. 1998). The study of purine nucleotide metabolism is very important for the understanding of disruptions in energy metabolism as the purine nucleotides participate in most energy-requiring metabolic reactions and act as coenzymes (Dudzińska and Hłyńczak 2004, Dudzińska et al. 2006). Adenine nucleotides: ATP, ADP and AMP are the characteristic compound in the erythrocyte energy metabolism in vertebrates (Ataullakhanov et al. 1996, Fokina et al. 2000). Adenosine triphosphate (ATP) is a well known as a major energy substance in all living systems. Scientists regarding its function during over past decades (Schwiebert and Zsembery 2003), have shown that ATP is a regulator of muscle contraction, platelet aggregation (North 2002), vascular tone (Burnstock 1972) and neurotransmission (Soto et al. 1996, Burnstock 2006) ATP concentration in a red blood cell is

Correspondence to: M. Suska, e-mail: fizjolzw@univ.szczecin.pl

a measure of metabolic and function of the cell. Energy delivered in the form of ATP is essential for metabolic processes. It is involved in the control of the correct shape of the erythrocytes, active transport, the control of the reduced level of iron in haemoglobin, and the synthesis of glutathione and pyridine enzymes in erythrocytes and pentose phosphate pathway (Siems et al. 2000). During the transition from the resting state of muscles to their maximal exercise, there is a great increase in energy demand (ATP consumption) (Arthur et al. 1992, Hogan et al. 1992)

Active and passive transport mechanisms are complex processes and the erythrocyte membrane is highly selective to ions, especially Na^+, K^+, Ca^{2+}, Mg^{2+} (Brugnara 1997, Pedersen 2002). Active transport through the erythrocyte membrane concerns Na^+, K^+, Ca^{2+}, Mg^{2+} ions passing against the concentration gradient with the use of energy stored in ATP. It occurs with the participation of enzymatic proteins belonging to ATPase of type P (Lutsenko and Kaplan 1995, Pedersen 2002). Na^+ and K^+ transport utilizes a lot of ATP, while Ca^{2+} transport requires markedly less energy. There is no consensus in the literature on the dependence of Na^+, K^+-ATPase (EC 3.6.1.37) on ATP. For this reason we also attempted to find out the relations between the ATP concentration and the activity of Na^+, K^+-ATPase (Brugnara 1997, Hoffman 1997, Siems et al. 2000).

This study aimed at determining relationships between the age of Wielkopolski horses (4-48 months) and HCT in whole blood and ATP concentration in whole blood and in the erythrocytes, and between Na^+, K^+-ATPase erythrocytes activity and serum concentrations of mineral components (based on Ca^{2+}, Mg^{2+}, Na^+, K^+ concentrations).

The results of this study may be of use for veterinarians and horse breeders to develop optimal training methods, as they may allow planning of physical effort of horses at optimum for energetic efficiency of erythrocytes. The study may also enable to determine physiological predisposition of horses towards various forms of exploitation. The results may serve as reference values.

Materials and Methods

Animals and blood sampling

The study was conducted on the Wielkopolski horse breed of the respiratory constitutional type. We examined male horses from the State-owned Stallion Stud in Łobez (Poland). In autumn, all the horses were fed in compliance with the National Research Institute of Animal Production feeding standards for livestock animals (Ryś 1981, Taylor et al. 1995). The diet included: oat grain (5-7 kg/per day), spring and winter grain shred (0.5-3 kg/per day), hay (5-7 kg/per day) and carrots (2-3 kg/per day), while fresh straw was used as a bedding. The horses had constant feeding times: 7-8 a.m., 1-2 p.m. and 6-7 p.m. Feeding was also coordinated with trainings times, so that horses were never trained earlier than 1-1.5 hour after meal. The feed was energetically balanced and contained all nutritional components, vitamins and minerals essential for keeping the horses in excellent condition (Table 1), with regard to energy consumption during training and work. The animals received water from the same source during the whole breeding period. Prior to morning feeding the horses were exercised, and after morning feeding they were taken for a 30 minute walk. Blood samples were collected from the external jugular vein (*vena jugularis externa*) to two test tubes: one with heparine (250 IU heparine from Polfa Poland) and the other one for the clot. To eliminate the influence of physical strain, diet, circadian and seasonal changes on the biochemical indices, blood samples were taken after a night rest, before the morning feeding, always in November. The studies were conducted on 40 clinically healthy horses (males) aged 4-48 months (Table 1).

Laboratory evaluation

The HPLC method was used to measure adenosine triphosphate nucleotide (ATP) concentrations

Table 1. Number of animals (n) in four age groups of Wielkopolski horses and approximate feed rations depending on equine height and age.

Group of horses	Number of animals (n)	Age (Month)	Horse height (cm)		Daily feed ration (kg)
A	8	4-6	Foals	<120	6.3-7.2
B	15	12	Yearlings	120-130	7.2-8.0
C	12	24	Colts	130-140 140-150	9.0-10.0 10.0-11.0
D	5	36-48	Stallions	150-160 >160	11.012.0 12.012.5

Table 2. ATP concentrations in whole blood (µmol l^{-1}) and the erythrocytes (µmol l^{-1}_{RBC}) of Wielkopolski horses aged from 4 to 48 months.

Group	ATP (µmol l^{-1}) in whole blood	Statistical Significance (P < 0.05)	ATP (µmol l^{-1}_{RBC}) in RBC	Statistical Significance (P < 0.05)	Relation ATP_{RBC} : : $ATP_{whole\ blood}$
	mean ± SD		Mean ± SD		
A	81.52 ± 7.86	A,C; A,D	275.56 ± 19.74	A,B; A,C; A,D; *	3.38
B	78.18 ± 5.36	B,C; B,D	238.03 ± 29.06	B,A; B,C; B,D; *	3.04 (↓10%)
C	71.71 ± 5.29	C,A; C,B	184.84 ± 22.76	C,A; C,B; C,D; *	2.57 (↓24%)
D	70.22 ± 4.27	D,A; D,B	178.30 ± 12.53	D,A; D,B;	2.54 (↓25%)

Legend: ± SD standard deviation; letters mark similarity between groups: * – statistical differences between the values in whole blood and the erythrocytes; ↓ decrease in concentration.

in whole blood and in the erythrocytes. Deproteinized blood samples (500 µl whole blood + 500 µl 1.3 M $HCLO_4$) were centrifuged (14 000 g for 10 minutes, at 4°C), and ATP concentration was immediately determined according to the method of Smolenski et al. (1990) with a Hewlett Packard series 1100 chromatographic system. Chromatographic data were registered and processed by HP Chemstation Software (Hewlett Packard). The results of ATP concentration mmol l^{-1} of whole blood were calculated considering hematocrit index value per mmol l^{-1}_{RBC}. The ATP concentrations obtained were expressed in µmol l^{-1} in whole blood and µmol l^{-1} in the erythrocytes (µmol l^{-1}_{RBC}).

Hematocrit (HCT) was measured by the standard method in heparin-covered microhematocrit tubes, using hematocrit centrifuge and a standard reading device.

Erythrocyte Na^+, K^+-ATPase (EC.3.6.3.9) activity was measured according to Chio et al. (1977). The enzyme activity was determined in erythrocytic membrane fragments based on the P_i amount released from ATP in the time unit per 1 mg of protein. The amount of released P_i phosphate groups was determined by colorimetric method according to Goldenberg and Fernandez (1966). Protein concentration was determined by Lowry's et al. (1951) method. The enzyme activity was expressed as µmol of P_i per mg protein per hour (µmol P_i mg h^{-1}).

The blood sampled for the clot was centrifuged (2000 turns/min/5 minutes). In the serum obtained (no traces of hemolysis), concentrations of Ca^{2+}, Mg^{2+}, N^+, K^+ were determined. Ca^{2+} and Mg^{2+} concentrations were determined by atomic absorption spectrophotometry with the use of SP 1900 Unicam spectrophotometer. Na^+ and K^+ were determined by flame photometry with the use of Flapho photometer.

Statistical evaluation

The results of the parameters examined are given as mean values and standard deviations (± SD). The statistical significance of differences was determined with mono and multifactor analysis of variance and the Duncan's test at P < 0.05. Statistica 6.0 software was used to carry out the statistical analysis.

Results

The study has shown a decrease in ATP concentration both in the erythrocytes and in whole blood of growing Wielkopolski horses aged from 4 to 48 months. Analysing ATP values of the four groups examined, the highest ATP concentration was found in whole blood (81.52 ± 7.86 µmol l^{-1}) and in the erythrocytes (275.56 ± 19.74 µmol l^{-1}_{RBC}) of the youngest horses (group A), and the lowest in stallions of group D (whole blood 70.22 ± 4.27 µmol l^{-1}; erythrocytes – 178.30 ± 12.53 µmol l^{-1}_{RBC}) (Table 2). Assuming that ATP concentration in group A is 100%, we calculated that ATP concentration in whole blood of group B decreased in relation to group A by 4.1%, in group C by 12.1% and in group D by 13.9%. Whereas ATP concentration in the erythrocytes of group B decreased in relation to group A by 13.6%, group C by 32.9% and group D by 35.3%. ATP concentration in whole blood and in the erythrocytes of the foals (4-6 month-old) was significantly (P < 0.05) higher as compared to that found in the older age groups (groups B, C, D). Among all the horse groups examined statistically significant (P < 0.05) differences were found.

Analyzing Na^+, K^+-ATPase activity (fmol P_i mg h^{-1}) of four age groups of Wielkopolski horses (groups: A, B, C, D) we found a statistically significant decrease in enzyme activity in erythrocytes in

Table 3. Ratios of ATP concentrations in whole blood and the erythrocytes to erythrocyte Na^+, K^+-ATPase activity in horses between 4 and 48 months of life.

Group	Relation	
	ATP in whole blood: Na^+, K^+-ATPase	ATP in RBC: Na^+, K^+-ATPase
A	108.69	367.41
B	144.7 (↑33.2%)	440.79 (↑20.0%)
C	199.8 (↑83.8%)	513.53 (↑39.8%)
D	206.5 (↑90.0%)	524.41 (↑42.7%)

↑ increase in concentration

Table 4. Na^+, K^+ and Ca^{2+} i Mg^{2+} (mmol l^{-1}) concentrations in blood serum of Wielkopolski horses between 4 and 48 month of life.

Group	Na^+ (mmol l^{-1})		K^+ (mmol l^{-1})		Relation Na^+ : K^+
	$\bar{x}$ + SD	min-max	$\bar{x}$ + SD	min-max	
A	146.0 ± 2.00	143.0-148.0	3.69 ± 0.15	3.50-4.00	39.46
B	144.7 ± 2.05	142.0-148.0	3.84 ± 0.11	3.70-4.00	37.68
C	143.5 ± 1.51	142.0-146.0	4.05 ± 0.17	3.90-4.20	35.43
D	142.0 ± 1.64	140.0-144.0	4.36 ± 0.11	4.20-4.50	32.56
Group	$Ca^{2\pm}$ (mmol l^{-1})		$Mg^{2\pm}$ (mmol l^{-1})		Relation $Ca^{2\pm}$: $Mg^{2\pm}$
	$\bar{x}$ ± SD	min-max	$\bar{x}$ ± SD	min-max	
A	3.41 ± 0.12	3.20-3.60	0.70 ± 0.01	0.69-0.72	4.87
B	3.27 ± 0.19	2.90-3.60	0.78 ± 0.02	0.75-0.82	4.20
C	3.17 ± 0.16	2.90-3.40	0.81 ± 0.01	0.79-0.83	3.91
D	3.00 ± 0.16	2.80-3.20	0.82 ± 0.01	0.80-0.83	3.65

horse age group (0.75-0.34 µmol l^{-1}). The highest Na^+, K^+-ATPase activity was found in foals (group A: 0.75 ± 0.06 µmol P_i mg h^{-1}), and the lowest in the oldest horses (group D: 0.34 ± 0.06 µmol P_i mg h^{-1}). Erythrocyte Na^+, K^+-ATPase activity in foals (group A) was significantly ($P < 0.05$) higher as compared to that observed in other equine groups (B, C, D). Assuming that Na^+, K^+-ATPase activity in group A is 100% (0.75 ± 0.06 µmol P_i mg h^{-1}) we found a decrease in enzyme activity in group B by 28.0% (0.54 ± 0.07 µmol P_i mg h^{-1}), in group C by 52.0% (0.36 ± 0.07 µmol P_i mg h^{-1}) and in group D by 54.7% (0.34 ± 0.06 µmol P_i mg h^{-1}). One-way analysis of variance and the Duncan's test revealed statistically significant differences in erythrocyte Na^+, K^+-ATPase activity among the horse groups examined.

The study has shown statistically significant relationships between ATP in whole blood or erythrocytes and Na^+, K^+-ATPase activity. The regression curve shows a decrease with age (4-48 months) in ATP levels in whole blood ($R^2 = 0.952$) and erythrocytes ($R^2 = 0.932$), as well as and in Na^+, K^+-ATPase activity ($R^2 = 0.914$) at $P < 0.05$.

The study revealed that the ratio of erythrocytes ATP concentration to whole blood concentration decreased during ageing from 3.34 µmol l^{-1} in foals (group A) to the value of 2.54 µmol l^{-1} in stallions (group D). The ratio of ATP concentration in the erythrocytes to the erythrocyte Na^+, K^+-ATPase activity increased from 367.4 to 524.4 in all the groups examined (A, B, C, D). A similar tendency was observed in the ATP concentration in whole blood and Na^+, K^+-ATPase activity ratio in all groups (A, B, C, D) of horses (108.7-206.5) (Table 3).

Analysing serum values of Na^+, K^+, Mg^{2+} and Ca^{2+} concentration in relation to age in groups A-D, we observed a slight decrease in Na^+ concentration in different age groups (A-D) of horses (146.0-142.0 mmol l^{-1}; max 2.7%), while K^+ concentration increased (3.69-4.36 mmol l^{-1}; max. 18.2%) (Table 4). We found that Ca^{2+} concentration in groups A-D decreased during the growth of horses (3.41-3.00 mmol l^{-1}), while Mg^{2+} concentration increased (0.70-0.082 mmol l^{-1}) (Table 4). There were not found statistically significant differences of Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations in the horses examined. We found a tendency in de-

crease of Na^+ to K^+ concentrations ratio and Ca^{2+} to Mg^{2+} concentrations ratio. Based on the regression coefficients (R^2), a strong relationship was found between Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations in blood serum of Wielkopolski horses of different age. Regression coefficient was determined for Na^+ and K^+ concentrations (R^2 = 0.988) and for Ca^{2+} and Mg^{2+} concentrations (R^2 = 0.942).

The study revealed an increase in HCT level during ageing, from 26.1% in 4 months old horses to 35% in 48 months old horses. The lowest HCT level was found in foals of group A of Wielkopolski horses (26.1%).

Correlation coefficients (r) revealed strong relationships between serum levels of K^+, Ca^{2+}, Mg^{2+} and Na^+, K^+ ATPase activity in horses from different age groups (group A, B, C, D). The study also revealed correlation (r = 0.908) between serum levels of Ca^{2+} and Mg^{2+} in group A, as well as between serum levels of K^+ and Ca^{2+} in group C. Negative correlations were found in Wielkopolski foals between Mg^{2+} concentration and erythrocyte Na^+, K^+ ATPase activity, as well as between K^+ concentration and Na^+, K^+ ATPase activity (r = -0.755). Significant correlation coefficients (r) were found for relationships between serum levels of Na^+ K^+, Ca^{2+}, Mg^{2+} and Na^+, K^+-ATPase activity at different age of horses examined (group A, B, C, D). Negative correlation (r = -0.991) was observed between Ca^{2+} and Mg^{2+} concentrations in horses of group C, while positive correlation (r = 0.953) occurred between K^+ and Na^+ concentrations in group B. Negative correlation was found between Na^+, K^+-ATPase activity and Ca^{2+} concentration (r = -0.959) in group B, as well as between Ca^{2+} and K^+ concentration in the 36 months old horses (r = -0.973).

Discussion

This study comprised Wielkopolski horses, a breed used for sport and recreation riding. This breed was developed by improving local horses with Mazury and Trakehner horses, and to a lower extent also with other half-breed horses of western origin. Wielkopolski horses are even-tempered, gentle and easy to school, and yet they inherited fantastic temperaments and vigour from their pure breed ancestors. The results of this study would be useful for veterinarians, horse breeders and horse riding centres for planning and carrying out training. Data gathered for Wielkopolski horses have been compared to similar data for Hanoverian and Ardennes horses (Table 5). According to the World Breeding Federation for Sport Horses (WBFSH), modern Hanoverian horses are among the world's best sport horses (dressage competitions and steeplechases). They are known for their excellent character, good temperament, perfect jumping abilities and elasticity. The Ardennes are strong workhorses distinguished by hardiness and used in agriculture. The Ardennes are distinguished by a digestive type of constitution. On the contrary, the Wielkopolski and the Hanoverian have a respiratory type of constitution, which is significantly related to age and effort.

Na^+, K^+-ATPase is a cell membrane enzyme which can be found in all mammalian cells. It is responsible for the transport of Na^+ ions from the cell to the extracellular area and K^+ ions in the opposite direction. In each catalytic cycle, 1 hydrolyzed ATP molecule provides energy for the transport of 3 Na^+ and 2 K^+ ions. ATP, like other adenine nucleotides (ADP and AMP) found in the erythrocytes, is a characteristic compound of the energy metabolism occurring inside. Moreover, it undergoes reversible changes in the process of glycolysis and in pentose phosphate pathway.

During the intensive growth of Wielkopolski horses from 4 to 48 months of life we found dynamic changes in Na^+, K^+ ATPase activity and ATP concentrations in whole blood and in the erythrocytes. Horses show comparatively low physiological ATP level in the erythrocytes (Dębski 1985, Miseta et al. 1993). According to Suska (2003), similar pattern of changes in erythrocyte Na^+, K^+ ATPase activity and ATP concentrations was observed also in the Hanoverian and Ardennes horses, and their maturation was also accompanied by reduction of ATP concentrations (Table 5). Her (Suska 2003) research in the Hanoverian horses also has shown similar pattern of changes in HCT level. She observed an increase in HCT level in the blood of growing Hanoverian horses aged from 12 to 24 months from 37.4% to 41.5%, but in the blood of 36-48 month old animals she observed a decrease of HCT level from 41.5% to 39.4%. The highest HCT level she found in blood of horses aged 24-48 months (39.4-41.5%) and in the present work we obtained the highest HCT level in the same age group. However the HCT peak value in our work was lower than in Suska (2003) study.

It is known that concentrations of adenine nucleotides (ATP, ADP and AMP) in vertebrate erythrocytes show high interspecies differences (Miseta et al. 1993). Changes in erythrocyte Na^+, K^+ ATPase activity and ATP concentrations may result from higher intensity of anabolic processes over the catabolic ones. Additionally, horses received salt to lick (max. 30 g per day). Reduction of ATP content in equine RBC depends on effort – its kind, intensity and duration, as well as on the organism's adaptation to effort

Table 5. ATP concentrations in erythrocytes of three horse breeds.

Equine age (months)	Erythrocyte ATP concentration (μmol l^{-1}_{RBC})		
	Wielkopolski horses	Hanoverian horses (Suska, 2003)	Ardennes horses (Suska, 2003)
	mean ± SD	mean ± SD	mean ± SD
4-8	275.56 ± 56.06	no data	no data
12	238.0 ± 29.06	214.9 ± 11.1 (↓10.3%)	no data
24	184.8 ± 22.76	173.5 ± 21.3 (↓6.1%)	no data
36-48	184.3 ± 12.53	155.0 ± 11.3 (↓13.0%)	148.7 ± 12.9 (↓16.1%)

↓ decrease in erythrocyte ATP concentration.

(Snow and Martin 1990). RBC in younger animals have more intensive metabolism, which indicates higher efficiency. It mainly depends on the presence of cell nucleus and its energy consumption. Serum concentrations of Na^+ and K^+ as well as Mg^{2+} and Ca^{2+} were also highly variable. The Ca/Mg ratio, and Na/K ratio, depend not only on availability from food, but are regulated by and play roles in general activity and exercise. These ions influenced activities of enzymes in RBC membrane and cytoplasm, and determined energetic processes occurring in them. According to Smith and Maguire (1993), Mg^{2+} activates glucokinases and type P ATPases. Serum levels of Mg^{2+} increased significantly with age in the Wielkopolski and Hanoverian horses (0.70-0.80 nmol l^{-1} and 0.76-0.80 nmol l^{-1}, respectively), which probably resulted from the Ca^{2+} and K^+ forage intake. Increased K^+ content may reduce Mg^{2+} absorption (Fisher et al. 1994). Concentrations of Mg^{2+}, Ca^{2+}, Na^+ and K^+ turned out to be significantly different in horses of the three breeds, aged 36-48 months. Highest concentrations of cations were found in the Wielkopolski horses: Mg^{2+} (0.80 ± 0.1 mmol l^{-1}), Ca^{2+} (3.0 ± 0.1 mmol l^{-1}), Na^+ (142.2 ± 1.6 mmol l^{-1}) and K^+ (4.4 ± 0.2 mmol l^{-1}) and the Hanoverian horses: Mg^{2+} (0.80 ± 0.1 mmol l^{-1}), Ca^{2+} (2.9 ± 0.1 mmol l^{-1}), Na^+ (142.3 ± 1.8 mmol l^{-1}) and K^+ (4.1 ± 0.2 mmol l^{-1}). In the Ardennes, cation levels were lower: Mg^{2+} (0.69 ± 0.1 mmol l^{-1}), Ca^{2+} (2.8 ± 0.1 mmol l^{-1}), Na^+ (138.4 ± 1.8 mmol l^{-1}) and K^+ (3.7 ± 0.2 mmol l^{-1}) (Suska 2003). Statistical analysis confirmed similarity of the three compared horse groups regarding age and gender. The concentration of Ca^{2+} and Mg^{2+} ions relative to the system of active transport, consisting of type P ATPase. It dependent on ATP and Mg^{2+} presence and responsible for the qualitative and quantitative location of Mg^{2+}, Ca^{2+}, Na^+ and K^+ on both sides of the erythrocytic membrane (Fu et al. 1998, Pedersen 2002). ATPases of type P belong to ATPases characterized by temporary enzyme phosphorylation in each catalytic cycle and cyclic conformational enzyme transformation from type E1 into E2 and vice versa (Jürgensen 1982). Adenine nucleotides pool varied depending on age, breed or sex of the horses examined (Suska et al. 2006). Whereas energy charge value in all the groups comprised within 0.89-0.92 range. Despite low ATP concentration in equine erythrocytes was observed very high adenylate energy charge value was observed. According to Miseta et al. (1993), sodium-potassium pump (Na^+, K^+ ATPase) is responsible for the active movement of K^+ to the erythrocytes and Na^+ export to the plasma. This process uses about 50% of the erythrocytic ATP available. The sodium-potassium pump activity is curbed by a decrease in ATP concentration in the cell. Our study has revealed that Na^+, K^+ ATPase activity decreases with the decrease in ATP concentration in the erythrocytes and whole blood. Comparison of our results with data reported by Suska (2003) indicates that horses of the Wielkopolski and the Hanoverian breeds, aged between 36 and 48 months, had equal activity of Na^+, K^+ ATPase (0.34 ± 0.4 ,mol $P_i \cdot$mg protein h^{-1}) and different from the Ardennes breed (0.22 ± 0.5 μmol Pi · mg protein h^{-1}). Annandale et al. (2005) found that ATP concentration, glucose-6-phosphate, lactate, ADP and AMP in skeletal muscles did not change during a 6-week study neither in healthy horses nor in the horses suffering from polysaccharide storage myopathy. Edner et al. (2007) studied an anaesthetic influence on the metabolism of healthy and sick horses. The study lasted for 7 days and was conducted before, during and after the anaesthetic application. It has shown that ATP concentration in the blood of sick horses decreased after the anaesthetic application. According to Miseta et al. (1993), erythrocyte Na^+, K^+ ATPase activity contributes to the interspecies and interbreed differences in sodium and potassium ions levels in the erythrocytes. According to Winnicka (2008), equine Na^+ concentrations are 139.1-156.5 mmol l^{-1}, and K^+ concentration 3.50-4.70 mmol l^{-1}. Na^+ transport in the erythrocytes depends also on chloride ion concentration, which is stimulated by urea (Speake and Gibson 1997). Relationships between Na^+, K^+ ATPase activity and Na^+ and

K^+ content in the erythrocytes, and ATP concentration previously was reported by Hoffman (1997) and Thomas and Fell (1998).

Maintaining appropriate serum concentrations of Ca^{2+} and Mg^{2+} is essential for proper RBC shape and metabolism. We found that serum levels of Ca^{2+} (3.23 ± 0.21 mmol l^{-1}) and Mg^{2+} (0.78 ± 0.04 mmol l^{-1}) in the horses examined corresponded with reference values reported for these animals (Edner et al. 2007). K^+, Na^+ ATPase plays a major role in H^+/CO_2 exchange during exercise. The levels of Ca^{2+} and Mg^{2+} ions depends on muscular activity. The role of Na^+, K^+ ATPase is to deliver energy for the H^+/CO_2 transport in the regulation of acid/base balance in erythrocytes during exertion. According to Winnicka (2008), average Mg^{2+} concentration in horse blood serum was 0.70 – 1.15 mmol l^{-1}, and Ca^{2+} 2.68-3.35 mmol l^{-1}. We found the decrease in serum concentration of Na^+ (146.0-142.0 mmol l^{-1}) and Ca^{2+} (3.41-3.00 mmol l^{-1}) with a simultaneous increase in Mg^{2+} (0.70-0.82 mmol l^{-1}) and K^+ (3.69-4.36 mmol l^{-1}) concentrations. A substantial decrease in Na^+, K^+ ATPase (0.75-0.34 µmol P_1 mg h^{-1}) activity was found in the horses between 36-48 and at the 4th month of life. The results obtained were higher than those presented by other authors (Krumrych and Wiśniewski 1993, Hłyńczak and Suska 1994, Danek et al. 1997). Wielkopolski foals (4-6-month-old) and yearlings (12-month-old) had significantly ($P < 0.05$) higher Na^+, K^+ ATPase activity compared to the stallions. Along with serum Na^+, K^+ ATPase activity drop we have observed a decrease of Na^+ concentration and an increase in K^+ concentration the horses examined.

Summing up, Na^+, K^+ ATPase activity and ATP concentration in the erythrocytes of the horses examined underwent dynamic changes during growth and development. A significant decrease in ATP concentration was observed both in the erythrocytes and in whole blood of Wielkopolski horses in periods between 4-6 and 36-48 months of life. ATP concentration in the erythrocytes decreased from the initial value assumed as 100% to 64.7%. Whereas ATP concentration in whole blood decreased to 86.1%. During the period of growth and development of the horses the decrease in Na^+, K^+ ATPase activity was observed proportional to the decrease in ATP concentration. Moreover, changes were found in the concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+} in the 4-6 and 36-48-months-old horses. The results obtained indicate that a decrease in Na^+ and Ca^{2+} concentrations was accompanied by an increase in K^+ and Mg^{2+} concentrations. In the period of equine intensive growth and development, age-dependent dynamic changes in erythrocyte Na^+, K^+ ATPase activity and serum levels of mineral constituents occurred.

The results of this study may be useful for veterinary clinical diagnostics as reference values for the Wielkopolski breed, and for selective breeding of horses with regard to different types of usage.

References

Annandale EJ, Valberg SJ, Essen-Gustavsson B (**2005**) Effects of submaximal exercise on adenine nucleotide concentrations in skeletal muscle fibers of horses with polysaccharide storage myopathy. Am J Vet Res 66: 839-845.

Arthur PG, Hogan MC, Bebout DE, Wagner PD, Hochachka PW (**1992**) Modeling the effects of hypoxia on ATP turnover in exercising muscle. J Appl Physiol 73: 737-742.

Ataullakhanov FJ, Vitvitskii VM, Komarova SV, Masharov EV (**1996**) Energy-dependent processes and metabolism of adenylates in human erythrocytes. Biokhimiia 61: 266--274.

Brugnara C (**1997**) Erythrocyte membrane transport physiology. Curr Opin Hematol 4: 122-127.

Burnstock G (**1972**) Purinergic nerves. Pharmacol Rev 24: 509-581.

Burnstock G (**2006**) Historical review: ATP as a neurotransmitter. Trends Pharmacol Sci 27: 166-176.

Chio SJ, Taylor MA, Abrams R (**1977**) Depression, ECT, and erythrocyte adenosine-triphosphatase activity. Biol Psychiatry 12: 75-81.

Collins DM, McCullough WT, Ellsworth ML (**1998**) Conducted vascular responses: communication across the capillary bed. Microvasc Res 56: 43-53.

Danek J, Wiśniewski E, Krumrych W (**1997**) Effect of sugar and calcium to feed on haematological and biochemical indices in stallions blood. Med Weter 53: 351-354.

Dębski B (**1985**) The effect of training and physical exercise on the energetic metabolism of equine erythrocytes. Zentralbl Veterinarmed A 32: 190-195.

Dudzińska W, Hłyńczak AJ (**2004**) Purine nucleotides of human erythrocytes – metabolism and regulation. Postepy Biochem 50: 353-362.

Dudzińska W, Hłyńczak AJ, Skotnicka E, Suska M (**2006**) The purine metabolism of human erythrocytes. Biochemistry (Mosc) 71(5): 467-475.

Edner AH, Nyman GC, Esseen-Gustavsson B (**2007**) Metabolism before, during and after anaesthesia in colic and healthy horses. Acta Vet Scand 15:49-34

Ellsworth ML, Forrester T, Ellis CG, Dietrich HH (**1995**) The erythrocyte as a regulator of vascular tone. Am J Physiol 269: H2155-H2161.

Fisher LJ, Dinn N, Tait RM, Shelford JA (**1994**) Effect of level of dietary potassium on the absorption and excretion of calcium and magnesium by lactating cows. Can J Anim Sci 74: 503-509.

Fokina KV, Yazykova MY, Danshina PV, Schmalhausen EV, Muronetz VI (**2000**) Participation of glyceraldehyde-3-phosphate dehydrogenase in the regulation of 2,3-diphosphoglycerate level in erythrocytes. Biochemistry (Mosc) 65: 463-468.

Fu Y, Wang S, Lu Z, Li H, Li S (**1998**) Erythrocyte and plasma Ca^{2+}, Mg^{2+} and cell membrane adenosine triphosphatase activity in patients with essential hypertension. Clin Med J (Engl) 111: 147-149.

Goldenberg H, Fernandez A (**1966**) Simplified method for the estimation of inorganic phosphorus in body fluids. Clin Chem 12: 871-882.

Halawa B (**1992**) The density of α-adrenergic receptors in lymphocytes and the rate of flow of sodium across cell lymphocytes membranes in the elderly people. Post Med Klin Dośw 1: 173-178.

Hłyńczak AJ, Suska M (**1994**) The level of magnesium, calcium, zinc and copper in horses serum of various breeds. Biul Magnezol 4: 72-74.

Hoffman JF (**1997**) ATP compartmentation in human erythrocytes. Curr Opin Hematol 4: 112-115.

Hogan MC, Arthur PG, Bebout DE, Hochachka PW, Wagner PD (**1992**) Role of O2 in regulating tissue respiration in dog muscle working in situ. J Appl Physiol 73: 728-736.

J8rgensen PL (**1982**) Mechanism of the Na^+, K^+ pump. Protein structure and conformations of the pure (Na^+ +K^+)-ATPase. Biochim Biophys Acta 694: 27-68.

Krumrych W, Wiśniewski E (**1993**) Influence of gender on the blood biochemical indices in horses. Med Weter 49: 327-328.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (**1951**) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275.

Lutsenko S, Kaplan JH (**1995**) Organization of P-type ATPases: significance of structural diversity. Biochemistry 34: 15607-15613.

McCullough WT, Collins DM, Ellsworth ML (**1997**) Arteriolar responses to extracellular ATP in striated muscle. Am J Physiol 272: H1886-H1891.

Miseta A, Bogner P, Berenyi E, Kellermayer M, Galambos C, Wheatley DN, Cameron IL (1993) Relationship between cellular ATP, potassium, sodium and magnesium concentrations in mammalian and avian erythrocytes. Biochim Biophys Acta 1175: 133-139.

North RA (**2002**) Molecular physiology of P2X receptors. Physiol Rev 82: 1013-1067.

Pedersen PL (**2002**) Transport ATPases in biological systems and relationship to human disease: a brief overview. J Bioenerg Biomembr 34: 327-332.

Ryś R (**1981**) Standards for livestock feed. 1st ed. PERiL Warsaw.

Schwiebert EM, Zsembery A (**2003**) Extracellular ATP as a signaling molecule for epithelial cells. Biochim Biophys Acta 1615: 7-32.

Siems WG, Sommerburg O, Grune T (**2000**) Erythrocyte free radical and energy metabolism. Clin Nephrol 53: S9-17.

Smith DL, Maguire ME (**1993**) Molecular aspects of Mg2+ transport systems. Miner Electrolyte Metab 19: 266-276.

Smoleński RT, Lachno DR, Ledingham SJ, Yacoub MH (**1990**) Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in heart for transplantation. J Chromatogr 527: 414-420.

Snow DH, Martin V (**1990**) Effects of exercise and adrenaline on equine erythrocyte ATP content. Res Vet Sci 49: 77-81.

Soto F, Garcia-Guzman M, Gomez-Hernandez JM, Hollmann M, Karschin C, Stuhmer W (**1996**) P2X4: an ATP-activated ionotropic receptor cloned from rat brain. Proc Natl Acad Sci USA. 93: 3684-3688.

Speake PF, Gibson JS (**1997**) Urea – stimulated K-Cl cotransport in equine red blood cells. Pflugers Arch 434: 104-112.

Sprague RS, Ellsworth ML, Stephenson AH, Lonigro AJ (**2001**) Participation of cAMP in a signal-transduction pathway relating erythrocyte deformation to ATP release. Am J Physiol Cell Physiol 281: C1158-C1164.

Suska M (**2003**) The energy metabolism of horses erythrocytes aged from 4 to 48 months old. Habilitation thesis. Szczecin, Poland, (in Polish).

Suska M, Skotnicka E, Dudzińska W, Orowicz W, Brzezińska M (**2006**) Adenylate nucleotides and 2,3-biphosphoglycerate concentration in erythrocytes of growing Wielkopolska stallions. Acta Vet Brno 75: 13-20.

Taylor LE, Ferrante PL, Kronfeld DS, Meacham TN (**1995**) Acid-base variables during incremental exercise in sprint-trained horses fed a high-fat diet. J Anim Sci 73: 2009-2018.

Thomas S, Fell DA (**1998**) A control analysis exploration of the role of ATP utilization in glycolytic-flux control and glycolytic-metabolite concentration regulation. Eur J Biochem 258: 956-967.

Winnicka A (**2008**) The reference values of basic laboratory research in veterinary medicine. 4th ed. SGGW, Warsaw.