

SECTION – SPORT SCIENCES

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CHANGES IN MORPHOLOGICAL-RHEOLOGICAL BLOOD PROPERTIES OF HUTNIK CLUB FOOTBALL PLAYERS

Authors' contribution:

- A. Study design/planning
- B. Data collection/entry
- C. Data analysis/statistics
- D. Data interpretation
- E. Preparation of manuscript
- F. Literature analysis/search
- G. Funds collection

Dominika Kolbiarz^{1 ABDE}, **Aneta Teległów**^{2 ABDEF},
Anna Ściśłowska-Czarnecka^{3 C}, **Jakub Marchewka**^{4 EF},
Jacek Głodzik^{5 DE}, **Krzysztof Żaba**^{6 EF},
Katarzyna Filar-Mierzwa^{7 EF}

¹ M.A. student, University of Physical Education, Krakow, Poland² Department of Clinical Rehabilitation, University of Physical Education, Krakow, Poland³ Department of Anatomy, University of Physical Education, Krakow, Poland⁴ Department of Orthopaedics and Trauma Surgery, 5th Military Hospital, Krakow, Poland⁵ Department of Physiotherapy, University of Physical Education, Krakow, Poland⁶ Ph.D. student, University of Physical Education, Krakow, Poland⁷ Department of Group Therapy, University of Physical Education, Krakow, Poland**Key words:** football, blood morphology, blood rheology**Abstract**

Study aim. The aim of the study was to investigate the effect of long-term football training on the morphological-rheological properties of the blood.

Study material. The study was conducted among a group of 16 footballers playing for the Hutnik Nowa Huta Club. The average age of subjects was 22 years. Practices are held 5 times a week, all year round with the exception of the last 3 weeks of December and the last 2 weeks of June. In addition, championship or sparring matches are played every weekend. Venous blood was collected at the end of the football season, in the morning on an empty stomach. Examination of morphological-rheological parameters of the blood was performed in the footballers and the control group.

Results. Compared to the control group, a statistically significant decrease in RBC, HGB, HCT, MCV, PLT and plasma viscosity were found for the footballer group. And a statistically significant increase in fibrinogen and MCHC as well. There were no statistically significant changes in the amount of WBC and MCH. Significant changes were also noticed in erythrocyte aggregation and elongation for the footballers. AI reduced, while AMP and T_{1/2} increased. Elongation index at shear-stress for the range of 0.30–1.13 [Pa] significantly increased, while for the value ranges of 2.19–59.97 [Pa], a decrease was observed in this parameter.

Conclusions. Long-term, regular football training does not cause pathological changes in the morphological-rheological properties of the blood.

Introduction

Football is the most popular sport in the world. Its earliest variant was already played in the Chinese army, B.C. [26]. The modern history of football began about 150 years ago in England [2]. Currently, the Interna-

tional Football Federation covers almost every part of the world, consisting of 209 national associations. It is responsible for the most prestigious sports tournament – the FIFA World Cup. [23]

The scientific branch dealing with the phenomena of blood flow through blood vessels is hemorheology.

Research focuses on whole blood, plasma and the cellular components. Analyzing the rheological properties of blood, we obtain information on how it behaves in the vascular system [16]. Blood flow is determined by its physicochemical properties, it is non-uniform, and its viscosity varies depending on shear rate. Plasma is a non-Newtonian liquid, the viscosity is determined by high-molecular weight protein content [14]. Blood rheology plays a very important role in the aggregation of erythrocytes, the phenomenon is dynamic and reversible. Erythrocytes are connected via macromolecular bridges forming single aggregates called Rouleaux which can create spatial branches [11]. The deformability of erythrocytes is based on the changes in their shape necessary to pass through a vessel with a diameter smaller than the erythrocytes themselves. This is a reversible process and does not affect the surface or volume and does not alter the properties or functions of erythrocytes [15].

The aim of the study was to investigate the effect of regular training on the morphological-rheological properties of the blood in football players.

Material and study methods

Study group

The study group was formed from a group of 16 footballers belonging to the Hutnik Nowa Huta Club. The men were aged 19–31 years, the mean age 22 years. Practices are held 5 times a week, all year round with the exception of the last 3 weeks of December and the last 2 weeks of June. In addition, championship or sparring matches are played every weekend.

The study took place on July 11, 2014 in the Laboratory of Locomotor Pathology at the University School of Physical Education in Krakow. The footballers arrived for examination in the morning, on an empty stomach. A qualified nurse collected the blood into Vacuette – type test tubes with potassium EDTA. The morphological-rheological indicators of the blood were tested. The study was authorized by the Bioethics Committee at the Regional Medical Chamber in Krakow.

The measurement of basic hematological indicators

The HORIBA ABX Micros 60 blood analyzer was used to measure basic morphological indicators:

- WBC [G/L] – number of white blood cells
- RBC [T/L] – number of red blood cells
- HGB [g/L] – hemoglobin
- T[L/L] – hematocrit
- PLT[G/L] – platelets
- MCV[fL] – mean corpuscular volume
- MCH[fmol] – average mass of corpuscular hemoglobin
- MCHC[mmol/L] – mean corpuscular hemoglobin concentration

Rheological blood testing

To study the aggregation and deformability of red blood cells, the LORCA lasik-optical analyzer constructed by Max R. Hardeman was used. The instrument has two cylinders in between which the blood is placed. The outer cylinder rotates relative to the inner one. In this method, the red blood cells scatter the laser light which is in a stationary cylinder [22].

Elongation index determination

For the determination of the elongation index, a 25 μ l blood sample was taken and put into 5 ml of 0.14 mM polyvinylpyrrolidone (PVP, M = 360000) solution. The measurement was performed at 37°C. The sample was placed between the cylinders and submitted to centrifugal force. The laser light passing through the red blood cells is diffracted. The compressed computer system records the scattering of the laser beam, and then computes the elongation index (EI) according to the following formula:

$$EI = (A - B) / (A + B),$$

A – is the length of the red blood cell

B – is the width of the red blood cell

The results of the elongation index (EI) were given in the range of 0.30 to 59.97 of shear forces expressed in Pascals [Pa]. The collected measurements determined the degree of erythrocyte flexibility [10] [22].

Aggregation index determination

To test the aggregation index, 1 ml of blood was put into the chamber located between two cylinders. The computer launched rotational movement of the cylinder, 120 s at a shear rate of $> 400 \text{ s}^{-1}$. After ten 10 seconds, the cylinder stops and aggregation of red blood cells occurs. Its measurement is the change in laser light intensity, until its maximal value within a range of 0.5–2.0 sec. After reaching 2 seconds to 1 minute or longer, erythrocyte aggregates are subjected to different shear rates from 6 to 700 s^{-1} [9] [22].

The computer calculated the following parameters defining the kinetics of erythrocyte aggregation:

- AI [%] – aggregation index calculated from the formula:

$$AI = A/A + B \times 100 \%$$

A – area above selectogram
 B – area below selectogram
- AMP [au] – degree of total aggregation
- $T_{1/2}$ [s] – half-life of total aggregation

Biochemical testing

Determination of fibrinogen concentration

Fibrinogen concentration [g/l] was determined using the Chrom – 7 coagulometer. The Bio-Ksel PT reagent containing thromboplastin with calcium chloride was

added to the plasma which resulted in plasma coagulation and clot formation. Conversion of fibrinogen to fibrin occurred. The standard for fibrinogen is from 2 to 5 g/l.

Determination of blood plasma viscosity

Examination of plasma viscosity [mPa] was performed using the Myrenne viscometer (model: D-52159, Germany). 0.5 ml of plasma, obtained after centrifugation of the morphotic components of the blood, was put into the measurement capillary. The measured value is the time in which the tested string of plasma covered the distance from the L3 to L4 light-points, thanks to constant pressure. The device was standardized using standard Myrenne NP1 and NP2 solutions before the measurement In order to obtain high accuracy (approx. ± 3%). NP1 was a standard solution for the lower range of measurements with the viscosity of 1.10 [mPa]. NP2 was the standard solution for high range measurements.

Statistical analysis

The results were obtained using Microsoft Office Excel. Analysis of the study took into account, inter alia, the number of people in the group, the mean and standard deviation. To compare data between the group of foot-

ballers and the control group, the Tukey test was used. Statistically significant values were found at the significance level of $p < 0.05$.

Study results

The analysis of morphological and rheological indicators of the blood:

1. HGB [g/L], HCT [L/L], MCV [fL], MCH [fmol], MCHC [mmol/L]

Comparing the group of footballers to the control group, the following statistically significant changes were found:

- average concentration of HGB [g/L] decreased by 8.52% in footballers
- average number of HCT [L/L] decreased by 13.79% in footballers
- average value of MCV [fL] decreased by 5.05% in footballers
- average value of MCHC [mmol/L] increased by 6.26% in footballers

Analyzing the changes in the average value of the MCH [fmol], no statistically significant changes were

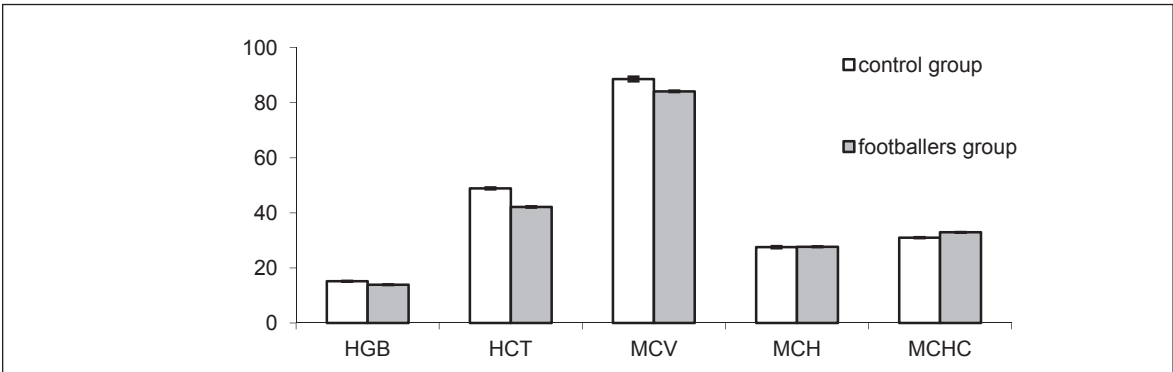


Fig. 1. Graph presenting average values of HGB [g/L], HCT [L/L], MCV [fL], MCH [fmol], MCHC [mmol/L] in footballers compared to the control group.

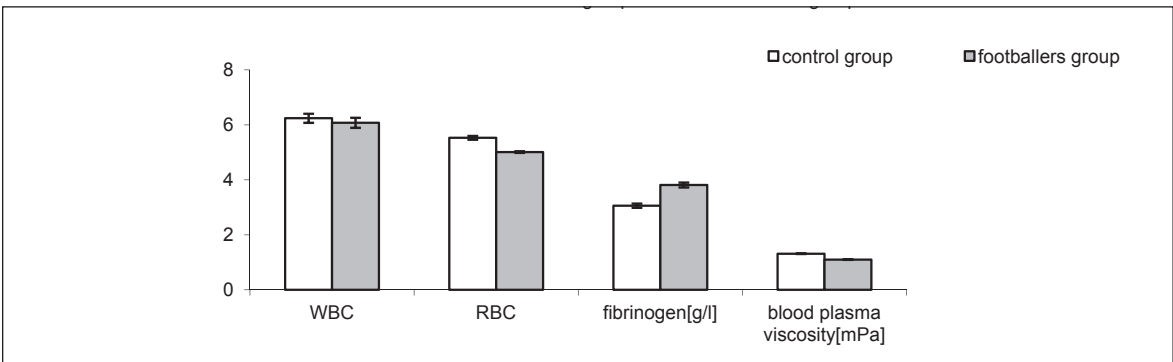


Fig. 2. Graph presenting average values of WBC [G/L], RBC [T/L], fibrinogen [g/l], blood plasma viscosity [mPa] in footballers compared to the control group.

found in the group of footballers compared to the control group.

2. WBC [G/L], RBC [T/L], fibrinogen [g/l], blood plasma viscosity [mPa]

Analyzing the changes in the average number of WBC [G/L], no statistically significant changes in the group of footballers were found compared to the control group.

Comparing the group of footballers to the control group, the following statistically significant changes were found:

- average number of RBC [T/L] decreased by 9.4% in footballers
- average value of fibrinogen [g/l] increased by 24.61% in footballers
- average value of blood plasma viscosity [mPa] decreased by 16.39% in footballers

3. PLT [G/L]

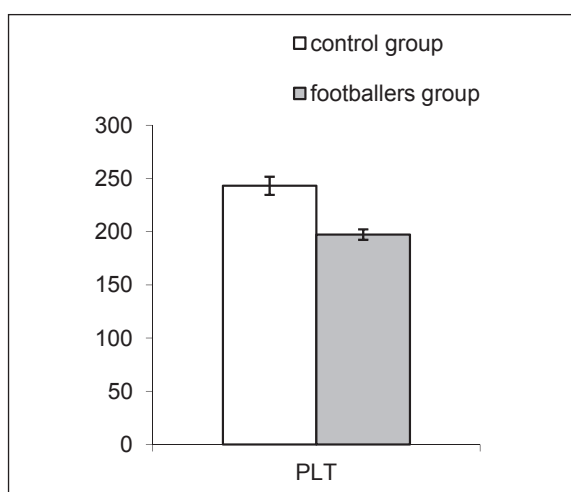


Fig. 3. Graph presenting average values of PLT [G/L] in footballers compared to the control group

Analyzing the average values in the number of PLT [G/L], statistically significant changes by 18.86% were noted in the group of footballers compared to the control group.

4. EI [Pa]

Comparing the group of footballers to the control group, the following statistically significant changes were found:

- average EI concentration at 0.30 [Pa] shear-stress increased by 179.76% in footballers
- average EI concentration at 0.58 [Pa] shear-stress increased by 235.53% in footballers
- average EI concentration at 1.13 [Pa] shear-stress increased by 51.32% in footballers
- average EI concentration at 2.19 [Pa] shear-stress reduced by 30.37% in footballers
- average EI concentration at 4.24 [Pa] shear-stress reduced by 29.34% in footballers
- average EI concentration at 8.23 [Pa] shear-stress reduced by 20.89% in footballers
- average EI concentration at 15.96 [Pa] shear-stress reduced by 14.29% in footballers
- average EI concentration at 31.04 [Pa] shear-stress reduced by 10.75% in footballers
- average EI concentration at 59.97 [Pa] shear-stress reduced by 6.85% in footballers

5. AI [%], AMP [au], $T_{1/2}$ [s]

Comparing the group of footballers to the control group, the following statistically significant changes were found:

- average AI [%] numerical values decreased by 15.31% in football players
- average AMP [au] numerical values increased by 69.14% in football players
- average $T_{1/2}$ [s] numerical values increased by 69.18% in football players

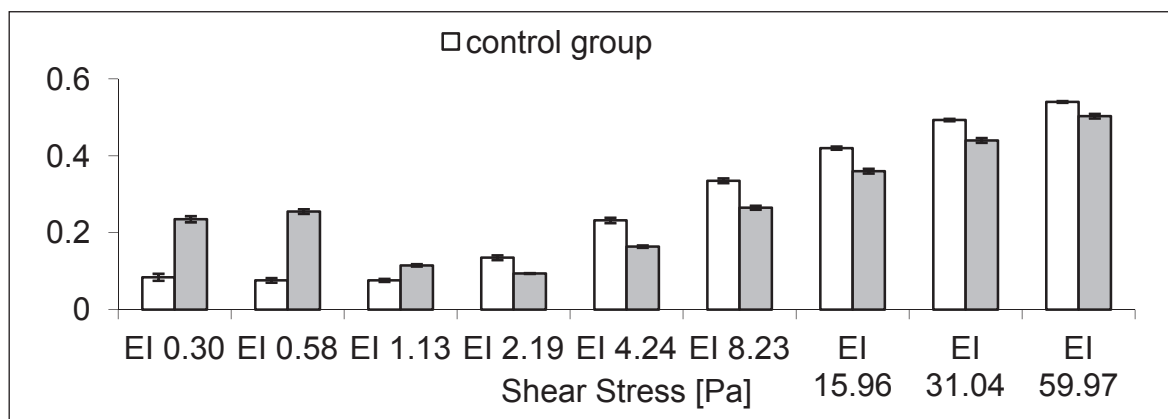


Fig. 4. Graph presenting average values of EI [Pa] in footballers compared to the control group

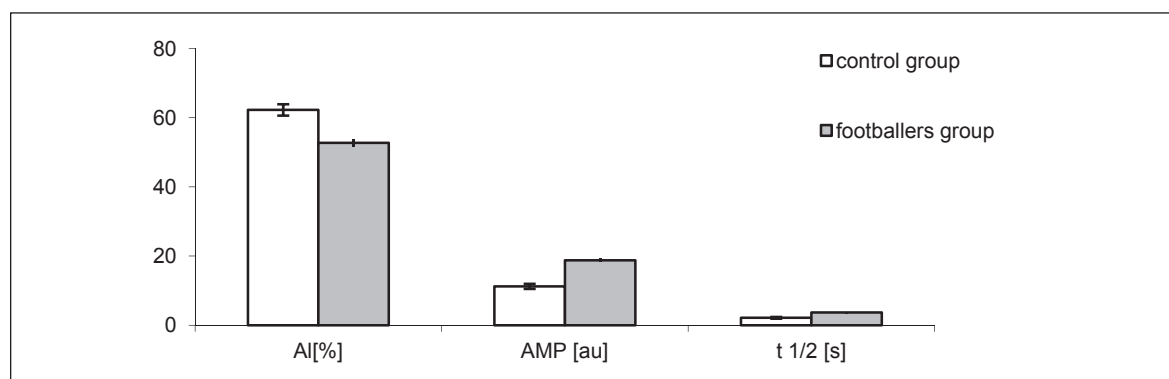


Fig. 5. Graph presenting average values of AI [%], AMP [au], $T_{1/2}$ [s] in footballers compared to the control group

Discussion

Association football belongs to a group of sports with a very particular training programme which escapes the simple distinction between endurance and strength training. A football match lasts 90 minutes and is divided into two halves, 45 minutes each; during this time, players engage in physical activity of both aerobic and anaerobic nature. Most of the time, a player's metabolism is aerobic, however, some distances must be covered with great speed, with anaerobic intensity and a heart rate at 80-90% of maximum heart rate [1] [17].

The objective of the present study is to demonstrate the effect of regular training on association football players' morphological and rheological properties of the blood. Many studies have already proved the effect of regular training on the properties of blood flow. Research indicates a decrease in haematocrit, haemoglobin concentration and a decrease in the number of red blood cells due to endurance training; the condition is explained by the increased volume of plasma occurring during and after physical exercise [18] [24]. However, the above-mentioned research only applies to strength or endurance training [20].

Research on the impact of association football training programmes on haematological parameters is limited. In their research, Bruno et al. (1991) showed that regular football training improves blood fluidity and has positive impact on blood rheology [4]. In their research, Edwards and Clark (2006) observed a significant decrease in the volume of plasma, measured after a non-professional (7.2%) and professional (11.6%) football match [7]. To my knowledge, the study by Edwards and Clark is the only one showing a decrease in plasma volume in response to years of exercise. Schumacher et al. (2002) explain this state as resulting from congestion of the blood caused by fluid loss, in turn caused by increased sweating resulting from intense exercise [20].

The decrease in plasma volume is also common in dehydrated athletes [6].

While studying 99 association football players, Varlet-Marie et al. (2011) showed, among other things, the correlation between $\dot{V}O_2$ max and haematocrit. The existence of the correlation confirms the results of earlier studies, performed on a smaller number of athletes. Another relation revealed in the aforementioned study concerns the influence of the appearance of lactate in the blood on the aggregation of red blood cells. The relationship is consistent with many previous reports by Varlet-Marie et al. and confirms that red blood cells are more likely to aggregate when the muscles at work release more lactate [25].

In my study, a statistically significant increase (24.60%) of fibrinogen as compared to the control group was proven. The impact of association football training on fibrinogen is poorly investigated, however, there is no doubt that fibrinogen and blood rheology are closely linked since the former is the main determinant of erythrocyte aggregation [19].

Furthermore, a statistically significant decrease in plasma viscosity, possibly attributable to increased plasma volume, was noticed. Karakoc et al. (2005) argue that the increase of lactate in the blood has no negative effects on plasma viscosity in football players since they develop a defence mechanism through regular training during the season. Analysis of the study by Karakoc et al. (2005) shows that blood viscosity tends to decrease as a result of this type of training: this is due to a decrease in mean red cell volume by 5.05%, also showed in my study [12].

My research on the aggregation and elongation of erythrocytes in association football players is pioneering. All these parameters were statistically significantly different from the control group. The study by Bilski et al. (2014) showed that physical exercise affects the rheology of blood in that it significantly changes the deformability of erythrocytes; the elongation index is also

statistically significantly reduced. These changes may be partly due to an increase in lactate levels in plasma after exercise. Lactate reduces the size of red blood cells, which in turn decreases their formability [3]. My research showed a decrease in the deformability by 2.19-59.97 [Pa] in the shear-stress interval; formability increased significantly for lower shear rates: 179.76% for EI 0.30 [Pa], 235.53% for EI 0.58 [Pa] and 51.32% for EI 1.13 [Pa].

Detailed mechanisms of correlation between erythrocyte aggregation and physical exercise are not known yet. Bilski et al. (2014) found a significant increase in AI and a decrease in the value of $T_{1/2}$ in an examination of 12 moderately active, healthy men on a cycle ergometer [3]. However, the present study showed a 15.31% reduction in AI and an increase in AMP and $T_{1/2}$ (69.14% and 69.18%, respectively), which proves that football training improves indicators of blood aggregation.

Kilgore et al. (2002) put forward the hypothesis that for the change in the number of erythrocytes, haemoglobin concentration, haematocrit, and the size of changes in plasma volume, a particular training program that includes endurance, strength, power, sprint and jumping is to be deemed mostly responsible [13]. The present study reported a statistically significant reduction in erythrocytes (9.40%), haemoglobin (8.51%) and haematocrit (13.79%); the studies by Ciorsac et al. (2010) and Silva et al. (2008) also found a statistically significant decrease in haematocrit at the end of the football season [5] [21].

Silva et al. (2008) published a study that demonstrated that association football training does not affect the immune system: leukocytes, lymphocytes, eosinophils and monocytes remained at a constant level [21]. In the present study, there were no statistically significant differences in the number of leukocytes in the football players compared to the control group.

The study by Filaire et al. (2003) verified the behaviour of haematological parameters for a particular football training programme. The study showed no significant changes in the number of platelets, and it lacks information about the concentration of haemoglobin and haematocrit [8]. In contrast, an analysis of original research showed a significant reduction in the number of platelets (18.86%) in football players compared to the control group.

Both my study and the study conducted by Silva et al. (2008) showed a statistically significant increase in the average haemoglobin concentration in red blood cells (6.26%) at the end of the football season [21].

From the conducted study, it may be concluded that training on a regular basis causes changes in morphological-rheological properties of the blood in football players. However, obtaining exact knowledge on the response requires further research.

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References

- [1] Bangsbo J: *The physiology of soccer – with special reference to intense intermittent exercise*. Acta Physiologica Scandinavica Supplementum. 1994; 151: 1–155.
- [2] Bednarz D: *Początki Futbolu*. Retro Futbol. 2014; 1: 8–11.
- [3] Bilski J, Teległów A, Pokorski J, Nitecki J, Pokorska J, Nitecka E et al.: *Effects of a Meal on the Hemorheologic Responses to Exercise in Young Males*. BioMed Research International, 2014; 862968: 7.
- [4] Brun JF, Fons C, Raynaud E, Fedou C, Orsetti A: *Influence of circulating lactate on blood rheology in professional football players*. Revista Portuguesa de Hemorreologia. 1991: 219–229.
- [5] Ciorsac A, Isvoran A, Ostafe V: *Hematological and biochemical parameters in Romanian junior football player*. Annals of West University of Timisoara Series of Chemistry. 2010; 19 (1): 33–40.
- [6] Dill DB, Costill DL: *Calculation of percentage change in volume of blood, plasma and red cells in dehydration*. Journal of Applied Physiology. 1974; 37: 247–248.
- [7] Edwards AM, Clark NA: *Thermoregulatory observations in soccer match play: professional and recreational level applications using an intestinal pill system to measure core temperature*. British Journal of Sports Medicine. 2006; 40: 133–138.
- [8] Filaire E, Lac G, Pequignot JM: *Biological, hormonal and psychological parameters in professional soccer players throughout a competitive season*. Perceptual and Motor Skills, 2003; 97: 1061–1072.
- [9] Hardeman MR, Dobbe JGC, Ince C: *The laser – assisted optical rotational cell analyzer (LORCA) as red blood cell aggreometer*. Clinical Hemorheology and Microcirculation, 2001; 25: 1–11.
- [10] Hardeman MR, Goedhart PT, Dobbe JGC, Lettinga KP: *Laser – assisted optical rotational cell analyser (LORCA), A new instrument for measurement of various structural hemorheological parameters*. Clinical Hemorheology and Microcirculation. 1994; 14 (4): 605–618.
- [11] Jaroszyński W, Kwiatkowski B, Boguś P: *Wpływ hematokrytu na stopień i kinetykę agregacji erytrocytów ludzkich. Badania metodą spektroskopii dielektrycznej*. Annales Academiae Medicae Gedanensis. 2010; 40: 19–31.

- [12] Karacok Y, Duzova H, Polat A, Emre MH, Arabac I: *Effectsoftraining periodon hemorheological variables regularly trained footballers*. *British Journal of Sports Medicine*. 2005; 39: 4.
- [13] Kilgore JL, Pendlay GW, Reeves JS, Kilgore TG: *Serum chemistry and hematological adaptations to 6 weeks of moderate to intense resistance training*. *Journal of Strength and Conditioning Research*. 2002; 16: 509–515.
- [14] Lerche D, Koch B, Vlastos G: *Flow behaviour of blood*. *Rheology*. 1993; 93: 105–112.
- [15] Maeda N: *Erythrocyte rheology in microcirculation*. *Japanese Journal of Physiology*. 1996; 46: 1–14.
- [16] Marcinkowska-Gapińska A, Kowal P: *Podstawy i znaczenie hemoreologii*. *Neuroskop*. 2005; 7.
- [17] Reilly T: *An ergonomics model of the soccer training process*. *Journal of Sports Sciences*. 2005; 23: 561–572.
- [18] Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ: *Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness*. *Medicine and Science in Sports and Exercise*. 2000; 32: 332–348.
- [19] Schmid-Schönbein H: *Hemorheology*, in: *Comprehensive Human Physiology From Cellular Mechanisms to Integration*. Greger R. and Windhorst U. (Eds), Springer, Berlin (Heidelberg/New York). 1996: 1747–1792.
- [20] Schumacher YO, Schmid A, Grathwohl D, Bultermann D, Berg A: *Hematological indices and iron status in athletes of various sports and performances*. *Medicine and Science in Sports and Exercise*. 2002; 34: 869–875.
- [21] Silva ASR, Santhiago V, Papoti M, Gobatto CA: *Hematological parameters and anaerobic threshold in Brazilian soccer players through a training program*. *International Journal of Laboratory Hematology*, 2008; 30: 158–166.
- [22] Słowińska L, Monkos K: *Kliniczne zastosowania laserowo-optycznego rotacyjnego analizatora krwinek czerwonych LORCA*. *Annales Academiae Medicae Silesiensis*, 2010; 64 (3–4): 42–47.
- [23] Szwarc A: *Rys historii piłki nożnej*. Pomorski Związek Piłki Nożnej, 2013.
- [24] Thirup P: *Haematocrit: within-subjected and seasonal variation*. *Sports Medicine*, 2003; 33: 231–243.
- [25] Varlet-Marie E, Brun JF, Fedou Ch, Raynaud de Mauvergerc E: *Blood rheology and body composition as determinants of exercise performance in male soccer players*. *Clinical Hemorheology and Microcirculation*. 2011; 49: 225–230.
- [26] <http://www.fifa.com>. The History of Football from FIFA.com. 24.05.2015.

Address for correspondence:

Aneta Teległów, PhD
 Department of Clinical Rehabilitation
 University of Physical Education, Krakow, Poland
aneta.teleglow@awf.krakow.pl

