RED BLOOD CELL DEFORMABILITY AND PHYSICAL ACTIVITY

Ivan Ivanov [1,2]

[1] National Sports Academy "Vassil Levski", Sofia, Bulgaria
[2] Institute of Mechanics, Bulgarian Academy of Sciences

ABSTRACT

Physical exertion and sport cause changes of different nature and degree (positive or negative, strong, weak, moderate) in blood and blood cells' biomechanical and fluid properties. The aim of this minireview was to attempt to summarize the main red blood cell deformability alterations during and after physical activity classified as follows: erythrocyte geometric shape; erythrocyte membrane rheological properties; biochemical and structural properties of erythrocyte membrane lipids and proteins; hemoglobin concentration; calcium ion concentration; intracellular diffusion and osmosis; nitric oxide and erythrocyte nitric oxide synthase; erythrocyte ATP synthase; erythrocyte age and degree of erythrocyte cell maturation; body and erythrocyte temperature. It is essential to study and evaluate the individual hemorheological response profile of erythrocyte deformability during physical activity because it is strongly personal. This is one of the major erythrocyte properties determining oxygen supply to working muscles. The multiple hemorheological changes during physical activity and their interconnectedness and strong personality make their differentiation very difficult. It is reasonable to weigh their effects (with specific quantitative measurements) to simplify the analysis and increase the likelihood of obtaining valid conclusions. But there will always be the probabilistic nature of these conclusions, determined by the complex and strictly individual picture of the hemorheological effects of physical exercises and activities.

OPEN ACCESS

Submitted: 19 November 2022
Revised: 12 December 2022
Accepted: 13 December 2022

ORCID
Ivan Ivanov
https://orcid.org/0000-0001-7519-9657

Cite this article as:
Ivanov, I. (2022). Red blood cell deformability and physical activity. *Journal of Applied Sports Sciences*, Vol.2, pp. 106 - 118. DOI: *10.37393/JASS.2022.02.8*

Keywords*: red blood deformability, erythrocyte morphological properties, blood flow, physical activity*

INTRODUCTION

One of the main functions of the cardiovascular system during exercise is to ensure a full supply of everything necessary for the intensively working muscles. A primary function of red blood cells is to transport oxygen (O_2) from the lung to the tissues and deliver metabolically produced carbon dioxide (CO_2) to the lung for exhalation. Hemoglobin in erythrocytes also contributes to the stable, functional capacity of the blood. The release of ATP (adenosine triphosphate) and nitric oxide (NO) from the red blood cells leads to vasodilatation (expansion of blood vessels) and, from there – to maximum, respectively, sufficient blood flow to the working muscles, organs, and tissues. The optimal erythrocyte functions for the human body are provided in the circulatory vascular bed by red blood cells with optimal micromechanical properties, namely rheological, morphological, m echanical, and biochemical (Ivanov, 2022; Mairbäurl, 2013).

The fact that blood viscosity is one of the main factors determining the behavior of blood flow in the macrocirculatory channel (arteries and veins) is also known. On the other hand, the micromechanical properties of blood cells (erythrocytes, leukocytes, platelets) determine the behavior of blood during its flow through the microcirculatory system (arterioles, capillaries, venules). In microcirculation, individual blood cells often pass through blood vessels with a smaller diameter than that of erythrocytes and leukocytes. In this aspect, in the microcirculatory bed, it is important

to determine what mechanical deformability the erythrocytes possess (Smith et al., 1999). It is accepted that erythrocytes mainly determine the blood flow behavior because they are the most numerous in a unit volume of blood. Their number depends on the sex and age of the individual, as well as other factors.

The movement of red blood cells in the microcirculatory bed determines the resistance to blood flow and the distribution of all types of blood cells in the microvascular network. Erythrocyte deformability is a major hemorheological factor requiring careful and in-depth study (Fedosov et al., 2014).

The purpose of this minireview was to summarize the main red blood cell deformability alterations during and after physical activity classified as follows: erythrocyte geometric shape; erythrocyte membrane rheological properties; biochemical and structural properties of erythrocyte membrane lipids and proteins; hemoglobin concentration; calcium ion concentration; intracellular diffusion and osmosis; nitric oxide and erythrocyte nitric oxide synthase; erythrocyte ATP synthase; erythrocyte age and degree of erythrocyte cell maturation; body and erythrocyte temperature.

TOPICS AND RESULTS

Morphological red blood cell characteristics

The number of erythrocytes in the human body is between 4.7 - 6.1x10^{12}/L, and their number depends on the sex, age, weight, health status, fitness status of the individual, as well as other external factors. Their shape is a biconcave disk with dimensions: diameter of about 8 μm, thickness on the periphery of about 2.5 μm, and in the middle of about 1.5 μm (Figure 1).

The average total surface of all erythrocytes of an average individual (3000–3200 m^2) is about 1500–2000 times larger than his body surface. Erythrocytes are enveloped by a lipoprotein cell membrane made up of four molecular layers. The erythrocyte membrane is permeable to important compounds, such as CO_2, HCO^{3-}, some molecules and ions, such as O_2, Cl^-, and impermeable to some cations. The primary function of erythrocytes is related to the transport of oxygen (O_2) and carbon dioxide (CO_2) from the lung to the cells and tissues and vice versa. In addition to gases, erythrocytes also transport water from the tissues to the lung alveoli. Their main cellular content is hemoglobin (Hb). Hb is composed of a protein part – globin- representing 96% of the molecule. The remaining 4% is a „heme“ prosthetic group.

Hemoglobin changes its shape depending on the number of oxygen molecules bound to it. The change in shape also results in a change in oxygen affinity. As the number of oxygen molecules bound to hemoglobin increases, hemoglobin‘s affinity for oxygen increases. When not bound to oxygen, hemoglobin is assumed to be in a tensed state (T-state) with a low affinity for oxygen. At the point where oxygen initially binds, hemoglobin changes shape to a relaxed state (R-state), which has a higher affinity for oxygen. During regular physical activity, the process of binding oxygen molecules from hemoglobin is of great importance.

According to their biochemical composition, biological properties, and cellular functions, red blood cells are essential for the proper and efficient functioning of the body.

Red blood cell deformability

Erythrocyte deformability is the ability of red blood cells to reversibly change their shape during flow in response to external forces. The deformability of red blood cells allows them to pass through narrow lumen microvessels while transporting O_2 and CO_2 to tissues and cells in the body.

Erythrocytes can deform from a normal doubly concave disc to an elongated shape due to changes in blood flow conditions and

other factors in the microcirculatory bed. The deformability of red blood cells is mainly determined by the viscosity of the cytoplasm, the properties of the cytoskeleton, and of the erythrocyte cell membrane. The intracellular viscosity of erythrocytes depends primarily on two factors - Hb concentration and water content, due to the effects of these substances on cellular osmolarity balancing. The shape and elasticity of the erythrocyte membrane are influenced by membrane protein complexes (Jordan et al., 1998; Kuhn et al., 2017).

Normal erythrocytes in healthy individuals deform primarily in four possible modes of cell deformation (Kuhn et al., 2017).

A. *Elongation* of the discoid shape under shear stress along the longitudinal axis.

B. *Rotation* of the erythrocyte cell membrane is its movement around the cell contents without significant changes in the erythrocyte shape. It has been shown that this movement forces the erythrocyte to orient itself in the center of the vascular lumen – along the axis of the vessel, which improves blood flow.

C. *Rocking* is membrane erythrocyte rotation combined with oscillations relative to the major axis of the ellipsoidal erythrocyte shape.

D. *Rollover* - facilitated by turbulent blood flow at high strain rates, seen primarily in larger blood vessels.

Regular physical exertion of different duration, frequency, and intensity is an important factor in modeling blood status as a fluid and affecting all its elements. When subjected to external stresses, human erythrocytes have the unique ability to undergo large deformations, allowing them to pass through capillaries and venules that are narrower than their resting cell diameter. Erythrocytes are one of the most deformable cells, and their deformation can take place in natural blood flow. The cellular phenomenon may occur without changes in the erythrocyte membrane but may also involve changes in erythrocyte shape and/or an increase in the area of their cell membranes (Kim et al., 2015) (Figure 1).

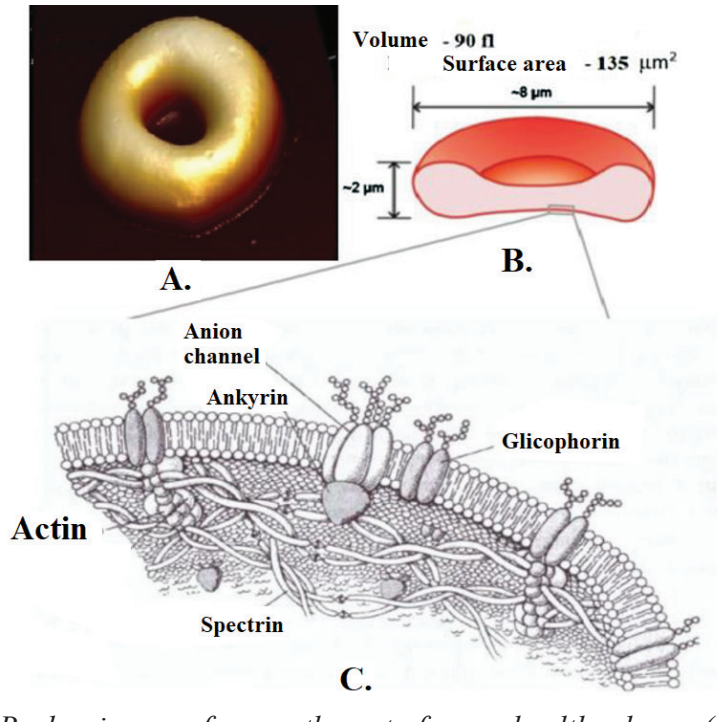

Figure 1. *A. ACM Bruker image of an erythrocyte from a healthy donor (Alexandrova-Uatanabe, 2021); B. Averaged morphological parameters of human erythrocyte (Kim et al., 2015); C. Schematic representation of the membrane structure of a human erythrocyte (Marinov, 2001)*

The deformability of red blood cells plays a vital role in the performance of their primary function – transporting gases (O_2 and CO_2) through blood circulation to tissues and cells in the body. A slight decrease in red blood cell deformability causes a significant increase in microvascular flow resistance and increases blood viscosity, as shown in Figure 2.

Red blood cell deformability alterations during and after physical activity

Healthy red blood cells are easily deformed in response to shear stress in the vascular lumen - during normal blood circulation. This means that healthy erythrocytes possess the quality of "excellent deformability," which facilitates their efficient passage through the microvessels (McMahon, 2019).

In the literature, there is a lot of evidence for RBC deformability alterations during and after physical activities and regular exercises with different frequencies, intensities, and durations (Barodka et al., 2014; Bor-Kucukatay et al., 2003; Bouix et al., 1998; Brzeszczynska et al., 2008; Green et al., 2004; Kuhn et al., 2017; Mairbäurl, 2013; McMahon, 2019; Jordan et al., 1998; Romagnoli et al., 2014; Tsuda et al., 2003; Berzosa et al., 2011; Nikolaidis et al., 2003; Heo et al., 2015; Connes et al., 2004a; Connes, et al., 2004b; Connes, et al., 2004c; Yalcin, et al., 2003; Hardeman et al., 1995; Dimmeler, Zeiher, et al., 2003; Grau et al, 2013; Mohanty et al., 2014; Connes et al., 2013; Tripette et al., 2011; Gleeson, 1998; Tomschi, 2018).

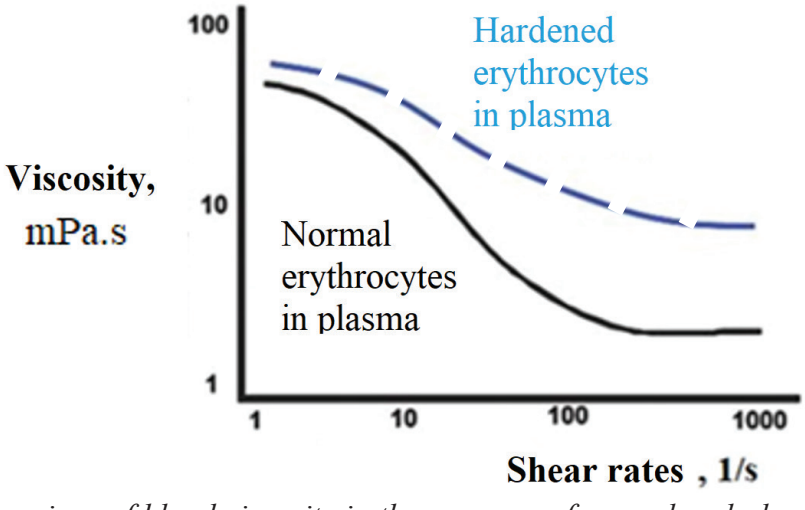

Figure 2. *Comparison of blood viscosity in the presence of normal and „hardened" (with reduced deformability) red blood cells suspended in blood plasma at different rates of deformation*

Geometric shape of erythrocytes

The shape of the human erythrocyte, a doubly concave disk at rest (Figure 1) (without the influence of external mechanical factors), is characterized by a specific but precise cell area/volume (S/V) ratio, which allows significant reversible elastic transformation at any arbitrary shape and ensures the achievement of substantial erythrocyte deformations (Mohandas et al., 1980; Safeukui et al., 2012).

Intense physical exertion changes the conditions of blood flow and thus affects the S/V ratio. Smith et al., 1999 reported the presence of increased erythrocyte deformability in elite cyclists (Australian national racers, n=9). Two weeks prior to blood sampling, athletes underwent training (25–29 hours/week covering 510–631 miles/week). This team of authors found a statistically significant increase in the hematological parameter MCV (mean cell volume, fl) of the studied group - compared to a control group without exercise (Smith et al.,

1999). An increase in this indicator indicates a change in the area/volume ratio of the individual erythrocyte, which is directly related to the deformability of red blood cells.

Another collective (Romagnoli et al., 2014) reported similar results of increased MCV parameter and erythrocyte deformability in a group of ten athletes aged 12–16 years.

Rheological properties of the erythrocyte membrane

Tsuda et al. (2003) reported that aerobic exercise (exercise within the aerobic threshold level performed twice weekly for 6 months) increased erythrocyte membrane deformability and improved membrane viscosity in hypertensive patients. The authors explain the improvement of erythrocyte rheological properties in hypertension with the beneficial effects of physical exercise.

Liu et al. (2018) presented that in male 24-hr ultra-marathoners, there are changes in the RBC membrane viscoelastic properties, a decisive factor of *RBC deformability* (Liu, et al., 2018). These authors obtained that *haptoglobin*, *RBC count*, hemoglobin, hematocrit, mean cell hemoglobin, plasma free hemoglobin, and unsaturated iron-binding capacity values of the recruited runners showed a statistically significant drop in the post-race values. The same study on RBC viscoelastic properties showed that 7 runners had increased viscosity (or less fluidity) after the run, while the 8 runners showed the opposite trend.

Biochemical and structural properties of erythrocyte membrane lipids and proteins

Author collective Jordan et al. (1998) demonstrated (through SEM examination) a disturbed structure of erythrocyte membranes after marathon running. They also found a loss of membrane material after the race compared to erythrocyte membranes before the race. Cell membrane areas of erythrocytes were increased by 30% ($p < .01$). Hemolysis was demonstrated with a 57% decrease in plasma haptoglobin values ($p < .001$). These data show the structural changes in the red blood cell membrane skeletons after the marathon race.

Brzeszczynska et al. (2008) reported that oxidative stress (the accumulation of free radicals that can damage various cells) that developed during an exercise session in untrained subjects (22 ± 2 years; 187 ± 7.84 cm; 87 ± 15, 6 kg; average BMI 25 ± 4.5 kg.m–2) eating a balanced diet causes erythrocyte changes in the form of:

- protein aggregation;
- changed organization of erythrocyte membranes, lipid rigidification due to lipid peroxidation.

Nemkov et al. (2021) described the metabolic and rheological differences in red blood cells sampled from well-trained males before and after a controlled, high-intensity 30 min cycling test. The authors obtained that RBC demonstrated decreased deformability and increased generation of some microparticles after the test (Nemkov et al., 2021).

Hemoglobin (Hb) concentration

In the literature, there is a lot of evidence for the Hb concentration influence on RBC deformability (Liu et al., 2018; Freitag et al., 2020; Grau et al., 2019).

The levels of the hematological and hematometric parameter MCHC (mean cell hemoglobin concentration) reflect the average concentration of hemoglobin in the erythrocyte. With increased MCHC, decreased deformability of red blood cells is observed. The reasons for the increase in this parameter can be determined by both sports and pathological processes (Clark et al., 1978). In addition, reduced deformability of erythrocytes is observed with their „aging“, which is also associated with an increased hemoglobin concentration in the erythrocyte cytoplasm.

A study by Radomski et al. (1980) on hematological changes in soldiers (marched 35 km/day for 6 days, at 35% of own VO_{2max}) showed a decrease in erythrocyte count (RBC) and decreased hematocrit values (Hct). After the 6th training day, the authors reported a decrease in hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean erythrocyte volume (MCV).

Calcium ion concentration (Ca^{2+})

The deformability of red blood cells and the elasticity of their membranes are highly dependent on the cellular concentration of calcium ions (Ca^{2+}) (Huisjes et al., 2018; Mytckan et al., 2018). An increase in the intracellular concentration of Ca^{2+} leads to a decrease in the deformability of red blood cells (Chien, 1987).

There are also conflicting results in the literature, ranging from higher to lower levels of erythrocyte Ca^{2+} concentration, including the lack of change when comparing active athletes to non-athletes (Romero & Romero, 1997).

Nikolaidis et al. (2003) reported the results of interesting observations on the dependence of erythrocyte deformability on calcium ion concentration in erythrocytes of adolescents compared to erythrocytes of adult individuals. This author‘s team reported significantly higher calcium concentrations in adolescents than adults. Regarding the influence of physical activity, however, all athletes included in the study (579 athletes were compared with 241 physically inactive participants) showed significantly higher concentrations of calcium in erythrocytes compared to the corresponding group of non-athletes.

Intracellular diffusion and osmosis (osmotic concentration)

Osmosis is the process of water movement and its entry into cells through a semi-permeable cell membrane. In this process, water molecules move from the less concentrated to the more concentrated intracellular environment. Therefore, osmosis serves to balance both sides of the semipermeable cell membrane by causing the solute-rich environment to be diluted by the solvent, which is water.

French authors (Connes et al., 2004b) investigated the influence of lactate anion on the rheological properties of blood and blood cells. They observed a decrease in erythrocyte deformability when the lactate concentration was artificially (addition of lactate anion in blood samples) or naturally increased (for example, during exercise). A reduction in erythrocyte deformability during exercise has been described in individuals with low physical fitness (Yalcin et al., 2003; Bouix et al., 1998). The author collectives Lipovac et al. (1985) and Reinhart et al. (2000) explained that in vitro addition of lactate or in vivo accumulation of lactate in the blood results in an increase in extra-erythrocyte osmolality.

Existing differences in data regarding the effect of lactate on erythrocyte deformability require further future research and analysis.

Nitric oxide and erythrocyte nitric oxide synthase

Nitric oxide (NO) is synthesized naturally and/or after drug stimulation in endothelial cells from a precursor - the amino acid L-arginine, with the participation of the enzyme endothelial nitric oxide synthase (eNOS) (Palmer et al., 1988). A major physiological stimulus for natural endothelial NO synthesis is increased blood flow through the vessel lumen (Pohl et al., 1986; Rubanyi et al., 1986). Released NO induces vasodilation, which normalizes shear stress (Dimmeler & Zeiher, 2003). At the same time, NO can contribute to the development of hyperemia during physical exertion and sports exercises, which are always associated with

increased blood pressure and increased heart rate. Repeated but moderate exposure to increased shear stress resulting from moderate exercise training can improve NO bioavailability in the body (Green et al., 2004).

The results of a study by Bor-Kucukatay et al. (2003) also demonstrate the regulatory role of NO, which can significantly influence and maintain normal red blood cell deformability – important for controlling and regulating blood flow in the body.

Erythrocyte ATP (adenosine triphosphate)

The maintenance of the geometric shape and deformability of red blood cells depends on the generation of erythrocyte ATP. A significantly reduced ATP concentration is known to contribute to a reduction in erythrocyte deformability.

McMahon (2019) explains that upon deformation, erythrocytes release ATP and a vasodilatory S-nitrosothiol (SNO) group – pre-formed on the hemoglobin (Hb) molecule by NO (Figure 3). The process co-occurs with the release of oxygen into the cells, allowing red blood cells to preferentially target where nutrient needs are most significant. The separated molecules ATP and SNO regulate the microvascular tone in the microcirculation and prevent some processes of intercellular adhesion (Figure 3).

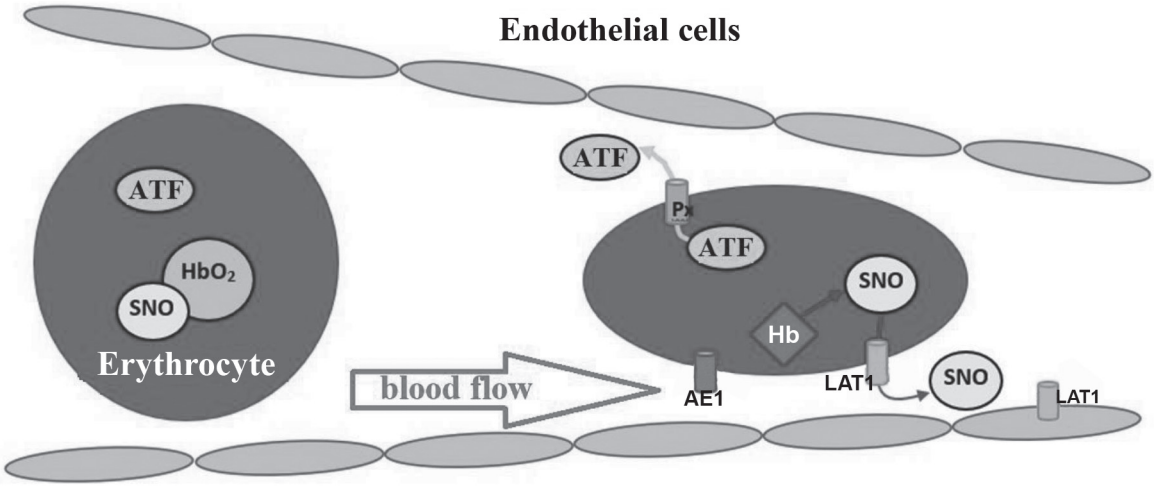

Figure 3. *Expression of vasoactive and anti-adhesive mediators during red blood cell deformation in flow (McMahon, 2019)*

The described physiological/pathophysiological phenomenon enables the adaptive and unhindered flow of erythrocytes downstream, optimizing O_2 delivery and carbon dioxide release (McMahon, 2019). The responsible mediator channel (transporter) for releasing ATP is pannexin 1 (Px1), and for SNO – LAT1 (L-amino acid transport system).

ATP is a macroergic chemical compound that contains energy-rich bonds. This makes it useful for the body of athletes and for all people. In other words, ATP is a compound that cells need to carry out their functions, ranging from DNA synthesis to sending chemical, intercellular signals and nerve impulses. ATP is constantly consumed and regenerated through metabolic pathways in the body, ensuring that the body functions and lives.

From the above, it is clear that, like other cells, red blood cells depend on ATP for energy. Erythrocytes can export ATP in response to hypoxia or after mechanical stress (McMahon, 2019). This means that during intense physical exertion, in which the microcirculation is increased, there will be an increased synthesis and release of ATP from erythrocytes. It is

assumed that the intensity of this process is strictly individual.

Erythrocyte age and degree of erythrocyte cell maturation and differentiation

It has been proven that the increased average age of erythrocytes is a biological factor for their reduced deformability. "Younger" erythrocytes are characterized by improved deformability and tissue oxygen delivery during exercise (Kim & Shin, 2015). Mohanty et al. (2014) demonstrated that oxidative stress plays an essential role in damaging the erythrocyte membrane and disrupting its natural deformability.

Recent studies have shown a linear relationship between red blood cell deformability and oxidative stress measured by the level of degraded blood products (Barodka et al., 2014).

The fact that the so-called endurance training can lead to the condition „sports anemia". Although under normal conditions, red blood cells have a lifespan of 120 days, the rate of cellular aging can be increased as a result of intense and prolonged exercise (Smith, 1995). However, the deficiency of erythrocytes is a rare phenomenon in athletes, and the so-called sports anemia is also due to other factors.

Body and erythrocyte temperature

During intense physical exertion, the heat released from the body can exceed 1000 W (Gleeson, 1998). Some of the released heat is stored, increasing the body temperature in depth by several degrees. An increase in body temperature is sensed by central and skin thermoreceptors, and this sensory information is processed by the hypothalamus to trigger appropriate effector responses.

Training and physical exercise improve body heat tolerance by increasing the sweat/temperature relationship sensitivity, lowering the basal temperature threshold for sweating, and increasing total blood volume (Gleeson, 1998).

Lowering the temperature from 37 °C to 4 °C causes a significant increase in the „shear" modulus of the erythrocyte membrane and changes its viscosity (Kim et al., 2015). These changes in the properties of the erythrocyte membrane lead to a significant decrease in the deformability of red blood cells with a reduction in temperature.

Human body hemorheological adaptation to physical activity

Human body hemorheological adaptation to physical activity is a strongly individual process, which is defined by biological and hemorheological factors, determining RBC deformability and whole hemorheological status before applied physical activity (Ivanov, 2022; Kim et al., 2015)

Physical exertion can change in different directions (positive, negative, neutral) the described factors for different individuals. Simultaneously, the main hemorheological alterations during and after physical activity with different frequencies, intensities, and durations are (Ivanov, 2022):

- changes in erythrocyte deformability and aggregation;
- change in the concentration of basic plasma components —fibrinogen, albumins, globulins, testosterone, etc.;
- changes in blood flow (through vasodilatation and change in overall blood viscosity);
- changes in blood volume;
- changes in the endothelial cells of the vascular walls;
- changes in blood pressure;
- changes as a result of tissue hypoxia;
- interacting, different in nature hemorheological changes.

CONCLUSIONS

The human body's physiological response to moderate and/or intense physical activity depends on the type, duration, intensity, and cyclicity of the physical exercises, as well as on the individual hemorheological, morphological, and fitness status of the individual.

Sports and physical exertion cause changes of different nature and degree (positive or negative, strong, weak, moderate) in the biomechanical and fluid properties of blood and blood cells (Ivanov, 2022).

Physical activity is a proven factor affecting erythrocyte deformability in several directions:

- erythrocyte geometric shape;
- rheological properties of the erythrocyte membrane;
- biochemical and structural properties of erythrocyte membrane lipids and proteins;
- hemoglobin (Hb) concentration;
- calcium ion concentration (Ca^{2+});
- intracellular diffusion and osmosis (osmotic concentration);
- nitric oxide and erythrocyte nitric oxide synthase;
- erythrocyte ATP synthase;
- erythrocyte age and degree of erythrocyte cell maturation and differentiation;
- body and erythrocyte temperature.

In the modern scientific literature, there are data on the proven influence of regular physical exercises on basic hematological, hematometric, and hemorheological indices (parameters) of erythrocytes, on whole blood viscosity (WBV) and blood plasma (PV), on basic biochemical plasma components, on blood pressure, on morphological and functional changes of endothelial cells in vascular walls, etc. (Sirufo et al., 2020; Filar-Mierzwa et al., 2019; Stupin et al., 2019; Härtel et al., 2019; Nemeth, 2022; Mugandani, 2019; Kachaunov, 2018; You, 2022; Ivanov, 2022; Liu et al., 2018; Nemkov et al., 2021; Liu et al., 2018; Freitag et al., 2020; Grau et al., 2019; Huisjes et al., 2018; Mytckan et al., 2018).

Extensive future research is needed to elucidate the individual cellular, tissue, and molecular mechanism of hemorheological changes in blood, blood cells, and blood plasma in the individual. It is essential to study and evaluate the individual hemorheological response profile of erythrocyte deformability during physical activity, as this is a major erythrocyte property determining oxygen supply to working muscles.

The multiple hemorheological changes during physical activity and their interconnectedness and strong personality make their differentiation very difficult. It is reasonable to weigh their effects (with specific quantitative measurements) to simplify the analysis and increase the likelihood of obtaining valid conclusions. But there will always be the probabilistic nature of these conclusions, determined by the complex and strictly individual character of the hemorheological effects of physical exercises and activities.

ACKNOWLEDGMENTS

The author thanks a lot to associate professor d-r Elissaveta Zvetkova from the Bulgarian Academy of Sciences for the valuable advice and in-depth analysis.

REFERENCES

Alexandrova-Uatanabe, A. (2021). Izsledvane na reologichnite, mehanichnite I morfologichnite svoistva na kryvta, neinite formeni elementi I parametri na hemokoagulacijata pri zaharen diabet tip 2. Disertacia za prisudena nauchno-obrazovatelna stepen "doctor", Institut po mehanika, BAN, Sofia, Bulgaria.

Barodka, V. M., Nagababu, E., Mohanty, J. G., Nyhan, D., Berkowitz, D. E., Rifkind, J. M., & Strouse, J. J. (2014). New insights

provided by a comparison of impaired deformability with erythrocyte oxidative stress for sickle cell disease. *Blood Cells, Molecules, and Diseases*, *52*(4), pp. 230-235.

Berzosa, C., Gómez–Trullén, E. M., Piedrafita, E., Cebrián, I., Martínez–Ballarín, E., Miana–Mena, F. J., ... & García, J. J. (2011). Erythrocyte membrane fluidity and indices of plasmatic oxidative damage after acute physical exercise in humans. *European journal of applied physiology*, 111(6), pp. 1127-1133.

Bor-Kucukatay, M., Wenby, R. B., Meiselman, H. J., & Baskurt, O. K. (2003). Effects of nitric oxide on red blood cell deformability. *American Journal of Physiology-Heart and Circulatory Physiology*, *284*(5), H1577-H1584.

Bouix, D., Peyreigne, C., Raynaud, E., Monnier, J. F., Micallef, J. P., & Brun, J. F. (1998). Relationships among body composition, hemorheology and exercise performance in rugbymen. *Clinical Hemorheology and Microcirculation*, *19*(3), pp. 245-254.

Brzeszczynska, J., Pieniazek, A., Gwozdzinski, L., Gwozdzinski, K., & Jegier, A. (2008). Structural alterations of erythrocyte membrane components induced by exhaustive exercise. *Applied Physiology, Nutrition, and Metabolism*, *33*(6), pp. 1223-1231.

Chien, S. (1987). Red cell deformability and its relevance to blood flow. *Annual Review of Physiology*; 49, pp. 177-92.

Clark, M. R., Mohandas, N., Caggiano, V., & Shohet, S. B. (1978). Effects of abnormal cation transport on deformability of desiccytes. *Journal of Supramolecular Structure*, *8*(4), pp. 521-532.

Connes, P., Bouix, D., Durand, F., Kippelen, P., Mercier, J., Prefaut, C., ... & Caillaud, C. (2004a). Is hemoglobin desaturation related to blood viscosity in athletes during exercise?. *International journal of sports medicine*, 25(08), pp. 569-574.

Connes, P., Bouix, D., Py, G., Caillaud, C., Kippelen, P., Brun, J. F., ... & Mercier, J. (2004b). Does exercise-induced hypoxemia modify lactate influx into erythrocytes and hemorheological parameters in athletes?. *Journal of Applied Physiology*, *97*(3), pp. 1053-1058.

Connes, P., Bouix, D., Py, G., Prefaut, C., Mercier, J., Brun, J. F., & Caillaud, C. (2004c). Opposite effects of in vitro lactate on erythrocyte deformability in athletes and untrained subjects. *Clinical hemorheology and microcirculation*, *31*(4), pp. 311-318.

Connes, P., Simmonds, M. J., Brun, J. F., & Baskurt, O. K. (2013). Exercise hemorheology: classical data, recent findings and unresolved issues. *Clinical hemorheology and microcirculation*, 53(1-2), pp. 187-199.

Dimmeler, S. and Zeiher, A. M. (2003). Exercise and cardiovascular health. Get active to AKTivate your endothelial nitric oxide synthase. *Circulation* 107, pp. 3118–3120.

Fedosov, D.A., Peltomäki, M. and Gompper, G. (2014). Deformation and dynamics of red blood cells in flow through cylindrical microchannels. *Soft matter*, *10*(24), pp. 4258-4267.

Filar-Mierzwa, K., Marchewka, A., Dąbrowski, Z., Bac, A., Marchewka, J., (2019). Effects of dance movement therapy on the rheo-logical properties of blood in elderly women. *Clin. Hemorheol. Microcirc. 72*, pp. 211–219.

Freitag, N., Böttrich, T., Weber, P. D., Manferdelli, G., Bizjak, D. A., Grau, M., ... & Schumann, M. (2020). Acute Low-Dose Hyperoxia during a Single Bout of High-Intensity Interval Exercise Does Not Affect Red Blood Cell Deformability and Muscle Oxygenation in Trained Men—A Randomized Crossover Study. *Sports*, 8(1), 4.

Gleeson, M. (1998). Temperature regulation during exercise. International Journal of Sports Medicine, 19(S 2), pp. S96-S99.

Grau, M., Pauly, S., Ali, J., Walpurgis, K., Thevis, M., Bloch, W., & Suhr, F. (2013). RBC-NOS-dependent S-nitrosylation of cytoskeletal proteins improves RBC deformability. *PloS one*, 8(2), e56759.

Grau, M., Jerke, M., Nader, E., Schenk, A., Renoux, C., Collins, B., ... & Prokop, A. (2019). Effect of acute exercise on RBC deformability and RBC nitric oxide synthase signalling pathway in young sickle cell anaemia patients. Scientific reports, 9(1), 1-10.

Green, D. J., Maiorana, A., O'Driscoll, G., & Taylor, R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *The Journal of physiology*, 561(1), pp. 1-25.

Hardeman, M. R., Peters, H. P. F., & Goedhart, P. T. (1995). Low hematocrit and plasma fibrinogen in trained athletes increase hemorheological tolerance for physical stress. *Clinical Hemorheology*, *3*(15), pp. 506.

Härtel, J.A., Müller, N., Herberg, U., Breuer, J., Bizjak, D.A., Bloch, W., Grau, M. (2019). Altered Hemorheology in Fontan Patients in Normoxia and After Acute Hypoxic Exercise. *Front. Physiol.*, 10, 1443. https://doi.org/10.3389/fphys.2019.01443.

Heo, Y., Jung, H., & Shin, S. (2015). Osmotic deformability of erythrocytes at various shear stresses. *Clinical Hemorheology and Microcirculation*, 59(3), pp. 211-218.

Huisjes, R., Bogdanova, A., Van Solinge, W. W., Schiffelers, R. M., Kaestner, L., & Van Wijk, R. (2018). Squeezing for life–properties of red blood cell deformability. *Frontiers in physiology*, *9*, 656.

Ivanov, I. (2022). Hemorheological Alterations and Physical Activity, *Applied Sciences* 12, no. 20: 10374. https://doi.org/10.3390/app122010374.

Jordan, J., Kiernan, W., Merker, H. J., Wenzel, M., & Beneke, R. (1998). Red cell membrane skeletal changes in marathon runners. *International journal of sports medicine*, *19*(01), 16-19.

Kachaunov, M. (2018). Post-competition blood lactate concentration in swimmers. *Journal of Applied Sports Sciences*, *1*, pp. 30-36.

Kim, J., Lee, H. and Shin, S. (2015). Advances in the measurement of red blood cell deformability: A brief review. *Journal of Cellular Biotechnology*, *1*(1), pp. 63-79.

Kuhn, V., Diederich, L., Keller IV, T. S., Kramer, C. M., Lückstädt, W., Panknin, C., ... & Cortese-Krott, M. M. (2017). Red blood cell function and dysfunction: redox regulation, nitric oxide metabolism, anemia. *Antioxidants & redox signaling*, *26*(13), pp. 718-742.

Lipovac, V., Gavella, M., Turk, Z., & Škrabalo, Z. (1985). Influence of lactate on the insulin action on red blood cell filterability. *Clinical Hemorheology and Microcirculation*, *5*(5), pp. 421-428.

Liu, C. H., Tseng, Y. F., Lai, J. I., Chen, Y. Q., Wang, S. H., Kao, W. F., ... & Chang, W. H. (2018). The changes of red blood cell viscoelasticity and sports anemia in male 24-hr ultra-marathoners. Journal of the Chinese Medical Association, 81(5), 475-481.

Mairbäurl, H. (2013). Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. *Frontiers in physiology*, *4*, p. 332.

Marinov, M. (2001). *Biofizika*. WM – OFSET, Sofia, pp. 214 – 236.

McMahon, T. J. (2019). Red blood cell deformability, vasoactive mediators, and adhesion. *Frontiers in physiology*, *10*, p. 1417.

Mohandas, N., Clark, M. R., Jacobs, M. S., & Shohet, S. B. (1980). Analysis of factors regulating erythrocyte deformability. *The Journal of clinical investigation*, *66*(3), 563-573.

Mohanty, J. G., Nagababu, E., & Rifkind, J. M. (2014). Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. *Frontiers in physiology*, 5, pp. 84.

Mugandani, S. C. (2019). Athletic performance enhancing ACE, ACTN3, AMPD1 genetic markers, fitness characteristics, c-reactive protein and uric acid of cricket, netball, rugby and soccer players: a review. *Journal of Applied Sports Sciences*, (1), pp. 131-149.

Mytckan, B. M., Verbovyi, V. P., Chovhan, R. Y., Zemska, N. O., Kryzanivskaya, O. F., Bublyk, S. A., ... & Baskevich, O. V. (2018). Influence of physical activity of the maximum aerobic power on hemo-dynamic and morpho-biochemical of change of erythrocytes of female volleyball players. *Pedagogics, psychology, medical-biological problems of physical training and sports*, (5), 272-279.

Nemeth, N. (2022). *Hemorheology and Metabolism*; MDPI: Basel, Switzerland.

Nemkov, T., Skinner, S. C., Nader, E., Stefanoni, D., Robert, M., Cendali, F., ... & D'Alessandro, A. (2021). Acute cycling exercise induces changes in red blood cell deformability and membrane lipid remodeling. International journal of molecular sciences, 22(2), 896.

Nikolaidis, M.G., Protosygellou, M.D., Petridou, A., Tsalis, G., Tsigilis, N. and Mougios, V. (2003). Hematologic and biochemical profile of juvenile and adult athletes of both sexes: implications for clinical evaluation. *International journal of sports medicine*, 24(07), pp. 506-511.

Palmer, R. M., Rees, D. D., Ashton, D. S., & Moncada, S. (1988). L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochemical and biophysical research communications*, *153*(3), pp. 1251-1256.

Pohl, U., Holtz, J., Busse, R., & Bassenge, E. (1986). Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension*, *8*(1), pp. 37-44.

Radomski, M. W., Sabiston, B. H., & Isoard, P. (1980). Development of „sports anemia" in physically fit men after daily sustained submaximal exercise. *Aviation, Space, and Environmental Medicine*, *51*(1), pp. 41-45.

Reinhart, W. H., Gaudenz, R., & Walter, R. (2000). Lactate and pyruvate increase blood viscosity. *JOURNAL DES MALADIES VASCULAIRES*, *25*, pp. 171-171.

Romagnoli, M., Alis, R., Martinez-Bello, V., Sanchis-Gomar, F., Aranda, R., & Gómez-Cabrera, M. C. (2014). Blood rheology effect of submaximal exercise on young subjects. *Clinical hemorheology and microcirculation*, 56(2), pp. 111-117.

Romero, P.J., & Romero, E.A. (1997). Differences in Ca^{2+} pumping activity between sub-populations of human red cells. *Cell calcium*, *21*(5), pp. 353-358.

Rubanyi, G. M., Romero, J. C., & Vanhoutte, P. M. (1986). Flow-induced release of endothelium-derived relaxing factor. *American Journal of Physiology-Heart and Circulatory Physiology*, *250*(6), H1145-H1149.

Safeukui, I., Buffet, P. A., Deplaine, G., Perrot, S., Brousse, V., Ndour, A., ... & Mohandas, N. (2012). Quantitative assessment of sensing and sequestration of spherocytic erythrocytes by the human spleen. *Blood, The Journal of the American Society of Hematology*, *120*(2), pp. 424-430.

Sirufo, M.M., Ginaldi, L., De Martinis, M. (2020). Nailfold capillaroscopic findings in a semi-professional volleyball player. *Clin. Hemorheol. Microcirc.*, *74*, pp. 281–285. https://doi.org/10.3233/CH-190667.

Smith, J.A. (1995). Exercise, training and red blood cell turnover. *Sports medicine*, 19(1), pp. 9-31.

Smith, J. A., Martin, D. T., Telford, R. D. & Ballas, S.K. (1999). Greater erythrocyte deformability in world-class endurance athletes. *American Journal of Physiology-Heart and Circulatory Physiology*, 276(6), pp. H2188-H2193.

Stupin, M., Kibel, A., Stupin, A., Selthofer-Relatić, K., Matić, A., Mihalj, M., Mihaljevic, Z., Jukic, I., Drenjančević, I. (2019). The physiological effect of n-3 polyunsaturated fatty acids (n-3 PUFAs) intake and exercise on hemorheology, microvascular function, and physical performance in health and cardiovascular diseases; Is there an interaction of exercise and dietary n-3 PUFA intake? *Front. Physiol.*, *10*, 1129.

Tomschi, F., Bloch, W., & Grau, M. (2018). Impact of type of sport, gender and age on red blood cell deformability of elite athletes. International journal of sports medicine, 40(01), 12-20.

Tripette, J., Hardy-Dessources, M. D., Beltan, E., Sanouiller, A., Bangou, J., Chalabi, T., ... & Connes, P. (2011). Endurance running trial in tropical environment: A blood rheological study. Clinical hemorheology and microcirculation, 47(4), pp. 261-268.

Tsuda, K., Yoshikawa, A., Kimura, K. & Nishio, I. (2003). Effects of mild aerobic physical exercise on membrane fluidity of erythrocytes in essential hypertension. *Clinical and experimental pharmacology and physiology*, 30(5-6), pp. 382-386.

Yalcin, O., Erman, A., Muratli, S., Bor-Kucukatay, M., & Baskurt, O. K. (2003). Time course of hemorheological alterations after heavy anaerobic exercise in untrained human subjects. *Journal of Applied Physiology*, 94(3), pp. 997-1002.

You, J. A. (2022). Study on the Effect of a Regular Exercise Habit on Health-Related Quality of Life in Adults with Cerebral Palsy. *Appl. Sci.*, *12*, 9068. https://doi.org/10.3390/app12189068.

Corresponding author:

Ivan M. Ivanov, PhD
Anatomy and Biomechanics Department
National Sports Academy "Vassil Levski"
21, Acad. Stefan Mladenov str.
Sofia, Bulgaria
E-mail: ivanmirchev@abv.bg