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Rheological and Biochemical Properties of Blood in Runners: A Preliminary Report

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Abstract: Purpose: Physical activity induces numerous modifications in the morphological, rheological, and biochemical properties of blood. The purpose of this study was to evaluate changes in blood rheological and biochemical indicators among runners. Also, we assessed how the rheological and biochemical properties of blood in people who practised running characterised the range and direction of exercise modifications and allowed for the diagnosis of transient adaptive effects. Methods: This study included 12 athletes who regularly trained in middle- and long-distance running (6–8 times a week) and presented a high sports level (national and international class). The athletes performed a 30 min warm-up consisting of 15 min of jogging and exercises. After a 10 min rest, they completed a 3 km run with submaximal effort. Blood samples were collected at baseline and after the effort. Results: No statistically significant changes were revealed in erythrocyte, leukocyte, platelet, iron, ferritin, transferrin, erythropoietin, or C-reactive protein concentrations in the examined runners. The same applied to the elongation index at a shear stress within the range of 0.30–60.00 Pa, amplitude and total extent of aggregation, aggregation half-life, and aggregation index. A significant increase (within standard limits) was only observed in fibrinogen concentration after running. Conclusions: The lack of post-exercise changes in blood rheological and biochemical indicators in the investigated runners points at an efficient haemorheological system. This, in turn, reflects well-executed training and remarkably well-trained adaptive systems responsible for regeneration.

Keywords: athletes; blood rheology; iron; ferritin; erythropoietin

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1. Introduction

Physical training induces adaptive changes in the blood cell system, and the magnitude of these changes depends on the level of training and on the effort that the body is subjected to [1]. The values of haematological indicators are lower in well-trained athletes of endurance disciplines than in those of strength disciplines or non-trained individuals [2]. Subjects presenting high physical activity typically exhibit high erythrocyte deformability, which is mainly associated with erythrocyte form and the viscoelastic properties of the erythrocyte membrane [3]. Physical capacity depends on efficient blood flow through arteries, veins, and capillaries. With an average diameter of 6–8 µm and a thickness of 1.7–2.5 µm, erythrocytes must pass through a network of minute capillaries 1–2.5 µm in diameter to fulfil their transport function [4]. Blood flow is affected by blood rheological properties, which are determined by a number of factors. The most essential ones include whole-blood viscosity, plasma viscosity, haematocrit, red blood cell deformability, red blood cell aggregation, and fibrinogen concentration [5]. Blood viscosity is mainly related to the amount and molecular structure of plasma proteins and to water content. Traditionally, studies concerning exercise haemorheology documented reduced blood fluidity (hyperviscosity) during short-term exercise and improved blood fluidity in conditions of regular exercise practise (haemorheologic fitness). This gave rise to the concepts of 'the triphasic effects of exercise', 'the paradox of haematocrit', and 'the haemorheological paradox of lactate'. It must be admitted, though, that some training studies do not corroborate this classical perception and indicate that blood viscosity phenomena do not necessarily follow a straight paradigm resulting from the Hagen–Poiseuille law [6].

The blood of athletes training in endurance disciplines is characterised by a lower density than that of non-training individuals [7]. It remains unclear, however, to what extent, from the point of view of both health and athletic performance, a reduction in blood viscosity can be beneficial to the human body [5]. Tomschi et al. [8] subjected young men with different levels of training and physical capacity to a cardiac stress test. The results demonstrated that highly trained individuals had a greater number of young red blood cells and presented a higher red blood cell deformability. There were no differences in mean platelet volume, red blood cell volume, haematocrit, and mean cell volume between groups with different levels of physical capacity. Neither the moderate nor the intensive stress test changed the deformability of red blood cells. Plasma viscosity only increased after intense effort and remained unchanged during moderate effort. Haematological changes were observed in both stress tests. It appears that erythrocyte alterations under a single effort are marginal, but training modifies red blood cell function. In turn, Bilski et al. [9] revealed an increase in the aggregation index, a reduction in aggregation half-life, and a decrease in the elongation index as a result of physical effort.

Evaluations of haemorheological properties can detect symptoms of physical training overload at an early stage. In addition, these can be applied to optimise the training load in athletes [10]. Teległów et al. [11] observed that despite the differences in blood rheological properties and biochemical markers among triathletes (1.5 km swimming, 36 km cycling, and 10 km mountain running or 3.8 km swimming, 180 km cycling, and 44 km running), these indicators remained within the normal ranges for the general population and might be indicative of normal functioning of the subjects' bodies. Determining effort intensity, or, in other words, the response to effort, by means of blood lactate concentration allows for the appropriate selection of training loads in high-performance athletes. A study monitoring 8-week direct competition conditioning in middle-distance athletes was also conducted. Applying predefined intensity thresholds with lactate concentration analysis is also a means of monitoring training [12].

The purpose of the presented study was to evaluate changes in blood rheological and biochemical indicators among runners who were in the final phase before preparing for the Polish championships and presented with very good physical performance. Also, we assessed how the rheological and biochemical properties of blood in people who practised running characterised the range and direction of exercise modifications and allowed for the diagnosis of transient adaptive effects.

2. Material and Methods

2.1. Characteristics of the Research Group

This study included 12 athletes who regularly trained in middle- and long-distance running (6–8 times a week) and presented a high sports level (national and international class) (Table 1). Among them, 10 competitors achieved an average result in an 800 m run of 1:55.2 (1:49.4–2:05.8) and 2 achieved 3:56.67 and 4:11.0 for a distance of 1500 m. Tests were not available during the competition season, as the competitors would have been interrupting their training cycle before the Polish Cross-Country Championships. The players represented two sports clubs, Krakow Athletics Team and AZS AWF Krakow, which represent the best competitions in this part of Poland.

Table 1. Characteristics of the studied runners.

Parameter	Mean ± Standard Deviation
Age, years	24.927 ± 1.929
Body mass, kg	69.75 ± 6.717
Body height, cm	179.75 ± 6.137

The analysed males were Poland's top athletes, winners of numerous medals at national championships in various age categories. Half of them were former or current international representatives of Poland. One athlete was invited to study at and received a scholarship to a university in the USA for his achievements in the junior category. Each of the study participants competed in Polish championships, academic or within their age categories, in the year of this research.

The coaches who took care of the examined athletes on a daily basis presented a very high professional level. Two of them hold Class I and one holds the Master Class of the Polish Athletics Association. Each has led the Polish national team in middle- or long-distance running or in race walking. One of the coaches is known to have a very rich sporting background. He is a triple Olympian, bronze medallist at world championships, and silver medallist at European championships. He won 34 Polish championship titles in various age categories. He was also awarded the Silver Cross of Merit by the President of the Republic of Poland.

2.2. Intervention

The athletes performed a 30 min warm-up consisting of 15 min of jogging and exercises. After a 10 min rest, they completed a 3 km run with submaximal effort. Heart rate was measured by using pulse oximeters: Polar (Vantage V, Vantage M) and Garmin (Fenix 6). In addition, 2 min after the effort, plasma lactate concentration was determined with a Lactate Scout device. The mean post-exercise lactate level was 8.0 mmol/L (6.6–9.2 mmol/L) with a mean heart rate of 191 bpm (202–184 bpm).

A medical doctor and a nurse supervised all the interventions. Blood samples of 10 mL were collected from all runners from the cubital vein in a sitting position into Vacuette EDTA K2 vacuum tubes, at baseline and after the effort, by a qualified nurse. Rheological blood indicators were evaluated in the Blood Physiology Laboratory of the University of Physical Education in Krakow and in the Diagnostyka S.A. laboratory in Krakow, Poland.

2.3. Ethical Considerations

Our investigations conformed to the tenets of the Declaration of Helsinki. This study was approved by the Ethical Committee at the Regional Medical Chamber in Krakow, Poland (approval No.: 70/KBL/OIL/2011). Informed consent was obtained from all subjects involved in the study. The authors declare no potential conflicts of interest.

2.4. Morphological, Biochemical, and Rheological Assessments

Complete blood counts were determined by using an ADVIA 2120i analyser (Siemens Healthineers, Erlangen, Germany). This included white blood cell count ($\times 10^9$/L), neutrocyte count ($\times 10^9$/L), lymphocyte count ($\times 10^9$/L), monocyte count ($\times 10^9$/L), eosinocyte count ($\times 10^9$/L), basophil count ($\times 10^9$/L), red blood cell count ($\times 10^{12}$/L), haemoglobin concentration (g/dL), haematocrit (%), mean corpuscular volume (fL), mean corpuscular haemoglobin (pg), mean corpuscular haemoglobin concentration (g/dL), red blood cell distribution width (fL), platelet count ($\times 10^9$/L), mean platelet volume (fL), procalcitonin concentration (%), and platelet distribution width (fL).

Parameters of blood rheology, such as aggregation index (%), amplitude and total extent of aggregation (arbitrary units), half-life of total aggregation (s), and deformability of red blood cells (elongation index), were tested with a Lorrca Maxsis analyser (Lorrca, RR Mechatronics, The Netherlands); the method described by Hardeman and Baskurt [13] was applied. Lorrca is a functional analyser that automatically determines numerous red blood cell rheological parameters. The mean elongation index was plotted with the shear stress values within the range of 0.30–60.00 Pa. Fibrinogen concentration (g/L) was assessed with a BCS Siemens coagulation analyser.

A Roche/Hitachi Cobas c 501 module 6000 biochemical analyser (Roche Diagnostics, Basel, Switzerland) served to measure the following blood biochemical indicators in plasma: iron (µmol/L), transferrin (mg/dL), ferritin (ng/mL), creatine kinase MB isoenzyme activity (U/L), creatine kinase MB isoenzyme mass (ng/mL), erythropoietin (mIU/mL). To evaluate C-reactive protein (an acute-phase protein) concentration, the immunonephelometric method was applied with a BN ProSpec nephelometer ((Siemens Healthcare Diagnostics Inc., Erlangen, Germany)).

2.5. Statistical Analysis

The data are presented as mean values and standard deviation. The normality of the variable distribution was verified with the Shapiro–Wilk test. Dependent variables were compared by using Student's *t*-test for related variables. A significance level of $\alpha = 0.05$ was assumed in the analyses. The analyses were performed with the Statistica 13 software package (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

The complete blood count results are presented in Table 2. No statistically significant changes were observed between the examinations at baseline and after the effort.

Table 2. Morphological parameters (mean values ± standard Deviation) in the examined runners before and after the effort.

Indicator	Before	After	*p*-Value for Student's *t*-Test
RBC, 10^{12}/L	5.008 ± 0.317	5.049 ± 0.313	>0.05
HGB, g/L	15.025 ± 0.838	15.167 ± 0.819	>0.05
HCT, %	44.117 ± 2.424	44.508 ± 2.647	>0.05
MCV, fL	88.192 ± 3.238	88.242 ± 3.758	>0.05
MCH, pg	30.033 ± 1.120	30.067 ± 1.290	>0.05
MCHC, g/dL	34.075 ± 0.884	34.100 ± 1.370	>0.05
RDW-SD, fL	40.508 ± 2.065	40.525 ± 2.202	>0.05
RDW-CV, %	12.617 ± 0.506	12.775 ± 0.954	>0.05
WBC, 10^9/L	5.892 ± 1.396	6.026 ± 1.534	>0.05
NEU, 10^9/L	2.884 ± 0.890	3.263 ± 1.430	>0.05
LYM, 10^9/L	2.238 ± 0.606	1.990 ± 0.546	>0.05
MONO, 10^9/L	0.537 ± 0.136	0.562 ± 0.155	>0.05
EOS, 10^9/L	0.192 ± 0.090	0.171 ± 0.083	>0.05
BASO, 10^9/L	0.041 ± 0.017	0.041 ± 0.015	>0.05
NEU, %	48.583 ± 7.865	52.600 ± 9.892	>0.05
LYM, %	38.233 ± 7.039	34.458 ± 9.893	>0.05
PLT, 10^9/L	217.417 ± 47.773	216.583 ± 39.014	>0.05
PDW, fL	13.250 ± 2.796	13.625 ± 2.816	>0.05
MPV, fL	10.892 ± 1.141	10.933 ± 1.140	>0.05
P-LCR, %	31.842 ± 9.272	32.625 ± 9.450	>0.05
PCT, %	0.250 ± 0.052	0.242 ± 0.051	>0.05

RBC—red blood cell; HGB—haemoglobin; HCT—haematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular haemoglobin; MCHC—mean corpuscular haemoglobin concentration; RDW-SD—red blood cell distribution width standard deviation; RDW-CV—red blood cell distribution width coefficient of variation; WBC—white blood cell; NEU—neutrocyte; LYM—lymphocyte; MONO—monocyte; EOS—eosinocyte; BASO—basophil; PLT—platelet; PDW—platelet distribution width; MPV—mean platelet volume; P-LCR—platelet-large cell ratio; PCT—procalcitonin.

The assessment of red blood cell deformability indicators is illustrated in Table 3. No statistically significant changes were determined in the elongation index between the baseline and post-exercise examinations. As for the red blood cell aggregation parameters, no statistically significant changes were revealed in the aggregation half-life or aggregation index. However, a statistically significant difference was observed between the baseline and post-effort status in fibrinogen concentration. After running, the mean fibrinogen level increased from 2.382 to 2.595 g/L ($p = 0.024$).

Table 3. Red blood cell rheological parameters (mean values ± standard deviation) in the examined runners before and after the effort.

Indicator	Before	After	*p*-Value for Student's *t*-Test
EI at 0.30 Pa	0.051 ± 0.006	0.050 ± 0.006	>0.05
EI at 0.58 Pa	0.148 ± 0.011	0.147 ± 0.011	>0.05
EI at 1.13 Pa	0.233 ± 0.011	0.232 ± 0.012	>0.05
EI at 2.19 Pa	0.339 ± 0.009	0.337 ± 0.012	>0.05
EI at 4.24 Pa	0.434 ± 0.009	0.434 ± 0.011	>0.05
EI at 8.23 Pa	0.512 ± 0.008	0.512 ± 0.011	>0.05
EI at 15.95 Pa	0.563 ± 0.007	0.563 ± 0.008	>0.05
EI at 30.94 Pa	0.599 ± 0.008	0.599 ± 0.007	>0.05
EI at 60.00 Pa	0.629 ± 0.007	0.628 ± 0.005	>0.05
AMP, au	35.675 ± 2.530	36.440 ± 2.665	>0.05
AI, %	48.296 ± 8.058	47.775 ± 8.750	>0.05
T1/2, s	4.533 ± 1.566	4.689 ± 1.606	>0.05
FIBR, g/L	2.382 ± 0.257	2.595 ± 0.302	0.024 *

EI—elongation index; AMP—amplitude and total extent of aggregation; AI—aggregation index; T1/2—aggregation half-life; FIBR—fibrinogen; * significant difference ($p < 0.05$).

There were no statistically significant changes in the blood biochemical indicators between the examinations at baseline and after the effort (Table 4).

Table 4. Selected biochemical parameters (mean values ± standard deviation) in the examined runners before and after the effort.

Indicator	Before	After	*p*-Value for Student's *t*-Test
Iron, µmol/L	17.425 ± 7.826	16.792 ± 4.591	>0.05
Transferrin, mg/dL	261.917 ± 30.315	268.833 ± 33.499	>0.05
Ferritin, ng/mL	57.917 ± 28.916	59.017 ± 28.477	>0.05
CK-MB activity, U/L	18.700 ± 4.506	19.158 ± 4.937	>0.05
CK-MB mass, ng/mL	5.341 ± 2.960	6.661 ± 4.406	>0.05
Erythropoietin, mIU/mL	9.938 ± 3.703	9.758 ± 3.740	>0.05
hs-CRP, mg/L	0.331 ± 0.177	0.563 ± 0.784	>0.05

CK-MB—creatine kinase MB isoenzyme; hs-CRP—high-sensitivity C-reactive protein.

4. Discussion

Physical activity induces numerous modifications in the morphological, rheological, and biochemical properties of blood. Investigating these blood properties allows us to characterise the actual extent and direction of exercise changes in runners. The results obtained in the presented study indicate that the morphological, rheological, and biochemical blood indicators did not change in the athletes. Most likely, as a result of the applied training, the bodies of trained athletes adapt their haemodynamic response to the current conditions and the tissue demand for blood flow. The selection of appropriate training loads, as well as the quick regeneration after the analysed training units, confirm the very high level of training and regeneration. Even submaximal and maximal competition efforts did not result in a significant disturbance in the biochemical or morphological blood parameters. This only confirms that outstanding athletes, at a high sporting level, present a much higher adaptability to physical effort and display enormous regeneration efficiency.

Ernst [14] conducted one of the first studies to explore the influence of regular physical activity on blood rheological properties. Blood samples were collected from 14 training individuals, and a comparison with a control group of 12 sedentary males was performed.

The author investigated plasma viscosity and the degree of erythrocyte deformability. In the experimental group, plasma viscosity was reduced and erythrocyte deformability was improved. After 3 months of training, the control group presented similar blood rheological parameters to the group of athletes. The three suggested conclusions were as follows: (1) in athletes, beneficial blood rheological properties may enhance blood flow in working muscles, increasing work output; (2) the received outcomes support the concept of a link between blood rheological properties and arteriogenesis; (3) regular physical activity may increase blood flow in patients with ischaemic vascular diseases. According to Brun et al. [6], moderately high haematocrit and erythrocyte stiffness values induced by high-intensity exercise are likely to induce physiological vasodilation, thus improving cardiovascular adaptation. As observed by Tripette et al. [15], a 10,000 m run had no effect on the athletes' blood rheological properties, which corroborates the findings of our study. Brun et al. [16,17] state that the mechanism causing disturbances in erythrocyte deformability during prolonged physical effort with high oxygen consumption consists of oxidative stress resulting from the production of free oxygen radicals, originating in the mitochondria, leukocytes, or temporary tissue hypoxia. It has been reported that during exercise, an increase in erythrocyte stiffness is observed, followed by a gradual return to baseline. Another cause of impaired deformability is a rise in lactate concentration above 4 mmol/L as a product of anaerobic glycolysis. Such a trend was noticed in the present study. Nevertheless, the erythrocytes of a trained individual are adapted to cope with lactate (and oxidative stress), which manifests as no reduction in deformability during exercise [18].

In turn, Nader et al. [19] determined blood parameters in competitive runners covering a distance of 10 km. The mean corpuscular volume increased, and haemoglobin concentration, as reflected by the mean corpuscular haemoglobin concentration indicator, decreased after exercise, which may suggest poor blood cell hydration. The deformability of red blood cells increased, and the level of red blood cell aggregation and plasma viscosity decreased, while haematocrit remained unchanged. The effort did not affect eryptosis markers, and the 10 km run did not have an impact on blood cell ageing. The increased deformability of red blood cells occurred, according to the researchers, as a result of rehydration, which led to a decrease in blood viscosity.

In a study involving endurance athletes, Nader et al. [20] reported a difference between cycling and running effort. A cycle ergometer test resulted in a rise in blood viscosity and red blood cell aggregation and had no significant effect on red blood cell deformability. During the run, blood viscosity did not change, but red blood cell deformability increased. This dissimilarity in the rheological properties of red blood cells while running and cycling may be due to a decrease in the arterial partial pressure of oxygen (hypoxaemia) during running.

Robert et al. [21] observed different rheological properties of blood in runners taking part in shorter- and longer-distance mountain runs. Red blood cell deformability at high shear forces and haematocrit decreased after a long-distance ultramarathon (171 km), but did not change after a 40 km run. Haemoglobin concentration and haemolysis rate remained unchanged over both distances. Erythrocyte aggregation and blood viscosity only increased for the shorter run. Lower blood viscosity in mountain ultramarathon runners facilitates blood flow to the muscles.

In a study involving runners who participated in a 24 h ultramarathon, Liu et al. [22] reported decreased concentrations of erythrocytes, haemoglobin, haematocrit, mean corpuscular haemoglobin, free haemoglobin, and iron (unsaturated iron-binding capacity) after the race completion. Reticulocyte and ferritin concentrations were significantly higher after the effort. Of the 19 runners, 15 exhibited changes in the elasticity and viscosity of red blood cells, which were associated with altered concentrations of red blood cells, as well as haemoglobin and haematocrit.

In turn, Spodaryk and Kopec [23] pointed out that endurance training modified serum ferritin concentration, as well as iron stores, in 17 male marathoners of the Polish national team. In the athletes preparing for the main sports season, a gradual reduction in ferritin level was found (from 64.2 ± 17.8 to 53.8 ± 17.8 μg/L), and iron stores reached the lowest level of 3.3 ± 0.9 mg/kg in the competition period (the end of the season). At the same time, no considerable differences in the main haematological parameters (haemoglobin concentration, haematocrit, erythrocyte count, and erythrocyte indicators) were reported during the consecutive periods of the training season. In the competition period, the iron store reduction was significant but not indicative of prelatent anaemia.

The presented study revealed no statistically significant changes in erythrocyte, leukocyte, platelet, iron, ferritin, transferrin, erythropoietin, or C-reactive protein concentrations in the examined runners who were in the final phase before preparing for the Polish championships and presented with very good physical performance. The same applied to the elongation index at a shear stress within the range of 0.30–60.00 Pa, amplitude and total extent of aggregation, aggregation half-life, and aggregation index. The absence of post-exercise changes in the rheological and biochemical indicators in our study is indicative of an efficient haemorheological system with a cooperating cardiovascular system. As implied by Neuhaus and Gaehtgens [3], this is largely due to the careful maintenance of extravascular and intravascular water balance and adequate electrolyte control. Simultaneously, the observed condition may reflect the harmlessness of the administered load or at least the ability to perform a prolonged run without signs of overtraining [6]. Athletes aged 12–18 years who practise cyclical sports (marathon running, skiing), both during the period of high-intensity training and at rest, are characterised by a lower arteriovenular coefficient than power sports representatives, the results being 0.53 ± 0.14 and 0.81 ± 0.1, respectively ($p < 0.05$). This phenomenon is due to the considerable dilation of a section of the capillary bed [4]. The oxygen supply system of muscle cells during exercise ensured efficient blood flow, represented by the rheological and biochemical blood indicators in this study. The lack of changes at the rheological level illustrates functional improvement. A significant increase (within standard limits) was only observed in fibrinogen concentration after the run. Fibrinogen is among the most crucial plasma proteins produced in the liver, and plasma proteins are essential for erythrocyte rouleaux formation. Increased levels of plasma proteins, especially fibrinogen, extend red blood cell aggregation with exercise [13], but this trend was not observed in our study. Brun et al. [16] consider the impact of training on fibrinogen concentration to be controversial as it depends on the molecule genetic subtype, which may explain the lack of negative correlations between fibrinogen levels and fitness or insulin sensitivity.

Tomschi et al. [8] subjected young males with different levels of training and physical capacity to a cardiac stress test. They reported that highly trained individuals were characterised by more young red blood cells and higher erythrocyte deformability. In groups with different levels of physical capacity, there were no differences in the platelet volume, red blood cell volume, haematocrit, or mean cell volume. The moderate and intensive cardiac stress tests did not modify erythrocyte deformability, a result similar to that obtained in the present study. Plasma viscosity only increased after intense effort, while remaining unchanged during moderate effort. Haematological changes were observed in both cardiac stress tests. It seems that erythrocyte changes influenced by a single effort are marginal, but training modifies red blood cell function. Freitag et al. [24] also did not report statistically significant changes in red blood cell deformability (elongation index) during high-intensity interval effort under hyperoxia and in normal ambient conditions. They observed a decrease in the ratio of elongation index values at half and maximum shear forces under normal conditions, as well as no change under hyperoxia.

In summary, there are many unresolved issues related to blood rheology in athletes and its positive and negative adaptive changes following physical effort in runners. The differences in research results may also be due to the use of varying research methods, instruments, and degrees of effort, or even to the subjects' age or sex. The existing research provides the basis for future studies that will aim to determine the relationship between training intensity and blood rheological properties in athletes compared with a control group. Of note, recent reports by Tønnessen et al. [1] provide novel information on the quantitative and qualitative aspects of training session patterns with different intensities applied by Norwegian athletes successful in Olympic endurance sports.

As pointed out by Brun et al. [16], when exploring blood rheological properties, one has to consider the short-, intermediate-, and long-term influence of exercise. These authors concluded that the most beneficial rheological changes were brought about by the first and the second types of exercise.

5. Practical Applications

The unchanged blood rheological and biochemical indicators in runners suggest that the application of proper athlete training by coaches results in improved sports performance. These findings are valuable for both athletes and coaches. There is a need to control sports preparation and medical conditions in runners. Rheological testing should constitute a routine check of training effects in this group.

6. Conclusions

The lack of post-exercise changes in blood rheological and biochemical indicators in the runners observed in the presented study points at an efficient rheological system. This, in turn, reflects well-executed training and remarkably well-trained adaptive systems responsible for regeneration.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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